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Identification, SAR Studies, and X-ray Co-crystallographic Analysis of a Novel Furanopyrimidine Aurora Kinase A Inhibitor

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Herein we reveal a simple method for the identification of novel Aurora kinase A inhibitors through substructure searching of an in-house compound library to select compounds for testing. A hydrazone fragment conferring Aurora kinase activity and heterocyclic rings most frequently reported in kinase inhibitors were used as substructure queries to filter the in-house compound library collection prior to testing. Five new series of Aurora kinase inhibitors were identified through this strategy, with IC_{50} values ranging from ~300 nM to ~15 μM, by testing only 133 compounds from a database of ~125000 compounds. Structure–activity relationship studies and X-ray co-crystallographic analysis of the most potent compound, a furanopyrimidine derivative with an IC_{50} value of 309 nM toward Aurora kinase A, were carried out. The knowledge gained through these studies could help in the future design of potent Aurora kinase inhibitors.

Introduction

The identification of a lead molecule for any given molecular target is an important step in drug discovery programs. Many researchers traditionally use high-throughput screening (HTS) of large compound libraries to identify a lead compound for drug development. However, HTS of large libraries is a time- and resource-consuming process, so for the work presented herein, a faster and more economical knowledge-based approach was used to selectively screen an in-house HTS library consisting of 125000 compounds.

Protein kinases are second only to G-protein-coupled receptors as the most sought-after drug targets, and account for 20–30% of all drug discovery efforts.[1] Currently more than 500 human protein kinases have been identified. Protein kinases catalyze the transfer of a phosphate group from adenosine triphosphate (ATP) to a tyrosine or serine/threonine residue of the given substrate protein.[2,3] The phosphorylated substrate protein is thus activated for the particular role it plays among various cell-signaling processes; thus, protein kinases act as switches for many cell-signaling processes that regulate cell proliferation and differentiation. Small molecules designed to act as ATP-competitive inhibitors could disrupt protein kinase activity, thereby controlling the cell-signaling process, which is aberrant in many disease states.[3] In particular, aberrant protein kinase activity is linked to the development, progression, and prognosis of various cancers; this has prompted the development of small-molecule inhibitors that target such aberrant protein kinase activity. For example, Bcr-Abl activity is blocked by Imatinib, which is used for the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GISTs); EGFR kinase activity is inhibited by Gefitinib, which is used in the treatment of non-small-cell lung cancer (NSCLC).[4,5] Because of the successful launch of eight small-molecule inhibitors that target protein kinases for various cancer therapies (Figure 1), and given that many inhibitors are currently at various stages of clinical trials, the development of protein kinase inhibitors has taken center stage.

All protein kinases for which structural information is so far available adopt the same fold, with N- and C-terminal lobes connected through a short strand of amino acids, referred to as the hinge region. ATP binds to the cleft formed between the two lobes and forms hydrogen bonds with residues of the hinge region.[6] Most of the kinase inhibitors that have been developed, or are under development, are ATP-competitive in-

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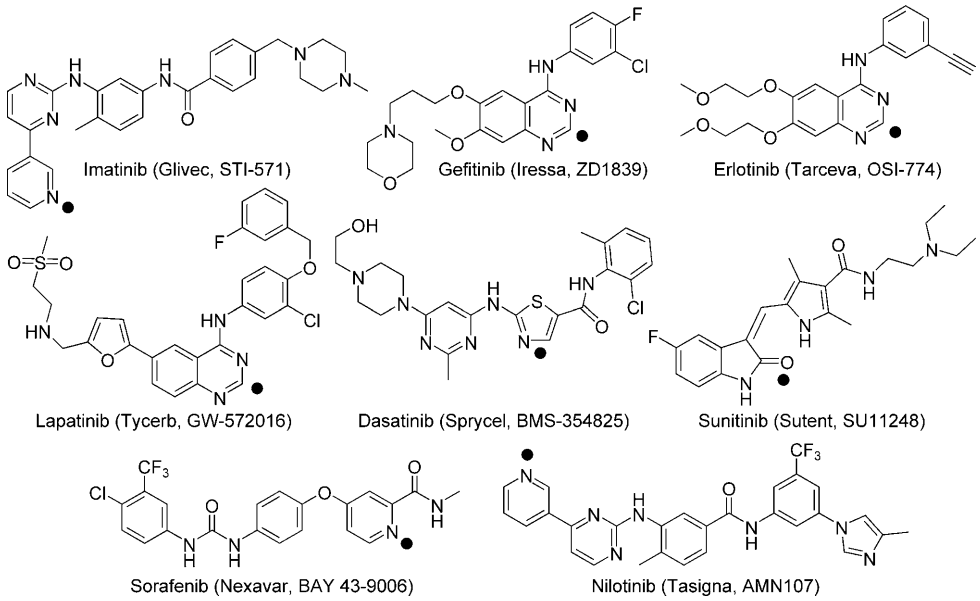


Figure 1. Eight kinase inhibitors marketed for the treatment of cancer: Imatinib (Novartis, targets Bcr-Abl and cKIT, approved for CML and GISTs); Gefitinib (AstraZeneca, targets EGFR, approved for NSCLC); Erlotinib (Roche, targets EGFR, approved for NSCLC); Lapatinib (GSK, targets EFGR and HER2, approved for breast cancer); Dasatinib (BMS, targets Bcr-Abl and Src, approved for CML); Sunitinib (Pfizer, targets Raf, VEGFR, KIT, and FLT3, approved for renal cell carcinoma (RCC) and GIST); Sorafenib (Bayer, targets Raf, VEGFR-2, VEGFR-3, PDGFR, cKIT, FLT3, and RET, approved for hepatocellular carcinoma (HCC)); and Nilotinib (Novartis, targets Bcr-Abl, approved for CML). Black dots indicate the heterocyclic ring that interacts with the kinase hinge region through one or more hydrogen bonds, as shown by X-ray co-crystallographic studies.

hibitors in that they bind to the ATP binding pocket of the kinase, mimicking the ATP–kinase interaction (Figure 2). As evidenced from X-ray co-crystal structure data, these kinase inhibitors have specific hydrogen bond acceptor and donor components that interact with the hinge region formed between the two terminal lobes of the kinase; they mimic the hydrogen bonds formed between the kinase and the adenine ring of ATP. This interaction between the kinase hinge region and the inhibitors is essential and is part of the kinase inhibitor pharmacophore. All the inhibitors developed so far have a hinge binding motif and establish at least one hydrogen bond between the inhibitor and protein kinase. Most of this hydrogen bond acceptor and donor interaction is provided by the heterocyclic portion of the inhibitors (Figure 1).[7–10] In addition to the ATP binding pocket, protein kinases have other hydrophobic and solvent-exposed hydrophilic regions that are not occupied by ATP. The size, shape, and amino acid composition of these regions differ markedly between protein kinases, and interaction between these regions and the inhibitor determines the selectivity of the particular inhibitor for the kinase in question.[7]

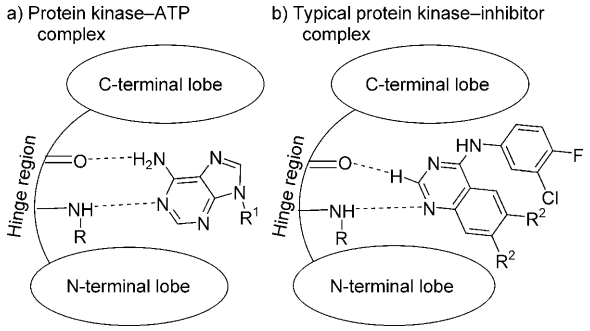


Figure 2. For clear visualization of the protein–ligand interaction, the full structures of ATP and Gefitinib are abbreviated with R^1 and R^2 groups. a) Protein kinase–ATP binding through hydrogen bond acceptor and donor elements between the adenine ring of ATP and the kinase hinge region amino acids; ATP binds in the cleft formed between the two lobes of the kinase. b) Typical protein kinase–inhibitor (in this case, EGFR–Gefitinib) binding through hydrogen bond acceptor and donor elements; Gefitinib binds in the cleft formed between the two lobes of the kinase, thereby blocking ATP access to the catalytic site.

Aurora kinase isoforms A, B, and C belong to the serine/threonine subclass of protein kinases and are key regulators of mitosis required for genomic stability. The levels of both Aurora kinases A and B are upregulated in various cancers and have been shown to promote oncogenic transformation in cell lines in vitro.[11] The dysregulation of Aurora kinase activity has been linked to genetic instability as well as defects in centrosome function, spindle assembly, chromosome alignment, and cytokinesis, all of which can lead to tumorigenesis.[11] Preclinical proof-of-concept studies with VX-680,[12] the first Aurora kinase inhibitor to enter clinical trials, showed tumor regression in various animal models of cancer, validating Aurora kinase as a drug target for cancer treatment. Currently more than ten ATP-competitive inhibitors, including PHA-739358, SNS-314, AZD1152, and AT-9283, are at different stages of clinical development for the treatment of various cancers (Figure 3).[13–16] Despite these positive advances, two of the Aurora kinase inhibitors, VX-680 and MLN8054, were recently dropped from clinical trials. VX-680 was found to cause QT prolongation in phase II clinical trials; this underscores the remaining need and opportunities for the development of further lead compounds as Aurora kinase inhibitors.

X-ray co-crystal structures of VX-680,[17] PHA-739358,[18] SNS-314,[19] and AT-9283[20] in complex with Aurora kinase A reveal the typical protein kinase–inhibitor hydrogen bonding pattern between the inhibitor heterocycle and the kinase hinge region (Figure 3). Based on our knowledge of the nature of the hinge interaction, the type of heterocyclic systems most commonly found in protein kinase inhibitors in general, and Aurora kinase inhibitors in particular, we have started a program to selectively screen, by rationally selecting compounds from our in-house compound library, for Aurora kinase A inhibitory activity.

