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Development of *o*-Chlorophenyl Substituted Pyrimidines as Exceptionally Potent Aurora Kinase Inhibitors

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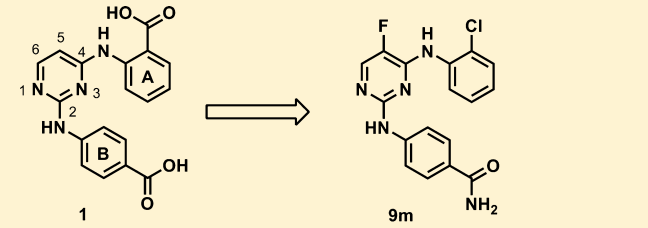
Supporting Information

ABSTRACT: The *o*-carboxylic acid substituted bisanilinopyrimidine **1** was identified as a potent hit (Aurora A IC_{50} = 6.1 ± 1.0 nM) from in-house screening. Detailed structure−activity relationship (SAR) studies indicated that polar substituents at the para position of the B-ring are critical for potent activity. X-ray crystallography studies revealed that compound **1** is a type I inhibitor that binds the Aurora kinase active site in a DFG-in conformation. Structure−activity guided replacement of the A-ring carboxylic acid with halogens and incorporation of fluorine at the pyrimidine 5-position led to highly potent inhibitors of Aurora A that bind in a DFG-out conformation. B-Ring modifications were undertaken to improve the solubility and cell permeability. Compounds such as **9m** with water-solubilizing moieties at the para position of the B-ring inhibited the autophosphorylation of Aurora A in MDA-MB-468 breast cancer cells.



Aurora A IC_{50} = 6.1 ± 1.0 nM (*in vitro*)
Aurora A IC_{50} > 100 μM (*in vivo*)

Aurora A IC_{50} = 14.4 ± 1.7 nM (*in vitro*)
Aurora A IC_{50} = 0.3 - 1.0 μM (*in vivo*)

INTRODUCTION

The Aurora kinases are a family of serine−threonine kinases that play an important role in the regulation of cell division. They are overexpressed in a variety of cancer types and are implicated in many aspects of tumor development. The three members of the Aurora family Aurora A, B, and C share a high degree of structural homology in their kinase domain, but each kinase plays a different role in the control of mitosis. Aurora A and B have received the most attention to date as anticancer targets.[1] Aurora A regulates the cell cycle and is associated with late S phase and entry into the M phase. It is essential for many processes including centrosome maturation and separation, chromosome alignment, and mitotic spindle formation.[2] Aurora A is frequently overexpressed in tumors and has characteristics of an oncogene.[3,4] Aurora B plays important roles in M phase to ensure correct chromosome-microtubule alignment and attachment and chromosomal cytokinesis. Aurora B is also overexpressed[5] in many cancer types but does not have oncogenic properties. Both Aurora A[6−10] and Aurora B[11−14] have been validated as targets for anticancer drugs in preclinical models. Intense activity has led to the development of numerous inhibitors[15−17] that display selectivity to either Aurora A or Aurora B or that are dual inhibitors. Many have been or are now in clinical development.[18−22] Selected examples are shown in Figure 1. The pyrimidine derivative VX-680 (tozasertib) developed by Vertex and Merck is a potent pan-Aurora inhibitor.[23] Clinical development was halted after QTC prolongation was observed. As one of the first reported Aurora inhibitors, VX-680 has been an important tool for studying Aurora biology. Other compounds remain in clinical trials including the orally bioavailable Aurora A selective inhibitor MLN8054[24−26] and its second generation analogue MLN8237[27−30] (Figure 1). The AstraZeneca quinazoline AZD-1152 (barasertib)[31,32] in phase II trials is a potent and selective Aurora B inhibitor administered via intravenous infusion. The 3-aminopyrazole PHA-739358[33−36] (Figure 1) developed by Nerviano and Pfizer is in phase II trials for CML and metastatic prostate cancer. The development of Aurora inhibitors continues to attract attention[37−41] and ultimately will lead to therapeutic benefit in the clinic. The bisanilinopyrimidines are a class of compounds that has shown unusual high selectivity for Aurora A over Aurora B.[42] The pyrimidine scaffold[43−45] has been used by many groups to develop novel Aurora kinase inhibitors.

In this report we describe our efforts in identifying novel and highly potent Aurora A inhibitors using the bisanilinopyrimidine scaffold. To this end, we screened our in-house 20 000

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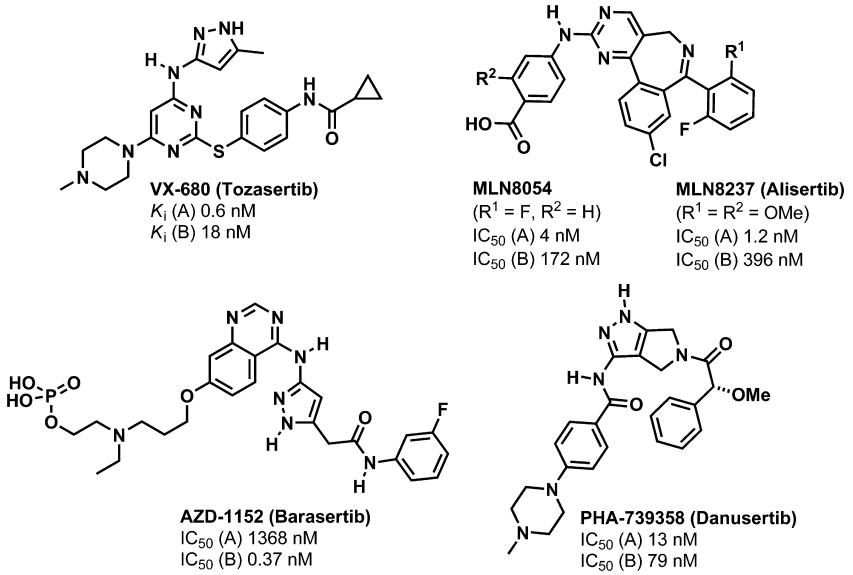

Figure 1. Structures of selected Aurora inhibitors evaluated in the clinic. Inhibition data are shown for Aurora A and B.

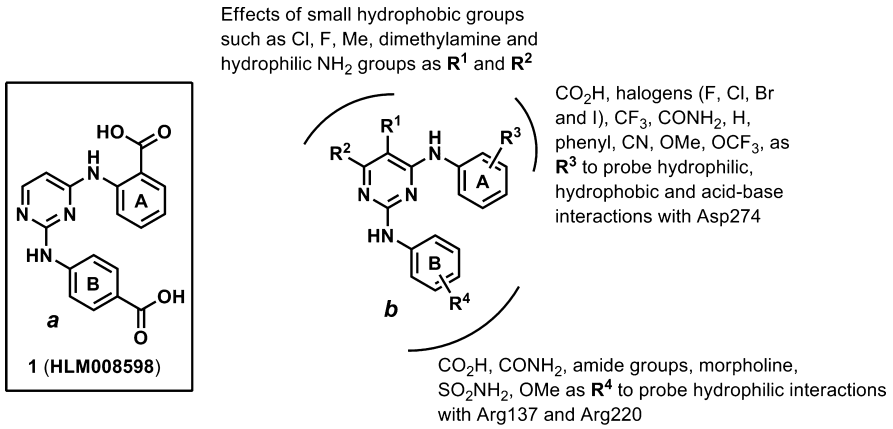

Figure 2. (a) Compound **1** (hit) identified from in-house ChemDiv library as an Aurora A inhibitor. (b) Synthetic modifications for library synthesis.

membered ChemDiv library using a Z-lyte assay and identified the bisanilinopyrimidine bis-carboxylic acid **1** (HLM008598, Figure 2) as a hit. Optimization of compound **1** was undertaken initially via SAR guided focused library synthesis followed by rational design based on co-crystal structures of **1** and related analogues bound to Aurora A.

CHEMISTRY

The bisanilinopyrimidine carboxylic acid **1** was resynthesized in-house to confirm the structure and activity against Aurora A and B. It has not previously been reported as an Aurora kinase A inhibitor. The general synthetic route used for preparation of compound **1** (Figure 2a) and 2,4-bisanilinopyrimidine focused libraries **3** and **4** from readily available building blocks is shown in Scheme 1. The displacement of the 4-chloro group of 2,4-dichloropyrimidine by various anilines is already widely reported in the literature.[42,46−48] The hit **1** was resynthesized by hydrolysis (method h, Scheme 1) of the methyl ester **3w**, which was prepared from **2a** and methyl *p*-aminobenzoate (method m, Scheme 2). Attempts to directly synthesize **1** from **2a** and *p*-aminobenzoic acid via method a resulted in formation of decarboxylated byproduct **3b** (**1**:**3b**, ∼5:1, observed by NMR and HPLC−MS analysis). Attempts to purify the mixture were not successful. For the library synthesis of analogues of **1** the 2,4-dichloropyrimidine was initially reacted with the requisite commercially available anilines using literature reported protocols[46,49,50] to obtain the intermediate library **2** (Scheme 1) with A-ring substituents such as carboxylic acid, carboxylic acid ester, amide groups, and chlorine functional groups at the ortho position. In this step, aqueous hydrochloric acid was used predominantly as the solvent with microwave assisted heating (method a) to obtain the required analogues **2**. The *o*-chloro analogues **2k** and **2l** were synthesized in good yields using aqueous acid at room temperature (method b) as shown in Scheme 1.[46] The 5- and 6-aminopyrimidine building blocks **2h** and **2i** were obtained from commercially available 5- and 6-amino-2,4-dichloropyrimidine starting materials, respectively, in moderate yields. The library **2** members were further functionalized under acid catalysis (methods d−g) to provide the library **3** in good yields.[46] Although the pyrimidine scaffold is featured in many kinase inhibitors, only one member of library **3**, i.e., compound **3c**, has been reported as a kinase inhibitor.[50] Intermediates **2h** and **2i** were converted to **3u** and **3v** using methods f and e, respectively, to obtain ethyl esters as intermediates. Similarly, ethyl esters **3s** and **3t** were obtained from **2m** and **2n**, respectively, using method f. Intermediates **3s**−**v** were hydrolyzed (method h) to obtain **4a**−**d** as final compounds.

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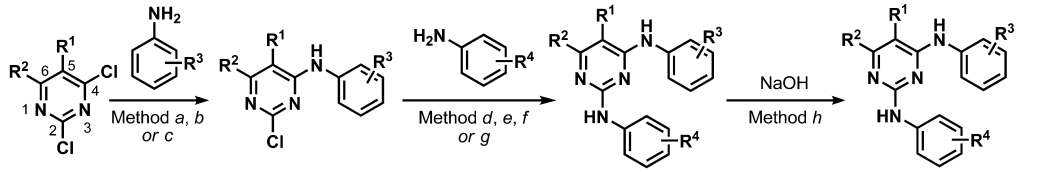
Scheme 1. Synthetic Route to Bisanilinopyrimidine Carboxylic Acid Library[a]



Library 2

2a, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$ (method *a*, 68%)
2b, $R^1 = R^2 = H$, $R^3 = o\text{-}CONH_2$ (method *a*, 63%)
2c, $R^1 = R^2 = R^3 = H$ (method *a*, 26%)
2d, $R^1 = R^2 = H$, $R^3 = p\text{-}CO_2H$ (method *a*, 78%)
2e, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2CH_3$ (method *a*, 45%)
2f, $R^1 = H$, $R^2 = CH_3$, $R^3 = o\text{-}CO_2H$ (method *a*, 41%)
2g, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}CO_2H$ (method *a*, 55%)
2h, $R^1 = NH_2$, $R^2 = H$, $R^3 = o\text{-}CO_2H$ (method *a*, 57%)
2i, $R^1 = H$, $R^2 = NH_2$, $R^3 = o\text{-}CO_2H$ (method *c*, 43%)
2j, $R^1 = CH_3$, $R^2 = H$, $R^3 = o\text{-}CO_2H$ (method *a*, 52%)
2k, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}Cl$ (method *b*, 47%)
2l, $R^1 = R^2 = H$, $R^3 = o\text{-}Cl$ (method *b*, 87%)
2m, $R^1 = Cl$, $R^2 = H$, $R^3 = o\text{-}CO_2H$ (method *a*, 42%)
2n, $R^1 = R^2 = Cl$, $R^3 = o\text{-}CO_2H$ (method *a*, 32%)
2o, $R^1 = Cl$, $R^2 = H$, $R^3 = o\text{-}Cl$ (method *b*, 60%)

Library 3

1, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$ (from **3w**, method *h*, 85%)
3a, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = o\text{-}CONH_2$ (method *d*, 53%)
3b, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = H$ (method *d*, 54%)
3c, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 =$ 4-morpholino (method *e*, 32%)
3d, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = o\text{-}CO_2H$ (method *d*, 60%)
3e, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2CH_3$, $R^4 = p\text{-}CO_2CH_3$ (method *d*, 32%)
3f, $R^1 = R^2 = R^3 = R^4 = H$ (method *d*, 49%)
3g, $R^1 = R^2 = H$, $R^3 = o\text{-}CONH_2$, $R^4 = p\text{-}CONH_2$ (method *d*, 48%)
3h, $R^1 = R^2 = R^3 = H$, $R^4 = p\text{-}CO_2H$ (method *d*, 71%)
3i, $R^1 = R^2 = H$, $R^3 = R^4 = p\text{-}CO_2H$ (method *d*, 99%)
3j, $R^1 = CH_3$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$, (method *g*, 36%)
3k, $R^1 = H$, $R^2 = CH_3$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$ (method *d*, 53%)
3l, $R^1 = R^2 = H$, $R^3 = o\text{-}Cl$, $R^4 = p\text{-}CO_2H$, (method *e*, 43%)
3m, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = H$, (method *e*, 94%)
3n, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$, (method *e*, 59%)
3o, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}Cl$, $R^4 = p\text{-}CO_2H$, (method *m*, scheme 2, 52%)
3p, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}Cl$, $R^4 = H$, (method *m*, Scheme 2, 51%)
3q, $R^1 = H$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = m\text{-}CO_2H$, (method *d*, 72%)
3r, $R^1 = F$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = m\text{-}CO_2H$, (method *m*, scheme 2, 66%)
3s, $R^1 = Cl$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2Et$, (method *f* (ii), 90%)
3t, $R^1 = R^2 = Cl$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2Et$, (method *f* (ii), 17%)
3u, $R^1 = NH_2$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2Et$ (method *f (i)*, 72%)
3v, $R^1 = H$, $R^2 = NH_2$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2Et$ (method *e*, 33%)
3w, $R^1 = R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2Me$ (method *m*, scheme 2, 76%)

4a, $R^1 = NH_2$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$ (from **3u**, method *h*, 65%)
4b, $R^1 = H$, $R^2 = NH_2$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$ (from **3v**, method *h*, 79%)
4c, $R^1 = Cl$, $R^2 = Cl$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$ (from **3t**, method *h*, 32%)
4d, $R^1 = Cl$, $R^2 = H$, $R^3 = o\text{-}CO_2H$, $R^4 = p\text{-}CO_2H$ (from **3s**, method *h*, 72%)

[a]Reagents and conditions. Method a: HCl (0.1 M, aq, 1-3 mL/mmol), microwave, 100 °C, 30 min. Method b: HCl (0.1 M, aq, 3 mL/mmol), rt, 3−5 days. Method c: HCl (0.1 M, aq, 1.5 mL/mmol), sealed tube, 100 °C, 24 h. Method d: HCl (0.1 M, aq, 3−6 mL/mmol), microwave, 160 °C, 15 min. Method e: EtOH: HCl (1 M, aq, 1:1, 4 mL/mmol), microwave, 160 °C, 15 min to 1 h. Method f: (i) HCl (4 M in dioxane, 0.5 mL/mmol), 2-butanol (3 mL/mmol), sealed tube, 120 °C, overnight (24 h), 72% or (ii) EtOH, sealed tube, 120 °C, overnight to 4 days. Method g: THF/HCl (1 M, aq) (1:2, 6 mL/mmol), microwave, 160 °C, 15 min. Method h: THF/NaOH (2 M, aq) (1:2, 4−7 mL/mmol), sealed tube, 85−100 °C, 0.5−16 h.

The high in vitro potency (Table 1) observed with compounds **3n** (with 5-fluoropyrimidine moiety), **3l**, and **3o** (*o*-chloro analogues, Scheme 1) prompted us to further investigate this series. The synthesis of library **6** with hydrophobic groups (Cl, Br, I, CF_3, phenyl) and hydrogen-bond acceptor groups (OMe, CN, OCF_3) on the A-ring is described in Scheme 2. Synthesis of the key intermediates **5a−i** with F, Cl, Br, I, OCF_3, CF_3, OMe, CN, and phenyl (Scheme 2) was achieved from commercially available 2,4-dichloropyrimidine building blocks using literature reported protocols.[50−52] Intermediates **5j** and **5k** were synthesized via alkylation of **2l** using Cs_2CO_3 in acetonitrile (Scheme 2).[51,52] These intermediates were directly reacted via nucleophilic aromatic substitution with anilines to obtain the final library **6** possessing halogens (F, Cl, Br, and I), polar groups (CN), nonpolar groups (Ph, H), and polar hydrophobic groups (OCF_3, CF_3, OMe) in the ortho position of the A-ring in moderate to high yields (method l, m, or n in Scheme 2). The majority of the library members **6** also readily precipitated under the reaction conditions, and the purity of final compounds tested against Aurora A inhibitory activity was determined as >95% by HPLC (high performance liquid chromatography). The analogue **6k** with *p*-carboxylic acid and *m*-OH (i.e., salicylic acid moiety) was obtained from its precursor methyl ester **6u** via basic hydrolysis (method o in Scheme 2).

Using the synthetic routes and protocols shown in Schemes 1 and 2, we were able to explore detailed in vitro SAR toward Aurora A inhibition. Furthermore, we designed and synthesized new molecules, exploiting the structures of compounds **1**, **3l**, **3n**, and **3o** complexed with Aurora A to develop potent Aurora A inhibitors with desirable druglike properties for in vitro and in vivo studies.[53] Compounds **3l** and **3o** (Scheme 1), with *o*-chloro and *p*-carboxylic acid groups were further modified via introduction of water-solubilizing groups to improve solubility and cell permeability (Scheme 4). The solubilizing group was attached via an amide of the B-ring *p*-carboxylic acid, as this part of the molecule is solvent exposed in the co-crystal complexes[53] of **1** (see Figure 3) and **3o** with Aurora A. The building blocks **8a−c** were synthesized as shown in the Scheme 3 via acylation of commercially available *p*-nitrobenzoyl chloride with amines possessing water-solubilizing groups (morpholine, *N*,*N*-dimethylamine, and methyl ether) followed by the hydrogenation of the nitro group in high yields.[54] The final bisanilinopyrimidines with water-solubilizing groups **9** (Scheme 4) were prepared by reaction of intermediates **2k** and **2l** (Scheme 1) with appropriate anilines in moderate yields and >95% HPLC purity. The library **9**

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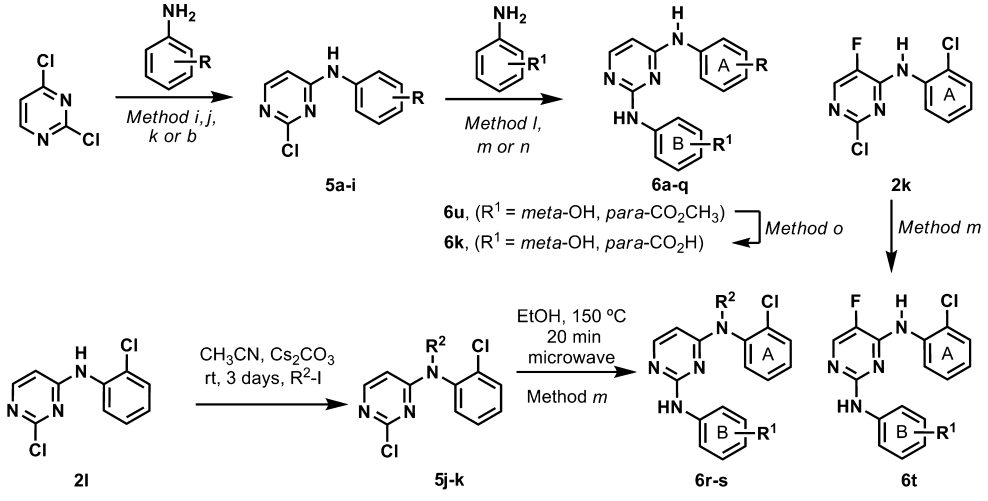
Scheme 2. Synthetic Route to Bisanilinopyrimidine Library 6 with Halogens (F, Cl, Br, and I), CN, Nonpolar Groups (H, Ph), and Polar Hydrophobic Groups (OCF_3, CF_3, and OMe) on the A-Ring[a]



Library 5

5a, R = *ortho*-F, 59% (*method i*)
5b, R = *ortho*-Cl and 4-F, 46% (*method i*)
5c, R = *ortho*-OCF_3, 48% (*method i*)
5d, R = *ortho*-OMe, 60% (*method j*)
5e, R = *ortho*-CN, 22% (*method k*)
5f, R = *ortho*-CF_3, 38% (*method k*)
5g, R = *ortho*-Br 63% (*method b*)
5h, R = *ortho*-I, 79% (*method b*)
5i, R = *ortho*-Ph, 54%, (*method i*)
5j, R^2 = Me, 84%
5k, R^2 = Et, 71%

Library 6

6a, R = *ortho*-F, R^1 = *para*-CO_2H, 69% (*method m*)
6b, R = *ortho*-CF_3, R^1 = H, 84% (*method m*)
6c, R = *ortho*-Cl and 4-F, R^1 = *para*-CO_2H, 69% (*method m*)
6d, R = *ortho*-OCF_3, R^1 = *para*-CO_2H, 58% (*method m*)
6e, R = *ortho*-OMe, R^1 = *para*-CO_2H, 68% (*method m*)
6f, R = *ortho*-OMe, R^1 = H, 76% (*method m*)
6g, R = *ortho*-CN, R^1 = H, 24% (*method m*)
6h, R = *ortho*-CF_3, R^1 = *para*-CO_2H, 82% (*method m*)
6i, R = *ortho*-Br, R^1 = *para*-CO_2H, 47% (*method m*)
6j, R = *ortho*-Cl, R^1 = *para*-CH_2CO_2H, 70% (*method l*)
6k, R = *ortho*-Cl, R^1 = *para*-CO_2H and *meta*-OH, 62% from **6u** (*method o*)
6l, R = *ortho*-F, R^1 = H, 55% (*method m*)
6m, R = *ortho*-I, R^1 = *para*-CO_2H, 32% (*method m*)
6n, R = *ortho*-CN, R^1 = *para*-CO_2H, 15% (*method m*)
6o, R = *ortho*-Cl, R^1 = *meta*-CO_2H, 67% (*method m*)
6p, R = *ortho*-Cl, R^1 = *para*-$CONH_2$, 70% (*method l*)
6q, R = *ortho*-Ph, R^1 = *para*-CO_2H, 67% (*method m*)
6r, R^1 = *para*-CO_2H, R^2 = Me, 80% (*method m*)
6s, R^1 = *para*-CO_2H, R^2 = Et, 71% (*method m*)
6t, R^1 = *meta*-CO_2H, 57% (*method m*)
6u, R = *ortho*-Cl, R^1 = *para*-CO_2Me and *meta*-OH, 56% (*method n*)

[a]Reagents and conditions. Method i: *n*-BuOH, DIPEA, 125 °C. Method j: *n*-BuOH, Na_2CO_3, 100 °C. Method k: DMF, NaH, rt, overnight (14−16 h). Method l: EtOH (1 drop of 1M HCl), microwave, 160 °C, 15 min, 56−75%. Method m: EtOH or MeOH, 150 °C, 20 min, microwave. Method n: cat. HCl, THF, reflux 14 h. Method o: THF, NaOH (1.8 M, aq, 5 equiv), THF, reflux, 14 h. Method b: HCl (0.1 M, aq, 3 mL/mmol), rt, 3−5 days.

with water-solubilizing moieties in the B-ring and an *o*-chloro A-ring comprises novel compounds that have not previously been reported as kinase inhibitors.

