Cdc7 Kinase Inhibitors: Pyrrolopyridinones as Potential Antitumor Agents. 1. Synthesis and Structure–Activity Relationships

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Cdc7 kinase is an essential protein that promotes DNA replication in eukaryotic organisms. Genetic evidence indicates that Cdc7 inhibition can cause selective tumor-cell death in a p53-independent manner, supporting the rationale for developing Cdc7 small-molecule inhibitors for the treatment of cancers. In this paper, the synthesis and structure–activity relationships of 2-heteroaryl-pyrrolopyridinones, the first potent Cdc7 kinase inhibitors, are described. Starting from 2-pyridin-4-yl-1,5,6,7-tetrahydro-pyrrolo[3,2-c]pyridin-4-one, progress toward a simple scaffold, tailored for Cdc7 inhibition, is reported.

Introduction

The inhibition of DNA synthesis has proven a useful strategy in the treatment of hyper-proliferative disorders.[1] However, because of the toxicity of current DNA replication inhibitors and because tumor cells can develop resistance to these drugs,[2] there is a great need for novel agents that tackle this process possibly by a different mechanism. In eukaryotic cells, DNA synthesis is initiated from a number of replication origins in the genome.[3] Cdc7[a] kinase is responsible for origin activation and the establishment of active replication forks. Indeed, Cdc7-dependent phosphorylation of one or more subunits of the minichromosome maintenance complex (MCM 2–7)[4,5] is thought to activate the helicase activity of the complex. Inhibition of Cdc7 kinase by both small interfering RNAs[6] and small-molecule inhibitors (Montagnoli et al., manuscript in preparation) causes a blockade of DNA synthesis in human cell lines. As a consequence, tumor cells are funnelled into the apoptotic pathway in a p53-independent manner, whereas normal cells are arrested in their progression through the cell cycle and are capable of surviving Cdc7 inhibition for long periods of time.[6] These findings support the notion that pharmacological inhibition of Cdc7 kinase can be an effective novel strategy for the development of oncologic therapeutics and that a small-molecule inhibitor of Cdc7 kinase could be a useful drug for the treatment of cancers.

This paper reports the discovery of the very first class of Cdc7 kinase inhibitors,[7,8] namely, the 2-heteroaryl-pyrrolopyridinones. The screening of our compound library (former Pharmacia-Upjohn collection) led to the identification of 2-pyridin-4-yl-1,5,6,7-tetrahydro-pyrrolo[3,2-c]pyridin-4-one **1** (Figure 1), a scaffold for MK2 inhibitors, originally developed at Pharmacia Corp.,[9] as a potent ATP mimetic inhibitor of Cdc7, with an IC_{50} of 10 nM in a Cdc7 kinase assay (using a complex Cdc7FL/Dbf4FL),[10] a K_i value of 3 nM, and greater than 20-fold selectivity versus a panel of other kinases. The biological properties of this compound will be described in a different work (Montagnoli et al., manuscript in preparation).

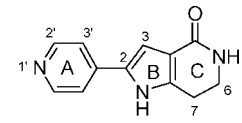

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Figure 1. Structure of pyrrolopyridinone **1**.

Starting from scaffold **1**, structure–activity relationship (SAR) studies, designed to identify modifications and substituents that might increase potency against Cdc7, were carried out.

The inhibitory activity of putative Cdc7 inhibitors was determined by a method of assay based on the use of a Dowex resin (see the Experimental Section). For the purposes of determining selectivity, the compounds were evaluated in an in-house panel of about 35 Tyr and Ser-Thr kinases. A few of these, namely, the Cdk2 and GSK3β kinases, consistent with their similarity in their ATP binding pockets,[11,12] were the most frequently affected. Other kinases were only sporadically inhibited by the compounds presented here. The synthesis of different analogues of **1**, sequentially modified at rings A, B and C, has allowed us to highlight the major features contributing to the inhibition of the Cdc7 enzyme.

Chemistry

The preparation of the compounds appearing in this paper is based on the following different protocols:

1. The Hantzsch reaction between piperidinediones **56** or **57** and bromoketones **64** (Scheme 1) was utilized to prepare compounds **1**, **3**−**8**, **15**, **16**, **31**, **34**, **37**, **38**, **40**, **43**, **49**, and **50**.

According to the reaction, heteroarylbromoketones **64a**−**l** were allowed to react with piperidinedione **56** or **57** in the presence of either ammonium acetate or a suitable amine

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[a] Abbreviations: Cdc7, cell division cycle 7; MCM, minichromosome maintenance complex; MK2 or MAPKAPK-2, mitogen-activated protein kinase−activated protein kinase-2; Cdk, cyclin-dependent kinase; GSK, glycogen synthase kinase; CK, casein kinase; CMGC kinases, cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs), and CDK-like kinases (CLKs); CDI, 1,1′-carbonyl diimidazole; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole; PAMPA, parallel artificial membrane permeation assay.

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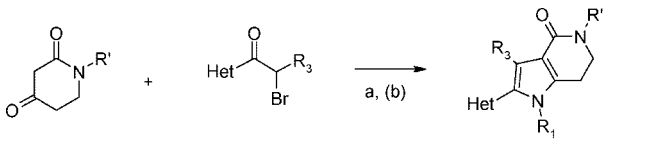
Scheme 1. Synthesis of Pyrrolopyridinones[a]



56 R' = Boc
57 R' = H

64
R_3 = H
a Het=pyridin-4-yl
b Het=2-aminopyrimidin-4-yl
c Het=pyridin-3-yl
d Het=3-fluoropyridin-4-yl
e Het=pyrimidin-4-yl
f Het=1-*t*Boc azaindol-4-yl
g Het=purin-4-yl
h Het=phenyl
i Het=2-phenylamino-pyrimidin-4-yl

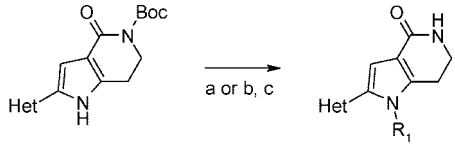
R_3 = methyl
l Het=pyridin-4-yl

1, 3-8, 15, 16, 31, 34, 37, 38, 40, 43, 49, 50

R_1 = H, alkyl, cycloalkyl, aryl, heterocyclyl

[a] Conditions: (a) NH_4OAc or R_1-NH_2, EtOH, rt; (b) TFA, CH_2Cl_2, rt or 4N HCl in dioxane, MeOH, rt.

Scheme 2. N-Alkylation of Pyrrole[a]



49 Het=2-aminopyrimidin-4-yl
50 Het=pyridin-4-yl

22-30, 32, 33, 35, 36, 39

R_1 = optionally substituted alkyls, benzyl

[a] Conditions: (a) 1. 60% NaH, DMF, rt. 2. R_1Br, rt. (b) R_1Br, K_2CO_3, DMF, 65 °C. (c) TFA, CH_2Cl_2, rt or 4N HCl in dioxane, MeOH, rt.

R_1−NH_2, depending on the need to obtain free or N-alkylated pyrroles, respectively.

2. N-alkylation of the pyrrole ring (Scheme 2) afforded compounds **22**–**30**, **32**, **33**, **35**, **36**, and **39**.

The reaction occurs between the protected pyrrolopyridinones and the appropriate alkyl bromides, under basic conditions, in the presence of sodium hydride or potassium carbonate.

3. Modifications of rings A and C of already formed pyrrolopyridinones (Scheme 3) led to compounds **12**, **20**, **21**, **41**, **42**, and **44**–**48**.