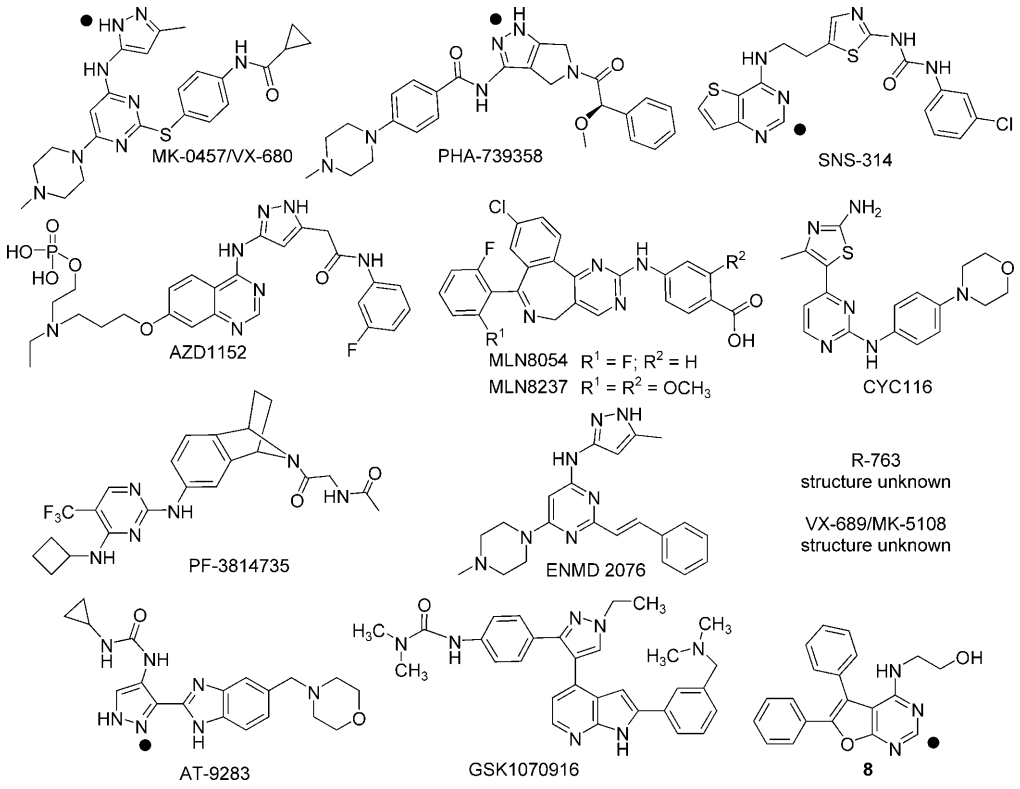


Figure 3. Aurora kinase inhibitors at various stages of clinical trials. MK-0457/VX-680 (phase II discontinued, pan-selective: Aurora A, B, C); PHA-739358 (phase II, pan-selective); SNS-314 (phase I, pan-selective); AZD1152 (phase II, Aurora B selective); MLN8054 (phase I discontinued, Aurora A selective); MLN8237 (phase I, Aurora A selective); CYC116 (phase I, pan-selective); PF-3814735 (phase I, Aurora A, B selective); GSK1070916 (phase I, Aurora B, C selective); AT-9283 (phase I/II, Aurora A, B); ENMD 2076 (phase I, Aurora A); R-763 (phase I); VX-689/MK-5108 (phase I). Black dots indicate the heterocyclic ring that interacts with the kinase hinge region through one or more hydrogen bonds, as shown by X-ray co-crystallographic studies.

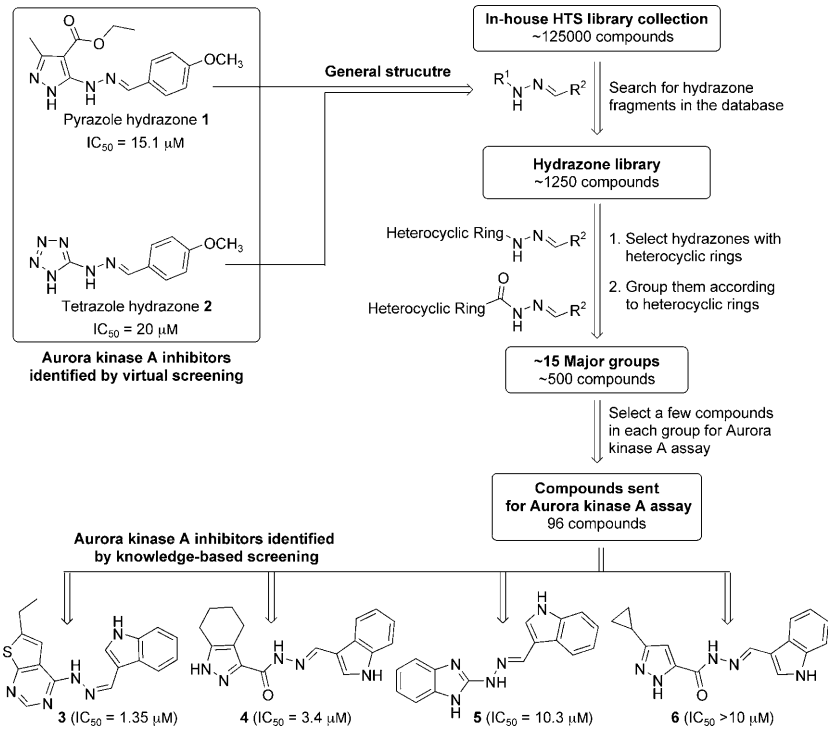
Herein we present our substructure search strategy to selectively screen a compound library to identify several Aurora kinase inhibitors, including a novel furanopyrimidine inhibitor, compound **8**. Structure–activity relationship (SAR) exploration of the hit and X-ray co-crystallographic analysis of the hit in complex with Aurora kinase A are also detailed.

Results and Discussion

Substructure searching

In our effort to develop novel Aurora kinase inhibitors, we recently reported the identification of pyrazole hydrazone **1** as an Aurora kinase inhibitor through virtual screening, and further reported our lead optimization efforts.[21] X-ray crystallographic studies of **1** and related analogues revealed that the pyrazole ring N and NH groups, along with the hydrazone linker NH group, form the essential hydrogen bond interaction with the hinge region amino acids of Aurora kinase. We also identified compound **2**, which has a different heterocycle (tetrazole) along with the hydrazone linker, that possesses Aurora kinase inhibitory activity. As it is well known from the vast amount of published data available for kinase inhibitors that certain structural motifs are shared among many of the inhibitors developed, we envisaged that structurally diverse analogues incorporating a hydrazone functionality would bind Aurora kinases. To test this possibility, we searched our in-house HTS library of ~125 000 compounds for a hydrazone fragment by using the substructure query build-in available in ISIS/Base. A total of 1250 compounds containing a hydrazone fragment were retrieved from the HTS library database. Visual examination of all of them revealed that this could be further divided into more than 15 subgroups (~500 compounds) based on the heterocyclic fragment (R^1) attached to the hydrazone functionality (Scheme 1). Compounds without heterocyclic components were omitted, as the kinase hinge binding motif is essential for efficacy.

One or two compounds from each subgroup were initially assayed for Aurora kinase inhibitory activity. The selected compounds were representative of that subgroup, with the R^2 group attached to the hydrazone/benzylidine portion containing either an indole, a dimethoxy- or monomethoxyphenyl group if available, as these groups in the benzylidine part of pyrazole series showed the best activity levels.[21] Compounds were initially screened for Aurora kinase inhibition at two concentrations: 50 and 10 μM. Once compounds with inhibitory activity were identified, all other compounds with the same heterocyclic (R^1) core structures were also tested for activity. A total of 96 compounds were assessed, out of which four different subgroup compounds possessed >30% Aurora kinase inhibition at 10 μM (table 1s, Supporting Information), and **3**–**6** are the most active compounds in each subgroup. Scheme 1



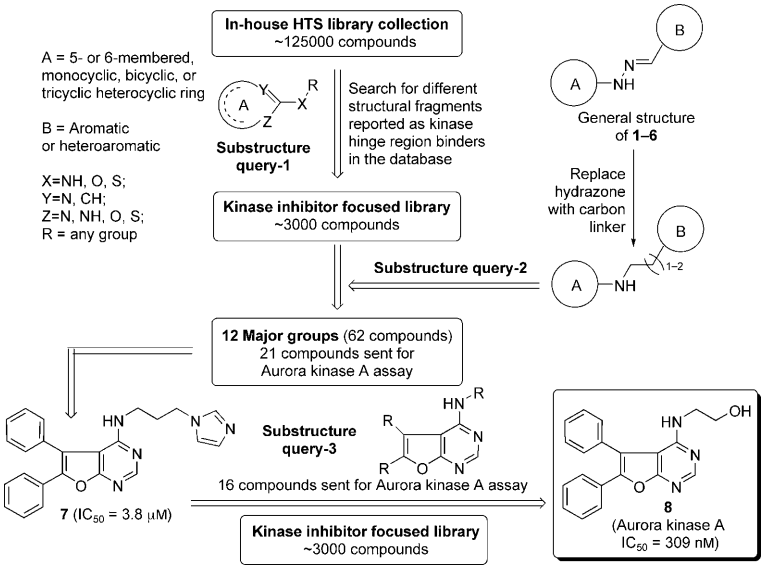
Scheme 1. Knowledge-based rational screening strategy applied to the HTS compound library, leading to the identification of new Aurora kinase A inhibitors **3**–**6** containing a hydrazone fragment. See the text for details of the screening strategy.

gives a schematic overview of the knowledge-based screening process and the most potent Aurora kinase inhibitors **3**–**6** with a hydrazone fragment identified through this strategy.