Synthesis of the *p*-tetrazole aniline building block **11** was achieved as shown in the Scheme 5 and used to generate compounds **12** (derivatives of **3l**, **3n**, and **3o** shown in Scheme 1). The starting material 4-nitrobenzonitrile was reacted with sodium azide (condition b, Scheme 5) to obtain intermediate **10** in high yield.[55] Hydrogenation of intermediate **10** using Pd−C as the catalyst provided the required building block **11** in good yield (condition c). Compound **11** was next coupled with intermediate **2k**, **2l** (Scheme 1), or **2o** to provide the final compounds **12a−d** (Scheme 5) with >95% purity (determined by HPLC). The final compounds **12** with a tetrazole moiety in the B-ring are reported for the first time.

RESULTS AND DISCUSSION

The high throughput screening (HTS) hit **1** was identified from our in-house 20 000 compound ChemDiv library as a potent and highly selective inhibitor for Aurora A (in vitro IC_{50} = 0.075 ± 0.039 μM) over Aurora B (in vitro IC_{50} = 5.4 ± 1.8 μM) using the Z-LYTE assay with LRRASLG as an Aurora substrate.[56,57] We verified the dose−response curve of the hit (compound **1**) using a coupled enzyme assay[58] (DiscoveRx) that measures ADP formation from the Aurora A phosphorylation of the same synthetic peptide LRRASLG, as described under methods. The determination of dose−response curve and IC_{50} value of the compound **1** using this coupled assay revealed in vitro Aurora A potency in the range of 6.1 ± 1.0 nM, and we used this assay to establish the SAR described in this study.

The bis-anilinopyrimidine scaffold, but not specifically compound **1**, has previously been reported for inhibitors of Aurora kinase[42,44] as well as other kinases such as JNK1,[50] FAK,[59] ephrin type B receptor 4 kinase,[60] CDK2, and CDK4.[61] For an HTS hit, compound **1** displayed an unusually high potency in the range of the most active Aurora A inhibitors reported to date. The suitability of this scaffold to focused library synthesis and availability of crystallization-grade protein

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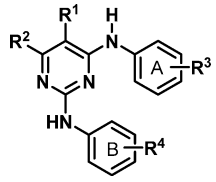
Table 1. Synthetic Modifications, Structure–Activity Relationship Studies, and in Vitro Activities of Bisanilinopyrimidine Libraries 3 and 4 against Aurora A



entry	compound	R^1	R^2	R^3	R^4	in vitro IC_{50}
1	**1**	H	H	*o*-CO_2H	*p*-CO_2H	6.1 ± 1.0 nM
2	**3a**	H	H	*o*-CO_2H	*o*-$CONH_2$	9.0 ± 6.8 μM
3	**3b**	H	H	*o*-CO_2H	H	79.4 ± 18 nM
4	**3c**	H	H	*o*-CO_2H	*p*-morpholine	57.6 ± 5.4 nM
5	**3d**	H	H	*o*-CO_2H	*o*-CO_2H	31.3 ± 5.9 μM
6	**3e**	H	H	*o*-CO_2Me	*p*-CO_2Me	24.6 ± 6.0 μM
7	**3f**	H	H	H	H	423 ± 66 nM
8	**3g**	H	H	*o*-$CONH_2$	*p*-$CONH_2$	38.2 ± 8.8 nM
9	**3h**	H	H	H	*p*-CO_2H	10 ± 1.6 nM
10	**3i**	H	H	*p*-CO_2H	*p*-CO_2H	256 ± 38 nM
11	**3j**	CH_3	H	*o*-CO_2H	*p*-CO_2H	281 ± 59 nM
12	**3k**	H	CH_3	*o*-CO_2H	*p*-CO_2H	>50 μM
13	**3l**	H	H	*o*-Cl	*p*-CO_2H	2.5 ± 0.3 nM
14	**3m**	F	H	*o*-CO_2H	all H	11.3 ± 1.7 nM
15	**3n**	F	H	*o*-CO_2H	*p*-CO_2H	3.9 ± 0.5 nM
16	**3o**	F	H	*o*-Cl	*p*-CO_2H	0.8 ± 0.16 nM
17	**3p**	F	H	*o*-Cl	H	19.9 ± 2.2 nM
18	**3q**	H	H	*o*-CO_2H	*m*-CO_2H	18.3 ± 3.4 nM
19	**3r**	F	H	*o*-CO_2H	*m*-CO_2H	5.1 ± 1.1 nM
20	**4c**	Cl	Cl	*o*-CO_2H	*p*-CO_2H	1.49 ± 0.196 μM
21	**4d**	Cl	H	*o*-CO_2H	*p*-CO_2H	3.17 ± 0.51 nM
22	**4a**	NH_2	H	*o*-CO_2H	*p*-CO_2H	376 ± 64 nM
23	**4b**	H	NH_2	*o*-CO_2H	*p*-CO_2H	>50 μM

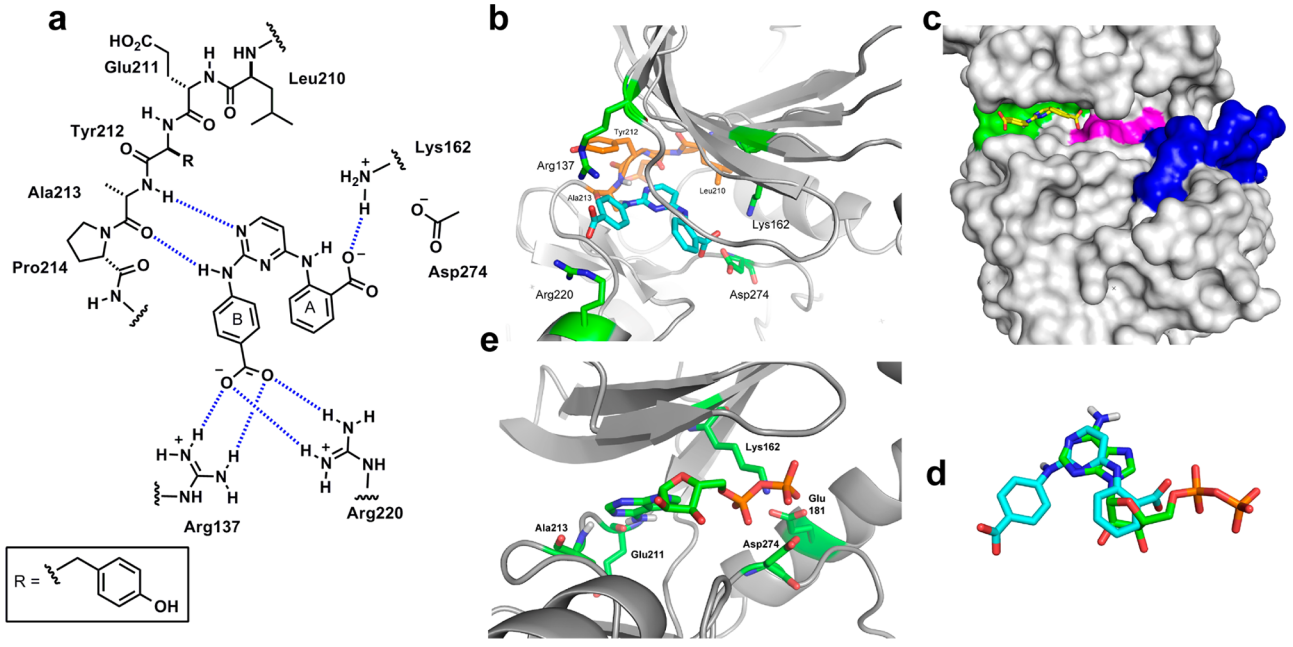


Figure 3. (a) Cartoon representing key binding interactions of hit (compound **1**) with Aurora A. (b) X-ray structure of compound **1** bound to Aurora A active site (compound **1**; cyan, carbon; red, oxygen; blue, nitrogen; PDB entry 3UP7). (c) Compound **1** co-crystallized with Aurora A shows hinge region (green), DFG-in (magenta), activation loop (blue) open conformation (compound **1**; yellow, carbon; red, oxygen; blue, nitrogen). (d) Overlay of ADP (carbons shown green) and compound **1** (carbons shown cyan). (e) ADP bound to Aurora A active site (PDB entry 4DEE).

prompted us to pursue the improvement of **1** by SAR studies and structure-based design.

In the beginning of this work, SAR studies were initiated while attempts were being made to co-crystallize compound **1** with

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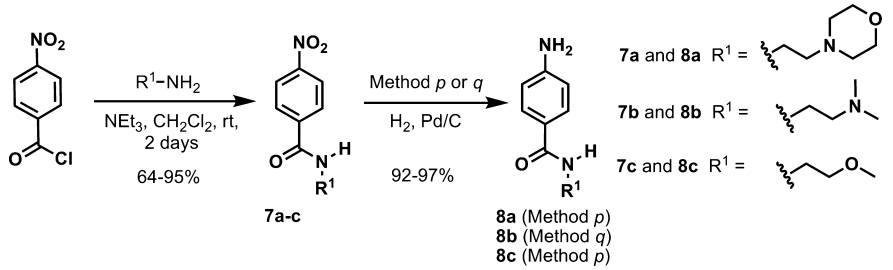
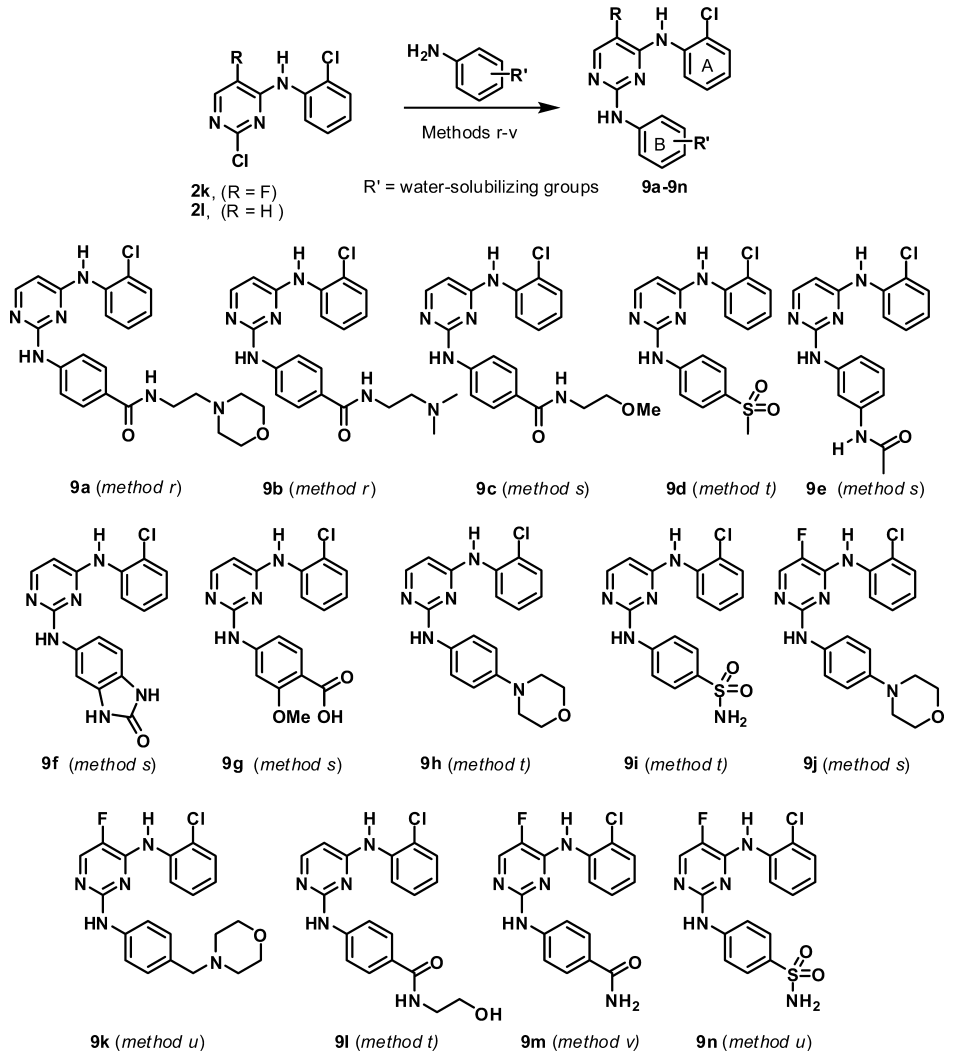
Scheme 3. Synthesis of Building Blocks with Water-Solubilizing Groups[a]



[a]Reagents and conditions. Method p: 10% Pd/C, EtOH, H_2, rt, overnight. Method q: H-Cube, 10% Pd/C, MeOH, 30 bar, four loops, rt.

Scheme 4. Synthesis of Bisanilinopyrimidines with Water-Solubilizing Groups in the B-Ring[a]



[a]Reagents and conditions. Method r: coonc HCl, *i*-PrOH, 170 °C, microwave, 20 min. Method s: EtOH, 150 °C, microwave, 20 min. Method t: EtOH, cat. HCl, 180 or 160 or 140 °C, microwave, 15 min. Method u: X-Phos (10 mmol %), bis(dibenzylideneacetone)palladium(0) (10 mmol %), K_2CO_3, *t*-BuOH, reflux,18 h. Method v: MeOH, 100 °C, sealed tube, 6 h.

Aurora A. Focused library synthesis based on **1** (Figure 2) was first undertaken varying four points of molecular diversity (R^1, R^2, R^3, and R^4; see Figure 2b) by systematically replacing or introducing the functional groups in the A and B rings. Replacement of the B-ring *p*-carboxylic acid in **1** by hydrogen or a morpholino as in **3b** or **3c** resulted in 13- or 10-fold loss of inhibitory activity, respectively, suggesting that the carboxylic acid is critical (entries 3 and 4, Table 1). Replacing both carboxylic acid moieties in **1** by hydrogens as in **3f** (entry 7, Table 1) resulted in 70-fold loss of potency, further emphasizing the importance of the carboxylic acid moieties. In addition, replacement of both carboxylic acid moieties by corresponding methyl esters as shown in **3e** (entry 6, Table 1) was detrimental and resulted in over 4000-fold loss of potency, demonstrating the importance of the negative charge of the acid moieties in the binding region. However, compound **3g** (entry 8, Table 1) with

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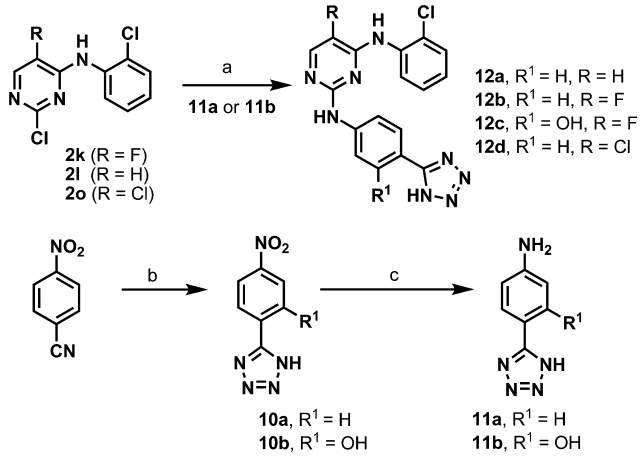
Scheme 5. Synthesis of Bisanilinopyrimidine Tetrazole Derivatives[a]



[a]Reagents and conditions. (a) **12a**: ethanol, microwave, 150 °C, 40 min, 37%. **12b**: ethanol, microwave, 170 °C, 40 min, 33%. **12c**: ethanol, microwave, 160 °C, 40 min, 36%. **12d**: ethanol, microwave, 150 °C, 40 min, 25%. (b) **10a** and **10b**: NaN_3, $Et_3N{\cdot}HCl$, toluene, 100 °C, 15 h, 92% and 95%. (c) **11a** and **11b**: H_2, Pd/C, methanol, rt, 20 h, 93% and 98%.

carboxamide in ortho and para positions of the A and B rings, respectively, was only 6-fold less potent than the compound **1**. Our SAR data showed that the positions of both carboxylic acid moieties in A and B rings are critical for activity. Moving the B-ring *p*-carboxylic acid moiety to the ortho position as in **3d** demonstrated 5000-fold loss of potency (entry 5, Table 1, IC_{50} = 31 300 nM). Compound **3q** (entry 18, Table 1, IC_{50} = 18.3 nM) with *m*-carboxylic acid in the B-ring was less detrimental with 3-fold loss of in vitro potency. Furthermore, moving the A-ring carboxylic acid moiety in compound **1** from the ortho to para position as in **3i** (entry 10, Table 1) resulted in 42-fold loss of potency. Replacement of the B-ring *p*-carboxylic acid by *o*-amide (compound **3a**) was also detrimental for Aurora A inhibitory activity, resulting in greater than 1000-fold loss of inhibitory activity (entry 2, Table 1). These observations suggested that the ortho position of the A-ring and para position of the B-ring are critical for inhibition of enzymatic activity and focused library synthesis.