In particular, the brominated aminopyrimidinyl derivative **12** was prepared by direct regioselective bromination of **49** with N-bromosuccinimide NBS at room temperature. Hydrogenolytic dehalogenation of lactam **51**[13] afforded compound **20**, whereas N-formylation of **1**, with $POCl_3$ in DMF at room temperature, led to the N-formyllactam **21**. N-alkylated (**41**, **42**, **44**, and **45**) and N-acylated (**46**–**48**) aminopyrimidinyl derivatives were obtained from pyrrolopyridinone **5** and its protected analogue **49**, the former by a reductive amination reaction, the latter by exhaustive acylation, followed by selective basic hydrolysis.

4. Finally, in Scheme 4, the synthesis of compounds **14**, **18**, and **19** is shown. Compound **14** and pyrrolopyrrolone **19** were synthesized once more through the Hantzsch reaction, and the furan analog **18** was obtained by oxidative cycloaddition[14] of the protected piperidinedione to 4-ethynylpyridine.

Compounds **1**,[7] **2**,[13] **10**,[13] **11**,[13] **13**,[15] **17**,[16] and **51** were available from the former Pharmacia-Upjohn collection, but their preparation also is described in the literature. Compound **9** is commercially available.

For the synthesis of piperidinediones, the procedure given in Scheme 5 was used.

In this procedure, Meldrum's acid was allowed to react with N-Boc-3-aminopropionic acid **54** to provide compound **55**, which was cyclized to protected piperidinedione **56** by refluxing in ethyl acetate. Piperidinedione **56** was optionally deprotected to **57** under acidic conditions, with either trifluoroacetic acid in dichloromethane or hydrochloric acid in dioxane.

Bromoketones **64a**−**l** are commercially available or were accessed through the halogenation of ketones or ethylenolethers **63**, as shown in Scheme 6. This scheme also outlines the preparation of **63**, which is not commercially available.

Results and Discussion

Having established Cdc7 kinase as a potentially viable target for oncologic studies, a synthetic chemistry program was initiated, based on testing of our internal collection of Cdk inhibitors. This was driven by the close sequence similarity of the two kinases and facilitated by a well-established in-house research project on Cdk inhibitors. This strategy, supported by a 65% successful hit rate achieved in a preliminary screening on 100 Cdk inhibitors, led to the prompt discovery of a few promising chemical classes, among which the pyrrolopyridinones, represented by compound **1**, gained a leading position due to their favorable characteristics. The latter include low-molecular-weight, good solubility, low plasma protein binding, and the possibility of facile synthetic extension of the series.

In the absence of the crystallographic structure of the target protein, we aimed to build a three-dimensional model of Cdc7 based upon homology with other kinases. In order to identify the more suitable kinase for modeling, the sequence of the Cdc7 kinase domain was aligned with the sequences of all known human kinases.[17] This alignment highlighted a peculiarity of Cdc7, namely, an insertion domain spanning from amino acid 203 to 370 that is partially conserved only in the mouse and *Xenopus* Cdc7 orthologues and not present in any other known kinase (Figure 2 in red box).[18]

When a similarity search was performed with this motif against a nonredundant protein database, no statistically significant hits were obtained. Thus, the lack of conservation of this Cdc7-specific motif within kinases and other known proteins might suggest that it has no function in the enzymatic activity of the kinase. Indeed, removal of most of this sequence resulted in an active enzyme, and recent work indicates that it contains a protein–protein interaction domain important for nuclear import.[19]

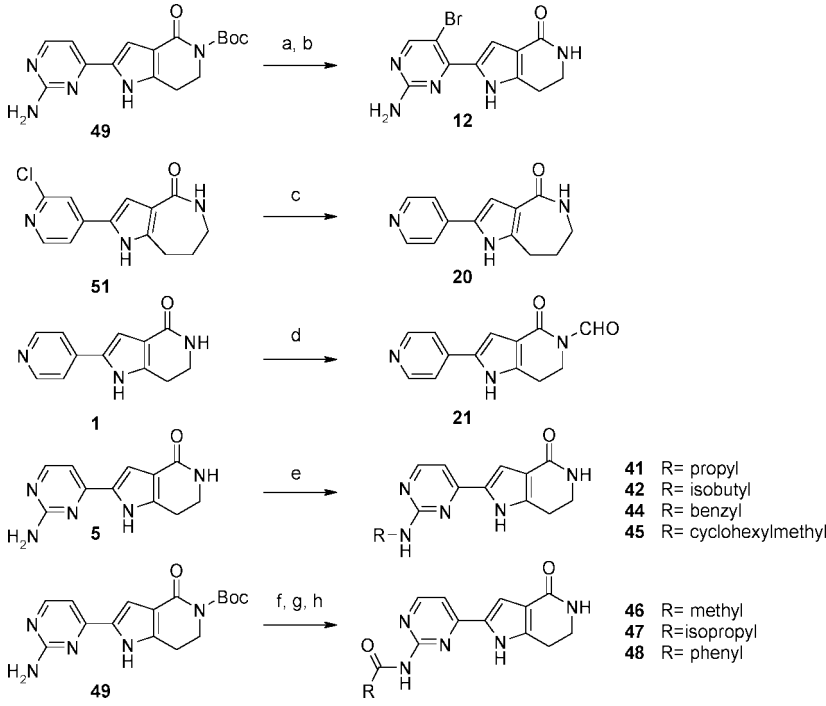
By repeating the similarity search with Cdc7Δ203–370 as a basic sequence, casein kinase 2 (CK2) and several members of the CMGC kinases, including cyclin-dependent kinase 2 (Cdk2), were the most important hits.

Because at the time of the analysis the CK2 structure was not available, a Cdc7 three-dimensional model was built starting from a Cdc7Δ203–370/Cdk2 alignment and Cdk2 structural information. The model was validated with early Cdc7 inhibitors and then used for a SAR rationalization, structure-based ligand design, and activity prediction. In this model, methionine 134 appears to be the gatekeeper residue.

Using this Cdc7 model, we explored how compound **1** would bind in the active site of the kinase and found that two different binding modes were possible: with either the pyridine nitrogen or the lactam group interacting with the hinge region (Figure 3).

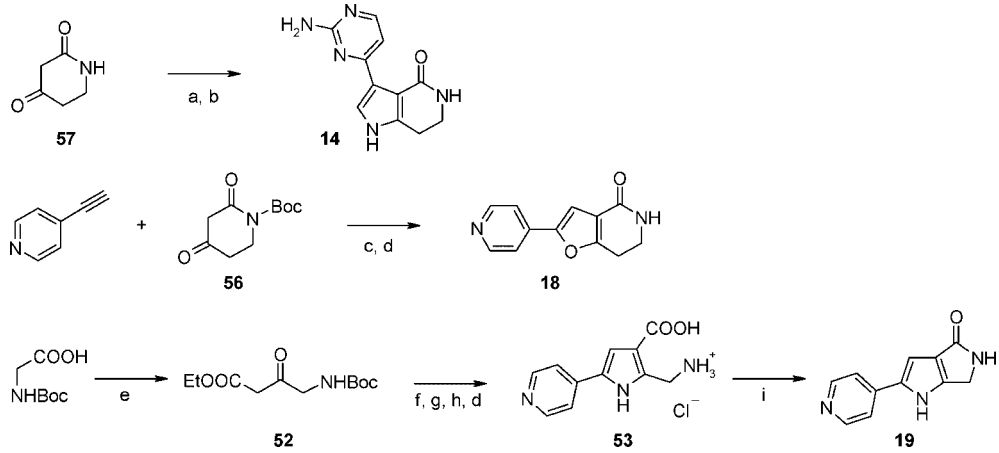
The first mode (A) presented a more favorable docking arrangement and better accounted for the subsequent SAR of this class (Figure 4). In the docking studies, molecules of water were included in the binding site and appeared to play an

Scheme 3. Synthesis of Pyrrolopyridinones from Other Pyrrolopyridinones[a]



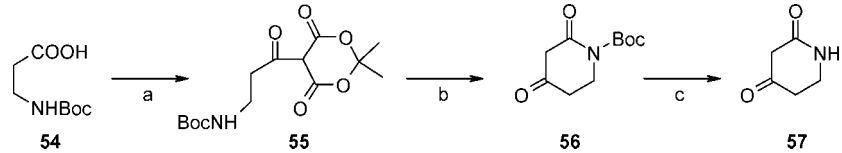
[a] Conditions: (a) NBS, DMF, rt; (b) 4N HCl in dioxane, MeOH, rt; (c) 10% Pd-C/H_2, MeOH, rt; (d) $POCl_3$, DMF, rt; (e) RCHO, $NaBH(OAc)_3$, DMF, TFA, rt; (f) RCOCl (2 equiv), TEA, THF, rt; (g) 1N NaOH, rt; (h) 4N HCl in dioxane, rt.