Thienopyrimidine compound **3** (Aurora kinase A IC_{50} = 1.35 μM) showed the highest potency, which is at least an order of magnitude more potent than the initial leads **1** and **2**, upon which the substructure search was executed. The next most active compound is tetrahydroindazole core compound **4**, which is at least threefold more active than **1**. Compounds **5** and **6**—with the benzimidazole and pyrazole cores, respectively—possess ranges of activity similar to that of **1**. The most active compounds in each of the four series identified contain a 3-substitued indole ring in the hydrazone part as the R^2 group, which was also found to be the most preferred substituent in the pyrazole series we investigated previously.[21]

With the successful identification of several new Aurora kinase inhibitor lead compounds through a simple substructure search protocol based on two virtual screening leads **1** and **2**, we were interested to know if this strategy could be used to identify a diverse set of compounds without a hydrazone fragment. Several research groups have realized a commonality in protein kinase inhibitors and have found general structural features most common in them; they have used this information to develop various expert systems (kinase-targeted libraries, virtual screening, etc.) that could be employed to identify and develop novel kinase inhibitors.[22–25] A recent publication reports the analysis of kinase inhibitor structures (~20000 compounds), and the authors identified "kinase-privileged fragments", such as the bisarylanilines, as the most common structural feature of protein kinase inhibitors.[9] They also identified several heterocyclic ring systems such as pyridines, pyrimidines, pyrazoles, quinazolines, and indoles to be present with greater frequency in kinase inhibitor scaffolds than in non-kinase-inhibiting small molecules that all contain one or more heteroatoms such as N, S, or O.[9]

Accordingly, we filtered our in-house compound library using the following criteria: heterocyclic ring, 5- or 6-membered, mono- or polycyclic containing at least one N, O, or S atom (Scheme 2, Substructure query-1). With this filter we generated a kinase-biased library of ~3000 compounds. Because this is still a large collection of compounds for in vitro screening, we set out to further narrow the field for the screening test. For this, we used the knowledge gained from the newly identified Aurora kinase inhibitors **1**–**6**. We started with the general structure of hydrazone-fragment-containing compounds **1**–**6**, in



Scheme 2. Knowledge-based rational screening strategy applied to the HTS compound library, leading to the identification of novel furanopyrimidine Aurora kinase A inhibitors **7** and **8**. See the text for details of the screening strategy.

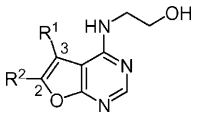
which two rings, A and B, are linked through the hydrazone group; next we assumed that replacement of the hydrazone linker with a linker of two to three methylene units would provide a similar type of molecular arrangement necessary for binding to Aurora kinase. With this assumption, we searched the kinase-biased library of ~3000 compounds using substructure query-2, in which ring B could be either 5- or 6-membered aromatic or heteroaromatic. Based on these criteria, 62 compounds were retrieved from the kinase-biased library. Visual inspection of all candidates allowed their division into 10 major groups depending on the heterocyclic ring system (A) present. A total of 21 compounds (figure 1s, Supporting Information) were selected from this set and were submitted to the Aurora kinase assay. This led to the identification of compound **7** as an Aurora kinase A inhibitor, which has a furanopyrimidine core and an IC_{50} value of 3.8 μM. With **7** thus identified as an Aurora kinase A inhibitor, compounds with a furanopyrimidine core were retrieved (16 compounds) from the kinase-biased library through substructure query-3 and tested for Aurora kinase inhibition. Most of the tested compounds exhibited varying levels of kinase inhibition (table 2s, Supporting Information); compound **8** was the most potent, with an IC_{50} value of 309 nM (Scheme 2).

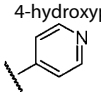
Four of the five Aurora kinase inhibitors identified in this study, compounds **3**–**6**, have never been reported as kinase inhibitors. However, the most active compound **8** identified in this study has been reported earlier as a weak inhibitor of Chk1 kinase (IC_{50} ~26 μM).[26] It is well known that many kinase inhibitors are active toward more than one kinase due to the close similarity between ATP binding pockets, as discussed above. We were therefore interested to know whether the new compounds identified in this study are selective for Aurora kinase inhibition. For this purpose, we screened **3**–**6** and **8** at a concentration of 10 μM for inhibition of two tyrosine kinases: epidermal growth factor receptor (EGFR) kinase and FMS-like tyrosine kinase 3 (FLT3), and the results are shown in table 3s of the Supporting Information. These compounds were found to inhibit EGFR kinase by <15%; however, they inhibited FLT3, with **4** and **6** showing ~70% inhibition at 10 μM. FLT3 is inhibited by most Aurora kinase inhibitors reported, due to the high degree of similarity in their binding pockets.[16] Inhibition of FLT3 might be beneficial, as FLT3 is also a target for acute myelogenous leukemia (AML).[27] Our present results, along with previous reports,[26] suggest that the furanopyrimidine hit **8** is a selective Aurora kinase A inhibitor over EGFR kinase, Chk1, PKA, and CDK1, with additional inhibition at FLT3. This prompted us to further explore the potential of the furanopyrimidine series for drug development.

SAR study of furanopyrimidine 8

Structural optimization of the hit **8** was initiated to understand the structure–activity relationship (SAR) in this novel series. Compounds **8**–**24** were initially synthesized, and their ability to inhibit Aurora kinase A at a concentration of 10 μM was determined; the results are listed in Table 1 and are expressed as percentage inhibition. In addition, for those compounds showing >90% inhibition, IC_{50} values were also determined. Initial attempts were made to determine the importance of the two phenyl rings on the furan ring. Removal of either phenyl ring led to loss of activity for compounds **9** and **10**. In particular, the phenyl group at the 2-position of the furan ring is more essential for maintaining activity than the 3-position phenyl group, as compound **10** showed complete loss of activity (**9**: 76.6% inhibition at 50 μM; **10**: 35.2% inhibition at 50 μM). Moreover, replacement of these phenyl groups by a bromine atom results in maintenance of activity, as shown by compound **11**, which retains the phenyl group at the 2-position of the furan ring, whereas compound **12** is inactive. This clearly demonstrates the importance of the phenyl ring at the 2-position of the furan ring for interacting with Aurora kinase. Having identified that the 3-position phenyl ring can be re-

Table 1. Effect of replacing the phenyl groups of **8** on Aurora kinase A inhibition.



Compd	R^1	R^2	Inhibition [%][a,b]	IC_{50} [nM][b]
8	Ph	Ph	98.3	309
9	H	Ph	17.3	–
10	Ph	H	0.0	–
11	Br	Ph	90.8	751
12	Ph	Br	36.0	–
13	CH_3	Ph	71.0	–
14	C_2H_5	Ph	95.8	709
15	C≡CH	Ph	89.6	–
16	4-methoxyphenyl	Ph	68.5	–
17	4-nitrophenyl	Ph	85.7	–
18	4-hydroxyphenyl	Ph	96.1	245
19	4-acetamidophenyl	Ph	95.7	272
20	4-methoxyphenyl	4-methoxyphenyl	93.3	639
21	4-hydroxyphenyl	4-hydroxyphenyl	95.6	159
22		Ph	83.7	–
23		Ph	94.7	732
24			99.7	619

[a] Determined at a compound concentration of 10 μM. [b] Values are expressed as the mean of at least two independent determinations and are within ±15%.

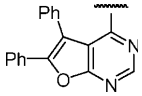
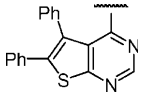
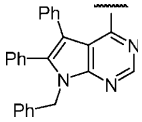
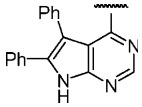
placed by a bromo group with only a 2.5-fold decrease in activity, we further explored other substitutions at this position. Introduction of alkyl groups such as methyl (compound **13**) and ethyl (compound **14**), or an alkynyl group such as acetylene (compound **15**), however, did not improve activity relative to **8**.

As only the phenyl group substitution at the 3-position of the furan ring showed maximum activity, and replacement with other groups had thus far led to loss of activity, exploration of the effect of substitution on this phenyl ring was initiated. Thus, the introduction of a 4-methoxy (compound **16**) or 4-nitro (compound **17**) group led to a slight decrease in activity, whereas the introduction of a 4-hydroxy (compound **18**) or 4-acetamido (compound **19**) group resulted in retention of Aurora kinase inhibition levels. We next introduced 4-methoxy (compound **20**) and 4-hydroxy (compound **21**) groups in both phenyl rings and found that compound **21**, with two hydroxy groups, has a twofold higher potency than unsubstituted compound **8**. However, replacement of either one or both phenyl groups with a heterocyclic ring such as pyridine (compound **22**) or furan (compounds **23** and **24**) did not improve activity relative to **8**.

We next focused our efforts on the effect of replacing the furan ring with thiophene (compound **25**) or pyrrole (compound **27**) on Aurora kinase inhibition (Table 2). Replacing the oxygen atom with sulfur decreased the activity of **25**, while replacement with an NH group enhanced activity threefold for **27** relative to **8**. However, the benzyl-protected pyrrole derivative **26** lost activity completely, demonstrating the crucial role of the heteroatom in the bicyclic ring core structure in maintaining Aurora kinase inhibition.

The most potent Aurora kinase A inhibitor identified in this study, compound **27**, was evaluated for the ability to inhibit Aurora kinase isoforms B and C, as well as the structurally related FLT3 kinase; the results are listed in tables 3s and 4s of the Supporting Information. Compound **27** inhibited Aurora kinase A more strongly than either the B or C isoforms, and also showed FLT3 kinase inhibition. As it has been established that both Aurora kinase isoform-selective as well as isoform-nonselective inhibitors possess anticancer activity in preclinical animal models and are also at various stages in clinical trials,[16] the new Aurora kinase inhibitors disclosed herein warrant further study.

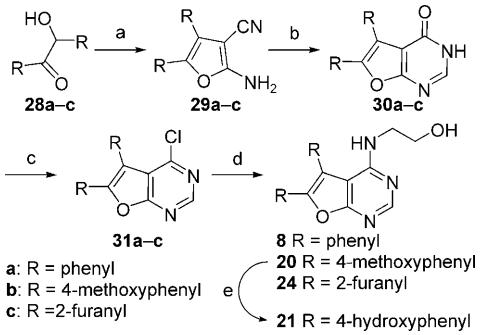
Table 2. Effect of replacing the furan ring of **8** on Aurora kinase inhibition.

Compd	R	Inhibition [%][a,b]	IC_{50} [nM][b]
8		98.3	309
25		60.1	–
26		2.2	–
27		100	104

[a] Determined at a compound concentration of 10 μM. [b] Values are expressed as the mean of at least two independent determinations and are within ±15%.