The X-ray co-crystal structure of **1** bound to Aurora A supports the above findings (Figure 3). Analysis of this structure shows the *p*-carboxylic acid group of the B-ring forming key H-bond interactions with the solvent exposed residues Arg137 and Arg220 (Figure 3a and Figure 3b). As expected of a bisanilinopyrimidine, compound **1** is a type I[62] kinase inhibitor that targets the ATP binding site (Figure 3c). Compound **1** binds to Aurora A in an active DFG-in conformation, as shown in Figure 3c (DFG motif shown in magenta). The activation loop (shown blue in Figure 3c) is oriented away from the ATP binding site. The pyrimidine scaffold and the amine moiety of the B-ring establish H-bonding with the hinge region (residues 211−213, Figure 3a and Figure 3b). Analysis of ADP/ATP bound to Aurora A indicated highly conserved residues Lys162, Asp274, and Glu181 undergoing electrostatic interactions with ADP phosphate moieties in the active site (Figure 3e). Compound **1** bound to Aurora A shows that these key residues in the active site are now in contact with the *o*-COOH moiety of the A-ring (Figure 3b). The *o*-carboxylic acid of the A-ring in compound **1** is in the vicinity (3.5 Å) to form an electrostatic interaction with Lys162 (Figure 3b). The carboxylic acid moiety of the Asp274 of the DFG motif (Figure 3a and Figure 3b) is also in close distance (3.2 Å) with Lys162. These key interactions contribute to the high in vitro potency of the compound **1**. Clearly the apparent disfavored interaction between the *o*-COOH moiety of **1** and the side chain carboxylic acid moiety of the Asp274 must be compensated by the presence of the Lys162. This is consistent

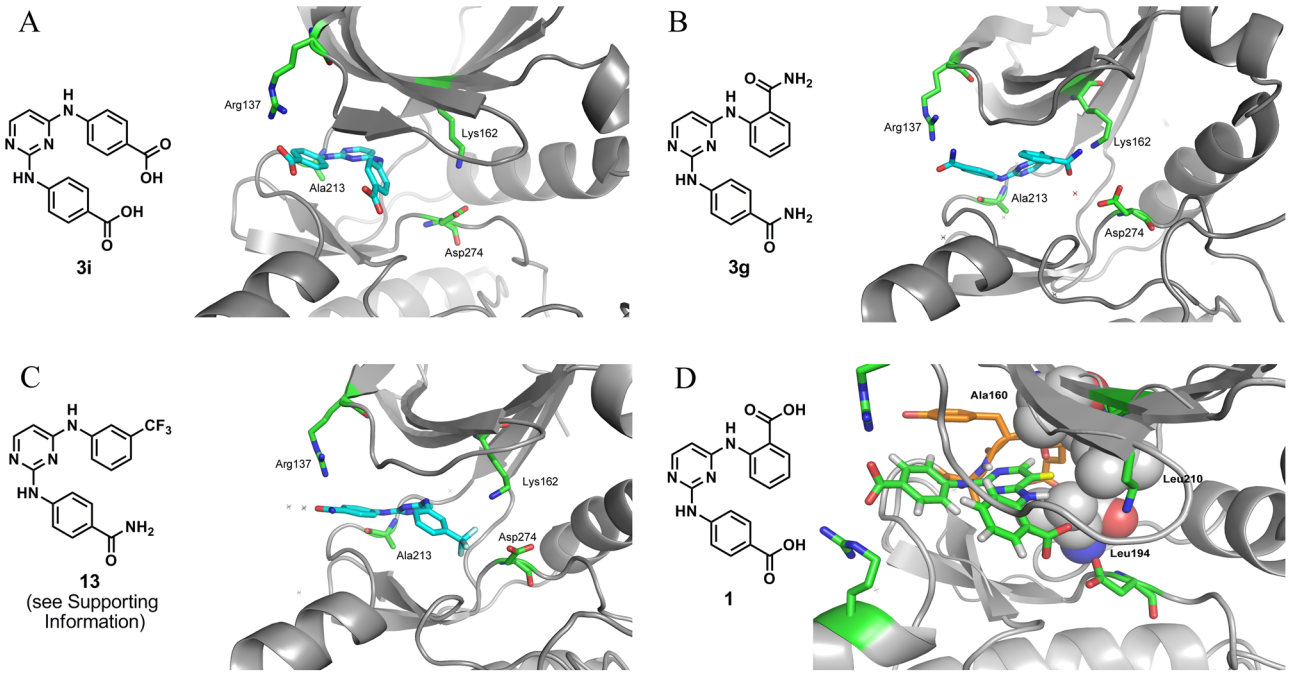


Figure 4. X-ray structures of compounds **3i** (A), **3g** (B), and **13** (C) (see Supporting Information) bound to Aurora A active site (PDB entries 4DEA, 4DED, and 4DEB, respectively). Compound **1** bound to Aurora A (D). The R^1 hydrogen is shown in yellow and Leu194, Leu210, and Ala160 are shown as spheres to show the narrow space where R^1 is occupying in the binding region.

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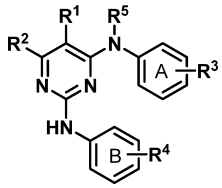
Table 2. Structure–Activity Relationship Studies and in Vitro Inhibitory Activities of Library 6 against Aurora A



entry	compound	R^1	R^2	R^3	R^4	R^5	IC_{50} (nM)[a]
24	**6a**	H	H	*o*-F	*p*-CO_2H	H	3.7 ± 0.7
25	**6b**	H	H	*o*-CF_3	all H	H	1773 ± 142
26	**6c**	H	H	2-Cl-4-F	*p*-CO_2H	H	2.0 ± 0.2
27	**6d**	H	H	*o*-OCF_3	*p*-CO_2H	H	28 ± 4.8
28	**6e**	H	H	*o*-OMe	*p*-CO_2H	H	4.0 ± 0.2
29	**6f**	H	H	*o*-OMe	all H	H	46.6 ± 8.4
30	**6g**	H	H	*o*-CN	all H	H	560 ± 70.3
31	**6h**	H	H	*o*-CF_3	*p*-CO_2H	H	35.1 ± 4.0
32	**6i**	H	H	*o*-Br	*p*-CO_2H	H	2.1 ± 0.4
33	**6j**	H	H	*o*-Cl	*p*-CH_2-CO_2H	H	3.3 ± 1.5
34	**6k**	H	H	*o*-Cl	*p*-CO_2H, *m*-OH	H	6.6 ± 0.6
35	**6l**	H	H	*o*-F	all H	H	284 ± 11.3
36	**6m**	H	H	*o*-I	*p*-CO_2H	H	35 ± 3.3
37	**6n**	H	H	*o*-CN	*p*-CO_2H	H	43 ± 8.0
38	**6o**	H	H	*o*-Cl	*m*-CO_2H	H	18.7 ± 1.5
39	**6p**	H	H	*o*-Cl	*p*-$CONH_2$	H	30.2 ± 1.4
40	**6q**	H	H	*o*-phenyl	*p*-CO_2H	H	149 ± 23
41	**6r**	H	H	*o*-Cl	*p*-CO_2H	CH_3	8.5 ± 1.2
42	**6s**	H	H	*o*-Cl	*p*-CO_2H	CH_3-CH_2	50.2 ± 2.7
43	**6t**	F	H	*o*-Cl	*m*-CO_2H	H	4.5 ± 1.1

[a]Determined in-house using the DiscoveRx format.

with the fact that compound **3f** (entry 7, Table 1) where both A and B rings contain unsubstituted phenyl was 70-fold less active than parent compound **1**, and the dimethyl ester **3e** lost the in vitro inhibitory activity (IC_{50} = 24.6 μM, entry 6, Table 1). These observations further confirm that the key interactions observed from the X-ray structure are important for inhibitory activity (Figure 3b). The loss of inhibitory activity observed with **3d** (entry 5, Table 1) in our SAR studies is consistent with the X-ray structure of compound **1** bound to Aurora A where ortho-substituted B-ring causes steric clash with the main chain residues Ala213 and Pro214 (Figure 3a and Figure 3b). However, the activity of compound **3q** is retained with carboxylic acid in the meta position and improved when fluorine is added as in **3r** (entries 18 and 19, Table 1). It is also likely that the *m*-COOH is able interact with Arg220 and Arg137.

During further investigation of this series, several X-ray structures were obtained in order to improve our understanding of the binding modes of this class of compound with Aurora A (Figure 4). Substitution of both carboxylic acid groups in compound **1** by primary amides (Figure 4B) reduced the inhibitory activity 6-fold (entry 8, **3g**, Table 1). The biscarboxylic acid **3i** (Figure 4A) where the A-ring *o*-carboxylic acid is moved to the para position was 42-fold less potent than parent molecule (Table 1, entry 10, IC_{50} = 256 nM). This decrease in inhibitory activity is probably due to lack of key binding interactions of the *p*-carboxyl or *o*-carboxamide groups in the A-ring with the Lys162 active site residue (Figure 4A and Figure 4B, compounds **3i** and **3g** bound to Aurora A). In contrast, removal of the *o*-carboxylic group as in **3h** (entry 9, Table 1) retains activity, reflecting both the loss of positive interaction with Lys162 and negative interaction with Asp274. The co-crystal structure of **3i** (Figure 4) does not explain the differences in activities of **3h** and **3i**. Compound **13** (Figure 4C), where A-ring has a CF_3 in the meta position, is a weaker binder (IC_{50} = 371 nM; see Supporting Information Table S3 for IC_{50} data of additional compounds) and has the trifluoromethyl group positioned in the region that binds the diphosphate group of ADP (see Figure 3e). Thus, the presence of a hydrophobic group close to Asn261 and Glu260 does not contribute to binding affinity of Aurora A. Overall meta substituents (not reported here) in the A-ring did not improve the activity.

The surprising observation that replacement of the *o*-carboxylic acid of the A-ring in compound **1** with hydrogen as in **3h** did not result in great loss of potency prompted us to further probe SAR at this position. Therefore, library **6** (Scheme 2) was synthesized to improve our understanding of the binding modes of Aurora A to bisanilinopyrimidines with different groups in the ortho position of the A-ring. To this end we synthesized analogues of **1** by replacing the carboxylic acid in the ortho position of the A-ring by fluoro, chloro, bromo, iodo, trifluoromethyl, trifluoromethoxy, methoxy, cyano, and phenyl as ortho substituents (**6a–q**, Scheme 2). Compounds with halogens (Cl, F, Br) at ortho position of the A-ring and *p*-COOH moiety in the B-ring [compounds **3l** (Table 1) and **6a** and **6i** (Table 2), respectively] improved potency by 1.5- to more than 3-fold, whereas compounds with bulky halogenated groups such as OCF_3 and CF_3 (entries 27 and 31, compounds **6d** and **6h**, respectively, in Table 2) were much less active at inhibiting Aurora A. Compound **6q** (entry 40, Table 2) with an *o*-phenyl group was 24-fold less potent than compound **1**, further indicating that bulky groups are not tolerated in this region. Compound **6n** (IC_{50} = 43 nM, entry 37, Table 2) that possess an

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Table 3. In Vivo and in Vitro Aurora A Activities of Bisanilinopyrimidines with Water-Solubilizing Moieties

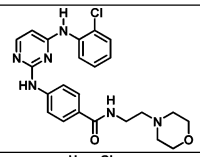
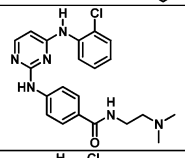
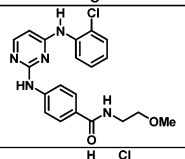
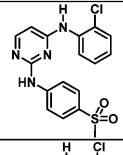
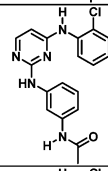
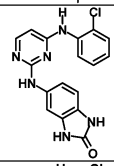
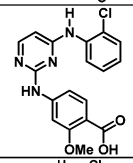
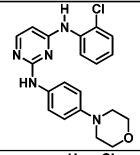
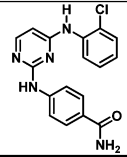
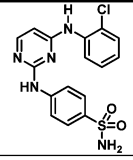
entry	Compound ID	*Aurora A in vitro* IC_{50} (nM)[a]	*Aurora A in vivo* IC_{50} (μM) [Inhibition of Aurora A T288]	*Aurora B in vitro* IC_{50} (nM)[b]	*Aurora B in vivo* IC_{50} (μM) [Inhibition of H3-Ser10]
44	**9a** 	216 ± 10.3	1-3	232	1-10
45	**9b** 	28.1 ± 5.5	<1	93.9	1-10
46	**9c** 	18 ± 2.8	1-3	123	1-10
47	**9d** 	44.9 ± 4.7	<1	180	> 10
48	**9e** 	71.2 ± 9.0	1-3	174	>10
49	**9f** 	316 ± 44.2	3-10	361	1-10
50	**9g** 	116.5 ± 10.6	3-10	1510	>10
51	**9h** 	27 ± 7.6	<1	194	<1
52	**6p** 	30.2 ± 1.4	<1	50.2	<1
53	**9i** 	253 ± 41.6	<1	43.3	<1

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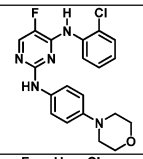
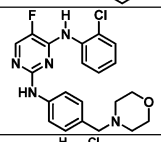
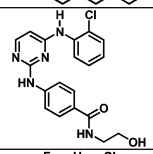
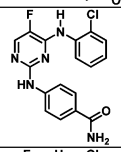
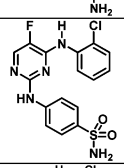
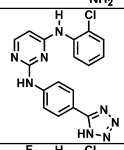
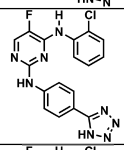
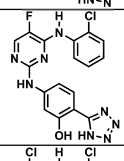
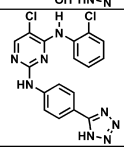
Table 3. continued

entry	Compound ID	*Aurora A in vitro* IC_{50} (nM)[a]	*Aurora A in vivo* IC_{50} (μM) [Inhibition of Aurora A T288]	*Aurora B in vitro* IC_{50} (nM)[b]	*Aurora B in vivo* IC_{50} (μM) [Inhibition of H3-Ser10]
54	**9j** 	21.4 ± 2.5	<1	25.6	<1
55	**9k** 	23.2 ± 1.6	<1	30.0	<1
56	**9l** 	12.3 ± 1.1	<1	40.4	<1
57[c]	**9m** 	14.4 ± 1.7	<1	10.7	<1
58	**9n** 	21.2 ± 2.2	<1	56.6	>10
59	**12a** 	3.1 ± 0.16	> 10	226	> 10
60	**12b** 	2.9 ± 0.30	3-10	83.7	>10
61	**12c** 	17.3 ± 2.3	>10	n.d.	>10
62	**12d** 	3.0 ± 0.53	3-10	106	1-10

[a]Determined in-house using the DiscoveRx format. [b]Measured by Reaction Biology using a ^{33}P assay. [c]The IC_{50} of **9m** in the Reaction Biology ^{33}P assay for **9m**, generated at the same time, was 5.7 nM for Aurora A and 15.6 nM for Aurora B.

o-CN group is more than 17-fold less active than **3l**. The in vitro activity of **3l** (IC_{50} = 2.5 ± 0.3 nM, entry 13, Table 1) with *o*-Cl and a *p*-COOH in B-ring was further improved when R^1 is fluorine in compound **3o** (IC_{50} = 0.8 ± 0.16 nM, entry 16, Table 1). The exceptional potency of this compound is thought to be derived from its novel binding mode, recently described by us.[53] Compound **3o** binds to Aurora A in an inactive DFG-out conformation with the activation loop folded back over the ATP binding site, effectively encapsulating the inhibitor. This binding mode is a consequence of the presence of the *o*-chloro group, which we postulate induces a dipole in the N-flanking alanine adjacent to the DFG motif.[53] The contrasting binding modes of **1** and **3o** is striking given their high structural similarity and provides chemical tools for studying the phenotypical consequences of these two binding modes. The *p*-COOH moiety in the B-ring was important to maintain the Aurora A inhibitory activity in library **6** (Scheme 2). The removal of the COOH moiety resulted in loss of in vitro potency as observed with **6b**, **6f**, **6g**, and **6l** (entries 25, 29, 30, and 35, Table 2). The loss of in vitro inhibitory activity in compounds lacking a *p*-COOH moiety was a general trend observed with libraries **3** and **6** and highlights the importance of H-bond interactions associated with Arg137

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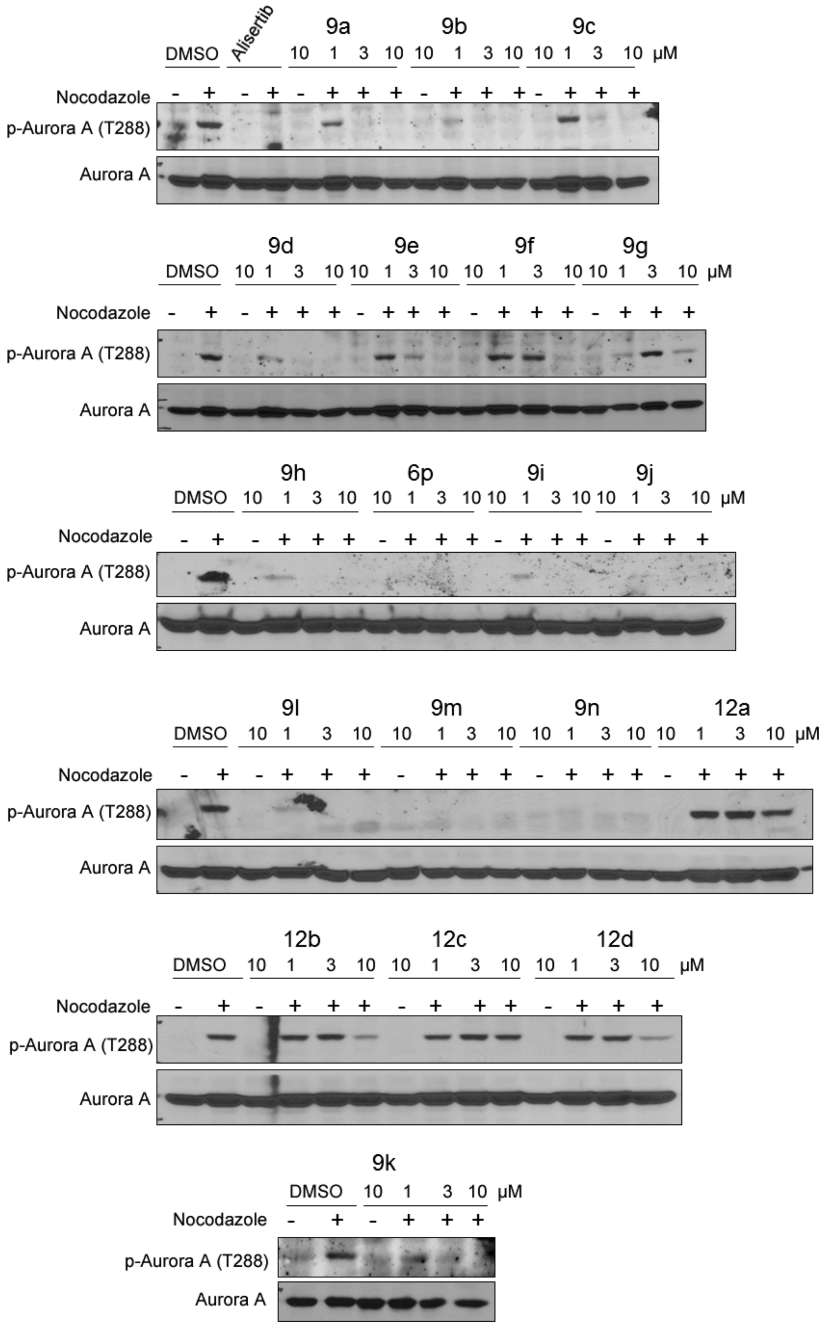

Figure 5. Inhibition of phosphorylation of Thr288 on Aurora A in MDA-MB-468 cells by bisanilinopyrimidines with water-solubilizing moieties. Inhibition at three concentrations (1, 3, and 10 μM) for each compound is shown. Alisertib (0.3 μM) was used as a positive control.

and Arg220. Switching the position of B-ring COOH moiety from para to meta position as in compound **6o** (entry 38, Table 2) reduced the inhibitory activity compared to **3o** (entry 16, Table 1). Modification of B-ring *p*-COOH to *p*-$CONH_2$ in compound **6p** reduced the in vitro activity by 5-fold (entry 39, Table 2). Compounds **6r** and **6s** (entries 41 and 42, Table 2) with N-alkylated moieties retained the activity; the *N*-methyl derivative was highly potent (IC_{50} = 8.5 ± 1.2 nM), while the *N*-ethyl derivative was less active (IC_{50} = 50.2 ± 2.7 nM). Compound **6t** (entry 43, Table 2) with *m*-COOH and fluorine as R^1 was 4-fold more potent compared to compound **6o** where R^1 is hydrogen. Overall our SAR indicated that fluorine or chlorine as R^1 in this series is beneficial and improves in vitro and in vivo Aurora A activity (see Table 3).

Alignment of the Aurora A–ADP complex with compound **1** indicated R^1 and R^2 in compound **1** as potential sites for synthetic modifications (Figure 3a,d,e). The R^1 position (Figure 2b) of the pyrimidine ring is in proximity (~4 Å) to the gatekeeper residue Leu210, and R^2 is close to Glu211 (~3.5 Å) (Figure 3a and Figure 3b). To exploit this narrow space, we first introduced small groups such as methyl (entry 11, **3j**, Table 1), amine (entry 22, **4a**, Table 1), chlorine (entries 20 and 21, **3s** and **3t**, respectively, Table 1), and fluorine (entries 15 and 17, **3n–p**) as R^1. The methyl and NH_2 derivatives (compounds **3j** and **4a**, respectively (entries 11 and 22, Table 1)) did not contribute to increased activity most probably because of the steric effect of these groups, making them unable to establish desired interactions. In contrast, compounds with fluorine (**3n**, **3o**

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(entries 15−16, Table 1)) and chlorine (**4d** (entry 21, Table 1)) were among the most potent inhibitors with IC_{50} values between 0.8 and 4 nM (entries 15, 16, and 21, Table 1). It is likely that fluorine and chlorine as R^1 (Figure 2b) undergo van der Waals interactions with the side chains of the hydrophobic pocket of Ala160, Leu194, and Leu210 (Figure 4D) as observed in the structures of fluorobisanilinopyrimidine inhibitors from Genentech.[42] Substitution of the R^2 position (Figure 2b) of the pyrimidine moiety with methyl, chloro, and amine as shown in compounds **3k**, **4c**, and **4b** (entries 12, 20, and 23, respectively, Table 1) was detrimental for inhibitory activity probably because of steric clash with the backbone carbonyl of Glu211. By contrast, as mentioned above, synthetic modifications at R^3 (Figure 3a and Figure 3b) are largely tolerated, since the region around the DFG is less confined than that opposite the hinge region.

We examined the isoform selectivity of the most potent compound **3o**. The inhibition of Aurora B activity was determined using the Reaction Biology ^{33}P kinase Hotspot assay. The IC_{50} of **3o** for Aurora A and Aurora B, using this assay format, was 0.2 and 42.9 nM, respectively. Similarly the IC_{50} of **1** for Aurora A and Aurora B was 5.1 and 31 nM, respectively. Significant levels of Aurora A selectivity was also observed for the potent compound **3h** (IC_{50}(Aurora A) = 8.9 nM and IC_{50}(Aurora B) = 193 nM).