Scheme 4. Synthesis of Miscellaneous Compounds[a]



[a] Conditions: (a) EtOH, NH_4OAc, rt; (b) 2-bromo-1-(2-aminopyrimidin-4-yl)ethanone (**64b**), rt; (c) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, 0 °C; (d) 4N HCl in dioxane, MeOH, rt; (e) CDI, $KOOCCH_2COOEt$, $MgCl_2$, THF, rt, then 50 °C; (f) 2-bromo-1-pyridin-4-ylethanone (**64a**), 60% NaH, 0 °C; (g) NH_4OAc, AcOH, THF, rt; (h) NaOH, EtOH, water, reflux; (i) EDC hydrochloride, HOBt, N-ethyldiisopropylamine, CH_2Cl_2, DMF, rt.

Scheme 5. Synthesis of Piperidinediones[a]

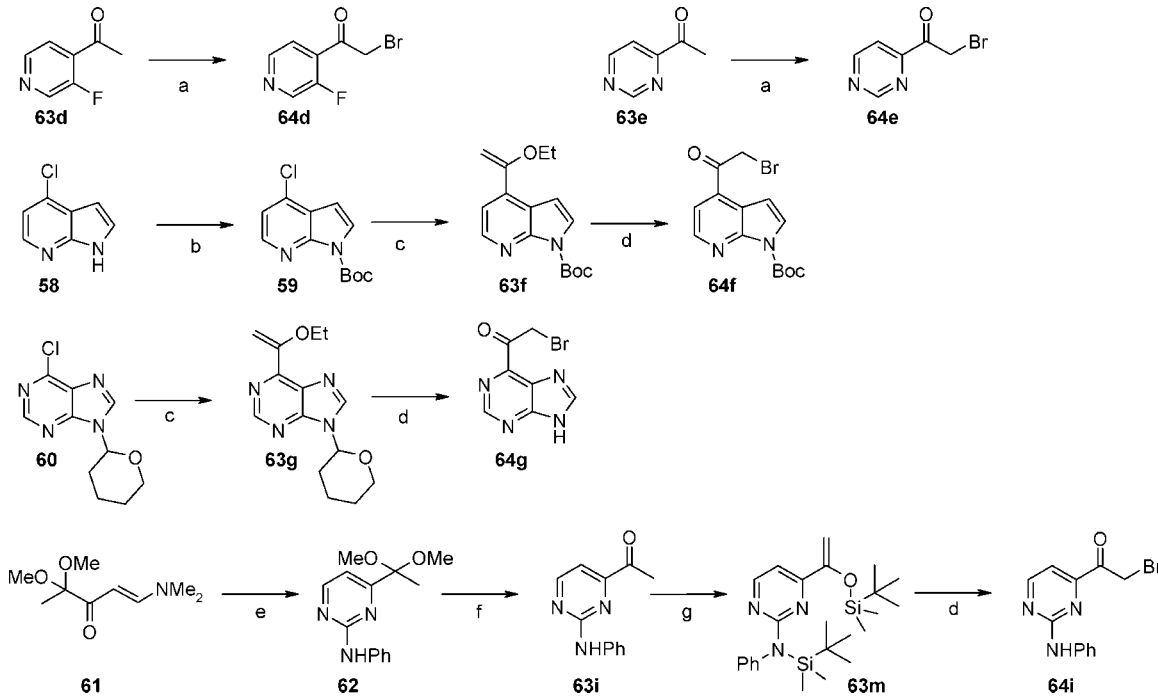


[a] Conditions: (a) Meldrum's acid, CH_2Cl_2, EDC hydrochloride, DMAP, 0 °C to rt; (b) EtOAc, reflux; (c) TFA, CH_2Cl_2, rt or 4N HCl in dioxane, rt.

important role in the binding of compound **1** and, in general, all of its close derivatives.

Our initial work focused on exploring variants of the three-ring systems that constitute pyrrolopyridinone **1**, involving the pyridinyl ring A, the pyrrole nucleus B, and the lactam ring C, and studying the effect these parts of the molecule have on potency. First, we investigated if the pyridine ring could be profitably replaced by other rings and if position 2 of the pyrrole is optimal for the attachment of the heteroaryl moiety. The set of compounds presented in Table 1 offers the opportunity to answer these questions. In most analogues, a nitrogen at position 1′ is present, a condition considered essential for activity, as duly demonstrated by compounds **2**, **8**, and **9**, in which, respectively, the repositioning or the absence of the nitrogen

Scheme 6. Synthesis of Bromoketones **64**[a]



[a] Conditions: (a) 48% HBr, Br_2, AcOH, 60 °C; (b) Boc_2O, DMAP, CH_3CN, rt; (c) 1-ethoxyvinyl tri-*n*-butyltin, $Pd(PPh_3)_4$, DMF, 100 °C; (d) NBS, THF, rt; (e) phenylguanidine carbonate, NaOEt, EtOH, reflux; (f) 88% HCOOH, rt; (g) *tert*-butyldimethylsilyl trifluoromethanesulfonate, TEA, CH_2Cl_2, rt.

atom, or the removal of ring A, led to inactive derivatives, consistent with the proposed binding mode (Figure 4). Moreover, moving the heteroaryl ring from position 2 to the adjacent position 3 resulted in the inactive compound **14**, possibly a consequence of the radical change in the geometry of the molecule.