Synthesis

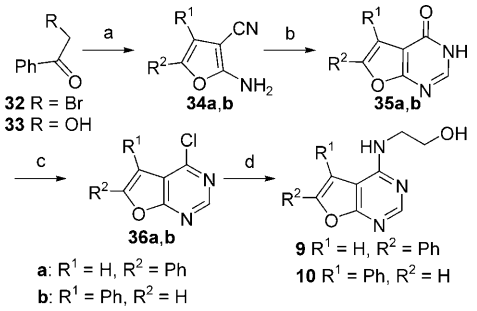
Compounds **8**, **20**, and **24**, with symmetric substitution on the furan ring, were synthesized from the appropriate benzoins through modification of published procedures.[26,28,29] In brief, cyclization of the appropriate benzoin compounds **28a–c** with malononitrile under basic conditions afforded the required symmetrically substituted furans **29a–c**, which were cyclized and chlorinated to establish the bicyclic furo[2,3-*d*]pyrimidine ring intermediates **31a–c**. Nucleophilic reaction with 2-aminoethanol gave the desired compounds **8**, **20**, and **24**. The 4-hydroxy derivative **21** was prepared from the 4-methoxy derivative **20** by demethylation using boron tribromide (Scheme 3).



Scheme 3. Reagents and conditions: a) malononitrile, DMF, Et_2NH, RT, 16 h, 40–67%; b) HCO_2H, Ac_2O, 0 °C→reflux, 16 h; c) $POCl_3$, neat, 70 °C, 3 h, 41–60% for two steps; d) 2-aminoethanol, *n*BuOH, MWI, 175 °C, 1 h, 63–94%; e) BBr_3, CH_2Cl_2, 0 °C→RT, 2 h, 54%.

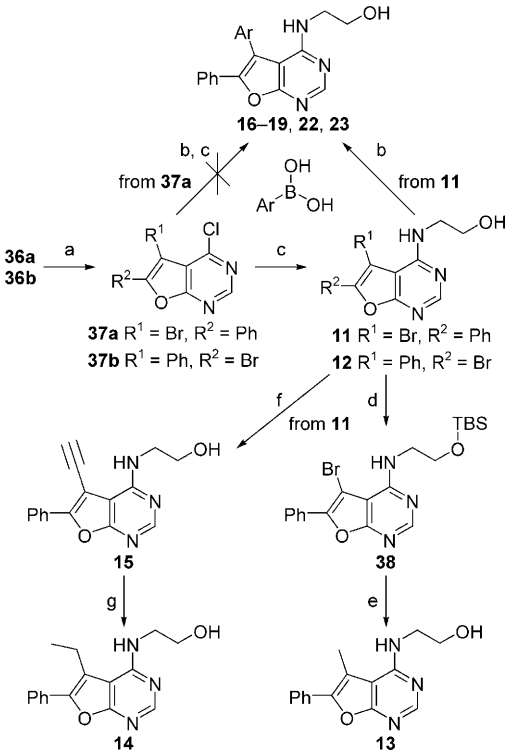
For the preparation of compounds **9** and **10** with a bromo substituent at either the 2- or 3-position of the furan ring, a modified reported procedure was used.[30,31] 2-Bromoacetophenone (**32**) or 2-hydroxyacetophenone (**33**) were condensed with malononitrile, followed by cyclization to afford the furan intermediates **34a**,**b**. A reaction sequence similar to that for the synthesis of compound **8** was then followed to construct the bicyclic furanopyrimidine system to give the desired compounds **9** and **10** (Scheme 4).

For the preparation of compounds **11**–**19**, **22**, and **23**, with various substitutions at the 3-position of the furan ring, the intermediates **36a**,**b** were brominated using *N*-bromosuccinimide to give **37a**,**b**, which could be converted into **11** and **12** by reaction with 2-aminoethanol. Attempts to carry out Suzuki coupling of **37a** with appropriately substituted boronic acids



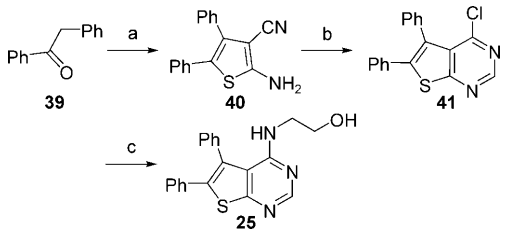
Scheme 4. Reagents and conditions: a) malononitrile, DMF, Et_2NH, RT, 16 h, 38–71%; b) HCO_2H, Ac_2O, 0 °C→reflux, 8 h; c) $POCl_3$, neat, 70 °C, 3 h, 37–75% for two steps; d) 2-aminoethanol, *n*BuOH, MWI, 175 °C, 1 h, 64–80%.

failed; however, Suzuki coupling was carried out with amino alcohol **11** to give compounds **16**–**19**, **22**, and **23**. An acetylene group was introduced at the 3-position of the furan ring through Sonagashira coupling of trimethylsilylacetylene with **11**, followed by TMS deprotection to give acetylene **15**. Reduction of the triple bond of **15** gave the ethyl derivative **14**. For the preparation of the methyl derivative **13**, TBDMS-protected **38** was cross-coupled with methyl zinc chloride using Negishi coupling[32] (Scheme 5).



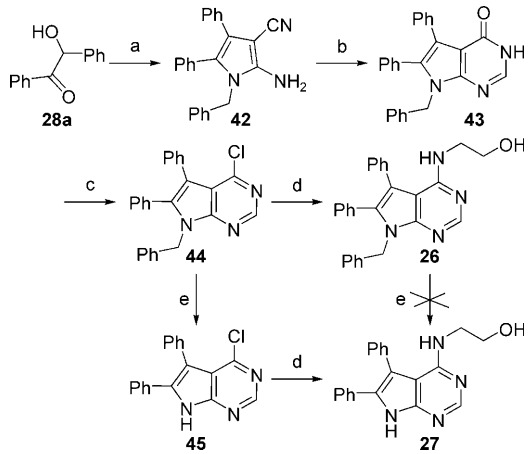
Scheme 5. Reagents and conditions: a) NBS, DMF, RT, 3–18 h, 68–85%; b) $ArB(OH)_2$, $Pd(OAc)_2$, PPh_3, Na_2CO_3, dioxane, H_2O, 100 °C, 3 h, 42–80%; c) 2-aminoethanol, *n*BuOH, MWI, 175 °C, 1 h, 28–85%; d) TBDMSCl, imidazole, DMF, RT, 1 h, 98%; e) 1. $Pd(PPh_3)_4$, CH_3ZnCl, THF, reflux, 24 h, 52%, 2. TBAF, CH_2Cl_2, RT, 16 h, 95%; f) 1. $(CH_3)_3SiC{\equiv}CH$, $Pd(PPh_3)_2Cl_2$, CuI, DIPEA, DMF, 66 °C, 16 h, 57%, 2. K_2CO_3, MeOH, RT, 16 h, 85%; g) PtO_2, H_2 (1 atm), EtOAc/MeOH, RT, 1 h, 70%.

For the preparation of thienopyrimidine compound **25**, a modified Gewald reaction was used.[33] Briefly, deoxybenzoin **39** was condensed with malononitrile and then cyclized in the presence of sulfur to construct the thiophene compound **40**, which could be further elaborated to give **25**, as in the case of **8** (Scheme 6).



Scheme 6. Reagents and conditions: a) $CH_2(CN)_2$, S_8, Et_3N, EtOH, reflux, 16 h, 64%; b) 1. HCO_2H, Ac_2O, 0 °C→reflux, 16 h, 2. $POCl_3$, neat, 70 °C, 3 h, 20% for two steps; c) 2-aminoethanol, *n*BuOH, reflux, 16 h, 23%.

For the preparation of pyrrolopyrimidine compounds **26** and **27**, benzoin (**28a**) was condensed with benzylamine, followed by cyclization with malononitrile, resulting in the construction of pyrrole compound **42**.[34] Cyclization with formic acid followed by chlorination gave the bicyclic pyrrolopyrimidine compound **44**, which, upon reaction with 2-aminoethanol, afforded **26**. As attempts to debenzylate this compound using aluminum trichloride failed, **44** was debenzylated to give **45**, which was treated with 2-aminoethanol to give the desired pyrrolopyrimidine **27** (Scheme 7).



Scheme 7. Reagents and conditions: a) 1. benzylamine, HCl, $PhCH_3$, reflux, 24 h, 72%, 2. $CH_2(CN)_2$, $PhCH_3$, reflux, 24 h, 22%; b) HCO_2H, reflux, 6 h, 62%; c) $POCl_3$, reflux, 3 h, 46%; d) 2-aminoethanol, *n*BuOH, reflux, 16 h, 25–48%; e) $AlCl_3$, $PhCH_3$, reflux, 2 h, 39%.

X-ray co-crystal analysis of the compound 8–Aurora kinase A complex

Concurrent with the above synthetic explorations, we attempted to solve the structure of the co-crystal complex between compound **8** and the Aurora kinase A protein. Co-crystalliza-

tion was carried out as detailed earlier by our research group,[21] by using an Aurora kinase A construct and the complex structure solved by X-ray crystallography with a resolution of 2.35 Å. The complex structure (Figure 4) reveals that the hit

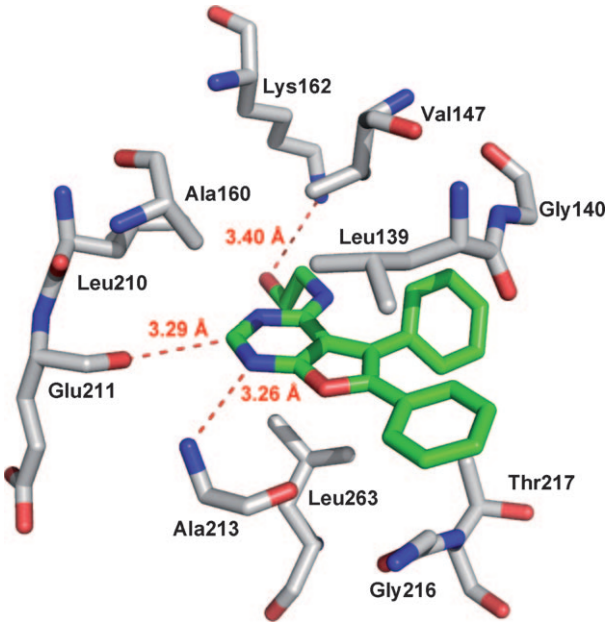


Figure 4. X-ray co-crystal complex of compound **8** with Aurora kinase A (PDB ID: 3K5U). Red lines represent hydrogen bonding interactions between the inhibitor and protein, with distances indicated.