Having several potent analogues of bisanilinopyrimidines in hand, we next focused on our attention to obtaining potent Aurora inhibitors that are cell permeable and able to inhibit Aurora A kinase in intact cells. However, the potent compounds **3l**, **3n**, **3o**, **4d**, **6a**, **6j**, **6k**, and **6r** that showed low nanomolar activities in the enzymatic assay showed poor aqueous solubility and poor activity in intact cells. Introduction of water-solubilizing groups at the para position of the B-ring was explored to improve both the solubility and cell permeability. Therefore, substitution of the *p*-carboxylic acid in the B-ring with groups that contain a variety of neutral polar moieties (Scheme 4) was employed to exploit H-bond interactions with Arg137 and Arg220. Direct replacement of the *p*-COOH by the carboxylic acid isostere tetrazole moiety[63] provided compounds **12a−d**, which retained in vitro potency similar to that of **3l** (entries 59−62, Table 3 and Scheme 5) with good aqueous solubility (49 μg/mL in DMEM buffer at pH 7.4). We were able to obtain the X-ray structure of compound **12a** (Scheme 5) bound to Aurora A, and compound **12a** adopted a binding conformation similar to that of compound **3l**.[53] Our primary aim with B-ring modifications described in Schemes 4 and 5 was to obtain a compound with chlorine at the ortho position of the A-ring and a water-solubilizing moiety at the para position of the B-ring.

Determination of the Effects of Aurora Kinase Inhibitors on Autophosphorylation of Aurora A on Thr288 and Phosphohistone H3 (Ser10) Levels in Breast Cancer Cells. We next determined the activity of the most potent Aurora kinase inhibitors in intact cells by assessing the inhibition of phosphorylation of Ser10 on Histone H3 and of Aurora A Thr 288 as surrogates for Aurora B[32] and A,[24,30] respectively. MDA-MB-468 breast cancer cells were treated with the inhibitors at various concentrations for 2 h and processed for immunoblotting as described in the Experimental Section. For Aurora A the cells were first synchronized by pretreatment with nocodazole prior to treatment with inhibitors. The hit **1** (Aurora A and B IC_{50} values 6.1 and 31 nM, respectively), which contains a carboxylic acid moiety on each of the A-ring and the B-ring as well as the most potent compound **3o** (in vitro Aurora A and B IC_{50} values 0.8 and 42.9 nM, respectively), had little effect on histone H3 Ser10 phosphorylation levels (Supporting Information Figure S3). This suggested that the carboxylic acid moiety may hinder the ability of these series of compounds to be taken up by cells. We therefore set out to improve the ability of these compounds to inhibit Aurora activity in intact cells by varying the substituents on the B-ring. The inhibition of Aurora A and B in vitro and cellular inhibition of Aurora A Thr288 and histone H3 phosphorylation levels are summarized in Figure 5. Replacement of the B-ring carboxylate with the acid bioisostere[63] tetrazole such as in **12a**, **12b**, **12c**, and **12d** did not improve cellular activity. The tetrazole series retained greater than 30-fold Aurora A/B in vitro selectivity observed for carboxylic acids **3o** and **3h**. In contrast, compounds with chlorine on the A-ring, substituting the B-ring carboxylate with a carboxamide (**6p**, Table 3), sulfonamide (**9i**, Table 3), or morpholino (**9h**, Table 3), greatly improved their activity and resulted in suppression of histone H3 and Aurora A Thr288 phosphorylation with concentrations as low as 1 μM. This is consistent with the observed lower selectivity of these compounds that significantly inhibit Aurora B in the in vitro assay. Similarly, compounds with *o*-chlorine on the A-ring and fluorine as R^1 on the pyrimidine ring and substitutions of the carboxylic acid moiety with carboxamide (**9m**, Table 3), morpholino (**9j**, Table 3), methylene morpholino (**9k**, Table 3), sulfonamide (**9n**, Table 3) also greatly improved the ability to suppress histone H3 phosphorylation and Aurora A autophosphorylation. Other substitutions of the B-ring *p*-carboxylic acid that resulted in improvements in cellular activity included **9a**, **9b**, **9c**, **9l**, and **9f** (Table 3). Taken together, these results demonstrate that in vitro Aurora A inhibitors that contain carboxylic groups are inactive in intact cells but that those where the B-ring carboxylic moiety was replaced by a carboxamide, sulfonamide, or morpholino group are highly potent at inhibiting, in intact cells, the phosphorylation of the Aurora A and B kinase substrates.

Compound **9m** was subjected to limited profiling using the Reaction Biology ^{33}P kinase Hotspot kinase profiling service (Table 4).[64] At a test concentration of 1 μM, **9m** was most potent

Table 4. Selectivity Profile of Compound 9m and Control VX-680 against a Panel of Kinases at 1 μM

	% inhibition at 1 μM	
kinase	**9m**	VX-680
ABL2/ARG	75	98
AKT1	8	3
Aurora A	99	100
Aurora B	93	98
Aurora C	84	86
CDK1/cyclin B	90	5
CDK2/cyclin A	94	7
CHK1	8	16
CHK2	30	4
FLT3	95	102
JAK2	94	81
NEK11	21	11
NEK2	3	−3
NEK6	11	−5
PLK1	64	0
RET	70	96
ROCK1	−5	−2
TRKA	39	95
WEE1	16	2

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against Aurora A (99% inhibition). Aurora B, CDK1, CDK2, FLT3, and JAK2 were also inhibited by >85%. JAK2 and FLT3 are often cross-inhibited by Aurora inhibitors, including VX-680 which was included as a control. The Aurora kinases while not strictly members of the AGC kinase class[65,66] are structurally related[67] to this group. The AGC kinases ROCK1 and AKT1 were not significantly inhibited by **9m**. The IC_{50} of the most potent compounds in the in vitro assay, **3l** and **3o**, were also tested in the Hotspot assay (see Supporting Information) and had IC_{50} of 0.74 and 0.2 nM, respectively (VX-680 had an IC_{50} of 0.24 nM) (see Figure S1, Supporting Information). The IC_{50} of **3o** against JAK2 in this assay was 91 nM, showing that the high potency against Aurora A is matched by high selectivity (over 450-fold vs JAK2) (see Figure S2, Supporting Information). The selectivity of **3o** was high when tested against the University of Dundee panel of 131 kinases (see Table S2, Supporting Information). Aurora A showed the highest inhibition (97%) when tested at 0.5 μM. Only 7 of the 131 kinases (including Aurora A and B) were inhibited by greater than 80%. These include the tyrosine kinase JAK2, in agreement with the Reaction Biology data, and the serine/threonine kinases EIF2AK3, GSK3β, NUAK1, and PIM3. The 12 members of the AGC kinase class tested showed no greater than 35% inhibition.

CONCLUSIONS

The screening hit **1** has provided an excellent starting point for the design and synthesis of highly potent cell permeable Aurora A and B inhibitors. The X-ray structure of **1** bound to Aurora A revealed key interactions that were exploited for focused library synthesis and rational design. As expected of a bisanilinopyrimidine, **1** is a type 1 kinase inhibitor that targets the ATP binding site and binds in a DFG-in conformation. In the course of our study, several X-ray structures were obtained and a novel DFG-out binding mode was discovered with compounds, such as **3o**, that contain a halogen at the ortho position of the A-ring.[53] Through SAR, X-ray structures, and focused library synthesis, we found that fluorine on the 5-position of the pyrimidine ring in combination with chlorine at the ortho position of the A-ring greatly increases the in vitro potency. The carboxylic acid in the para position of the B-ring is important for in vitro potency as exemplified by the DFG-out inhibitor **3o**; several compounds **9g−m** with water-solubilizing moieties at the para position of the B-ring inhibited the auto-phosphorylation of Aurora A and the phosphorylation of the Aurora B substrate histone H3 in MDA-MB-468 breast cancer cells. The carboxamide **9m** displayed selectivity against a small panel of kinases. This selectivity was also observed for the carboxylic acid **3o** when screened against a larger panel of kinases.

EXPERIMENTAL SECTION

Protein Expression and Purification. Aurora A kinase (123−390) was subcloned into a modified pET28 plasmid, overexpressed in *E. coli*, and purified through a combination of affinity, ion exchange, and size exclusion chromatography as described previously.[53]

In Vitro Enzyme Activity Assay. The synthetic peptide LRRASLG served as a substrate for Aurora A. Formation of ADP from ATP was quantified using a coupled enzyme assay (DiscoveRx, Fremont, CA) as described previously.[53] Inhibitor was added to the assay solution, and the reaction was initiated through the addition of 75 μM ATP and 2 mM peptide substrate. IC_{50} values were obtained by fitting the data to eq 1,

$$A = \frac{1}{1 + \left(\frac{[I]}{IC_{50}}\right)^{n}} \quad (1)$$

where A is the remaining activity, [I] is the concentration of the inhibitor, and n is the Hill slope coefficient. The VX-680 compound was used as a standard for IC_{50} determinations.[23]

Protein Crystallography. Aurora A (123−390) was crystallized in the presence of 2 mM inhibitor compound using the precipitant PEG3350 as described previously.[53] X-ray diffraction data were recorded at −180 °C using Cu Kα radiation generated by a Rigaku Micro-Max 007-HF rotating anode (MSC, The Woodlands, TX) using a CCD Saturn 944+ in the Moffitt Structural Biology Core facility. Data were reduced with XDS.[68] The structures were solved by molecular replacement using the MolRep program from CCP4[69] with PDB entry 3FDN as the search model. CNS[70] was employed for refinement, and model building was performed using Coot.[71] Figures were prepared using PYMOL.[72] The data and refinement statistics for the co-crystal structures are provided in the Supporting Information (Table S1). The atomic coordinates and structure factors for Aurora A in complex with compounds **1**, **3i**, **3g**, **13**, and ATP have been deposited under accession numbers 3UP7, 4DEA, 4DED, 4DEB, and 4DEE, respectively.

Determination of the Effects of Aurora Kinase Inhibitors on Autophosphorylation of Aurora A on Thr288 and Phosphohistone H3 (Ser10) Levels in Breast Cancer Cells. MDA-MB-468 cells (American Type Culture Collection) were maintained in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (FBS) (Invitrogen, U.S.) at 37 °C and 5% CO_2. The cells were plated in 6 cm dishes at a density of 2×10^5 cells/dish. Aurora A activity was determined by measuring autophosphorylation of Aurora A on Thr288 in cells that were synchronized by treatment with nocodazole (100 ng/mL) (Sigma) for 20 h prior to the treatment with Aurora inhibitors, whereas Aurora B activity was determined by measuring phosphorylation of histone H3 on Ser10 (pHisH3-Ser10). Cells were then treated with the compounds (0−10 μM). DMSO was used as a vehicle control, and MLN8237 (alisertib) (0.3 μM) or VX-680 (tozasertib) (0.5 μM) (Selleck Chemical LLC) was used as a positive control. Cells were harvested after 2 h of treatment and processed for SDS−PAGE and Western blotting as described previously.[73] Briefly, following electrotransfer, the membranes were blocked at room temperature for 1 h with TBS containing 5% (w/v) milk and then washed with a mixture of TBS containing 0.2% Tween 20 (Sigma). The membranes were then gently shaken at 4 °C overnight with anti-phospho Aurora A (Thr288) antibody (3079, Cell Signaling), anti-phospho-histone H3 (Ser-10) antibody (9701, Cell Signaling), anti-Aurora A antibody (4718, Cell Signaling), or anti-GAPDH monoclonal antibody (E10086CF, Covance) diluted in TBS containing 5% BSA. The membranes were then incubated with HRP conjugated anti-rabbit or anti-mouse IgG antibody (Jackson ImmunoResearch Lab) at room temperature for 1 h followed by washing with Tween 20−PBS. The membranes were washed again with PBS and developed with the ECL system (Perkin-Elmer) as described previously.[74]

Chemistry. General. All reagents were purchased from commercial suppliers and used without further purification. Melting points were determined using a Barnstead international melting point apparatus and remain uncorrected. Proton NMR spectra were recorded on an Agilent-Varian Mercury 400 MHz spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent. ^{13}C NMR spectra are recorded at 100 MHz. The ^{13}C spectrum of **1** was recorded at 150 MHz, using an Agilent VNMRS 600 spectrometer with cold probe (University of South Florida Center for Drug Discovery and Innovation). All coupling constants are measured in hertz (Hz), and the chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm) relative to TMS (δ 0), which was used as the internal standard. High resolution mass spectrometry was carried out on an Agilent 6210 LC−MS (ESI-TOF). Low resolution mass spectrometry (LRMS) was performed on an Agilent single quad G1956A (Department of Chemistry, University of South Florida). Microwave reactions were performed in CEM 908005 model and Biotage initiator 8 machines. All final compounds were purified to ≥95% purity as determined HPLC analysis 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 mm × 4.6 mm, 5 μm) and Agilent Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm). 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, isopropanol, 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. Synthetic protocols and analytical data for compounds **2a**−**o**, **5a**−**k**, **7a**−**c**, **8a**−**c**, **10a,b**, and **11a,b** are reported in the Supporting Information.

***N*⁴-(2-Carboxyphenyl)-*N*²-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (1, Method h in Scheme 1).** A suspension of **3w** (0.040 mg, 0.100 mmol) in NaOH (0.2 mL, 4 M) and THF (0.5 mL) was refluxed in a sealed tube at 85 °C for 2 h. When the mixture was cooled, THF was removed and water (2 mL) was added to the mixture, followed by addition of HCl (1M) to acidify (pH 2) the mixture. The solid obtained was filtered and washed with water (3 mL × 2) and MeOH (3 mL × 2) and dried under high vacuum to afford **1** as a white solid (0.033 mg, 85%), mp 256−258 °C. HPLC 98% (t_R = 7.80 min, 45% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.57 (br s, 1H), 10.68 (s, 1H), 9.93 (s, 1H), 8.44 (appt, J = 5.6 Hz, 1H), 8.15 (d, J = 6.0 Hz, 1H), 7.98 (dd, J = 8.0 Hz, 1H), 7.84−7.79 (m, 4 H), 7.60 (t, J = 7.6 Hz, 1H), 7.17 (t, J = 7.6 Hz, 1H), 6.46 (d, J = 5.6 Hz, 1H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 169.85, 167.77, 160.93, 158.28, 155.08, 144.94, 141.45, 134.18, 131.90, 130.81, 123.92, 123.22, 122.95, 119.22, 118.83, 101.18; LRMS (ESI−) m/z 351.11 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{15}N_4O_4$ (M − Cl)$^+$ 351.1088, found 351.1092.

***N*⁴-(2-Carboxyphenyl)-*N*²-(4-carbamoylphenyl)pyrimidine-2,4-diamine Hydrochloride (3a, Method d in Scheme 1).** A mixture of **2a** (0.050 g, 0.175 mmol) and 2-aminobenzamide (0.027 g, 0.199 mmol) in HCl (1.0 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The precipitate obtained upon cooling the mixture was filtered and washed with water (5 mL) and acetone (5 mL × 2) to obtain pure compound **3a** (0.036 g, 53%) as a light yellow solid, mp 226 °C (dec). HPLC 96% (t_R = 3.55 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, J = 7.6 Hz, 1H), 8.63 (appd, J = 7.6 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 8.03 (dd, J = 8.0, 1.4 Hz, 1H), 7.84 (td, J = 7.6, 1.2 Hz, 1H), 7.72−7.69 (m, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.40 (t, J = 7.6 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 6.83 (d, J = 8.0 Hz, 1H); LRMS (ESI+) m/z 333.1 (M − NH_2 − HCl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{15}N_5O_2$ (M − NH_2 − HCl)$^+$ 333.0982, found 333.1002.

***N*⁴-(2-Carboxyphenyl)-*N*²-(phenyl)pyrimidine-2,4-diamine Hydrochloride (3b, Method d in Scheme 1).** A mixture of **2a** (0.100 g, 0.349 mmol) and aniline (0.038 g, 0.409 mmol) in HCl (1.0 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The precipitate formed upon cooling the mixture was filtered and washed with water (5 mL) and hot methanol (5 mL × 2) to obtain **3b** (0.065 g, 54%) as a white solid, mp 233 °C (dec). HPLC 91% (t_R = 17.27 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 9.34 (s, 1H), 8.65 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 5.6 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.70 (d, J = 8.4 Hz, 2H), 7.55 (t, J = 6.8 Hz, 1H), 7.25 (t, J = 8.0 Hz, 2H), 7.08 (t, J = 7.6 Hz, 1H), 6.93 (t, J = 6.8 Hz, 1H), 6.35 (d, J = 5.6 Hz, 1H); LRMS (ESI−) m/z 305.1 (M − H − HCl)$^-$; HRMS (ESI+) m/z calculated for $C_{17}H_{15}N_4O_2$ (M − Cl)$^+$ 307.1189, found 307.1187.

***N*⁴-(2-Carboxyphenyl)-*N*²-(4-morpholinophenyl)pyrimidine-2,4-diamine Hydrochloride (3c, Method e in Scheme 1).** A mixture of **2a** (0.125 g, 0.437 mmol) and 4-morpholinoaniline (0.089 g, 0.500 mmol) in a solution of EtOH/1 M HCl (1:1, 2 mL) was heated in a microwave reactor at 160 °C for 20 min. Addition of EtOAc (0.5 mL) gave a precipitate. The precipitate was filtered and dried under vacuum to afford the desired compound **3c** (0.060 g, 32%) as a light yellow solid, mp 162 °C (dec). HPLC 97% (t_R = 2.99 min, 50% MeOH in 0.1% DEA in water, 20 min); ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (brs, 1H), 8.14 (dd, J = 8.0, 1.6 Hz, 1H), 7.88 (d, J = 7.2 Hz, 1H), 7.64−7.54 (m, 5H), 7.35 (t, J = 7.6 Hz, 1H), 6.56 (d, J = 7.2 Hz, 1H), 4.06 (appt, J = 4.6 Hz, 4H), 3.14 (appt, J = 4.7 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.36, 162.30, 152.53, 144.03, 137.78, 133.70, 131.71, 126.68, 126.62, 124.49, 123.42, 119.53, 100.29, 65.36, 52.16; LC−MS (ESI−) m/z 390.15 (M − H − HCl)$^-$; HRMS (ESI−) m/z calculated for $C_{21}H_{20}N_5O_3$ (M − H − HCl)$^-$ 390.1572, found 390.1577.

***N*⁴-(2-Carboxyphenyl)-*N*²-(2-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (3d, Method d in Scheme 1).** A suspension of 2,4-dichloropyrimidine (0.149 g, 1.00 mmol) and 2-aminobenzoic acid (0.274 g, 2.00 mmol) in HCl (3.0 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The precipitate obtained was filtered and washed with water (10 mL), followed by washing with acetone (5 mL × 2) to obtain the desired compound **3d** (0.232 g, 60%) as a white solid, mp 254 °C (dec). HPLC 99% (t_R = 3.53 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 8.91 (d, J = 8.0 Hz, 1H), 8.22 (dd, J = 8.0, 1.2 Hz, 1H), 8.04 (dd, J = 8.0, 1.2 Hz, 1H), 7.97−7.93 (m, 2H), 7.76 (td, J = 7.6, 1.6 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.54−7.47 (m, 2H), 7.11 (appd, J = 8.0 Hz, 1H); LRMS (ESI−) m/z 331.0 (M−OH − HCl)$^-$; HRMS (ESI+) m/z calculated for $C_{18}H_{13}N_4O_3$ (M − HO − HCl)$^+$ 333.0982, found 333.0997.

***N*⁴-(2-Carboxymethylphenyl)-*N*²-(4-carboxymethylphenyl)-pyrimidine-2,4-diamine Hydrochloride (3e, Method d in Scheme 1).** To a mixture of methyl 4-aminobenzoate (0.166 g, 1.099 mmol) and **2e** (0.263 g, 0.876 mmol) was added HCl (3.0 mL, 0.1 M). The reaction mixture was heated in a microwave reactor at 160 °C for 15 min. The crude product that was precipitated was filtered, dried under vacuum, and purified using SiO_2 chromatography (gradient elution 0−20% EtOAc in hexane) to obtain the desired product **3e** (0.116 g, 32%) as a white solid, mp 196−198 °C. HPLC 99% (t_R = 5.82 min, 60% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H), 9.73 (s, 1H), 8.37 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 6.0 Hz, 1H), 7.94 (dd, J = 8.0, 1.6 Hz, 1H), 7.84 (d, J = 9.2 Hz, 2H), 7.80 (d, J = 9.2 Hz, 2H), 7.64−7.60 (m, 1H), 7.18 (appt, J = 7.2 Hz, 1H), 6.45 (d, J = 6.0 Hz, 1H), 3.81 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.25, 166.69, 160.74, 159.58, 157.54, 146.04, 141.41, 134.30, 131.39, 130.62, 123.02, 122.94, 121.99, 118.58, 118.39, 101.12, 52.97, 52.35; LRMS (ESI+) m/z 379.1 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{20}H_{19}N_4O_4$ (M − Cl)$^+$ 379.1401, found 379.1402.

***N*⁴,*N*²-Diphenylpyrimidine-2,4-diamine Hydrochloride (3f, Method d in Scheme 1).** A mixture of 2,4-dichloropyrimidine (0.050 g, 0.336 mmol) and aniline (0.063 g, 0.677 mmol) in HCl (1.0 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The product obtained was purified using SiO_2 chromatography (gradient elution 0−20% EtOAc in hexane) to afford the desired product **3f** (0.048 g, 49%) as a white solid, mp 144 °C (dec). HPLC 96% (t_R = 5.33 min, 60% MeOH in 0.1% TFA in water, 40 min); ^{1}H NMR (400 MHz, CD_3OD) δ 7.85 (d, J = 6.0 Hz, 1H), 7.59 (d, J = 7.6 Hz, 2H), 7.55 (dd, J = 7.6 Hz, 2H), 7.27 (d, J = 7.6 Hz, 2H), 7.23 (d, J = 7.6 Hz, 2H), 7.05−6.98 (m, 2H), 6.20 (d, J = 6.0 Hz, 1H); LRMS (ESI+) m/z 263.1 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{16}H_{15}N_4$ (M − Cl)$^+$ 263.1291, found 263.1293.