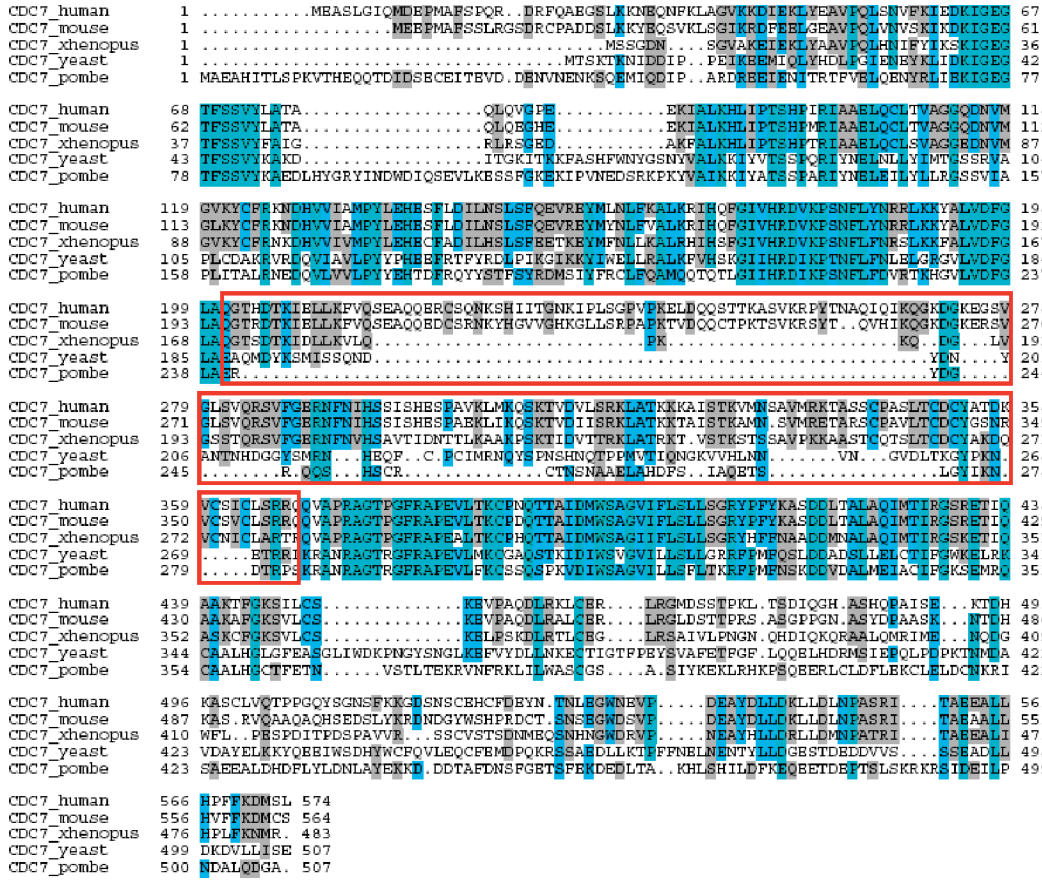


Figure 2. Alignment of Cdc7 orthologues showing the characteristic motif of human Cdc7 (hereafter called Cdc7-insertion domain) poorly conserved in other species and not found in other kinases.

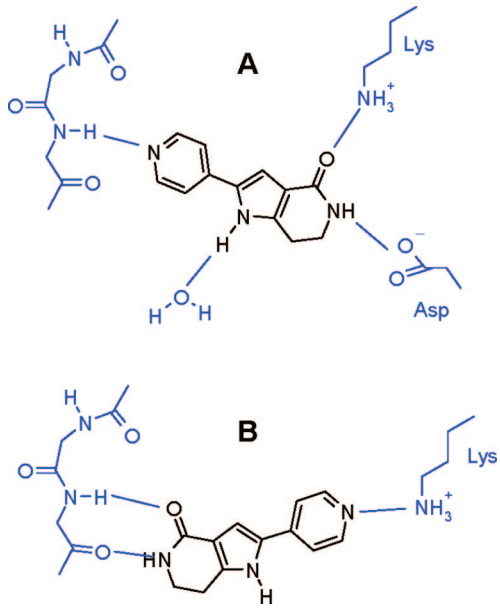


Figure 3. Possible binding modes of pyrrolopyridinone **1** in the homology model of Cdc7 kinase.

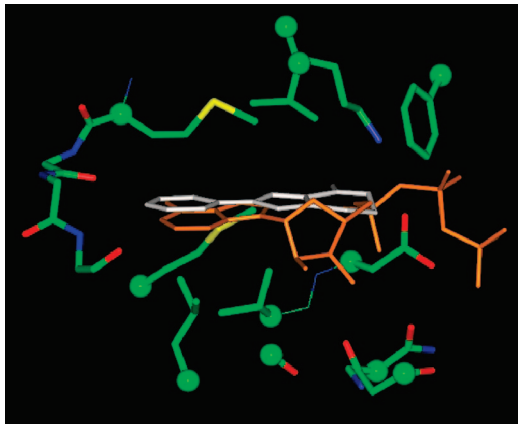


Figure 4. Predicted binding site of Cdc7 homology model with insertion of ATP (orange) and pyrrolopyridinone **1** (white). The sulfur atom of the gatekeeper *Met* 134 is in yellow.

Some compounds with a substituent at different positions of the heteroaryl moiety (ring A, Figure 1) were also examined. It is noteworthy that, while substituents at position 3′ or 5′ (fluoro in **3**, bromo in **12**, and vinylidene in **13**, retained or increased activity, a substituent at position 2′ resulted in different outcomes. In fact, a chloro substituent (**10**) showed less activity and represents a disturbance to binding, and the presence of an amino group also is somewhat detrimental in pyridine (**11** vs **1**) but slightly favorable in the pyrimidine (**5** vs **4**). However, its influence is unclear when it is embedded in a ring (**6** vs **7**), the differences presumably depending on the electronic distribution in the system. In conclusion, as is apparent from Table 1, derivatives **3**, **4**, **5**, **6**, **11**, **12**, and **13** increase or retain activity when compared to **1**.

Next, we concentrated our studies on substitutions on the central core of the pyrrolopyridinone **1** (Table 2).

In Table 2, pyrrole, pyrazole, and furan derivatives demonstrate that, when the pyrrole NH is alkylated (**16**) or when the whole ring is replaced with a different heteroaromatic system (**17**–**18**), a significant loss in potency is experienced. By retaining the pyridine as the A ring system (see Figure 1), the presence of hydrogen on the nitrogen of the pyrrole is a necessary determinant for activity. The influence of N-alkylation of pyrrole on activity (**16**) deserves attention and will be examined in more detail later. An alkyl substituent at position 3 (**15**) appears to be detrimental, because it causes a decrease in activity. This is most likely due to its unfavorable interaction with the large gatekeeper residue methionine (*Met* 134, Figure 4) in the narrow hydrophobic pocket toward which it points. In contrast, the presence of a vinylidene group, bridging the pyrrole with the adjacent pyridine ring (**13**), demonstrated a considerable improvement of activity, probably due to an optimized fitting of this flat moiety within the hydrophobic pocket.

Finally, we examined modifications of the lactam ring of compound **1** (Table 3).

Six- and five-membered lactam rings (compounds **1** and **19**, respectively) showed good inhibitory activity on the enzyme, whereas the azepinone analogue **20** did not. The free NH of the lactam seems important for activity, likely because the hydrogen is well placed for binding to aspartate. This is evidenced by the fact that the N-formyl derivative **21** is poorly active.

In summary, from the investigation of variations in rings A, B, and C, several compounds have emerged that display good inhibitory activity against the Cdc7 enzyme. Also, as outlined in Table 4, they show good activity against the A-2780 ovarian cancer cell line.

These compounds vary in their ring A substitution pattern, the region which binds well to the hinge region of the enzyme. Ring C can adopt only two profitable geometries, and ring B has to be a pyrrole. When challenged for cellular activity, only a few compounds (**3**, **5**, **12**, and **13**, Table 4) were as active, approximately, as the reference compound **1**. Of these, those carrying a substituent at position 3′ or 5′ (fluoro in **3**, bromo in **12**, and vinylidene in **13**) suffer from a severe loss of selectivity, and only compound **5** shows a selectivity comparable to that of **1**, at least 20-fold versus other kinases.

In conclusion, on the basis of the potency against the Cdc7 enzyme, the antiproliferative activity on the A-2780 cell line, and selectivity, only the parent compound **1** and the 2-aminopyrimidin-4-yl analogue **5** were considered suitable for further structural modifications and were subjected to N-substitutions of the pyrrole ring (both series) and to functionalization of the amino group of the pyrimidine.