8 binds in the region formed between the C- and N-terminal lobes of the protein and occupies the ATP binding pocket of Aurora kinase A. The furanopyrimidine heterocyclic core forms two essential hydrogen bonds with the hinge region amino acid residues (Ala 213 and Glu 211) from N6 nitrogen (3.26 Å) and C7 hydrogen (3.29 Å). In addition to these two hydrogen bonds, another hydrogen bond is observed between the hydroxy group and the side chain of Lys 162 (3.40 Å). In addition to the three hydrogen bonds anchoring the molecule to the protein, extensive hydrophobic interactions with the surrounding amino acid residues was observed. The furanopyrimidine core has hydrophobic interactions with Leu 139, Val 147, Ala 160, and Leu 263. The alkyl chain bearing the hydroxy group undergoes hydrophobic interactions with Leu 210. In particular, the two phenyl groups form strong hydrophobic contacts with residues Leu 139, Gly 140, Val 147, Gly 216, and Thr 217. The X-ray co-crystal structure of **8** in complex with Chk1 has already been reported. The main difference between the Aurora and Chk1 structures is that the side chain hydroxy group forms an intramolecular hydrogen bond with N8 of the pyrimidine ring in Chk1 and lacks interaction with the Lys residue.[26]

As apparent from the above description, the two phenyl groups of compound **8** show extensive hydrophobic contacts. These contacts explain why compounds **9** and **10**, which lack phenyl groups, are inactive, as such hydrophobic interactions are absent in these cases. Moreover, when the phenyl groups were replaced with other hydrophobic groups, the inhibitory activity was either impaired or retained, with the exception of compound **21**, which showed improved activity (Table 1). As replacement of the furan ring with pyrrole (in **27**) resulted in a threefold improvement in activity relative to **8** (Table 2), the X-ray co-crystal complex with compound **8** was used to gather structural biology insight into the role played by the NH group. The distance between the oxygen atom in the furan ring of **8** and the oxygen atom in the main chain of the kinase residue Ala 213 is 3.15 Å. It is possible that upon replacing the oxygen with an NH group, it forms an additional hydrogen bond with the hinge residue, and this may contribute to the improved activity of **27** over that of **8**. This can also explain the inactivity of compound **26**, as the bulky *N*-benzyl group could effect an unfavorable steric clash between ligand and protein. Notably, our attempts to co-crystallize compound **27** with Aurora kinase A in order to gain direct evidence for the interaction between the NH group and the protein failed.

Conclusions

We used preexisting knowledge to select compounds for screening for inhibitory activity toward Aurora kinase A. This enabled us to identify the potent Aurora kinase A inhibitor compound **8**, with an IC_{50} value of 309 nM, by sampling only 133 compounds from a library of ~125 000 compounds. Substructure searching has been used by medicinal chemists to identify analogues of known active core structures, but has not been documented as a stand-alone tool in the identification of a potent compound with activity in the nanomolar range, particularly in the kinase domain. Application of a similar substructure search strategy for inhibitors of other kinases is therefore possible. For example, EGFR kinase inhibitors (e.g., Gefitinib, Erlotinib, and Lapatinib; Figure 1) are known to have an aniline group attached to a hinge-binding heterocyclic core;[7,8,27] this information can be exploited through an appropriate substructure search. The use of specific structural information gained in-house, such as the hydrazone fragment information for Aurora kinase inhibition in our case, could help in the identification of novel inhibitors. Computer-aided virtual screening, the basis of which is substructure/similarity searching, has been shown to effectively decrease the number of compounds sampled to identify potent lead compounds, relative to high-throughput screening of a large library of compounds. However, the use of virtual screening and related technologies depends on the availability of resources such as specialized hardware and software, and also on having personnel with specialized training in computational chemistry. The approach presented herein could be used by organic and medicinal chemists who are involved in optimizing lead compounds in drug discovery projects using simple, everyday chemistry tools such as ISIS/Draw to identify potential hits more effectively, either separately or in conjunction with other hit identification techniques such as virtual screening.

In summary, the novel Aurora kinase A inhibitors **3**–**8** identified in this study could be used as starting points for the development of novel Aurora kinase inhibitors as anticancer

agents. To aid in this process, the furanopyrimidine compound **8** was co-crystallized with Aurora kinase A, and the key ligand–protein interactions were mapped. This structural biology insight, along with the SAR information revealed herein, could be used in the further design of potent Aurora kinase inhibitors.

Experimental Section

General methods

All commercial chemicals and solvents were reagent grade and were used without further treatment unless otherwise noted. All reactions were carried out under an atmosphere of dry N_2. Reactions were monitored by TLC using Merck 60 F_{254} silica gel glass backed plates (5×10 cm); zones were detected visually under UV light (λ 254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230–400 mesh ASTM). ^{1}H NMR spectra were obtained with a Varian Mercury-300 or Varian Mercury-400 spectrometer operating at 300 and 400 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to the solvent peak or $(CH_3)_4Si$. HRMS data were measured with a Finnigan (MAT-95XL) electron impact (EI) mass spectrometer. LC–MS data were measured on an Agilent MSD-1100 electrospray ionization (ESI)-MS–MS system.

Substructure searching

The in-house HTS compound collection was purchased from ChemDiversity, and the structure information was stored in Accord Enterprise Client database 6.0.0. Using the in-build substructure search option, compounds were retrieved as SDF (structure data file) format, then imported into MDL ISIS/Base 2.5 database and further analyzed before selecting compounds for testing.

Chemistry

2-Amino-4,5-diphenylfuran-3-carbonitrile 29a. Diethylamine (13.8 g) was added dropwise over a period of 30 min to a mixture of benzoin (**28a**, 10.0 g, 47.17 mmol) and malononitrile (3.8 g, 57.58 mmol) in DMF (30 mL) at 0 °C (the reaction temperature should not exceed 40 °C). After the resulting mixture was stirred at room temperature for 16 h, H_2O (100 mL) was added. The resulting precipitate was filtered, washed with a sufficient amount of H_2O, then with *n*-hexane, and dried. The solid was recrystallized from EtOH to provide a yellowish-brown solid product of **29a** (6.0 g, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47–7.34 (m, 8H), 7.28–7.18 (m, 2H), 4.94 (br, 2H); LC–MS (ESI) *m/z*: 261.1 $[M+H]^+$.

4-Chloro-5,6-diphenylfuro[2,3-*d*]pyrimidine (31a). A mixture of **29a** (2.0 g, 7.69 mmol) and formic acid (24 mL) was cooled to 0 °C, and acetic anhydride (24 mL) was added dropwise. The resulting mixture was stirred for 1 h. The reaction mixture was then warmed to 100 °C and stirred for 16 h. The reaction mixture was cooled, and H_2O was added (40 mL). The precipitate was filtered and washed thoroughly with H_2O and *n*-hexane to give **30a** (2.1 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94 (s, 1H), 7.56–7.52 (m, 4H), 7.46–7.43 (m, 3H), 7.32–7.28 (m, 3H), 7.22 (s, 1H); LC–MS (ESI) *m/z*: 289.1 $[M+H]^+$. A mixture of **30a** (3.0 g, 10.41 mmol) and $POCl_3$ (30 mL) was heated at 70 °C for 3 h. The reaction mixture was cooled in an ice bath and neutralized by the careful addition of a saturated solution of $NaHCO_3$. The resulting mixture was extracted with EtOAc; the organic layer was concentrated, and the crude compound was purified by silica gel column chromatography using a mixture of *n*-hexane/EtOAc (95:5) to give white solid **31a** (2.0 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.77 (s, 1H), 7.61–7.58 (m, 2H), 7.52–7.46 (m, 5H), 7.35–7.32 (m, 3H); LC–MS (ESI) *m/z*: 307.0 $[M+H]^+$.

2-(5,6-Diphenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 8. 4-Chloro-5,6-diphenylfuro[2,3-*d*]pyrimidine (**31a**, 100 mg, 0.33 mmol) and 2-aminoethanol (33 mg, 0.54 mmol) in *n*-butanol (1 mL) were sealed in a microwave tube and subjected to microwave irradiation (MWI) at 175 °C for 1 h. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography using a mixture of CH_2Cl_2/MeOH (40:1) to give **8** (80 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.41 (s, 1H), 7.58–7.48 (m, 7H), 7.30–7.28 (m, 3H), 5.18 (br, 1H), 3.74 (t, *J* = 4.5 Hz, 2H), 3.62–3.58 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.8 (C), 157.5 (C), 153.0 (C), 147.8 (C), 132.1 (C), 130.1 (CH), 129.9 (CH), 129.4 (CH), 129.0 (CH), 128.8 (CH), 126.6 (CH), 115.1 (C), 103.7 (C), 77.4 (CH), 63.1 (CH_2), 44.9 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{20}H_{17}N_3O_2$: 331.1321, found 331.1320.

2-Amino-4,5-bis-(4-methoxyphenyl)furan-3-carbonitrile 29b. Compound **29b** was prepared in 40% yield from bis-4-methoxybenzoin (**28b**) similar to **29a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37–6.91 (m, 8H), 6.80 (br, 2H), 3.85 (s, 3H), 3.80 (s, 3H); LC–MS (ESI) *m/z*: 321.1 $[M+H]^+$.