***N*⁴-(2-Carbamoylphenyl)-*N*²-(4-carbamoylphenyl)-pyrimidine-2,4-diamine Hydrochloride (3g, Method d in Scheme 1).** A mixture of **2b** (0.248 g, 0.871 mmol) and 4-aminobenzamide (0.136 g, 1.00 mmol) in HCl (3.0 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The precipitate was filtered and washed with water (10 mL), hot MeOH (10 mL), hot THF (10 mL), dioxane (5 mL), DMF (5 mL), and MeOH (10 mL) sequentially to obtain pure **3g** (0.160 g, 48%) as a light yellow solid, mp 257−260 °C. HPLC 97% (t_R = 3.32 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H disappeared on D_2O shake), 9.65 (s, 1H disappeared on D_2O shake), 8.57 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 5.2 Hz, 1H), 7.98 (dd, J = 7.6, 1.6 Hz, 1H), 7.83−7.73 (m, 5H becomes 4H on D_2O shake), 7.63−7.58 (m, 1H), 7.17 (brs, 1H disappeared on D_2O shake), 7.12 (t, J = 8.4 Hz, 1H), 6.42 (d, J = 6.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.31, 168.30, 160.55, 159.67, 157.36, 144.07, 142.41, 134.35, 131.96, 128.81, 127.22, 122.25, 121.83, 118.50, 117.57, 101.05; LRMS (ESI+) m/z 349.1 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{17}N_6O_2$ (M − Cl)$^+$ 349.1408, found 349.1407.

***N*⁴-(Phenyl)-*N*²-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (3h, Method d in Scheme 1).** A mixture of **2c** (0.020

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g, 0.082 mmol) and 4-aminobenzoic acid (0.013 g, 0.097 mmol) in HCl (1.5 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The solid obtained upon cooling the mixture was filtered, washed with water (5 mL), and dried under vacuum to obtain the desired compound **3h** (0.020 g, 71%) as a white solid, mp 215 °C (dec). HPLC 99% (t_R = 7.28 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 7.2 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 7.6 Hz, 2H), 7.41 (t, J = 7.6 Hz, 2H), 7.28 (t, J = 7.6 Hz, 1H), 6.46 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.44, 161.79, 152.70, 144.17, 141.82, 137.81, 130.87, 129.50, 126.80, 126.15, 123.30, 121.17, 100.58. LRMS (ESI−) m/z 305.0 $(M - Cl)^-$; HRMS (ESI+) m/z calculated for $C_{17}H_{15}N_4O_2$ $(M - Cl)^+$ 307.1190, found 307.1187.

***N*^{4},*N*^{2}-Di(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (3i, Method d in Scheme 1).** A mixture of **2d** (0.100 g, 0.350 mmol) and 4-aminobenzoic acid (0.055 g, 0.401 mmol) in HCl (1.5 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The precipitate obtained upon cooling the mixture was filtered and washed with water (10 mL), followed by quick wash with acetone (5 mL × 2) to give pure **3i** (0.136 g, 99%) as a white solid, mp 281−283 °C. HPLC 98% (t_R = 4.97 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (brs, 1H), 10.46 (brs, 1H), 8.09 (d, J = 6.8 Hz), 7.90 (d, J = 6.0 Hz, 2H partially overlapping) 7.88 (d, J = 6.0 Hz, 2H partially overlapping), 7.79 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 6.50 (d, J = 6.4 Hz, 1H); LRMS (ESI−) m/z 349.0 $(M - H - HCl)^-$; HRMS (ESI+) m/z calculated for $C_{18}H_{15}N_4O_4$ $(M - Cl)^+$ 351.1088, found 351.1082.

***N*^{4}-(2-Carboxyphenyl)-*N*^{2}-(4-carboxyphenyl)-5-methylpyrimidine-2,4-diamine Hydrochloride (3j, Method g in Scheme 1).** A mixture of **2j** (0.115 g, 0.383 mmol) and 4-aminobenzoic acid (0.179 g, 1.307 mmol) in HCl (1 M) /THF (2:1, 3.0 mL) was heated in a microwave reactor at 160 °C for 15 min. The precipitate obtained upon cooling the mixture was filtered and washed with water (5 mL) and MeOH (5 mL × 2). The solid obtained was slurried in DMF (2 mL), filtered, and washed with MeOH (5 mL), acetone (5 mL), and DCM (5 mL) sequentially to obtain the desired compound **3j** (0.055 g, 36%) as a white solid, mp 213 °C (dec). HPLC 97% (t_R = 5.17 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (brs, 1H disappeared on D_2O shake), 11.00 (s, 1H disappeared on D_2O shake), 9.68 (s, 1H disappeared on D_2O shake), 9.01 (d, J = 8.4 Hz, 1H), 8.08 (s, 1H), 8.03 (d, J = 7.9 Hz, 1H), 7.85−7.80 (m, 4H), 7.60 (t, J = 7.9 Hz, 1H), 7.11 (t, J = 7.6 Hz, 1H), 2.16 (s, 3H); LC−MS (ESI+) m/z 365.13 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{19}H_{17}N_4O_4$ $(M - Cl)^+$ 365.1244, found 365.1240.

***N*^{4}-(2-Carboxyphenyl)-*N*^{2}-(4-carboxyphenyl)-6-methylpyrimidine-2,4-diamine Hydrochloride (3k, Method d in Scheme 1).** A mixture of **2f** (0.263 g, 0.877 mmol) and 4-aminobenzoic acid (0.137 g, 1.00 mmol) in HCl (3.0 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The precipitate obtained was filtered and washed with water (10 mL), MeOH (10 mL × 2), DMSO (1 mL, quick wash), and acetone (5 mL) to obtain the desired compound **3k** (0.187 g, 53%) as a white solid, mp 243 °C (dec). HPLC 90% (t_R = 5.11 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 9.73 (s, 1H), 8.55 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 7.58 (t, J = 7.2 Hz, 1H), 7.11 (t, J = 7.2 Hz, 1H), 6.33 (s, 1H), 2.28 (s, 3H); LC−MS (ESI−) m/z 363.11 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{19}H_{15}N_4O_4$ $(M - H - HCl)^-$ 363.1099, found 363.1107.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (3l, Method e in Scheme 1).** A mixture of **2l** (0.094 g, 0.340 mmol) and 4-aminobenzoic acid (0.107 g, 0.781 mmol) in EtOH/1 M HCl (2.0 mL, 1:1) was heated in a microwave reactor at 160 °C for 15 min. The mixture was cooled to room temperature. The precipitate obtained was filtered, washed with water (2 mL), and MeOH (2 mL) sequentially to afford the desired compound **3l** (0.055 g, 43%) as a white solid, mp 234 °C (dec). HPLC 99% (t_R = 4.32 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 10.00 (s, 1H), 9.76 (s, 1H), 8.06 (d, J = 6.0 Hz, 1H), 7.70−7.68 (m, 3H), 7.63−7.58 (m, 3H), 7.43 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.4 Hz, 1H), 6.40 (d, J = 6.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.36, 163.18, 152.30, 145.07, 141.91, 134.61, 130.72, 130.64, 130.47, 129.67, 129.52, 128.56, 126.30, 120.01, 95.58; LC−MS (ESI+) m/z 341.09 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}ClN_4O_2$ $(M - Cl)^+$ 341.0800, found 341.0810.

***N*^{4}-(2-Carboxyphenyl)-*N*^{2}-phenyl-5-fluoropyrimidine-2,4-diamine Hydrochloride (3m, Method e in Scheme 1).** A mixture of **2g** (0.134 g, 0.441 mmol) and aniline (0.140 g, 1.50 mmol) in 1:1 ratio of EtOH/1 M HCl (2.0 mL) was heated in a microwave reactor at 160 °C for 30 min. The precipitate formed upon cooling the mixture was filtered and washed with MeOH (5 mL), acetone (5 mL) and dried under vacuum to afford the desired compound **3m** (0.150 g, 94%) as a white solid, mp 240 °C (dec). HPLC 99% (t_R = 4.21 min, 70% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.50 (s, 1H), 9.41 (s, 1H), 8.96 (d, J = 8.4 Hz, 1H), 8.21 (d, J = 3.1 Hz, 1H), 8.04 (dd, J = 7.9, 1.5 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 7.60 (t, J = 8.4 Hz, 1H), 7.27 (t, J = 8.4 Hz, 2H), 7.12 (t, J = 7.6 Hz, 1H), 6.94 (t, J = 7.3 Hz, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −166.03 (s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.83, 156.07 (d, J = 3.0 Hz), 149.60 (d, J = 10.0 Hz), 142.26, 141.68 (d, J = 245 Hz), 141.52, 141.33, 141.25, 134.72, 132.02, 129.05, 122.15 (d, J = 21.0 Hz), 120.74, 119.80, 116.24; LC−MS (ESI+) m/z 325.12 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}FN_4O_2$ $(M - Cl)^+$ 325.1095, found 325.1093.

***N*^{4}-(2-Carboxyphenyl)-*N*^{2}-(4-carboxyphenyl)-5-fluoropyrimidine-2,4-diamine Acid (3n, Method e in Scheme 1).** A mixture of **2g** (0.134 g, 0.44 mmol) and 4-aminobenzoic acid (0.206 g, 1.50 mmol) in 1:1 ratio of EtOH/HCl (2.0 mL, 1 M) was heated in a microwave reactor at 160 °C for 30 min. The precipitate formed upon cooling the mixture was filtered and washed with saturated $NaHCO_3$ (3 mL) and water (5 mL). The solid obtained was slurried in hot DMF (3 mL), filtered, washed with MeOH (5 mL), and dried under vacuum to afford the desired compound **3n** (0.096 g, 59%) as a white solid, mp 287−290 °C. HPLC 99% (t_R = 14.73 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.42 (s, 1H disappeared on D_2O shake), 9.83 (s, 1H disappeared on D_2O shake), 8.94 (d, J = 8.6 Hz, 1H), 8.29 (appd, J = 2.7 Hz, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 7.63 (t, J = 7.2 Hz, 1H), 7.16 (t, J = 7.5 Hz, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −164.42 (s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.76, 167.82, 155.42 (d, J = 3.5 Hz), 149.76 (d, J = 10 Hz), 145.58, 142.09 (d, J = 247 Hz), 142.00, 141.25 (d, J = 18 Hz), 134.70, 132.03, 130.87, 123.39, 122.57, 120.93, 118.13, 116.65; LC−MS (ESI+) m/z 369.10 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{14}FN_4O_4$ $(M + H)^+$ 369.0994, found 369.0994.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-(4-carboxyphenyl)-5-fluoropyrimidine-2,4-diamine Hydrochloride (3o, Method d in Scheme 1).** A mixture of **2k** (0.300 g, 1.12 mmol) and 4-aminobenzoic acid (0.153 g, 1.12 mmol) in ethanol (1.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The reaction mixture was cooled and stirred at room temperature for 48 h. The white precipitate was isolated by filtration and washed with ethyl acetate (5 mL). The product was suspended in ethyl acetate (5 mL) and sonicated for 5 min and filtered to provide **3o** (0.232 g, 52%) as a white powder, mp 308 °C (dec). HPLC 99% (t_R = 7.57 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.44 (s, 1H), 9.59 (s, 1H), 9.38 (s, 1H), 8.15 (d, J = 3.4 Hz, 1H), 7.62−7.52 (m, 6H), 7.45−7.36 (m, 2H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −164.92; ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.77, 155.67 (d, J = 3.0 Hz), 151.48 (d, J = 12.0 Hz), 145.80, 141.60 (d, J = 20.0 Hz), 141.46 (d, J = 245.0 Hz), 136.04, 131.63, 130.55, 130.40, 130.19, 128.44, 128.38, 122.66, 117.37; LC−MS (ESI+) m/z 359.07 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{13}ClFN_4O_2$ $(M - Cl)^+$ 359.0706, found 359.0709.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-phenyl-5-fluoropyrimidine-2,4-diamine Hydrochloride (3p, Method m in Scheme 2).** A mixture of **2k** (0.061 g, 0.207 mmol) and aniline (0.020 g, 0.207 mmol) in EtOH (2.0 mL) was heated in a microwave reactor at 150 °C for 20 min. The precipitate formed upon cooling the mixture was filtered and quickly washed with MeOH (1 mL) to afford the desired product **3p** (0.037 g, 51%) as a white solid, mp 145−148 °C. HPLC 99% (t_R = 9.43 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H disappeared on D_2O shake), 9.12 (s, 1H disappeared on D_2O shake), 8.08 (d, J = 4.0 Hz, 1H), 7.59−7.56 (m, 2H), 7.44 (d, J = 8.4

Hz, 2H), 7.39 (td, J = 7.6, 1.6 Hz, 1H), 7.31 (td, J = 7.6, 1.6 Hz, 1H), 7.02 (t, J = 7.2 Hz, 2H), 6.77 (t, J = 7.6 Hz, 1H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −166.49; ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.97, 151.42 (d, J = 12 Hz), 141.40, 141.24 (d, J = 20 Hz), 141.11 (d, J = 244 Hz), 136.05, 131.18, 130.35, 129.80, 128.76, 128.29, 128.13, 121.33, 118.80; LC−MS (ESI+) m/z 315.08 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{16}H_{13}ClFN_4$ $(M - Cl)^+$ 315.0807, found 315.0812.

N^4-(2-Carboxyphenyl)-N^2-(3-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (3q Method d in Scheme 1). A mixture of **2a** (0.050 g, 0.175 mmol) and 3-aminobenzoic acid (0.024 g, 0.175 mmol) in HCl (0.1 mL, 0.1 M) was heated in a microwave reactor at 160 °C for 15 min. The solution was cooled and the precipitate obtained was filtered and washed quickly with hot methanol (3 mL) to obtain the desired product **3q** (0.056 g, 72%) as a white solid, mp 275 °C (dec). HPLC 99% (t_R = 6.31 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.85 (brs, 1H), 10.73 (s, 1H), 9.52 (s, 1H), 8.67 (d, J = 8.4 Hz, 1H), 8.27 (s, 1H), 8.12 (d, J = 5.7 Hz, 1H), 7.96 (d, J = 7.9 Hz, 2H), 7.54−7.47 (m, 2H), 7.51 (t, J = 7.7 Hz, 2H), 7.05 (t, J = 7.6 Hz, 1H), 6.36 (d, J = 5.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.49, 168.16, 160.41, 159.99, 157.52, 142.75, 141.52, 134.46, 131.93, 131.72, 129.22, 124.08, 122.79, 121.79, 121.25, 120.71, 116.79, 100.98; LC−MS (ESI−) m/z 349.10 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{18}H_{14}N_4O_4$ $(M - H - HCl)^-$ 349.0942, found 397.0940.

N^4-(2-Carboxyphenyl)-N^2-(3-carboxyphenyl)-5-fluoropyrimidine-2,4-diamine Hydrochloride (3r Method m Scheme 2). A mixture of **2g** (0.100 g, 0.329 mmol) and 3-aminobenzoic acid (0.048 g, 0.350 mmol) in HCl (1.0 mL, 0.1 M) was heated in a microwave reactor at 150 °C for 20 min. The mixture was cooled to room temperature and the precipitate obtained was filtered and washed quickly with ethanol (3 mL) to obtain the desired product **3r** (0.089 g, 66%) as a white solid, mp 272 °C (dec). HPLC 99% (t_R = 14.34 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.07 (brs, 1H), 11.48 (s, 1H), 9.61 (s, 1H), 8.97 (d, J = 8.4 Hz, 1H), 8.31−8.23 (m, 2H), 8.03 (d, J = 7.9 Hz, 1H), 7.60−7.48 (m, 2H), 7.39 (t, J = 7.9 Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.87, 168.29, 155.90, 149.69 (d, J = 9.7 Hz), 151.37 (d, J = 11.5 Hz), 141.91 (d, J = 235.3 Hz), 142.22, 141.54, 134.89, 132.04, 131.77, 129.32, 123.78, 122.87, 122.29, 120.62, 120.37, 116.19; ^{19}F NMR (376 MHz, DMSO) δ −165.34. Anal. Calcd for $C_{18}H_{14}ClFN_4O_4$: C, 53.41; H, 3.49; N, 13.84. Found: C, 53.22; H, 3.37; N, 13.57.

N^4-(2-Carboxyphenyl)-N^2-(4-ethoxycarbonylphenyl)-5-chloropyrimidine-2,4-diamine Hydrochloride (3s, Method f(ii) in Scheme 1). A mixture of **2m** (0.063 g, 0.193 mmol) and ethyl 4-aminobenzoate (0.043 g, 0.260 mmol) in ethanol (0.8 mL) was heated in a sealed tube at 120 °C (oil bath temperature) overnight. The resulting precipitate was filtered, washed with ethanol (1 mL × 2), Et_2O (3 mL), and hexane (2 mL) sequentially, and dried under vacuum to afford the title compound **3s** (0.079 g, 90%) as a white solid, mp 221 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.77 (s, 1H), 11.46 (s, 1H), 9.97 (s, 1H), 8.93 (d, J = 8.3 Hz, 1H), 8.34 (s, 1H), 8.04 (dd, J = 1.5, 7.9 Hz, 1H), 7.86 (d, J = 9.1 Hz, 2H), 7.83 (d, J = 9.1 Hz, 2H), 7.64−7.60 (m, 1H), 7.19−7.15 (m, 1H), 4.26 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H); LC−MS (ESI−) m/z 412.09 $(M + H)^+$; HRMS (ESI−) m/z calculated for $C_{20}H_{18}ClN_4O_4$ $(M + H)^+$ 413.1011, found 413.0989.

N^4-(2-Carboxyphenyl)-N^2-(4-ethoxycarbonylphenyl)-5,6-dichloropyrimidine-2,4-diamine Hydrochloride (3t, Method f(ii) in Scheme 1). A mixture of **2n** (0.091 g, 0.257 mmol) and ethyl 4-aminobenzoate (0.044 g, 0.266 mmol) in ethanol (1.0 mL) was heated in a sealed tube at 110 °C (oil bath temperature) for 4 days. The resulting precipitate was filtered, washed with ethanol (1 mL × 2), and suspended in methanol (1 mL). The mixture was sonicated and the solid was filtered and dried under vacuum to afford the title compound **3t** (0.022 g, 17%) as a white solid, mp 206 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.52 (s, 1H), 10.25 (s, 1H), 8.74 (s, 1H), 8.04 (dd, J = 1.5, 7.9 Hz, 1H), 7.86 (d, J = 8.8 Hz, 2H), 7.75 (d, J = 8.9 Hz, 2H), 7.64−7.60 (m, 1H), 7.23−7.20 (m, 1H), 4.27 (q, J = 7.1 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H); LC−MS (ESI−) m/z 447.07 $(M - Cl)^+$; HRMS (ESI−) m/z calculated for $C_{20}H_{17}Cl_2N_4O_4$ $(M - Cl)^+$ 447.0621, found 447.005 98.

5-Amino-N^4-(2-carboxyphenyl)-N^2-(4-ethoxycarbonylphenyl)pyrimidine-2,4-diamine Hydrochloride (3u, Method f(i) in Scheme 1). This was prepared by using a method described Gray and co-workers.[47] A mixture of **2h** (0.080 g, 0.266 mmol), ethyl 4-aminobenzoate (0.099 g, 0.600 mmol), and HCl (0.15 mL, 4 M in dioxane) in 2-butanol (1.0 mL) was heated in a sealed tube at 120 °C (oil bath temperature) for 24 h. After the mixture was cooled to room temperature, the suspension obtained was filtered and washed with water (5 mL), MeOH (3 mL) to afford the desired compound **3u** (0.082 g, 72%) as a yellow solid, mp 262 °C (dec). HPLC 99% (t_R = 15.48 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.87 (s, 1H), 9.18 (s, 1H), 7.94−7.84 (m, 5H), 7.80 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 8.4 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.04 (t, J = 7.5 Hz, 1H), 4.26 (q, J = 7.0 Hz, 2H), 1.30 (t, J = 7.0 Hz, 3H); LC−MS (ESI+) m/z 376.15 $(M - OH - HCl)^+$; HRMS (ESI+) m/z calculated for $C_{20}H_{18}N_5O_3$ $(M - OH - HCl)^+$ 376.1404, found 376.1405.

6-Amino-N^4-(2-carboxyphenyl)-N^2-(4-ethoxycarbonylphenyl)pyrimidine-2,4-diamine Hydrochloride (3v, Method e in Scheme 1). A mixture of **2i** (0.265 g, 0.880 mmol) and ethyl 4-aminobenzoate (1.65 g, 10.00 mmol) in EtOH/1 M HCl (1:1, 12 mL) was heated in a microwave reactor at 160 °C for 1 h. The mixture was cooled to room temperature, and the solid obtained was filtered, washed with MeOH (5 mL), and slurried in acetone (10 × 5 mL) until no impurity was shown by NMR, affording the desired compound **3v** (0.125 g, 33%) as a beige solid, mp 280 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H disappeared on D_2O shake), 9.36 (s, 1H disappeared on D_2O shake), 8.85 (appd, J = 7.6 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H), 7.83 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.4 Hz, 2H), 7.47 (t, J = 8.4 Hz, 1H), 6.94 (t, J = 8.0 Hz, 1H), 6.54 (brs, 2H disappeared on D_2O shake), 5.54 (s, 1H), 4.26 (q, J = 7.0 Hz, 2H), 1.29 (t, J = 7.0 Hz, 3H); LC−MS (ESI+) m/z 394.15 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{20}H_{20}N_5O_4$ $(M - Cl)^+$ 394.1510, found 394.1509.