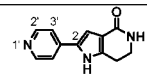
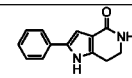
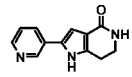
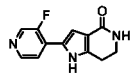
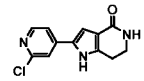
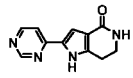
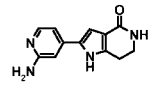
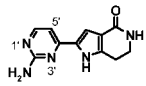
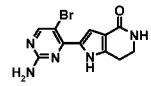
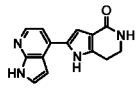
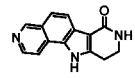
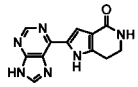
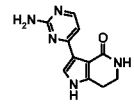
As anticipated, N-substitution of the pyrrole hampered activity in the pyridyl series. On the contrary, the aminopyrimidinyl homologues tolerated N substitution, providing some derivatives as potent as the unsubstituted precursor (Table 5).

For this reason, we extended our investigations to several other compounds of the latter series, and these substantially confirmed our previous results with only two exceptions (Table 6).

One possible explanation of the difference between the two series is that the pyrrole binds to the protein backbone by the intermediacy of one or two molecules of water. This binding possibility, definitively lost in the pyridinyl series, can be restored in aminopyrimidines (Figures 3A and 5) by the NH_2 of the pyrimidine ring, provided that R_1 is not a lipophilic bulky substituent, as evidenced especially by compounds **37** and **38**.

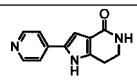
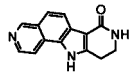
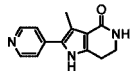
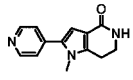
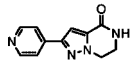
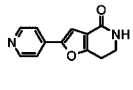
Most N-substituted aminopyrimidinyl analogues, when provided with a short lipophilic chain or a small rigid residue (compounds **26**, **28**, **29** and **31**–**35**), showed IC_{50} values in the low nanomolar range, whereas with larger cyclic groups, activity declined. The ideal chain length seems to be C_2-C_3, and the presence of a hydrophilic terminus is less favorable (compound **27**). The more hydrophilic piperazinyl ring behaved better than

Table 1. SAR: Substituent Variations in the Pyrrole Ring at Position 2 (IC50, μM)[a]

Cpd	Structure	Cdc7	Cpd	Structure	Cdc7
1		0.010 ± 0.004	**8**		>5
2		>5	**9**		>5
3		0.009 ± 0.005	**10**		0.147 ± 0.032
4		0.020 ± 0.004	**11**		0.028 ± 0.006
5		0.007 ± 0.001	**12**		0.004 ± 0.001
6		0.014 ± 0.004	**13**		0.002 ± 0.001
7		0.178 ± 0.044	**14**		>5

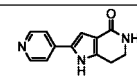
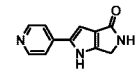
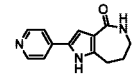
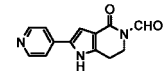
[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$).

Table 2. SAR: Variations of the Central Core B (IC_{50}, μM)[a]

Cpd	Structure	Cdc7
1		0.010 ± 0.004
13		0.002 ± 0.001
15		0.045 ± 0.012
16		0.404 ± 0.065
17		0.210 ± 0.004
18		0.201 ± 0.001

[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$).

Table 3. SAR: Variations of the Lactam Ring C (IC_{50}, μM)[a]

Cpd	Structure	Cdc7
1		0.010 ± 0.004
19		0.027 ± 0.001
20		0.158 ± 0.074
21		0.863 ± 0.087

[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$).

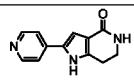
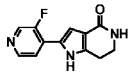
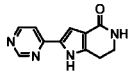
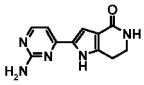
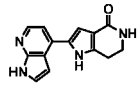
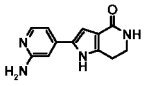
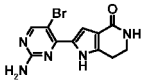
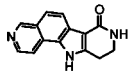
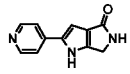
cyclohexyl (see **40** vs **37**), possibly a sign of binding to water or to a polar residue in the enzyme.

On the whole, N-substitution of the pyrrole provided very active compounds that regrettably proved equipotent across a broad spectrum of kinases, a finding that caused us to abandon the N-substituted pyrroles in favor of free pyrroles.

Having examined variations in the three main parts of the molecule, modifications at the amino group of pyrimidine were investigated. As shown in Table 7, alkylations (**41**, **42**, **44**, and **45**), arylation (**43**), and acylations (**46**–**48**) led to a much reduced activity, the only exception being the phenyl derivative (**43**), which is quite active on Cdc7 but poorly selective. In conclusion, functionalization of the amino group of the aminopyrimidinyl ring must be avoided in order to preserve the most favorable profile of biological activity.

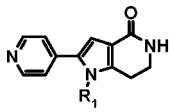
This refining process led to the definition of the basic scaffold **bs** (Figure 6) characterized by structural requirements that provide the best balance between activity and selectivity: (1) an unsubstituted pyridin-4-yl or 2-aminopyrimidin-4-yl rings at position 2 of the pyrrole ring, (2) a pyrrole ring with a free NH and no other substitutions, and (3) a six-membered lactam, ideal for size and rigidity, with free NH. Scaffold **bs** is characterized

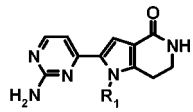
Table 4. SAR: Comparison of the Most Interesting Scaffolds (IC_{50}, μM)[a]

Cpd	Structure	Cdc7	A-2780[b]
1		0.010 ± 0.004	1.1
3		0.009 ± 0.005	0.2
4		0.020 ± 0.004	5.5
5		0.007 ± 0.001	1.7
6		0.014 ± 0.004	>10
11		0.028 ± 0.006	>10
12		0.004 ± 0.001	0.8
13		0.002 ± 0.001	0.1
19		0.027 ± 0.001	4.7

[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$). [b] IC_{50}values are reported as the mean of 2–3 experiments with a coefficient of variation below 35%.

Table 5. SAR: Variations of the Pyrrole Alkylating Group R_1 (IC_{50}, μM)[a]





Cpd	Cdc7	R_1	Cpd	Cdc7
1	0.010 ± 0.004	H	**5**	0.007 ± 0.001
16	0.404 ± 0.065		**25**	0.088 ± 0.055
22	0.067 ± 0.004		**26**	0.008 ± 0.005
23	3.3		**27**	0.138 ± 0.055
24	0.160 ± 0.044		**28**	0.005 ± 0.003

[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$).

by a low-molecular-weight (around 200), good solubility in water (>225 μM at pH 7),[20] and good metabolic stability (intrinsic clearance lower than 20 mL/min/kg in rat hepatocytes)[20] but low cellular permeability (P_{app} lower than 2.10^{-6} cm/s) in the PAMPA assay.[21] The latter property may account for the moderate cellular activity displayed against the A-2780 cell line. A further SAR study, related to the functionalization of the unexplored positions 6 and 7, will be presented in due course.

Conclusions

In this paper, the first known inhibitors of Cdc7 kinase are revealed. Starting from the basic framework of **1**, the SARs associated with the molecule were investigated. These show that most functionalizations are detrimental to biological properties. Strong Cdc7 inhibitory activity is associated with a central unsubstituted pyrrole nucleus fused with a five- or six-membered lactam and carrying at position 2 an appropriate, optionally substituted, heteroaromatic ring. Regarding selectivity, more stringent conditions are required of ring A, in that it can only be achieved with a pyridin-4-yl ring, unsubstituted at position 3′, or with a pyrimidin-4-yl residue, unsubstituted at position 5′, and optionally bearing a primary amino function at position 2′ as the substituent.

This study led to the definition of the simple pharmacophore **bs**, as a potent inhibitor of Cdc7 yet selective versus other tested kinases (Montagnoli et al., manuscript in preparation). This scaffold seems to possess the best geometry and a favorable entropy in its interaction with the Cdc7 binding site. The decreased potency of most substituted analogues might be due to distortion of this ideal geometry or to the positioning of the ligands away from the best binding mode presented by the naked scaffolds.