4-Chloro-5,6-bis-(4-methoxyphenyl)furo[2,3-*d*]pyrimidine 31b. Compound **31b** was prepared in 41% yield over two steps from **29b**, similar to **31a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.71 (s, 1H), 7.57–7.52 (m, 2H), 7.38–7.33 (m, 2H), 7.04–7.00 (m, 2H), 6.87–6.22 (m, 2H), 3.90 (s, 3H), 3.81 (s, 3H); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{20}H_{15}ClN_2O_3$: 366.0771, found: 366.0758.

2-[5,6-Bis-(4-methoxyphenyl)furo[2,3-*d*]pyrimidin-4-ylamino]ethanol 20. Compound **20** was prepared in 94% yield from **31b**, similar to **8**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.35 (s, 1H), 7.49–7.44 (m, 2H), 7.41–7.37 (m, 2H), 7.08–7.03 (m, 2H), 6.83–6.79 (m, 2H), 5.14 (t, *J* = 5.4 Hz, 1H), 4.16 (br, 1H), 3.90 (s, 3H), 3.80 (s, 3H), 3.74 (t, *J* = 5.1 Hz, 2H), 3.57 (dt, *J* = 5.4, 5.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.4 (C), 159.8 (C), 159.7 (CH), 157.7 (C), 153.0 (C), 147.3 (CH), 131.0 (CH), 127.7 (CH), 124.0 (CH), 122.0 (C), 115.0 (C), 113.9 (CH), 112.7 (C), 103.4 (C), 62.8 (CH_2), 55.3 (CH_3), 55.2 (CH_3), 44.3 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{22}H_{21}N_3O_4$: 391.1532, found: 391.1536.

5′-Amino-[2,2′;3′,2″]terfuran-4′-carbonitrile 29c. Compound **29c** was prepared in 67% yield from **28c**, similar to **29a**. ^{1}H NMR (300 MHz, CD_3OD): δ = 7.58 (dd, *J* = 1.8, 0.6 Hz, 1H), 7.54 (dd, *J* = 1.8, 0.6 Hz, 1H), 6.88 (dd, *J* = 3.6, 0.6 Hz, 1H), 6.70 (dd, *J* = 3.6, 0.6 Hz, 1H), 6.54 (dd, *J* = 3.6, 1.8 Hz, 1H), 6.52 (dd, *J* = 3.6, 1.8 Hz, 1H); LC–MS (ESI) *m/z*: 241.0 $[M+H]^+$.

4-Chloro-5,6-difuran-2-ylfuro[2,3-*d*]pyrimidine (31c). Compound **31c** was prepared in 41% yield over two steps from **29c**, similar to **31a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.76 (s, 1H), 7.65 (dd, *J* = 1.8, 0.9 Hz, 1H), 7.57 (dd, *J* = 1.8, 0.6 Hz, 1H), 6.94 (dd, *J* = 3.6, 0.6 Hz, 1H), 6.76 (dd, *J* = 3.6, 0.9 Hz, 1H), 6.61 (dd, *J* = 3.6, 0.9 Hz, 1H), 6.55 (dd, *J* = 3.6, 0.9 Hz, 1H); LC–MS (ESI) *m/z*: 287.1 $[M+H]^+$.

2-(5,6-Difuran-2-ylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 24. Compound **24** was prepared in 63% yield from **31c**, similar to **8**. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.29 (s, 1H), 7.76 (dd, *J* = 1.8, 0.6 Hz, 1H), 7.69 (dd, *J* = 1.8, 0.6 Hz, 1H), 6.98 (dd, *J* = 3.6, 0.6 Hz, 1H), 6.94 (dd, *J* = 3.6, 0.6 Hz, 1H), 6.67 (dd, *J* = 3.6, 1.8 Hz, 1H), 6.64 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.80–3.69 (m, 4H); ^{13}C NMR (150 MHz, CD_3OD): δ =

165.2 (C), 157.8 (C), 154.1 (CH), 144.8 (C), 144.7 (C), 143.5 (CH), 142.3 (CH), 139.6 (C), 112.4 (CH), 104.9 (C), 104.4 (C), 63.0 (CH_2), 44.5 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{16}H_{13}N_3O_4$: 311.0906, found: 311.0924.

2-[5,6-Bis-(4-hydroxyphenyl)furo[2,3-*d*]pyrimidin-4-ylamino]ethanol (21). BBr_3 (0.36 mL, 1 M solution in CH_2Cl_2) was added, while stirring, to a solution of **20** (20 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) cooled to 0 °C. The reaction mixture was allowed to reach room temperature while it was stirred for 2 h. H_2O (5 mL) was added to the reaction mixture, and the precipitated material was purified by silica gel column chromatography using a mixture of CH_2Cl_2/MeOH (95:5) to give **21** (10 mg, 54%). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.22 (s, 1H), 7.36–7.27 (m, 4H), 6.99–6.94 (m, 2H), 6.72–6.67 (m, 2H), 3.63–3.52 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD): δ = 165.1 (C), 159.4 (C), 159.3 (C), 153.8 (CH), 148.9 (C), 132.2 (CH), 129.0 (CH), 123.7 (C), 122.1 (C), 117.6 (CH), 116.4 (CH), 114.2 (C), 104.7 (C), 61.3 (CH_2), 44.0 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{20}H_{17}N_3O_4$: 363.1219, found: 363.1230.

2-Amino-5-phenylfuran-3-carbonitrile 34a. Compound **34a** was prepared in 38% yield from 2-bromoacetophenone (**32**), similar to **29a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.50–7.47 (m, 2H), 7.39–7.33 (m, 2H), 7.27–7.25 (m, 1H), 6.54 (s, 1H), 4.86 (br, 2H); LC–MS (ESI) *m/z*: 185.0 $[M+H]^+$.

4-Chloro-6-phenylfuro[2,3-*d*]pyrimidine 36a. Compound **36a** was prepared in 75% yield over two steps from **34a**, similar to **31a**. Compound **35a**: 80% yield from **34a**. ^{1}H NMR (300 MHz, CD_3OD): δ = 9.00 (br, 1H), 8.36 (br, 1H), 7.67 (d, *J* = 7.5 Hz, 2H), 7.44–7.38 (m, 2H), 7.32–7.25 (m, 1H), 7.10 (s, 1H); LC–MS (ESI) *m/z*: 235.0 $[M+Na]^+$. Compound **36a**: 94% yield from **35a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (s, 1H), 7.93–7.89 (m, 2H), 7.55–7.26 (m, 3H), 7.09 (s, 1H); LC–MS (ESI) *m/z*: 231.0 $[M+H]^+$.

2-(6-Phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 9. Compound **9** was prepared in 80% yield from **36a**, similar to **8**. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.21 (s, 1H), 7.82–7.78 (m, 2H), 7.47–7.36 (m, 3H), 7.19 (s, 1H), 3.81–3.77 (m, 2H), 3.72–3.68 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD): δ = 166.6 (C), 159.1 (C), 154.3 (CH), 153.3 (C), 130.7 (C), 130.0 (CH×2), 125.5 (CH), 104.6 (C), 99.0 (CH), 61.7 (CH_2), 44.6 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{14}H_{13}N_3O_2$: 255.1008, found: 255.0993.

2-Amino-4-phenylfuran-3-carbonitrile 34b. Compound **34b** was prepared in 71% yield from 2-hydroxyacetophenone (**33**), similar to **29a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.57–7.53 (m, 2H), 7.43–7.30 (m, 3H), 6.98 (s, 1H), 4.92 (br, 2H); LC–MS (ESI) *m/z*: 207.0 $[M+Na]^+$.

4-Chloro-5-phenylfuro[2,3-*d*]pyrimidine 36b. Compound **36b** was prepared in 37% yield over two steps from **34b**, similar to **31a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.81 (s, 1H), 7.77 (s, 1H), 7.56–7.47 (m, 5H); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{12}H_7ClN_2O$: 230.0247, found: 230.0249.

2-(5-Phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 10. Compound **10** was prepared in 64% yield from **36b**, similar to **8**. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.29 (s, 1H), 7.70 (s, 1H), 7.55–7.43 (m, 5H), 3.70–3.61 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD): δ = 167.4 (C), 159.4 (C), 154.6 (CH), 139.4 (CH), 132.2 (C), 130.5 (CH), 129.6 (CH×2), 122.6 (C), 101.3 (C), 61.2 (CH_2), 44.1 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{14}H_{13}N_3O_2$: 255.1008, found: 255.1008.

5-Bromo-4-chloro-6-phenylfuro[2,3-*d*]pyrimidine 37a. *N*-Bromosuccinimide (1.16 g, 6.50 mmol) was added portionwise to 4-chloro-6-phenylfuro[2,3-*d*]pyrimidine (**36a**, 1.0 g, 4.33 mmol) in 20 mL DMF. After the resulting mixture was stirred at room temperature for 3 h, H_2O (100 mL) was added. The resulting precipitate was filtered and washed with H_2O. The crude compound was purified by silica gel column chromatography using a mixture of EtOAc/*n*-hexane (1:10), to provide **37a** (1.14 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.75 (s, 1H), 8.20–8.16 (m, 2H), 7.55–7.51 (m, 3H); LC–MS (ESI) *m/z*: 308.9 $[M+H]^+$, 310.9 $[M+2+H]^+$.

2-(5-Bromo-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 11. Compound **11** was prepared in 85% yield from **37a**, similar to **8**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.36 (s, 1H), 8.08–8.05 (m, 2H), 7.52–7.40 (m, 3H), 6.53 (br, 1H), 3.94–3.89 (m, 2H), 3.54 (br, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.0 (C), 157.7 (C), 154.3 (CH), 147.0 (C), 129.5 (CH), 128.7 (CH), 128.3 (C), 126.6 (CH), 102.7 (C), 88.8 (C), 62.8 (CH_2), 44.3 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{14}H_{12}BrN_3O_2$: 333.0113, found: 333.0107.

6-Bromo-4-chloro-5-phenylfuro[2,3-*d*]pyrimidine 37b. Compound **37b** was prepared in 68% yield from **36b**, similar to **37a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.34 (s, 1H), 7.53–7.47 (m, 5H); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{12}H_6BrClN_2O$: 307.9352, found: 307.9368.