N^4-(2-Carboxyphenyl)-N^2-(4-methoxycarbonylphenyl)-pyrimidine-2,4-diamine Hydrochloride (3w, Method m in Scheme 2). A suspension of **2a** (0.060 g, 0.210 mmol) and methyl 4-aminobenzoate (0.032 g, 0.210 mmol) in MeOH (1 mL) was heated in a microwave reactor at 150 °C for 20 min. When the mixture was cooled, the resulting precipitate was filtered and washed with MeOH (2 mL) to afford **3w** (0.064 mg, 76%) as a yellow solid which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.40 (br s, 1H), 10.74 (s, 1H), 10.11 (s, 1H), 8.33 (br d, J = 5.6 Hz, 1H), 8.14 (d, J = 6.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.84−7.76 (m, 4 H), 7.63 (t, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6 Hz, 1H), 6.50 (d, J = 6.4 Hz, 1H), 3.80 (s, 3H); LC−MS (ESI+) m/z 365.13 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{19}H_{17}N_4O_4$ $(M - Cl)^+$ 365.1244, found 365.1243.

5-Amino-N^4-(2-carboxyphenyl)-N^2-(4-carboxyphenyl)-pyrimidine-2,4-diamine (4a, Method h in Scheme 1). A suspension of **3u** (0.069 g, 0.161 mmol) in NaOH/THF (2 M, 0.44 mL/0.2 mL) was heated at 100 °C (oil bath temperature) in a sealed tube for 30 min. A solution of HCl (1 M) was added to acidify the solution to pH 1−2 after removing THF. The solid obtained was filtered and washed with water (5 mL), saturated $NaHCO_3$ (3 mL), water (5 mL), acetone (3 mL), and MeOH (3 mL) sequentially to afford the desired compound **4a** (0.038 g, 65%) as a brown solid, mp 160 °C (dec). HPLC 96% (t_R = 4.19 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (brs, 1H disappeared on D_2O shake), 8.86 (brs, 1H), 7.99 (brs, 1H), 7.88−7.78 (m, 5H), 7.50 (brs, 1H), 7.01 (brs, 1H); LC−MS (ESI+) m/z 366.12 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{16}N_5O_4$ $(M + H)^+$ 366.1197, found 366.1198.

6-Amino-N^4-(2-carboxyphenyl)-N^2-(4-carboxyphenyl)-pyrimidine-2,4-diamine (4b, Method h in Scheme 1). A suspension of **3v** (0.070 g, 0.163 mmol) in NaOH (0.45 mL, 2 M) and THF (0.25 mL) was heated at 100 °C (oil bath temperature) in a sealed tube for 16 h. The THF in the mixture was evaporated, and HCl (1 M) was added to acidify (pH 1−2) the mixture. The solid obtained was filtered, washed with water (3 mL), saturated aqueous $NaHCO_3$ (3 mL), and water (3 mL) sequentially, and dried to afford the desired compound **4b** (0.047 g, 79%) as a yellow solid, mp 220 °C (dec). HPLC

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95% (t_R = 5.20 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H disappeared on D_2O shake), 9.78 (s, 1H disappeared on D_2O shake), 8.54 (brs, 1H), 7.96 (appd, J = 5.2 Hz, 1H), 7.82−7.80 (m, 2H), 7.59−7.53 (m, 3H), 7.34−7.10 (m, 3H), 5.60 (s, 1H); LC−MS (ESI+) 366.13 m/z $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{16}N_5O_4$ $(M + H)^+$ 366.1197, found 366.1194.

N^4-(2-Carboxyphenyl)-N^2-(4-carboxyphenyl)-5,6-dichloropyrimidine-2,4-diamine Hydrochloride (4c, Method h in Scheme 1). A mixture of **3t** (0.018 g, 0.034 mmol) in THF (0.3 mL) and NaOH (0.1 mL, 2 M) was heated in a sealed tube at 110 °C (oil bath temperature) overnight. The THF was then removed under reduced pressure, and HCl (1 M aqueous, 0.5 mL) was added to the residue. The resulting precipitate was filtered and washed with water (2 mL) and dried under vacuum. The solid obtained was then slurried in methanol (1 mL), filtered, and dried under vacuum to afford the title compound **4c** (0.005 g, 32%) as a white solid, mp 230 °C (dec). HPLC 84% (t_R = 10.84 min, 20% MeOH, 80% water (with 0.1% DEA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (s, 1H), 11.47 (s, 1H), 10.21 (s, 1H), 8.72 (s, 1H), 8.04 (dd, J = 7.9, 1.4 Hz, 1H), 7.84 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.65−7.54 (m, 1H), 7.23−7.20 (m, 1H); LC−MS (ESI−) m/z 417.02 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{18}H_{13}Cl_2N_4O_4$ $(M - H - HCl)^-$ 417.0163, found 417.0160.

N^4-(2-Carboxyphenyl)-N^2-(4-carboxyphenyl)-5-chloropyrimidine-2,4-diamine Hydrochloride (4d, Method h in Scheme 1). A mixture of **3s** (0.058 g, 0.119 mmol) in THF (0.4 mL) and NaOH (2 M aqueous, 0.2 mL) was heated in a sealed tube at 110 °C (oil bath temperature) overnight. The THF was then removed under reduced pressure, and HCl (1 M aqueous, 0.6 mL) was added to the residue. The resulting precipitate was filtered, washed with water (2 mL × 3), and dried under vacuum. The solid obtained was then slurried in DMF (2 mL), filtered, washed with DMF (5 mL), methanol (1 mL), and Et_2O (3 mL) sequentially, and dried under vacuum to afford the title compound **4d** (0.036 g, 72%) as a white solid, mp 261 °C (dec). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 11.45 (s, 1H), 9.90 (s, 1H), 8.87 (s, 1H), 8.30 (s, 1H), 8.01−7.55 (m, 6H), 7.13 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.57 167.72, 157.79, 155.85, 155.36, 145.16, 141.90, 134.51, 131.90, 130.84, 123.84, 122.82, 121.54, 118.76, 117.26, 107.11; HPLC 97% (t_R = 8.74 min, 15% MeOH, 85% water (with 0.1% DEA), 20 min); LC−MS (ESI−) m/z 383.04 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{18}H_{14}ClN_4O_4$ $(M - H - HCl)^-$ 383.0553, found 383.0552.

N^4-(2-Fluorophenyl)-N^2-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6a, Method m in Scheme 2). A mixture of 2-chloro-*N*-(2-fluorophenyl)pyrimidin-4-amine (**5a**) (0.096 g, 0.428 mmol) and 4-aminobenzoic acid (0.069 g, 0.503 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The mixture was filtered and the resulting precipitate was washed with EtOH (0.5 mL × 2) to provide the title compound **6a** (0.107 g, 69%) as a white solid, mp 264 °C (dec). HPLC 98.7% (t_R = 3.8 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (s, 1H), 11.04 (s, 1H), 10.87 (s, 1H), 8.13 (d, J = 7.0 Hz, 1H), 7.77 (d, J = 8.7 Hz, 2H), 7.66 (t, J = 7.9 Hz, 1H), 7.56 (d, J = 8.5 Hz, 2H), 7.46−7.36 (m, 2H), 7.33−7.25 (m, 1H), 6.58 (d, J = 6.7 Hz, 1H); ^{19}F NMR (400 MHz, DMSO-d_6) δ 121.12; ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.40, 163.00, 156.59 (d, J = 245 Hz), 152.55, 145.08, 141.92, 130.79, 129.14, (d, J = 9.27 Hz), 128.30, 126.43, 125.39, 125.04 (d, J = 12.15 Hz), 120.29, 116.88 (d, J = 18.72 Hz), 99.84; LC−MS (ESI−) m/z 323.10 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{12}FN_4O_2$ $(M - H - HCl)^-$ 323.0950, found 323.0974.

N^4-[2-(Trifluoromethyl)phenyl]-N^2-phenylpyrimidine-2,4-diamine Hydrochloride (6b, Method m in Scheme 2). A mixture of chloropyrimidine **5f** (0.076 g, 0.277 mmol) and aniline (0.03 mL, 0.328 mmol) in EtOH (0.4 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was removed under reduced pressure to provide an off-white solid. Ethyl acetate (3 mL) was added to the reaction mixture. The mixture was left at room temperature for 30 min and sonicated occasionally. The resulting precipitate was isolated by filtration and washed with ethyl acetate (1 mL × 5) and hexane (3 mL) to afford **6b** (0.085 g, 84%) as a white solid, mp 207 °C (dec). HPLC 100% (t_R = 9.1 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (s, 1H), 10.43 (s, 1H), 8.01 (apparent s, 1H), 7.86−7.80 (m, 2H), 7.71−7.48 (m, 2H), 7.27 (d, J = 7.2 Hz, 2H), 7.14 (t, J = 7.5 Hz, 2H), 7.03 (t, J = 7.3 Hz, 1H), 6.46 (s, 1H); LC−MS (ESI+) m/z 331.13 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}F_3N_4$ $(M - Cl)^+$ 331.1165, found 331.1170.

N^4-(2-Chloro-4-fluorophenyl)-N^2-(4-carboxyphenyl)-pyrimidine-2,4-diamine Hydrochloride (6c, Method m in Scheme 2). A mixture of chloropyrimidine **5b** (0.096 g, 0.372 mmol) and 4-aminobenzoic acid (0.058 g, 0.422 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The resulting precipitate was isolated by filtration and washed with EtOH (0.5 mL × 2), diethyl ether (2 mL), and hexane (2 mL) sequentially to provide the title compound **6c** (0.102 g, 69%) as a white solid, mp 268 °C (dec). HPLC 97.7% (t_R = 4.8 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 10.72 (s, 1H), 8.12 (d, J = 7.0 Hz, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.72−7.63 (m, 2H), 7.50 (d, J = 8.6 Hz, 2H), 7.40 (td, J = 8.5, 2.9 Hz, 1H), 6.53 (d, J = 7.0 Hz, 1H); ^{19}F NMR (400 MHz, DMSO-d_6) δ 112.66; ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.37 163.39, 161.22 (d, J = 246.3 Hz), 152.39, 145.37, 141.91, 131.87 (d, J = 11.12 Hz); LC−MS (ESI−) m/z 357.06 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{11}ClFN_4O_2$ $(M - H - HCl)^-$ 357.0560, found 357.0521.

N^4-[2-(Trifluoromethoxy)phenyl]-N^2-(4-carboxyphenyl)-pyrimidine-2,4-diamine Hydrochloride (6d, Method m in Scheme 2). A mixture of chloropyrimidine **5c** (0.074 g, 0.255 mmol) and 4-aminobenzoic acid (0.042 g, 0.306 mmol) in EtOH (0.5 mL) was heated with a microwave reactor at 150 °C for 20 min. The resulting precipitate was isolated by filtration and washed with EtOH (0.5 mL × 2), diethyl ether (2 mL), and hexane (2 mL) sequentially to provide the title compound **6d** (0.63 g, 58%) as a white solid, mp 228 °C (dec). HPLC 96.4% (t_R = 5.8 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.93 (s, 1H), 10.75 (s, 1H), 8.12 (d, J = 7.0 Hz, 1H), 7.77−7.73 (m, 3H), 7.63−7.39 (m, 5H), 6.58 (d, J = 6.7 Hz, 1H); ^{19}F NMR (400 MHz, DMSO-d_6) δ 57.09; ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.40, 163.23, 152.77, 145.96, 143.26, 142.04, 130.77, 130.16, 129.31, 129.10, 128.65, 126.30, 122.51, 120.22, 118.10 (q, J = 256 Hz), 99.76; LC−MS (ESI−) m/z 389.07 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{18}H_{12}F_3N_4O_3$ $(M - H - HCl)^-$ 389.0867, found 389.0818.

N^4-(2-Methoxyphenyl)-N^2-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6e, Method m in Scheme 2). A mixture of chloropyrimidine **5d** (0.075 g, 0.317 mmol) and 4-aminobenzoic acid (0.046 g, 0.328 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. Ethanol (0.5 mL) was added to the reaction mixture. The resulting precipitate was isolated by filtration and washed with EtOH (0.5 mL) and hexane (5 mL) to provide the title compound **6e** (0.8 g, 68%) as a white solid, mp 247.5−249.3 °C. HPLC 99.6% (t_R = 6.0 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H), 10.85 (s, 1H), 10.41 (s, 1H), 8.03 (d, J = 7.1 Hz, 1H), 7.77 (d, J = 8.6 Hz, 2H), 7.59−7.56 (m, 3H), 7.33 (t, J = 7.9 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H), 7.02 (t, J = 7.5 Hz, 1H), 6.50 (apparent s, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.42, 162.78, 153.64, 152.19, 144.09, 142.02, 130.81, 128.73, 127.42, 126.30, 125.47, 120.89, 120.17, 112.66, 99.85, 56.32; LC−MS (ESI−) m/z 335.11 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{18}H_{15}N_4O_3$ $(M - H - HCl)^-$ 335.1150, found 335.1153.

N^4-(2-Methoxyphenyl)-N^2-(phenyl)pyrimidine-2,4-diamine (6f, Method m in Scheme 2). A mixture of chloropyrimidine **5d** (0.105 g, 0.444 mmol) and aniline (0.05 mL, 0.548 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was removed under reduced pressure. Aqueous saturated $NaHCO_3$ (10 mL) was added to the residue and extracted with ethyl acetate (10 mL × 2). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by flash chromatography (10 g silica gel, hexane/EtOAc) to afford the title compound **6f** (0.100 g, 76%) as an off-white solid, mp 142.3−144.5 °C. HPLC 99.4% (t_R = 4.90 min, 60% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.54 (s, 1H), 7.99

(d, J = 8.0 Hz, 1H), 7.96 (d, J = 5.7 Hz, 1H), 7.69 (d, J = 7.6 Hz, 2H), 7.18−7.14 (m, 2H), 7.11−7.02 (m, 2H), 6.97−6.89 (m, 1H), 6.85 (t, J = 7.3 Hz, 1H), 6.32 (d, J = 5.8 Hz, 1H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 161.00, 160.08, 156.52, 149.55, 140.01, 128.97, 128.31, 123.69, 122.60, 121.50, 120.91, 120.33, 110.62, 98.24; LC−MS (ESI+) m/z 293.15 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{17}N_4O$ $(M + H)^+$ 293.1397, found 293.1393.

N^4-(2-Cyanophenyl)-N^2-(phenyl)pyrimidine-2,4-diamine (6g, Method m in Scheme 2). A mixture of chloropyrimidine **5e** (0.092 g, 0.398 mmol) and aniline (0.037 mL, 0.398 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was removed under reduced pressure. The solid obtained was slurried in ethyl acetate (3 mL), filtered, and washed with ethyl acetate (5 mL) and hexane (5 mL). The dried solid was dissolved in methanol (3 mL). Diisopropylethylamine (1 mL) was added, and the solvent was removed under reduced pressure. The solid obtained was slurried with water (5 mL), filtered, washed with water (10 mL × 2), ether (5 mL), slurried with methanol (1 mL), filtered, and dried under vacuum to provide the title compound **6g** (0.027 g, 24%) as an off-white solid, mp 170.0.2−172.7 °C. HPLC 100% (t_R = 5.1 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 9.15 (s, 1H), 8.07 (d, J = 5.7 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.77−7.66 (m, 2H), 7.59 (d, J = 8.8 Hz, 2H), 7.32 (t, J = 8.0 Hz, 1H), 7.11 (t, J = 7.9 Hz, 2H), 6.83 (t, J = 7.3 Hz, 1H), 6.28 (d, J = 5.7 Hz, 1H); LC−MS (ESI+) m/z 288.13 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}N_5$ $(M + H)^+$ 288.1244, found 288.1241.

N^4-[2-(Trifluoromethyl)phenyl]-N^2-(4-carboxyphenyl)-pyrimidine-2,4-diamine Hydrochloride (6h, Method m in Scheme 2). A mixture of chloropyrimidine **5f** (0.055 g, 0.2 mmol) and 4-aminobenzoic acid (0.03 g, 0.218 mmol) in EtOH (0.3 mL) was heated in a microwave reactor at 150 °C for 20 min. Ethanol (0.5 mL) was added to the reaction mixture and sonicated at room temperature for 5 min. The resulting precipitate was isolated by filtration and washed with EtOH (0.5 mL × 2), and hexane (3 mL × 2) to afford the title compound **6h** (0.067 g, 82%) as a white solid, mp 240.0−242.6 °C. HPLC 99.6% (t_R = 7.7 min, 50% MeOH in 0.1% TFA in water, 30 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 1H), 10.76 (s, 1H), 8.11 (d, J = 7.0 Hz, 1H), 7.90−7.85 (m, 2H), 7.69−7.64 (m, 4H), 7.38 (d, J = 8.5 Hz, 2H), 6.53 (d, J = 6.7 Hz, 1H); ^{19}F NMR (400 MHz, DMSO-d_6) δ 59.99 (s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.35, 164.21, 152.47, 145.71, 142.05, 135.37, 134.40, 131.77, 130.65, 129.09, 127. (q, J = 4.6 Hz), 126.62 (q, J = 29.5 Hz), 126.10, 124.03 (q, J = 271.5 Hz), 119.77; LC−MS (ESI−) m/z 373.08 $(M + H)^+$; LC−MS (ESI+) m/z 375.11 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{14}F_3N_4O_2$ $(M + H)^+$ 375.1063, found 375.1068.

N^4-(2-Bromophenyl)-N^2-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6i, Method m in Scheme 2). A mixture of chloropyrimidine **5g** (0.100 g, 0.350 mmol) and 4-aminobenzoic acid (0.048 g, 0.350 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The resulting precipitate was isolated by filtration and washed with EtOH (0.5 mL × 2) and dried under vacuum to provide the title compound **6i** (0.069 g, 47%) as a white solid, mp 240 °C (dec). HPLC 98% (t_R = 7.40 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.46 (s, 2H), 8.06 (d, J = 6.8 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.68 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.3 Hz, 1H), 7.51−7.47 (m, 3H), 7.32 (t, J = 8.3 Hz, 1H), 6.45 (s, 1H); ^{13}C NMR (100 MHz, d_6-DMSO) 167.43, 163,07, 153.17, 146.60, 142.37, 136.36, 133.80, 130.71, 129.95, 129.71, 129.19, 125.91, 121.27, 119.83, 99.45; LC−MS (ESI+) m/z 385.02 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}BrN_4O_2$ $(M - Cl)^+$ 385.0295, found 385.0292.

N^4-(2-Chlorophenyl)-N^2-(4-methylcarboxyphenyl)-pyrimidine-2,4-diamine Hydrochloride (6j, Method l in Scheme 2). A mixture of chloropyrimidine **2l** (0.120 g, 0.500 mmol) and 4-aminophenylacetic acid (0.076 g, 0.5 mmol) in EtOH (2 mL with 1 drop of 1 M hydrochloric acid) was heated in a microwave reactor at 160 °C for 15 min. A clear solution was obtained. Ethanol was removed from the mixture under vacuum, and the analysis of the crude NMR showed formation of 25% ethyl ester of **6j**. The crude material was stirred in THF (0.5 mL) and NaOH solution (1 mL, 2 M) at room temperature for 16 h. The THF was evaporated from the mixture, and HCl (1 M) was added to acidify the mixture (pH 1−2). The precipitate obtained was filtered, washed with water (5 mL), and dried under high vacuum to afford **6j** (0.137 g, 70%) as a beige solid, mp 132 °C (dec). HPLC 97% (t_R = 3.77 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.19 (s, 1H disappeared on D_2O shake), 9.30 (s, 1H disappeared on D_2O shake), 9.21 (s, 1H disappeared on D_2O shake), 8.01 (d, J = 6.0 Hz, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.54 (dd, J = 8.0, 1.4 Hz, 1H partially overlapping), 7.51 (d, J = 8.4 Hz, 2H partially overlapping with dd), 7.38 (apptd, J = 8.0, 1.4 Hz, 1H), 7.24 (apptd, J = 8.0, 1.4 Hz, 1H), 7.03 (d, J = 8.4 Hz, 2H), 6.30 (d, J = 6.0 Hz, 1H), 3.45 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.65, 162.04, 158.90, 155.15, 139.43, 136.41, 130.31, 129.87, 129.85, 128.56, 128.44, 128.10, 126.90, 119.66, 119.63, 98.71; LC−MS (ESI+) m/z 355.11 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{16}ClN_4O_2(M - Cl)^+$ 355.0956, found 355.0971.

N^4-(2-Chloromethyl)phenyl]-N^2-(4-carboxy-3-hydroxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6k, Methods n and o in Scheme 2). A mixture of chloropyrimidine **2l** (0.100 g, 0.416 mmol) and methyl 5-aminosalicylate (0.070 g, 0.416 mmol) in THF (10 mL) was stirred at room temperature for 10 min. Two drops of concentrated HCl were added, and the mixture was heated at reflux for 14 h. The solvent was removed under reduced pressure to provide a gray solid which was suspended in sodium bicarbonate (saturated aqueous 50 mL), sonicated for 10 min, filtered, and washed with water (10 mL) to provide a white powder. The crude material was washed with methanol (2 × 10 mL) and finally with ethyl acetate (20 mL) to give methyl 4-(4-(2-chlorophenylamino)pyrimidin-2-ylamino)-2-hydroxybenzoate (**6u**) (0.081 g, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 9.63 (s, 1H), 9.15 (s, 1H), 8.07 (s, 1H), 7.76 (s, 1H), 7.59−7.33 (3 × broad s, 3H), 7.25−7.10 (2 × broad s, 2H), 6.35 (s, 1H), 3.83 (s, 3H); LC−MS (ESI+) 371.09, m/z calculated for $C_{18}H_{16}ClN_4O_3$ $(M + H)^+$ 371.0905, found 371.0914.