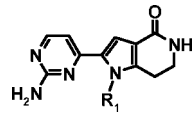
Further work, consisting of functionalizing the unexplored positions 6 and 7, will answer the question as to whether there are substituents that might impart better characteristics, in particular, increased intracellular activity, and, in general, a more appropriate druglike profile that seems to be obligatory for this demanding kinase.

Experimental Section

I. Chemistry. Unless otherwise noted, solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under an argon atmosphere. All final compounds were purified to >95% purity as determined by high-performance liquid chromatography (HPLC). Purity was measured by HPLC on a Waters X Terra RP 18 (4.6 × 50 mm, 3.5 μm) column using a Waters 2790 HPLC system equipped with a 996 Waters PDA detector and Micromass mod. A ZQ single quadrupole mass spectrometer, equipped with an electrospray ion source (ESI). Mobile phase A was an ammonium acetate 5 mM buffer (pH 5.5 with acetic acid/acetonitrile 95:5), and mobile phase B was H_2O/acetonitrile (5:95). The following conditions were used: a gradient from 10 to 90% B in 8 min and held at 90% B for 2 min; UV detection at 220 and 254 nm; a flow rate of 1 mL/min; an injection volume of 10 μL; full scan, mass range from 100 to 800 amu. The capillary voltage was 2.5 kV; the source temperature was 120 °C; the cone was 10 V. Masses are given as an *m*/*z* ratio.

When necessary, compounds were purified by preparative HPLC on a Waters Symmetry C18 (19 × 50 mm, 5 μm) column using a Waters preparative HPLC 600 equipped with a 996 Waters PDA detector and a Micromass mod. A ZMD single quadrupole mass spectrometer, with electro-spray ionization, in the positive mode, was used. Mobile phase A was water and 0.01% trifluoroacetic acid, and mobile phase B was acetonitrile. The following conditions were used: a gradient from 10 to 90% B in 8 min and held at 90% B for 2 min; a flow rate of 20 mL/min. Column chromatography

Table 6. Other N-Substituted Aminopyrimidinylpyrrole Derivatives[a]



Cpd	R_1	Cdc7	Cpd	R_1	Cdc7	Cpd	R_1	Cdc7
5	H	0.007 ± 0.001	**33**		0.013± 0.005	**37**		>5
29		0.012 ± 0.008	**34**		0.004 ± 0.001	**38**		>5
30		0.042 ± 0.024	**35**		0.003 ± 0.001	**39**		0.290 ± 0.126
31		0.014 ± 0.006	**36**		0.047 ± 0.025	**40**		0.120 ± 0.066
32		0.023 ± 0.011						

[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$).

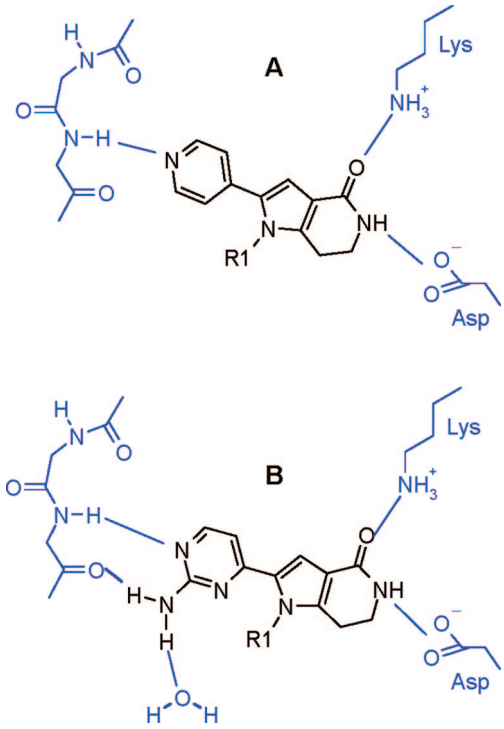


Figure 5. Supposed binding modes of N-alkylated pyridinyl pyrroles and aminopyrimidinyl pyrroles.

was conducted either under medium pressure on silica (Merck silica gel 40–63 μm) or on prepacked silica gel cartridges (Biotage) or on a Horizon system.

^{1}H NMR spectra were routinely recorded on a Varian Inova 400 spectrometer operating at 400 MHz and equipped with a 5 mm indirect detection PFG probe (1H{15N-31P}). Where reported, a Varian Inova 500 spectrometer, operating at 500 MHz and equipped with a 5 mm triple resonance PFG probe (1H{15N-31P}), or a Varian Mercury 300 spectrometer, operating at 300 MHz and equipped with a 5 mm switchable probe (15N-31P{1H,19F}) was used. All observed proton absorptions are reported as parts per million (ppm) downfield from tetramethylsilane or other internal reference in the appropriate solvent indicated. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet, bd = broad doublet, bt = broad triplet, td = triplet of doublet, ddd = doublet of doublet of doublet, m = multiplet), coupling constants, and number of protons.

Low-resolution mass spectral (MS) data were determined on a Finnigan MAT LCQ ion trap instrument, equipped with ESI.

High-resolution mass spectra (HRMS) were obtained on a Waters Q-TOF Ultima instrument, equipped with ESI, and using reserpine (MW 609.28065) for lock mass correction.

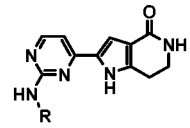
Elemental analyses were performed on a Carlo Erba EA1110 instrument, and C, H, and N values were within ± 0.4% of theoretical values, unless otherwise noted. Thin-layer chromatography was performed on Merck silica gel 60 plates coated with a 250 μM layer with a fluorescent indicator. Components were visualized by UV light (λ = 254 and 366 nm) and iodine vapors.

2-(Pyridin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one hydrochloride (1) and 4-oxo-(2-pyridin-4-yl)-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (50). A suspension of 2,4-dioxopiperidine-1-carboxylic acid *tert*-butyl ester (**56**; 2.6 g, 12.24 mmol) and 2-bromo-1-pyridin-4-ylethanone hydrobromide (**64a**; 2.6 g, 9.42 mmol) in ethanol (120 mL) was treated with ammonium acetate (2.9 g, 37.7 mmol) at room temperature. The resulting solution was stirred for 16 h. The mixture was concentrated under reduced pressure, diluted with ethyl acetate, and washed with 0.5 N NaOH (pH = 9). The aqueous layer was extracted with ethyl acetate (50 mL × 5). The collected organic layers were combined, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude material was flash-chromatographed (eluant: dichloromethane/ethanol 10:1), to provide **50** as a white solid (1.74 g, 59%).

^{1}H NMR (400 MHz DMSO-d_6): δ 1.47 (s, 9 H), 2.95 (t, J = 6.40 Hz, 2 H), 3.97 (t, J = 6.34 Hz, 2 H), 7.11 (d, J = 2.07 Hz, 1 H), 7.63 (d, J = 6.22 Hz, 2 H), 8.51 (d, J = 6.22 Hz, 2 H), 12.14 (bs, 1 H). HRMS $(M+H)^+$ calcd: 314.1499. Found: 314.1505.

The compound was dissolved in anhydrous methanol (30 mL); 4 N HCl in dioxane (7 mL) was added, and the mixture was stirred at room temperature for 2 h. After concentrating to dryness, the material was neutralized with 7 N ammonia in methanol, affording **1** as an off-white solid (1.2 g, 88%).

Table 7. SAR: Substitutions R at the Amino Group of Pyrimidine (IC_{50}, μM)[a]



Cpd	R	Cdc7	Cpd	R	Cdc7	Cpd	R	Cdc7
5	H	0.007 ± 0.001	**43**		0.007 ± 0.002	**46**		0.323 ± 0.037
41		0.259 ± 0.201	**44**		0.220 ± 0.094	**47**		1.397 ± 0.259
42		0.636 ± 0.501	**45**		0.655 ± 0.006	**48**		0.262 ± 0.066

[a] IC_{50} values are reported as the mean ± standard deviation ($n \geq 2$).