2-(6-Bromo-5-phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 12. Compound **12** was prepared in 28% yield from **37b**, similar to **8**. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.28 (s, 1H), 7.58–7.51 (m, 5H), 3.65–3.58 (m, 4H); ^{13}C NMR (150 MHz, CD_3OD): δ = 167.1 (C), 158.1 (C), 154.7 (CH), 131.1 (C), 130.5 (CH×2), 130.2 (CH), 124.0 (C), 121.0 (C), 102.9 (C), 61.1 (CH_2), 44.1 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{17}H_{14}BrN_5O$: 333.0113, found: 333.0116.

(5-Bromo-6-phenylfuro[2,3-*d*]pyrimidin-4-yl)-[2-(*tert*-butyldimethylsilanyloxy)ethyl]amine 38. Imidazole (0.51 g, 7.50 mmol) and *tert*-butyldimethylsilyl chloride (TBDMSCl, 0.54 g, 3.60 mmol) were added to a solution of **11** (1.0 g, 3.00 mmol) in DMF (20 mL) at room temperature and stirred for 1 h. H_2O was added to the reaction mixture, which was extracted with CH_2Cl_2; the organic layer was separated, washed with brine, and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using a mixture of CH_2Cl_2/MeOH (30:1) to give **38** (1.31 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.38 (s, 1H), 8.10–8.07 (m, 2H), 7.51–7.42 (m, 3H), 6.60 (t, *J* = 5.2 Hz, 1H), 3.89 (t, *J* = 5.2 Hz, 2H), 3.78 (q, *J* = 5.2 Hz, 2H), 0.92 (s, 9H), 0.09 (s, 6H); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{20}H_{26}BrN_3OSi$: 447.0978, found: 447.0996.

2-(5-Methyl-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 13. $Pd(PPh_3)_4$ (35 mg, 0.03 mmol) and CH_3ZnCl (0.9 mL, 2 M solution in THF) were added to a solution of **38** (140 mg, 0.30 mmol) in dry THF (5 mL) and held at reflux for 24 h. H_2O was added to the reaction mixture, which was extracted with EtOAc; the organic layer was separated, washed with brine, and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using a mixture of *n*-hexane/EtOAc (4:1) to give [2-(*tert*-butyldimethylsilanyloxy)ethyl]-(5-methyl-6-phenylfuro[2,3-*d*]pyrimidin-4-yl)amine (60 mg, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.37 (s, 1H), 7.73–7.69 (m, 2H), 7.49–7.34 (m, 3H), 5.75 (t, *J* = 5.1 Hz, 1H), 3.87 (t, *J* = 5.1 Hz, 2H), 3.75 (q, *J* = 5.1 Hz, 2H), 2.57 (s, 3H), 0.93 (s, 9H), 0.10 (s, 6H); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{21}H_{29}N_3O_2Si$: 383.2029, found: 383.2022. The above compound (30 mg, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), to which was added tetra-*n*-butylammonium fluoride (TBAF, 0.16 mL, 1 M solution in CH_2Cl_2); this was stirred at room temperature for 16 h. H_2O was added to the reaction mixture, which was extracted with CH_2Cl_2; the organic layer was separated, washed with brine, and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using a mixture of acetone/*n*-hexane (3:1) to give **13** (20 mg, 95%). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.20

(s, 1H), 7.70–7.67 (m, 2H), 7.50–7.36 (m, 3H), 3.81–3.70 (m, 4H), 2.56 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ = 165.9 (C), 159.8 (C), 154.1 (CH), 148.6 (C), 131.2 (C), 129.8 (CH), 129.5 (CH), 128.1 (CH), 111.1 (C), 105.3 (C), 61.7 (CH_2), 44.4 (CH_2), 11.2 (CH_3); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{15}H_{15}N_3O_2$: 269.1164, found: 269.1170.

2-(5-Ethynyl-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 15. $Pd(PPh_3)_2Cl_2$ (53 mg, 0.08 mmol), CuI (14 mg, 0.08 mmol), *N*,*N*-diisopropylethylamine (DIPEA, 5 mL), and $(CH_3)_3SiC{\equiv}CH$ (0.7 mL, 5.1 mmol) were added to a solution of **11** (500 mg, 1.50 mmol) in anhydrous DMF (5 mL) and heated at 65 °C for 16 h. H_2O was added to the reaction mixture, which was extracted with CH_2Cl_2; the organic layer was separated, washed with brine, and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using a mixture of *n*-hexane/EtOAc (1:1) to give 2-(6-phenyl-5-trimethylsilanylethynylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol (301 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.35 (s, 1H), 8.21–8.18 (m, 2H), 7.50–7.38 (m, 3H), 6.53 (t, *J* = 5.4 Hz, 1H), 3.92 (t, *J* = 4.5 Hz, 2H), 3.78 (q, *J* = 5.4 Hz, 2H), 3.55 (br, 1H), 0.34 (s, 9H); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{19}H_{21}N_3O_2Si$: 351.1403, found: 351.1393. The above compound (37 mg, 0.11 mmol) was dissolved in MeOH (1 mL), to which K_2CO_3 (44 mg, 0.32 mmol) was added, and the mixture was stirred at room temperature for 16 h. H_2O was added to the reaction mixture, which was extracted with CH_2Cl_2; the organic layer was separated, washed with brine, and concentrated under vacuum. The residue obtained was purified by silica gel column chromatography using a mixture of *n*-hexane/EtOAc (1:2) to give **15** (25 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.37 (s, 1H), 8.21–8.18 (m, 2H), 7.51–7.40 (m, 3H), 6.43 (t, *J* = 4.4 Hz, 1H), 3.92 (t, *J* = 4.8 Hz, 2H), 3.80 (td, *J* = 4.8, 4.4 Hz, 2H), 3.72 (s, 1H), 3.64 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2 (C), 158.0 (C), 154.5 (CH), 154.4 (C), 129.8 (C), 128.8 (CH), 128.7 (C), 125.7 (CH), 103.0 (C), 94.0 (C), 86.2 (C), 76.1 (C), 62.7 (CH_2), 44.3 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{16}H_{13}N_3O_2$: 279.1008, found: 279.1010.

2-(5-Ethyl-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 14. PtO_2 (2 mg) was added to a solution of **15** (17 mg, 0.06 mmol) in a mixture of EtOAc/MeOH (1:1, 2 mL) and hydrogenated at atmospheric pressure (using a balloon of H_2 gas) for 1 h at room temperature. The reaction mixture was filtered over Celite, the solvents were removed under vacuum, and the residue obtained was purified by silica gel column chromatography using a mixture of *n*-hexane/EtOAc (1:2) to give **14** (12 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.34 (s, 1H), 7.69–7.65 (m, 2H), 7.49–7.35 (m, 3H), 5.66 (t, *J* = 4.5 Hz, 1H), 4.09 (br, 1H), 3.92 (t, *J* = 4.5 Hz, 2H), 3.81 (q, *J* = 4.5 Hz, 2H), 2.89 (q, *J* = 4.5 Hz, 2H), 1.42 (t, *J* = 4.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.3 (C), 158.0 (C), 153.4 (CH), 147.3 (C), 129.8 (C), 128.8 (CH), 128.5 (CH), 126.9 (CH), 115.5 (C), 103.7 (C), 62.6 (CH_2), 44.3 (CH_2), 18.6 (CH_2), 15.4 (CH_3); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{16}H_{17}N_3O_2$: 283.1321, found: 283.1329.

2-[5-(4-Methoxyphenyl)-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino]ethanol 16. *p*-Methoxybenzene boronic acid (105 mg, 0.46 mmol), $Pd(OAc)_2$ (10 mg, 0.04 mmol), PPh_3 (44 mg, 0.16 mmol), and Na_2CO_3 (0.46 mL, 2 M solution) were added to a solution of **11** (140 mg, 0.42 mmol) in dioxane/H_2O (1:1, 4 mL) mixture under N_2 and heated at 100 °C for 3 h. After completion, the reaction mixture was cooled to room temperature, H_2O (10 mL) was added, and the reaction was extracted with CH_2Cl_2 (3×20 mL). Combined organic phases were dried over Na_2SO_4, concentrated under vacuum, and the residue obtained was purified by silica gel column chromatography using CH_2Cl_2/MeOH (30:1) to give **16** (81 mg, 53%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.33 (s, 1H), 7.52–7.49 (m, 2H), 7.38 (d, *J* = 8.1 Hz, 2H), 7.26–7.24 (m, 3H), 7.04 (d, *J* = 8.1 Hz, 2H), 5.20 (t, *J* = 5.4 Hz, 1H), 3.89 (s, 3H), 3.74 (t, *J* = 5.4, Hz, 2H), 3.57 (q, *J* = 5.4 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.5 (C), 159.9 (C), 157.9 (C), 153.5 (CH), 147.0 (C), 120.8 (CH), 129.4 (C), 128.4 (CH×2), 126.2 (CH), 123.7 (C), 115.1 (CH), 114.5 (C), 103.5 (C), 62.6 (CH_2), 55.3 (CH_3), 44.2 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{21}H_{19}N_3O_3$: 361.1426, found: 361.1412.

2-[5-(4-Nitrophenyl)-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino]ethanol 17. Compound **17** was prepared in 80% yield from *p*-nitrobenzene boronic acid and **11**, similar to **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.44 (s, 1H), 8.42–8.37 (m, 2H), 7.73–7.68 (m, 2H), 7.47–7.43 (m, 2H), 7.35–7.28 (m, 3H), 5.01 (t, *J* = 4.8 Hz, 1H), 3.77 (t, *J* = 4.8 Hz, 2H), 3.64 (q, *J* = 4.8 Hz, 2H), 3.18 (br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.1 (C), 157.6 (C), 154.0 (CH), 148.0 (C), 147.9 (C), 139.4 (C), 130.9 (CH), 129.3 (CH), 128.8 (CH), 128.4 (CH), 126.8 (CH), 124.8 (CH), 112.9 (C), 102.5 (C), 62.1 (CH_2), 43.9 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{20}H_{16}N_4O_4$: 376.1172, found: 376.1191.