To a stirred solution of ester **6u** (200 mg, 0.540 mmol) in THF (15 mL) was added sodium hydroxide (108 mg, 2.70 mmol) in water (1.5 mL). The mixture was heated under reflux for 14 h. The solvent was removed under reduced pressure to provide a white solid which was then dissolved in water (20 mL) and acidified to pH ∼6−7 by addition of HCl (1 M). The colorless precipitate was filtered, washed with water, and dried under vacuum. The crude solid was suspended in methanol (20 mL), sonicated for 10 min, filtered, washed with methanol (5 mL), and dried under vacuum to provide the title compound **6k** (118 mg, 62%) as an off-white powder, mp 202−204 °C. HPLC 100% (t_R = 8.3 min, 45% MeOH, 65% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.55 (brs, 1H), 11.29 (brs, 1H), 10.14 (brs, 1H), 9.93 (s, 1H), 8.07 (d, J = 6.7 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.25 (s, 1H), 7.02 (d, J = 8.5 Hz, 1H), 6.43 (d, J = 5.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.34, 162.82, 162.58, 155.69, 146.06, 135.35, 131.18, 130.58, 129.32, 128.64, 128.53, 128.25, 111.11, 107.27, 106.54, 99.78; LC−MS (ESI+) m/z 357.08 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}ClN_4O_3$ $(M - Cl)^+$ 357.0749, found 357.0751.

N^4-(2-Fluorophenyl)-N^2-4-phenylpyrimidine-2,4-diamine (6l, Method m in Scheme 2). A mixture of chloropyrimidine **5a** (0.073 g, 0.325 mmol) and aniline (0.04 mL, 0.438 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was removed under reduced pressure. Aqueous saturated $NaHCO_3$ (10 mL) was added to the residue and extracted with ethyl acetate (10 mL × 2). The combined organic phase was dried (Na_2SO_4), filtered, and concentrated to dryness. The residue was purified by flash chromatography (10 g silica gel, hexane/EtOAc) to afford the title compound **6l** (0.050 g, 55%) as an off-white solid, mp 133.2−134.6 °C. HPLC 99.5% (t_R = 4.4 min, 60% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H), 9.07 (s, 1H), 8.02 (d, J = 5.7 Hz, 1H), 7.97 (t, J = 7.9 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.32−7.23 (m, 1H), 7.22−7.07 (m, 4H), 6.86 (t, J = 7.3 Hz, 1H), 6.30 (d, J = 5.7 Hz, 1H); LC−MS (ESI+) m/z 281.11 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{16}H_{14}FN_4$ $(M - Cl)^+$ 281.1197, found 281.1209.

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N^4-(2-Iodophenyl)-N^2-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6m, Method m in Scheme 2). A mixture of chloropyrimidine **5h** (0.119 g, 0.358 mmol) and 4-aminobenzoic acid (0.048 g, 0.350 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was then removed under reduced pressure to provide an off-white solid. The solid was slurried in methanol (2 mL), filtered, washed with methanol (2 mL), and dried under vacuum to provide the title compound **6m** (0.053 g, 32%) as a white solid, mp 253 °C (dec). HPLC 99.7% (t_R = 7.80 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (bs, 1H), 10.72 (s, 1H), 10.62 (s, 1H), 8.07 (d, J = 7.0 Hz, 1H), 8.01 (dd, J = 1.2, 7.9 Hz, 1H), 7.67 (d, J = 8.5 Hz, 2H), 7.54−7.45 (m, 4H), 7.19−7.15 (m, 1H), 6.44 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.36, 163.09, 152.32, 145.44, 142.04, 139.99, 139.67, 130.68, 130.04, 129.94, 129.45, 126.12, 119.90, 99.24; LC−MS (ESI+) m/z 433.02 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}IN_4O_2$ (M − Cl)$^+$ 433.0156, found 433.0150.

N^4-(2-Cyanophenyl)-N^2-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6n, Method m in Scheme 2). A mixture of chloropyrimidine **5e** (0.094 g, 0.406 mmol) and 4-aminobenzoic acid (0.037 mg, 0.408 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The solvent was removed under reduced pressure. The compound was purified by reverse phase C-18 preparative HPLC (Eclipse XDB-C18 PrepHT 21.2 mm × 250 mm, 7 μm; 40% MeOH, 60% water (with 0.1% TFA), 20 min, 20 mL/min) to provide the title compound as its TFA salt. The solid obtained was suspended in methanol (9 mL) followed by the addition of HCl (4 M in dioxane, 1 mL). The solvent was then removed in a Genevac evaporator. The dried solid was slurried with acetonitrile (1 mL), filtered, washed with acetonitrile (1 mL), and dried under vacuum to provide the title compound **6n** (0.022 g, 15%) as an off-white solid, mp 222 °C (dec). HPLC 97.6% (t_R = 5.5 min, 40% MeOH, 60% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 10.43 (s, 1H), 8.14 (d, J = 6.8 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 7.72−7.68 (m, 3H), 7.55 (d, J = 7.3 Hz, 2H), 7.50 (t, J = 7.6 Hz, 1H), 6.50 (d, J = 6.8 Hz, 1H); LC−MS (ESI+) m/z 332.11 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{14}N_5O_2$ (M − Cl)$^+$ 332.1142, found 332.1139.

N^4-(2-Chlorophenyl)-N^2-(3-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6o, Method m in Scheme 2). A mixture of **2l** (0.100 g, 0.417 mmol) and 3-aminobenzoic acid (0.057 g, 0.417 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The precipitate obtained upon cooling the reaction mixture was filtered and washed quickly with ethanol (3 mL) to obtain the **6o** (0.097 g, 62%) as a white solid, mp 290−296 °C (dec). HPLC 100% (t_R = 7.58 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.03 (s, 1H), 10.49 (brs, 1H), 8.04 (d, J = 6.4 Hz, 1H), 7.88 (brs, 1H), 7.73 (appd, J = 7.6 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.57 (dd, J = 7.9, 1.5 Hz, 1H), 7.37 (td, J = 7.6, 0.8 Hz, 1H), 7.32−7.27 (m, 2H), 6.49 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5, 162.95, 153.3, 145.9, 138.08, 134.6, 132.1, 130.5, 129.6, 129.5, 129.1, 128.89, 128.36, 125.9, 125.5, 122.6, 99.5. Anal. Calcd for $C_{17}H_{14}Cl_2N_4O_2$: C, 54.13; H, 3.74; N, 14.85. Found: C, 53.78; H, 3.64; N, 14.66.

N^4-(2-Chlorophenyl)-N^2-(4-carbamoyl)pyrimidine-2,4-diamine Hydrochloride (6p, Method l in Scheme 2). A mixture of chloropyrimidine **2l** (0.100 g, 0.417 mmol) and 4-aminobenzamide (0.057 g, 0.417 mmol) in EtOH (2.0 mL with 1 drop of 1 M hydrochloric acid) was heated with a microwave reactor at 160 °C for 15 min. The mixture was filtered and the solid obtained was washed with MeOH (1 mL) and dried to afford the desired compound **6p** (0.110 g, 70%) as a white solid, mp 227 °C (dec). HPLC 99% (t_R = 3.70 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.75 (brs, 2H disappeared on D_2O shake), 8.09 (dd, J = 6.1, 3.5 Hz, 1H), 7.91 (brs, 1H disappeared on D_2O shake), 7.71−7.64 (m, 4H), 7.50−7.42 (m, 4H), 7.30 (brs, 1H disappeared on D_2O shake), 6.52 (brs, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.76, 163.15, 152.33, 144.97, 140.39, 134.55, 130.62, 130.29, 130.02, 129.54, 129.49, 128.90, 128.55, 119.98, 99.43; LC−MS (ESI+) m/z 340.10 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{15}ClN_5O$ (M − Cl)$^+$ 340.0960, found 340.0971.

N^4-(2-Biphenyl)-N^2-(4-carboxyphenyl)pyrimidine-2,4-diamine Hydrochloride (6q, Method m in Scheme 2). A mixture of chloropyrimidine **5i** (0.096 g, 0.34 mmol) and 4-aminobenzoic acid (0.054 g, 0.393 mmol) in EtOH (0.4 mL) was heated in a microwave reactor at 150 °C for 20 min. The resulting precipitate was filtered and washed with EtOH (0.5 mL × 3) to provide the title compound **6q** (0.098 g, 67%) as a white solid, mp 259 °C (dec). HPLC 100% (t_R = 8.7 min, 55% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 10.96 (s, 1H), 10.71 (s, 1H), 7.96 (d, J = 7.2 Hz, 1H), 7.73 (d, J = 8.3 Hz, 2H), 7.61−7.42 (m, 6H), 7.42−7.17 (m, 5H), 6.31 (apparent s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.41, 163.26, 152.00, 144.30, 142.07, 138.96, 134.37, 131.35, 130.75, 129.35, 129.09, 128.74, 128.17, 126.18, 119.89, 99.22; LC−MS (ESI−) m/z 381.13 (M − H − HCl)$^-$; HRMS (ESI−) m/z calculated for $C_{23}H_{17}N_4O_2$ (M − H − HCl)$^-$ 381.1357, found 381.113 66.

N^4-(2-Chlorophenyl)-N^2-(4-carboxyphenyl)-N^4-methylpyrimidine-2,4-diamine Hydrochloride (6r, Method m in Scheme 2). This was obtained as a white solid (0.127 g, 0.36 mmol, 80%) from **5j** (0.115 g, 0.452 mmol) and 4-aminobenzoic acid (0.062 g, 0.452 mmol) in the same manner as described for **6s**, mp 288 °C (dec). HPLC 98% (t_R = 8.39 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, CD_3OD) δ 8.17−8.04 (m, 2.57 H), 7.77−7.74 (m, 3H), 7.70−7.67 (m, 2H), 7.58−7.54 (m, 4H), 7.49−7.41 (m, 0.42 H), 7.17 (d, J = 8.8 Hz, 1H, major), 6.83 (d, J = 7.5 Hz, 0.41 H, minor), 5.80 (d, J = 7.4 Hz, 1H, major), 3.55 (s, 4H); LC−MS (ESI+) m/z 355.10 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{16}ClN_4O_2$ (M − Cl)$^+$ 355.0956, found 355.0962. Anal. Calcd for $C_{18}H_{16}Cl_2N_4O_2$: C, 55.26%; H, 4.12; N, 14.32. Found: C, 54.98%; H, 4.12; N, 14.18. .

N^4-(2-Chlorophenyl)-N^2-(4-carboxyphenyl)-N^4-ethylpyrimidine-2,4-diamine Hydrochloride (6s, Method m in Scheme 2). A solution of **5k** (0.151 g, 0.673 mmol) and 4-aminobenzoic acid (0.092 g, 0.674 mmol) in anhydrous ethanol (0.673 mL) was heated in a microwave reactor at 150 °C for 20 min. The resulting precipitate was isolated by filtration and washed with EtOH (1 mL × 2) and dried under vacuum to provide the title compound **6s** (0.172 g, 71%) as a white solid, mp 284 °C (dec). HPLC 100% (t_R = 13.96 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min). Two rotamers are present: ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, J = 8.8 Hz, 2H, major), 8.07 (d, J = 7.6 Hz, 0.4 H, minor), 7.75−7.66 (m, 5H), 7.63−7.54 (m, 5H), 7.15 (d, J = 8.8 Hz, 1H, major), 6.87 (d, J = 7.5 Hz, 0.5 H, minor), 5.74 (d, J = 7.4 Hz, 1H, major), 4.30−4.21 (m, 1H), 4.09−4.02 (m, 0.6 H, minor), 3.93−3.83 (m, 2H), 1.35 (t, J = 7.2 Hz, 1.5 H, minor), 1.29 (t, J = 7.2 Hz, 3H, major); LC−MS (ESI+) m/z 369.12 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{19}H_{18}ClN_4O_2$ (M − Cl)$^+$ 369.1113, found 369.1123. Anal. Calcd for $C_{19}H_{18}Cl_2N_4O_2$: C, 56.31%; H, 4.48; N, 13.82. Found: C, 56.30%; H, 4.45; N, 13.76.

N^4-(2-Chlorophenyl)-N^2-(3-carboxyphenyl)-5-fluoropyrimidine-2,4-diamine Hydrochloride (6t, Method m in Scheme 2). A mixture of **2k** (0.100 g, 0.350 mmol) and 3-aminobenzoic acid (0.048 g, 0.350 mmol) in EtOH (0.5 mL) was heated in a microwave reactor at 150 °C for 20 min. The reaction mixture was cooled and the precipitate obtained was filtered and washed quickly with ethanol (3 mL) to provide **6t** (0.080 g, 57%) as a white solid, mp 257 °C (dec). HPLC 98% (t_R = 9.45 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.74 (brs,1H), 9.32 (s, 1H), 9.18 (s, 1H), 8.12 (d, J = 3.5 Hz, 1H), 8.02 (s, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.40−7.36 (m, 2H), 7.29 (t, J = 7.6 Hz, 1H), 7.11 (t, J = 7.9 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.14, 156.0 (d, J = 2.9 Hz), 151.37 (d, J = 11.5 Hz), 141.75, 141.43 (d, J = 246.44 Hz), 141.39 (d, J = 18.4 Hz), 135.92, 131.54, 130.88, 130.38, 129.55, 128.90, 128.32, 128.03, 122.84, 122.18, 119.87; ^{19}F NMR (376 MHz, DMSO-d_6) δ −165.76; LC−MS (ESI−) m/z 357.06 (M − H − HCl)$^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{12}ClFN_4O_2$ (M − H − HCl)$^-$ 357.0560, found 357.0554.

N^4-(2-Chlorophenyl)-N^2-[4-*N*-(2-morpholinoethyl)-carbamoylphenyl]pyrimidine-2,4-diamine Dihydrochloride (9a, Method r in Scheme 4). A solution of **2l** (0.100 g, 0.42 mmol) and **8a** (0.105 g, 0.42 mmol) in isopropyl alcohol (8 mL) and

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concentrated HCl (0.315 mL) was heated in a microwave reactor at 170 °C for 20 min. The solvent was then removed under reduced pressure. The resulting solid was dissolved in methanol (2 mL) followed by the addition of diethyl ether (10 mL). The precipitate was filtered and dried under vacuum to afford the title compound **9a** (0.054 g, 29%) as white crystals, mp 173.0−174.6 °C. HPLC 99.0% (t_R = 5.53 min, 50% MeOH, 40% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.37−10.62 (m, 1H), 8.88 (s, 1H), 8.59−8.43 (m, 1H), 8.12 (s, 1H), 7.78 (s, 2H), 7.69−7.61 (m, 2H), 7.50 (s, 2H), 7.43 (s, 1H), 6.76−6.41 (m, 2H), 4.03−2.95 (m, 12H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.39, 163.08, 152.86, 145.97, 140.91, 134.67, 130.67, 130.15, 129.42, 129.24, 128.82, 128.60, 119.81, 99.46, 63.80, 56.32, 51.87, 34.28; LC−MS (ESI+) m/z 453.17 (M − HCl − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{23}H_{25}ClN_6O_2$ (M − HCl − Cl)$^+$ 453.1800, found 453.1797.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[4-*N*-(2-dimethylaminoethyl)-carbamoylphenyl]pyrimidine-2,4-diamine Dihydrochloride (9b, Method *r* in Scheme 4).** This was obtained as a white solid (0.02 g, 0.04 mmol, 8%) from **2l** (0.100 g, 0.48 mmol) and **8b** (0.116 g, 0.48 mmol) in a similar manner as described for **9a**, mp 143.1−145.8 °C. HPLC 98% (t_R = 5.10 min, 60% MeOH, 40% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 9.96 (s, 1H), 8.75 (s, 1H), 8.09 (s, 1H), 7.73 (s, 2H), 7.71−7.61 (m, 2H), 7.58−7.45 (m, 3H), 7.45−7.35 (m, 1H), 6.50 (s, 1H), 3.62−3.55 (m, 2H), 3.23 (d, J = 5.7 Hz, 2H), 2.80 (d, J = 4.0 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.50, 163.10, 152.72, 145.80, 140.87, 134.73, 130.67, 130.19, 129.45, 129.31, 128.61, 119.80, 99.50, 56.76, 43.01, 35.16; LC−MS (ESI +) m/z 411.18 (M − HCl − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{21}H_{24}ClN_6O$ (M − HCl − Cl)$^+$ 411.1695, found 411.1693.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[4-*N*-(2-methoxyethyl)-carbamoylphenyl]pyrimidine-2,4-diamine Hydrochloride (9c, Method s in Scheme 4).** This was obtained as a white solid (0.047 g, 0.12 mmol) from **2l** (0.092 g, 0.47 mmol) and **8c** (0.060 g, 0.47 mmol) in a similar manner as described for compound **6s**, mp 210.4−213.0 °C. HPLC 97% (t_R = 5.61 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, 2H), 8.44 (t, J = 5.6 Hz, 1H), 8.08 (d, J = 7.3 Hz, 1H), 7.68−7.62 (m, 4H), 7.49−7.38 (m, 3H), 6.52 (s, 1H), 3.41−3.37 (m, 4H), 3.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.06, 163.12, 152.41, 145.17, 140.32, 134.55, 130.60, 130.23, 130.08, 129.50, 129.42, 128.56, 119.98, 99.48, 71.50, 58.58, 58.55; LC−MS (ESI+) m/z 398.14 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{20}H_{21}ClN_5O_2$ (M − Cl)$^+$ 398.1378, found 398.1370.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[4-(methylsulfonyl)phenyl]-pyrimidine-2,4-diamine (9d, Method t in Scheme 4).** A solution of **2l** (0.098 g, 0.41 mmol) and 4-(methylsulfonyl)aniline (0.070 g, 0.41 mmol) in ethanol (1 mL) and 1 M HCl (aqueous, 1.0 mL) was heated in a microwave reactor at 180 °C for 15 min. The solvent was removed under reduced pressure. The solid obtained was slurred in a saturated solution of sodium bicarbonate, filtered, slurried with methanol (1 mL), then filtered to yield the title compound **9d** (0.077 g, 44%) as a white solid, mp 169.3−171.3 °C. HPLC 99% (t_R = 4.15 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 9.17 (s, 1H), 8.08 (d, J = 5.7 Hz, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 7.8, 1H), 7.65−7.51 (m, 3H), 7.41 (t, J= 7.6, 1H), 7.26 (t, J = 7.6 Hz, 1H), 6.35 (d, J = 5.7 Hz, 1H), 3.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.03, 159.47, 156.92, 146.25, 136.52, 132.20, 130.40, 129.03, 128.38, 128.34, 128.21, 127.12, 118.38, 99.77, 44.70; LC−MS (ESI+) m/z 375.06 (M + H)$^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{16}ClN_4O_2S$ (M + H)$^+$ 375.0677, found 375.0670.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-(3-acetamidophenyl)pyrimidine-2,4-diamine Hydrochloride (9e, Method s in Scheme 4).** This compound was prepared from **2l** (0.105 g, 0.44 mmol) and *N*-(3-aminophenyl)acetamide (0.066 g, 0.44 mmol) in a similar manner as described for **6s**. The solvent was then removed under reduced pressure. The resulting solid was dissolved in methanol (2 mL), followed by the addition of ethyl acetate (10 mL). A precipitate formed, and the mixture was sonicated for 30 min at room temperature. The precipitate was filtered and washed with ethyl acetate (10 mL) and hexane (20 mL), affording the pure product **9e** (0.166 g, 95%) as an off-white solid, mp 150 °C (dec). HPLC 100% (t_R = 5.30 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H), 10.45 (s, 1H), 10.00 (s, 1H), 8.00 (d, J = 5.0 Hz, 1H), 7.70−7.52 (m, 3H), 7.4−7.28 (m, 2H), 7.24−7.13 (m, 2H), 7.13−7.07 (m, 1H), 2.02 (s, 3H); LC−MS (ESI+) m/z 354.11 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{17}ClN_5O$ (M − Cl)$^+$ 354.1116, found 354.1119.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[6-(1*H*-benzo[*d*]imidazol-2(3*H*)-one)]pyrimidine-2,4-diamine Hydrochloride (9f, Method s in Scheme 4).** A solution of **2l** (0.105 g, 0.437 mmol) and 5-amino-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.065 g, 0.437 mmol) and ethanol (0.437 mL) was heated in a microwave reactor at 150 °C for 40 min. The resulting precipitate was filtered, dried under vacuum, and suspended in ethanol. The suspension was sonicated for 30 min, filtered, and dried under vacuum to provide the title compound **9f** (0.112 g, 66%) as an off-white solid, mp 263 °C (dec). HPLC 98% (t_R = 3.21 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (s, 2H), 7.65−7.63 (m, 1H), 7.54−7.52 (m, 1H), 7.35−7.28 (m, 2H), 7.04 (d, J = 7.4 Hz, 3H), 6.42 (s, 1H); LC−MS (ESI+) m/z 353.10 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}ClN_6O$ (M − Cl)$^+$ 353.0912, found 353.0913.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-(4-carboxyl-3-methoxyphenyl)-pyrimidine-2,4-diamine Hydrochloride (9g, Method s in Scheme 4).** A mixture of chloropyrimidine **2l** (0.100 g, 0.416 mmol) and 4-amino-2-methoxybenzoic acid (0.070 g, 0.418 mmol) in EtOH (0.5 mL) was heated with a microwave reactor at 150 °C for 20 min. The solvent was evaporated from the resulting thick mass under reduced pressure. The residue was suspended in ethyl acetate (8 mL) and sonicated for 10 min. The mixture was filtered and the solid was washed with ethyl acetate (8 mL) and ethyl acetate/methanol (1:1, 1 mL) to provide the title compound **9g** (0.068 g, 57%) as a gray solid, mp 160−162 °C. HPLC 95% (t_R = 4.5 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6), δ 12.69 (s, 1H), 10.42 (s, 1H), 10.24 (s, 1H), 7.95 (d, J = 6.2 Hz, 1H), 7.64−7.51 (m, 3H), 7.48 (d, J = 8.4 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.29 (t, J = 7.4 Hz, 1H), 6.98 (d, J = 8.8 Hz, 1H), 6.45 (s, 1H), 3.76 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 167.37, 162.97, 155.67, 152.95, 144.61, 134.39, 130.51, 129.61, 129.00, 128.35, 127.11, 124.90, 122.22, 113.36, 99.26, 56.70; LC−MS (ESI+) m/z 371.09 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{18}H_{16}ClN_4O_3$ (M − Cl)$^+$ 371.0905, found 371.0909.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[4-(*N*-morpholino)phenyl]-pyrimidine-2,4-diamine Hydrochloride (9h, Method t in Scheme 4).** A mixture of chloropyrimidine **2l** (0.120 g, 0.500 mmol) and 4-morpholinoaniline (0.089 g, 0.500 mmol) in EtOH (2 mL with 1 drop of 1 M of hydrochloric acid) was heated in a microwave reactor at 160 °C for 15 min. The reaction mixture was cooled to room temperature and the product precipitated. The mixture was filtered, and the product obtained was quickly washed with MeOH (1 mL), DCM (1 mL) and dried to afford **9h** (0.14 g, 67%) as a green solid, mp 136−138 °C. HPLC 99% (t_R = 7.43 min, 50% MeOH in 0.1% TFA water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H disappeared on D_2O shake), 10.63 (s, 1H disappeared on D_2O shake), 10.37 (s, 1H disappeared on D_2O shake), 7.94 (brs, 1H), 7.65−.60 (m, 2H), 7.43 (appt, J = 7.6 Hz, 1H), 7.36 (appt, J = 7.6 Hz, 1H), 7.22 (d, J = 8.1 Hz, 2H), 6.84 (brs, 2H), 6.45 (brs, 1H), 3.73 (appt, J = 4.4 Hz, 4H), 3.05 (brt, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.03, 152.56, 148.63, 144.36, 134.58, 130.53, 129.99, 129.37, 129.12, 128.43, 122.90, 116.05, 98.78, 66.65, 49.38; LC−MS (ESI+) m/z 382.15 (M − Cl)$^+$; HRMS (ESI+) m/z calculated for $C_{20}H_{21}ClN_5O$ (M − Cl)$^+$ 382.1429, found 382.1434.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[4-(aminosulfonyl)phenyl]-pyrimidine-2,4-diamine Hydrochloride (9i, Method t in Scheme 4).** A mixture of chloropyrimidine **2l** (0.120 g, 0.500 mmol) and 4-aminobenzenesulfonamide (0.086 g, 0.500 mmol) in EtOH (2 mL with 1 drop of 1 M HCl) was heated in a microwave reactor at 160 °C for 15 min. The precipitate formed upon cooling was filtered and washed with MeOH (2 mL), then slurried and sonicated in DCM (5 mL) and filtered. The product obtained was dried to afford **9i** (0.155 g, 75%) as a white solid, mp 215 °C (dec). HPLC 96% (t_R = 3.23 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 10.67 (s, 1H), 8.10 (d, J = 6.9 Hz, 1H), 7.67−7.63 (m, 2H), 7.61−7.56 (m, 4H), 7.47 (td, J = 7.7, 1.6 Hz, 1H), 7.41 (td, J = 7.7, 1.6 Hz, 1H), 7.28 (s, 2H), 6.53 (appd, J = 5.2 Hz, 1H); ^{13}C NMR (100 MHz,