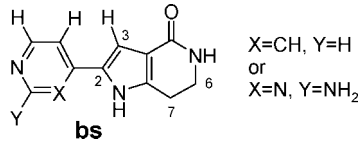


Figure 6. The basic scaffold **bs**.

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.94 (t, J = 6.83 Hz, 2 H), 3.45 (t, J = 6.83 Hz, 2 H), 7.30 (bs, 1 H), 7.59 (s, 1 H), 8.23 (d, J = 7.08 Hz, 2 H), 8.71 (d, J = 7.08 Hz, 2 H), 12.89 (bs, 1 H). HRMS $(M+H)^+$ calcd: 214.0975. Found: 214.0975. Anal. calcd ($C_{12}H_{11}N_3O$): C, H, N.

The following compounds were synthesized starting from the appropriate haloketone and according to the general procedure detailed above:

2-(3-Fluoro-pyridin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (3). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.91 (m, 2 H), 3.41 (m, 2 H), 7.08 (bs, 1 H), 7.19 (bs, 1 H), 7.90 (dd, J = 5.60 Hz, 7.19 Hz, 1 H), 8.48 (d, J = 5.00 Hz, 1 H), 8.70 (d, J = 4.15 Hz, 1 H), 12.15 (bs, 1 H). HRMS $(M+H)^+$ calcd: 232.0881. Found: 232.0876. Anal. calcd ($C_{12}H_{10}FN_3O$): C, H, N.

2-(Pyrimidin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one hydrochloride (4). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.87 (t, J = 6.83 Hz, 2 H), 3.44 (t, J = 6.83 Hz, 2 H), 7.19 (bs, 1 H), 7.37 (s, 1 H), 7.89 (d, J = 5.85 Hz, 1 H), 8.68 (d, J = 5.85 Hz, 1 H), 9.10 (s, 1 H). HRMS $(M+H)^+$ calcd: 215.0927. Found: 215.0932. Anal. calcd ($C_{11}H_{10}N_4O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one (5). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.91 (t, J = 6.71 Hz, 2 H), 3.36 (t, J = 6.71 Hz, 2 H), 7.27 (d, J = 6.70 Hz, 1 H), 7.29 (bs, 1 H), 7.46 (s, 1 H), 7.86 (bs, 2 H), 8.21 (d, J = 6.70 Hz, 2 H). HRMS $(M+H)^+$ calcd: 230.1036. Found: 230.1032. Anal. calcd ($C_{11}H_{11}N_5O.0.25\ H_2O$): C, H, N.

2-(1H-Pyrrolo[2,3-b]pyridin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one (6). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.95 (t, J = 6.71 Hz, 2 H), 3.47 (t, J = 6.71 Hz, 2 H), 7.10 (s, 1 H), 7.22 (bs, 1 H), 7.29 (s, 1 H), 7.61 (bd, 1 H), 7.71 (bt, 1 H), 8.38 (d, J = 6.10 Hz, 2 H), 12.26 (bs, 1 H). HRMS $(M+H)^+$ calcd: 253.1084. Found: 253.1076. Anal. calcd ($C_{14}H_{12}N_4O$): C, H, N.

2-(9H-Purin-6-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one (7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.91 (t, J = 6.83 Hz, 2 H), 3.42 (t, J = 6.83 Hz, 2 H), 7.16 (bs, 1 H), 7.77 (s, 1 H), 8.59 (s, 1 H), 8.80 (s, 1 H), 12.26 (bs, 1 H). HRMS $(M+H)^+$ calcd: 255.0989. Found: 255.0988. Anal. calcd ($C_{12}H_{10}N_6O$): C, H, N.

2-Phenyl-1,5,6,7-tetrahydro-pyrrolo[3,2-c]pyridin-4-one (8). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.83 (t, J = 6.89 Hz, 2 H), 3.38–3.44 (m, 2 H), 6.68 (d, J = 2.44 Hz, 1 H), 6.93 (t, J = 2.74 Hz, 1 H), 7.15–7.23 (m, 1 H), 7.37 (t, J = 7.80 Hz, 2 H), 7.64 (dd, J = 8.35, 1.16 Hz, 2 H), 11.59 (s, 1 H). HRMS $(M+H)^+$ calcd: 213.1022. Found: 213.1020. Anal. calcd ($C_{13}H_{12}N_2O$): C, H, N.

3-Methyl-2-(pyridin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one (15). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.66 (s, 4 H), 2.90 (t, J = 6.83 Hz, 3 H), 3.29–3.47 (m, 2 H), 7.24 (s, 1 H), 8.00 (d, J = 6.95 Hz, 2 H), 8.71 (d, J = 7.19 Hz, 2 H), 12.39 (s, 1 H). HRMS $(M+H)^+$ calcd: 228.1131. Found: 228.1134. Anal. calcd ($C_{13}H_{13}N_3O$): C, H, N.

2-(2-Phenylaminopyrimidin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one (43). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.7 (m, J = 17.1, 6.2, 5.1, 1.5, 0.9 Hz, 1 H), 2.8 (m, J = 17.1, 6.3, 5.1, 1.5, 0.9 Hz, 1 H), 3.5 (m, J = 12.2, 6.2, 5.1, 2.5 Hz, 1 H), 3.5 (m, J = 12.2, 6.3, 5.1, 2.5 Hz, 1 H), 6.9 (m, J = 7.5, 1.2 Hz, 1 H), 7.3 (m, J = 8.1, 7.5, 1.6, 0.4 Hz, 1 H), 7.3 (m, J = 8.1, 7.5, 1.6, 0.4 Hz, 1 H), 7.6 (m, J = 0.9 Hz, 1 H), 7.6 (m, J = 8.1, 2.5, 1.2, 0.4 Hz, 1 H), 7.6 (m, J = 8.1, 2.5, 1.2, 0.4 Hz, 1 H), 7.7 (m, J = 4.8 Hz, 1 H), 8.4 (m, J = 4.8 Hz, 1 H). HRMS $(M+H)^+$ calcd: 306.1349. Found: 306.1346. Anal. calcd ($C_{17}H_{15}N_5O$): C, 66.87; H, 4.95; N, 22.94. Found: C, 65.71; H, 4.64; N, 21.44.

2-(2-Aminopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydro-pyrrolo[3,2-c]pyridine-5-carboxylic Acid *tert*-Butyl Ester (49). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.48 (s, 9 H), 2.94 (m, 2 H), 3.96 (m, 2 H), 6.38 (bs, 2 H), 6.95 (d, J = 5.12 Hz, 1 H), 7.14 (s, 1 H), 8.19 (d, J = 5.12 Hz, 1 H), 12.00 (bs,1 H). HRMS $(M+H)^+$ calcd: 330.1561. Found: 330.1566. Anal. calcd ($C_{16}H_{19}N_5O_3$): C, H, N.