2-[5-(4-Hydroxyphenyl)-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino]ethanol 18. Compound **18** was prepared in 94% yield from **16**, similar to **21**. ^{1}H NMR (300 MHz, CD_3OD): δ = 8.26 (s, 1H), 7.52–7.48 (m, 2H), 7.32–7.26 (m, 5H), 6.99–6.95 (m, 2H), 3.62–3.54 (m, 5H); ^{13}C NMR (75 MHz, CD_3OD): δ = 165.5 (C), 159.6 (C), 159.2 (C), 154.5 (CH), 148.2 (C), 132.1 (CH), 130.8 (C), 129.6 (CH×2), 127.3 (CH), 123.3 (C), 117.7 (CH), 116.8 (C), 104.6 (C), 61.2 (CH_2), 44.0 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{20}H_{17}N_3O_3$: 347.1270, found: 347.1266.

2-[5-(4-Acetamidophenyl)-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino]ethanol 19. Compound **19** was prepared in 60% yield from *p*-acetamidobenzene boronic acid and **11**, similar to **16**. ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 10.20 (br, 1H), 8.33 (s, 1H), 7.80–7.77 (m, 2H), 7.45–7.41 (m, 4H), 7.38–7.29 (m, 3H), 5.42 (t, *J* = 5.2 Hz, 1H), 4.70 (t, *J* = 5.2 Hz, 1H), 3.47–3.42 (m, 4H), 2.10 (s, 3H); ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 168.7 (C), 164.5 (C), 157.4 (C), 154.0 (CH), 145.7 (C), 139.9 (C), 130.1 (CH), 129.2 (C), 128.9 (CH), 128.7 (CH), 126.0 (CH), 125.4 (C), 119.7 (CH), 115.2 (C), 102.4 (C), 59.2 (CH_2), 42.8 (CH_2), 24.2 (CH_3); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{22}H_{20}N_4O_3$: 388.1535, found: 388.1531.

2-(6-Phenyl-5-pyridin-3-ylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 22. Compound **22** was prepared in 53% yield from 3-pyridyl boronic acid and **11**, similar to **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.74–8.70 (m, 2H), 8.38 (s, 1H), 7.87–7.83 (m, 1H), 7.50–7.42 (m, 3H), 7.30–7.26 (m, 3H), 5.07 (t, *J* = 5.1 Hz, 1H), 4.07 (br, 1H), 3.75 (t, *J* = 5.1 Hz, 2H), 3.62 (q, *J* = 4.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.0 (C), 157.7 (C), 153.9 (CH), 150.3 (C), 149.9 (CH), 148.1 (C), 137.5 (CH), 129.0 (CH), 128.7 (CH), 128.7 (C), 126.6 (CH), 124.2 (CH), 111.0 (C), 102.9 (C), 61.9 (CH_2), 43.8 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{19}H_{16}N_2O_2$: 332.1273, found: 332.1266.

2-(5-Furan-2-yl-6-phenylfuro[2,3-*d*]pyrimidin-4-ylamino)ethanol 23. Compound **23** was prepared in 42% yield from 2-furanyl boronic acid and **11**, similar to **16**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.38 (s, 1H), 7.70–7.67 (m, 2H), 7.62 (dd, *J* = 1.5, 0.6 Hz, 1H), 7.41–7.36 (m, 3H), 6.56 (dd, *J* = 3.3, 1.5 Hz, 1H), 6.54 (dd, *J* = 3.3, 0.6 Hz, 1H), 6.43 (t, *J* = 4.8 Hz, 1H), 3.87 (t, *J* = 4.8 Hz, 2H), 3.74 (q, *J* = 4.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.2 (C), 157.8 (C), 153.9 (CH), 145.4 (C), 142.8 (CH), 129.4 (CH), 129.1 (CH), 128.6 (CH), 127.3 (CH), 112.1 (CH), 110.5 (CH), 105.2 (C), 102.1 (C), 62.9 (CH_2), 44.4 (CH_2); HRMS (EI): *m/z* $[M]^+$ calcd for $C_{18}H_{15}N_3O_3$: 321.1113, found: 321.1117.

2-Amino-4,5-diphenylthiophene-3-carbonitrile 40. A mixture of deoxybenzoin (**39**, 558 mg, 2.90 mmol), malononitrile (198 mg, 3.00 mmol), powdered sulfur (96 mg, 3.00 mmol), and triethylamine

(300 mg, 3.00 mmol) in EtOH were held at reflux for 16 h. The solvents were removed under vacuum, H_2O was added, the mixture was acidified with concentrated HCl, and the solid residue obtained was filtered. The crude product was purified by silica gel column chromatography using *n*-hexane/EtOAc (3:1) to give **40** (500 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.44–7.35$ (m, 10H); LC–MS (ESI) *m/z*: 277.1 $[M+H]^+$.

4-Chloro-5,6-diphenylthieno[2,3-*d*]pyrimidine 41. Compound **41** was prepared in 20% yield over two steps from **40**, similar to **31a**. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.88$ (s, 1H), 7.38–7.28 (m, 10H); LC–MS (ESI) *m/z*: 323.2 $[M+H]^+$.

2-(5,6-Diphenylthieno[2,3-*d*]pyrimidin-4-ylamino)ethanol 25. Compound **25** was prepared in 23% yield from **41**, similar to **8**. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.45$ (s, 1H), 7.45–7.17 (m, 10H), 3.75 (bs, 1H), 3.66–3.63 (m, 2H), 3.51–3.46 (m, 2H); LC–MS (ESI) *m/z*: 348.1 $[M+H]^+$.

2-Amino-1-benzyl-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile 42. A mixture of benzoin (1.18 g, 5.60 mmol), benzylamine (2.12 g, 19.80 mmol), and HCl (1 mL) in toluene was held at reflux for 24 h, after which time the solvents were evaporated under vacuum. The residue obtained was purified by silica gel column chromatography using *n*-hexane/EtOAc (3:1) to give 2-benzylamino-1,2-diphenylethanone (1.2 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.00–7.43$ (m, 15H), 6.31–6.29 (d, 1H), 4.24–4.09 (m, 2H); LC–MS (ESI) *m/z*: 302.1 $[M+H]^+$. A mixture of the above compound (1.20 g, 4.0 mmol) and malononitrile (316 mg, 4.8 mmol) in toluene was held at reflux for 24 h, and the solvents were then evaporated under vacuum. The residue obtained was purified by silica gel column chromatography using *n*-hexane/EtOAc (3:1) to give **42** (300 mg, 22%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.41–7.08$ (m, 15H), 4.93 (m, 2H); LC–MS (ESI) *m/z*: 350.1 $[M+H]^+$.

7-Benzyl-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ol 43. A solution of **42** (300 mg, 0.90 mmol) in formic acid (20 mL) was held at reflux for 6 h. After cooling the reaction mixture, ice H_2O (200 mL) was added, and the precipitate was filtered, washed well with H_2O, and dried to give **43** (200 mg, 62%). ^{1}H NMR (300 MHz, $[D_6]$DMSO): $\delta = 7.99$ (s, 1H), 7.35–6.81 (m, 15H), 5.31 (s, 2H); LC–MS (ESI) *m/z*: 378.1 $[M+H]^+$.

7-Benzyl-4-chloro-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 44. A solution of **43** (644 mg, 1.60 mmol) in $POCl_3$ (15 mL) was held at reflux for 3 h. After cooling the reaction mixture, ice H_2O (100 mL) was added, and the precipitate was collected by filtration and purified by silica gel column chromatography using *n*-hexane/EtOAc (3:1) to give **44** (310 mg, 46%). ^{1}H NMR ($CDCl_3$): $\delta = 8.74$ (s, 1H), 7.29–6.91 (m, 15H), 5.49 (m, 2H); LC–MS (ESI) *m/z*: 396.2 $[M+H]^+$.

2-(7-Benzyl-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamino)ethanol 26. 2-Aminoethanol (202 mg, 3.30 mmol) was added to a solution of **44** (115 mg, 0.30 mmol) in *n*-butanol (15 mL), and held at reflux for 16 h. The reaction mixture was evaporated, and the residue obtained was purified by silica gel column chromatography using *n*-hexane/EtOAc (3:1) to give **26** (30 mg, 25%). ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.24$ (s, 1H), 7.31–7.14 (m, 15H), 5.38 (s, 2H), 3.65–3.54 (m, 2H), 3.32–3.30 (m, 2H); LC–MS (ESI) *m/z*: 421.1 $[M+H]^+$.

4-Chloro-5,6-diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 45. A solution of **44** (202 mg, 0.50 mmol) and $AlCl_3$ (133 mg, 1.00 mmol) in toluene (10 mL) was held at reflux for 2 h. After cooling the reaction mixture, ice H_2O (50 mL) was added, and the separated solid was collected by filtration to give **45** (60 mg, 39%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.58$ (s, 1H), 7.47–7.24 (m, 11H); LC–MS (ESI) *m/z*: 306.3 $[M+H]^+$.

2-(5,6-Diphenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamino)ethanol 27. 2-Aminoethanol (1 mL, 16.60 mmol) was added to a solution of **45** (60 mg, 0.20 mmol) in *n*-butanol (10 mL) and held at reflux for 16 h. The reaction mixture was evaporated, and the residue obtained was purified by silica gel column chromatography using *n*-hexane/EtOAc (3:1) to give **27** (31 mg, 48%) ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.16$ (s, 1H), 7.43–7.23 (m, 10H), 3.61–3.48 (m, 2H), 3.34–3.30 (m, 2H); LC–MS (ESI) *m/z*: 331.1 $[M+H]^+$.

Enzyme inhibition assay

Aurora kinase A inhibition assays[21,35] and EGFR kinase inhibition assays[36] for the compounds were carried out as described by us previously.

X-ray co-crystal structure determination

Compound **8** was co-crystallized with Aurora kinase A construct, and the complex structure was solved in a manner similar to that described by us before.[21]

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