DMSO-d_6) δ 163.15, 152.43, 145.28, 140.86, 139.52, 134.61, 130.64, 130.38, 129.60, 129.50, 128.57, 127.10, 120.32, 99.66; LC−MS (ESI+) m/z 376.06 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{16}H_{15}ClN_5O_2S$ $(M - Cl)^+$ 376.0629, found 376.0634.

N^4-(2-Chlorophenyl)-N^2-[4-(*N*-morpholino)phenyl]-5-fluoropyrimidine-2,4-diamine (9j, Method s in Scheme 4). A mixture of chloropyrimidine **2k** (0.100 g, 0.387 mmol) and 4-morpholinoaniline (0.069 g, 0.387 mmol) in ethanol (1 mL) was heated in a microwave reactor at 150 °C for 20 min. The reaction mixture was cooled and stirred at room temperature for 30 min. The solvent was removed in vacuo. The residual solid was suspended in ethyl acetate (10 mL) and sonicated for 10 min, filtered, and washed with ethyl acetate (5 mL). The solid was suspended in sodium bicarbonate (saturated aqueous 50 mL), filtered, and washed with water (10 mL) to provide the title compound **9k** (0.92 g, 60%) as an off-white solid, mp 209−211 °C. HPLC 99% (t_R = 7.2 min, 50% MeOH, 50% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 9.36 (s, 1H), 8.11 (d, J_{H-F} = 4.2 Hz, 1H), 7.61−7.54 (m, 2H), 7.44−7.37 (m, 1H), 7.36−7.25 (m, 3H), 6.79 (s, 2H), 3.73 (s, 4H), 3.03 (s, 4H); ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 8.06 (d, J = 4.1 Hz, 1H), 7.59−7.51 (m, 2H), 7.39 (td, J = 7.6, 1.5 Hz, 1H), 7.32 (td, J = 7.7, 1.7 Hz, 1H), 7.27 (d, J = 9.0 Hz, 2H), 6.79 (d, J = 8.9 Hz, 2H), 3.71 (t, J = 4.0 Hz, 4H), 3.03 (t, J = 4.4 Hz, 4H); ^{19}F NMR (376 MHz, DMSO-d_6), δ −165.59; ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.38, 152.31 (d, J = 12.0 Hz), 141.63, 139.04, 139.19, 135.42, 134.20, 130.43, 129.76, 120.74, 117.11, 66.31, 50.71; LC−MS (ESI+) m/z 400.13 $(M - Cl)^+$; HRMS (ESI+) m/z calculated for $C_{20}H_{20}ClFN_5O$ $(M - Cl)^+$ 400.1335, found 400.1344.

4-(2-Chlorophenylamino)-2-(4-(morpholinomethyl)phenyl)-5-fluoropyrimidine (9k, Method u in Scheme 4). A mixture of chloropyrimidine **2k** (0.100 g, 0.387 mmol), 4-(morpholinomethyl)-aniline (0.087 g, 0.452 mmol), X-Phos (2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl) (0.019 g, 0.0387 mmol), bis-(dibenzylideneacetone)palladium(0) (0.022 g, 0.0387 mmol), potassium carbonate (0.117 g, 0.851 mmol) in *t*-BuOH (3.0 mL) was heated under reflux for 18 h. The solvent was evaporated from the resulting dark solution. The dark residue was purified by chromatography (SiO_2, ethyl acetate, and hexanes) to provide the title compound **9k** (0.062 g, 39%) as an off-white solid, mp 142−144 °C. HPLC 98% (t_R = 23.4 min, 50% MeOH, 50% water (with 0.1% TFA), 36 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.16 (s, 1H), 9.10 (s, 1H), 8.07 (d, J_{H-F} = 2.6 Hz, 1H), 7.58 (t, J = 6.8 Hz, 2H), 7.42−7.34 (m, 3H), 7.31 (t, J = 8.2 Hz, 1H), 6.94 (d, J = 7.7 Hz, 2H), 3.52 (s, 4H), 2.26 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 156.22 (d, J_{CF} = 2.8 Hz), 151.27 (d, J_{CF} = 11.4 Hz), 141.15 (d, J_{CF} = 245 Hz), 141.62 (d, J_{CF} = 18.9 Hz), 140.44, 136.11, 131.08, 130.33, 130.26, 129.73, 129.52, 128.26, 128.01, 118.57, 66.84, 62.79, 53.74; ^{19}F NMR (376 MHz, DMSO-d_6), δ −166.63 (s); LC−MS (ESI+) m/z 327.08 (M − morpholine); HRMS (ESI+) m/z calculated for $C_{21}H_{22}ClFN_5O$ $(M + H)^+$ 414.1491, found 414.1497 $(M + H)^+$, 327.0819 (M − morpholine).

N^4-(2-Chlorophenyl)-N^2-[4-*N*-(2-hydroxyethyl)-carbamoylphenyl]pyrimidine-2,4-diamine (9l, Method t in Scheme 4). A mixture of chloropyrimidine **2l** (0.100 g, 0.416 mmol) and 4-amino-*N*-(2-hydroxyethyl)benzamide (0.075 g, 0.416 mmol) in HCl (1 mL of 0.1 N aqueous) was heated in a microwave reactor at 140 °C for 30 min. The contents were then stirred in the microwave vial at room temperature for 30 min. The mixture was filtered, and the obtained solid was washed with water (20 mL) and sodium bicarbonate (saturated aqueous 50 mL). The crude product was purified by chromatography (silica gel, hexane/ethyl acetate) to provide **9l** as an off-white solid (0.087 g, 54%), mp 171−173 °C. HPLC 100% (t_R = 8.8 min, 40% MeOH, 60% water (with 0.1% TFA), 20 min); ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.28 (t, J = 6.2 Hz, 1H, peak disappeared upon D_2O shake), 8.00 (d, J = 5.9 Hz, 1H), 7.84 (dd, J = 8.0, 1.5 Hz, 1H), 7.71−7.64 (m, 4H), 7.50 (dd, J = 8.0, 1.5 Hz, 1H), 7.37 (td, J = 8.0, 1.5 Hz, 1H), 7.22 (td, J = 8.0, 1.5 Hz, 1H), 6.30 (d, J = 5.9 Hz, 1H), 3.70 (t, J = 5.5 Hz, 2H), 3.49 (q, J = 5.5 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6), δ 172.68, 166.63, 161.90, 159.62, 156.84, 144.10, 136.56, 130.31, 128.61, 128.27, 128.07, 126.92, 126.82, 117.93, 99.18, 60.56, 42.68; LC−MS (ESI+) m/z 384.12 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{19}H_{19}ClN_5O_2$ $(M + H)^+$ 384.1222, found 384.1219.

N^4-(2-Chlorophenyl)-N^2-(4-carbamoyl)-5-fluoropyrimidine-2,4-diamine (9m, Method v in Scheme 4). A mixture of chloropyrimidine **2k** (0.500 g, 1.935 mmol) and 4-aminobenzamide (0.265 g, 1.935 mmol) in methanol (3 mL) was heated in a 5 mL sealed pressure tube at 100 °C for 6 h. The white precipitate was isolated by filtration and washed with methanol (2 mL). The white solid was sonicated for 5 min in sodium bicarbonate (aqueous saturated, 10 mL). The mixture was filtered and the solid washed with water (10 mL) and finally with methanol (2 mL) to provide **9m** (0.439 g, 63%) as an off-white powder, mp 250−252 °C. HPLC 100% (t_R = 7.8 min, 50% MeOH, 50% water (with 0.1% formic acid), 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 (s, 1H), 9.31 (s, 2H), 8.12 (d, J_{H-F} = 3.6 Hz, 1H), 7.70 (s, 1H), 7.59 (dd, J = 8.6, 1.3 Hz, 1H), 7.56 (dd, J = 8.6, 1.3 Hz) overlapping 7.54 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 8.7 Hz, 2H), 7.42 (td, J = 7.7, 1.5 Hz, 1H), 7.35 (td, J = 7.6, 1.6 Hz, 1H), 7.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.57, 155.66 (d, J_{C-F} = 3.0 Hz), 151.35 (d, J_{C-F} = 11.7 Hz), 144.27, 142.54, 141.66 (d, J_{C-F} = 19.0 Hz), 140.09, 135.77, 131.27, 130.41, 129.79, 128.67, 128.41, 126.18, 117.31; ^{19}F NMR (376 MHz, DMSO-d_6), δ −165.45 (bd, J_{H-F} = 3.4 Hz) LC−MS (ESI+) m/z 358.09 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{17}H_{14}ClFN_5O$ $(M + H)^+$ 358.0865, found 358.0868.

N^4-(2-Chlorophenyl)-N^2-(4-aminosulfonyl)-5-fluoropyrimidine-2,4-diamine (9n, Method u in Scheme 4). A mixture of chloropyrimidine **2k** (free base) (0.100 g, 0.387 mmol), 4-amino-benzenesulfonamide (0.066 g, 0.0.387 mmol), X-Phos (2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl) (0.019 g, 0.0387 mmol), bis(dibenzylideneacetone)palladium(0) (0.022 g, 0.0387 mmol), and potassium carbonate (0117 g, 0.851 mmol) in *t*-BuOH (2.0 mL) was heated in a 5 mL sealed pressure tube at 100 °C for 64 h under argon. The solid was isolated by filtration and suspended in ethyl acetate (5 mL) and sonicated for 5 min. The mixture was filtered and the solid washed with ethyl acetate (5 mL). The crude product was dissolved in DMSO (2 mL) and filtered to remove undissolved material. The DMSO was removed in vacuo at 50 °C to provide the title sulfonamide **9n** (0.080 g, 53%) as a brown solid, mp 253−255 °C. HPLC 99% (t_R = 11.2 min, 40% MeOH, 60% water (with 0.1% TFA), 66 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 9.39 (s, 1H), 8.13 (d, J_{H-F} = 3.7 Hz, 1H), 7.61−7.52 (m, 4H), 7.46−7.38 (m, 3H), 7.35 (t, J = 8.0 Hz, 1H), 7.07 (s, 1H); ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ 8.09 (d, J_{H-F} = 3.5 Hz, 1H), 7.58 (dd, J = 7.9, 1.5 Hz, 1H), 7.54−7.48 (m, 3H), 7.44−7.39 (m, 3H), 7.35 (dt, J = 7.4, 1.7 Hz, 1H); ^{19}F NMR (376 MHz, DMSO-d_6 + D_2O), δ −164.56 (d, J = 3.1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) 155.67 (d, J_{C-F} = 2.8 Hz), 151.57, 151.46, 144.66, 142.73, 141.79, 141.60, 140.27, 136.06, 135.87, 131.65, 130.42, 130.19, 128.42, 126.85, 117.46; LC−MS (ESI+) m/z 394.05 $(M + H)^+$; HRMS (ESI+) m/z calculated for $C_{16}H_{14}ClFN_5O_2S$ $(M + H)^+$ 394.0535, found 394.0537.

N^4-(2-Chlorophenyl)-N^2-[4-(2*H*-tetrazol-5-yl)phenyl]-pyrimidine-2,4-diamine Hydrochloride (12a, Method a in Scheme 5). A mixture of **2l** (0.072 g, 0.299 mmol) and 4-(2*H*-tetrazol-5-yl)phenylamine (**11a**) (0.050 g, 0.310 mmol) in EtOH (2.0 mL) was heated in a microwave reactor at 150 °C for 40 min. The precipitate obtained upon cooling the reaction mixture was filtered and washed with EtOH (5 mL) to provide **12a** (0.095 g, 79%) as a yellow solid (^{1}H NMR analysis indicated the presence of trace impurities). The product was further purified by washing with methanol (5 mL) to give pure **12a** (0.045 g, 37%) as a yellow solid, mp 250 °C (dec). HPLC 100% (t_R = 6.21 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (s, 1H), 10.64 (s, 1H), 8.09 (d, J = 6.9 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.69−7.59 (m, 4H), 7.48 (t, J = 7.4 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 6.52 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.07, 153.08, 146.47, 140.76, 134.74, 130.64, 130.17, 129.48, 129.32, 129.31, 128.57, 128.21, 121.13, 119.58. Anal. Calcd for $C_{17}H_{14}Cl_2N_8$: C, 50.89; H, 3.52; N, 27.93. Found: C, 51.22; H, 3.50; N, 27.54. LC−MS (ESI−) m/z 363.08 $(M - H - HCl)^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{12}ClN_8$ $(M - H - HCl)^-$ 363.0879, found 363.0871.

N^4-(2-Chlorophenyl)-N^2-[4-(2*H*-tetrazol-5-yl)phenyl]-5-fluoropyrimidine-2,4-diamine Hydrochloride (12b, Method a in Scheme 5). A mixture of **2k** (free base) (0.076 g, 0.298 mmol) and 4-

(2*H*-tetrazol-5-yl)phenylamine (**11a**) (0.050 g, 0.310 mmol) in EtOH (2.0 mL) was heated in a microwave reactor at 170 °C for 40 min. The precipitate obtained upon cooling the reaction mixture was filtered and washed with EtOH (5.0 mL) to get **12b** (0.090 g, 72%) as a brown-yellow solid. The ^{1}H NMR spectrum showed the presence of a baseline impurity, and the product was further purified by washing with methanol (5 mL) to provide pure **12b** (0.041 g, 33%) as a brown-yellow solid, mp 223 °C (dec). HPLC 99% (t_R = 10.37 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.98 (s, 1H), 9.91 (s, 1H), 8.25 (d, J = 4.0 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.64 (d, J = 7.8 Hz, 1H), 7.62−7.56 (m, 3H), 7.50−7.38 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.74 (d, J = 3.0 Hz), 151.50 (d, J = 11.7 Hz), 144.37, 141.45 (d, J = 247.45 Hz), 141.68 (d, J = 17.6 Hz), 136.06, 131.58, 130.43, 130.15, 128.41, 127.85, 118.47; ^{19}F NMR (376 MHz, DMSO) δ −165.12 (s). Anal. Calcd for $C_{17}H_{13}Cl_2FN_8$: C, 48.70; H, 3.13; N, 26.73. Found: C, 49.09; H, 3.13; N, 26.48. LC−MS (ESI−) m/z 381.1 (M − H)$^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{11}ClFN_8$ (M − H)$^-$ 381.0785, found 381.0784.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[3-hydroxy-4-(2*H*-tetrazol-5-yl)-phenyl]-5-fluoropyrimidine-2,4-diamine Hydrochloride (12c, Method a in Scheme 5).** A mixture of **2k** (freebase) (0.072 g, 0.279 mmol) and tetrazole **11b** (0.050 g, 0.282 mmol) in EtOH/HCl (1:1, 2.0 mL, 0.1 M HCl) was heated in a microwave reactor at 160 °C for 40 min. The precipitate obtained upon cooling was filtered and rinsed with EtOH (5 mL) to obtain the product with baseline impurity (0.096 g, 77%) as a yellow solid. This solid was further purified by washing with methanol (5 mL) to provide pure **12c** (0.044 g, 36%) as a yellow solid, mp 214 °C (dec). HPLC 93% (t_R = 12.99 min, 50% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 9.44 (s, 1H), 9.24 (s, 1H), 8.13 (d, J = 3.3 Hz, 1H), 7.66−7.54 (m, 4H), 7.41 (t, J = 7.5 Hz, 1H), 7.33 (t, J = 7.4 Hz, 1H), 7.29−7.23 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) 156.34, 155.80 (d, J = 3.1 Hz), 151.46 (d, J = 11.5 Hz), 145.58, 141.61 (d, J = 247.2 Hz), 141.23 (d, J = 19.1 Hz), 135.90, 131.04, 130.42, 129.71, 129.25, 128.38, 128.20, 110.49, 105.32, 103.39; ^{19}F NMR (376 MHz, DMSO-d_6) δ −165.94 (s); LC−MS (ESI−) m/z 397.07 (M − H)$^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{11}ClFN_8O$ (M − H)$^-$ 397.0734, found 397.0730.

***N*^{4}-(2-Chlorophenyl)-*N*^{2}-[4-(2*H*-tetrazol-5-yl)phenyl]-5-chloropyrimidine-2,4-diamine Hydrochloride (12d, Method a in Scheme 5).** A mixture of chloropyrimidine **2o** (0.093 g, 0.3 mmol) and 4-(2*H*-tetrazol-5-yl)phenylamine (**11a**) (50 mg, 0.3 mmol) in EtOH (2 mL) was heated in a microwave reactor at 150 °C for 40 min. The precipitate obtained upon cooling was filtered and washed with EtOH (5 mL) to provide impure **12d** (105 mg, 81%) as a yellow color solid. Analysis of the ^{1}H NMR spectrum indicated the presence of trace impurities. The product was further purified by washing with hot ethyl acetate (5 mL) and hot methanol (5 mL) to provide pure **12d** (0.032 g, 25%) as a yellow solid, mp 238 °C (dec). HPLC 91% (t_R = 5.71 min, 70% MeOH in 0.1% TFA in water, 20 min); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 9.05 (s, 1H), 8.19 (s, 1H), 7.71−7.56 (m, 6H), 7.52−7.38 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.73, 157.53, 154.65, 143.69, 136.39, 131.71, 130.38, 130.32, 128.69, 128.49, 127.87, 119.04, 104.93; LC−MS (ESI−) m/z 397.05 (M − H)$^-$; HRMS (ESI−) m/z calculated for $C_{17}H_{11}Cl_2N_8$ (M − H)$^-$ 397.0489, found 397.0472.

■ ASSOCIATED CONTENT

Supporting Information

(i) Synthetic protocols and characterization data for **2a−o**, **5a−k**, **7a,b**, **8a,b**, **10a,b** and **11a,b**; (ii) scanned NMR spectra and HPLC and LC−MS reports for **1**, **3l**, **3n**, **3o**, **6c**, **6i**, **9h**, **9m**, **9n**, and **12a**; (iii) determination of co-crystal structures of **1**, **3i**, **3g**, **13**, and ADP with Aurora A; (iv) kinase profiling of **3o**; (v) IC_{50} data (Reaction Biology) for **9h**, **3l**, **3o** against Aurora A and JAK2; (vi) Aurora A IC_{50} data of compounds **13−24**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CML, chronic myeloid leukemia; DCM, dichloromethane; DEA, diethylamine; DMEM, Dulbecco's modified Eagle's medium; EI, electrospray ionization; ECL, enhanced chemiluminescence; EIF2AK3, eukaryotic translation initiation factor 2α kinase 3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GSK3β, glycogen synthase kinase 3β; HPLC, high performance liquid chromatography; HTS, high throughput screening; HRP, horseradish peroxidase; JAK2, Janus kinase 2; LC−MS, liquid chromatography−mass spectrometry; PBS, phosphate buffered saline; PIM3, provirus integration site for Moloney murine leukemia virus; NUAK1, SnF1-like kinase; SAR, structure−activity relationship; TLC, thin layer chromatography; TFA, trifluoroacetic acid

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