2-(2-Amino-5-bromo-pyrimidin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (12). To a solution of 2-(2-aminopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (**49**; 0.2 g, 0.6 mmol) in N,N-dimethylformamide (2 mL) at room temperature was added N-bromosuccinimide (0.11 g, 0.6 mmol) in N,N-dimethylformamide (1 mL) dropwise with stirring. After 3 h, water was added, and the aqueous solution was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated to a crude product that, after flash chromatography (eluant: 96:4 dichloromethane/ethanol), yielded 2-(2-amino-5-bromopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (0.09 g, 37%). To a solution of this compound in methanol (4 mL) was added 4 N HCl in dioxane (0.55 mL), and the mixture was stirred at room temperature for 1 h. After concentrating to dryness, the material was neutralized with 7 N ammonia in methanol, and the title compound was isolated as a pale yellow solid (0.05 g, 65%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.86 (m, 2 H), 3.3–3.5 (m, 2 H), 6.4–6.8 (bs, 2 H), 7.16 (bs, 1 H), 7.51 (s, 1 H), 8.35 (s, 1 H), 11.75 (s, 1 H). HRMS $(M+H)^+$ calcd: 308.0141. Found: 308.0135. Anal. calcd ($C_{11}H_{10}BrN_5O$): C, H, N.

3-(2-Aminopyrimidin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]-pyridin-4-one (14). A solution of piperidine-2,4-dione (**57**; 2.86 g, 25.3 mmol) and ammonium acetate (5.2 g, 67.5 mmol) in absolute ethanol (75 mL) was stirred at room temperature for 45 min; then, 1-(2-amino-pyrimidin-4-yl)-2-bromo-ethanone hydrobromide (**64b**; 5 g, 16.8 mmol) was added, and the reaction mixture stirred at room temperature for 18 h. After solvent removal, the residue was adsorbed onto a Horizon column and eluted with dichloromethane/methanol/30% aqueous ammonia in a ratio of 93:7:0.7 (then 90:10:1). In this way, the title compound was obtained (1.27 g, 33% yield). The main impurity is the 2-isomer **5**, which was isolated in 13% yield (0.49 g) from the less polar fractions.

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.97 (t, J = 7.70 Hz, 2 H), 3.64 (t, J = 7.70 Hz, 2 H), 7.05 (d, J = 5.24 Hz, 1 H), 7.23 (s, 2 H), 8.31 (d, J = 5.24 Hz, 1 H), 8.42 (s, 1 H), 9.71 (s, 1 H), 11.67 (s, 1 H). HRMS $(M+H)^+$ calcd: 230.1036. Found: 230.1037. Anal. calcd ($C_{11}H_{11}N_5O$): C, H, N.

2-Pyridin-4-yl-6,7-dihydro-5H-furo[3,2-c]pyridin-4-one (18). To a stirred, cooled (0 °C) suspension of 2,4-dioxopiperidine-1-carboxylic acid *tert*-butyl ester (**56**; 75 mg, 0.35 mmol) and 4-ethynylpyridine hydrochloride (490 mg, 3.5 mmol) in acetonitrile (15 mL) was added ceric ammonium nitrate (390 mg, 0.7 mmol) in three portions. After being stirred for 6 h, the solvent was evaporated, 5% K_2CO_3 (aq) was added, and the mixture was extracted with ethyl acetate and the extract dried over anhydrous sodium sulfate and concentrated. The crude residue was purified by flash chromatography (eluant: ethyl acetate). The N-Boc protected product (10 mg, 0.03 mmol) was dissolved in methanol (1 mL) containing 4 N HCl in dioxane (0.5 mL) and stirred for 24 h. The solvent was evaporated; 5% K_2CO_3 (aq) was added, and the mixture was extracted with ethyl acetate, dried over anhydrous sodium sulfate, and concentrated. The crude residue was purified by flash chromatography (eluant: 6:1 ethyl acetate/methanol) to yield the title compound (7 mg, 11% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.39 (t, J = 7.44 Hz, 2 H), 3.55 (dt, J = 7.44, 2.07 Hz, 2 H), 7.40 (s, 1 H), 4.47 (d, J = 4.63, 2 H), 8.50 (d, J = 4.76, 2 H), 8.57 (bs, 1H). HRMS $(M+H)^+$ calcd: 215.0815. Found: 215.0812.

2-(Pyridin-4-yl)-5,6-dihydro-1H-pyrrolo[3,4-b]pyrrol-4-one (19). A solution of N-Boc glycine (2 g, 11.4 mmol) and 1,1′-carbonyldiimidazole (2.8 g, 17 mmol) in 18 mL of anhydrous tetrahydrofuran was shaken at room temperature for 3 h. Magnesium chloride (2.18 g, 22.8 mmol) and potassium ethylmalonate (3.9 g, 22.8 mmol) in anhydrous tetrahydrofuran (20 mL) were added. The temperature was brought to 50 °C and the suspension shaken for 18 h, then filtered. The solvent was removed, and the raw product was dissolved in ethyl acetate and washed with 5% sodium bisulfate (aq, × 3), sodium bicarbonate (aq), and then brine. The organic solution was dried over anhydrous sodium sulfate, and the solvent was removed. The crude product was purified by chromatography (eluant: 15:85 ethyl acetate/*n*-hexane, then 80:20), yielding 4-*tert*-butoxycarbonylamino-3-oxobutyric acid ethyl ester (**52**; 2 g, 71%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15 (t, J = 7.03 Hz, 3 H), 1.37 (s, 9 H), 3.55 (s, 2 H), 3.82 (d, J = 5.86 Hz, 2 H), 4.06 (q, J = 7.33 Hz, 2 H), 7.07 (t, 1 H).

A mixture of **52** (1 g, 4.1 mmol) and 60% sodium hydride (0.41 g, 10.2 mmol) in anhydrous tetrahydrofuran (20 mL) was stirred for 1 h at room temperature and then cooled to 0 °C. A suspension of 2-bromo-1-(pyridin-4-yl)ethanone hydrobromide (**64a**; 1.4 g, 5 mmol) in anhydrous tetrahydrofuran (10 mL) was added dropwise and the mixture stirred at 0 °C for 4 h. After solvent removal, the residue was dissolved in ethanol/acetic acid (1:1, 30 mL), and ammonium acetate (1.27 g, 16.4 mmol) was added. The solution was stirred at room temperature for 5 h. After concentration, the crude product was dissolved in ethyl acetate, washed with brine (× 3), and dried over anhydrous sodium sulfate. The solvent was removed, and the impure product was purified by chromatography (eluant: 98:2 dichloromethane/methanol), thus providing 2-(*tert*-butoxycarbonylaminomethyl)-5-(pyridin-4-yl)-1H-pyrrole-3-carboxylic acid ethyl ester (0.5 g, 35% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.30 (t, J = 7.07 Hz, 3 H), 1.41 (s, 9 H), 4.22 (d, J = 7.07 Hz, 2 H), 4.50 (d, J = 5.49 Hz, 2 H), 6.91 (s, 1 H), 7.15 (d, J = 2.68 Hz, 1 H), 7.70 (dd, J = 4.63, 1.59 Hz, 2 H), 8.52 (dd, J = 4.63, 1.59 Hz, 2 H), 11.94 (s, 1 H).

To a solution of 2-(*tert*-butoxycarbonylaminomethyl)-5-(pyridin-4-yl)-1H-pyrrole-3-carboxylic acid ethyl ester (0.2 g, 0.6 mmol) in ethanol/water 3:1 (30 mL) was added sodium hydroxide (0.6 g). The solution was heated to reflux overnight, partially concentrated, and brought to pH 6–7 with 2 N HCl. The precipitate was filtered and dried, yielding 2-(*tert*-butoxycarbonylamino-methyl)-5-pyridin-4-yl-1H-pyrrole-3-carboxylic acid (0.19 g, quantitative yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.36 (s, 9 H), 4.47 (s, 2 H), 7.04 (s, 1 H), 7.19 (s, 1 H), 7.67 (d, J = 5.86 Hz, 2 H), 8.45 (d, J = 6.15 Hz, 2 H), 12.06 (bs, 1 H).

To a solution of 2-(*tert*-butoxycarbonylamino-methyl)-5-(pyridin-4-yl)-1H-pyrrole-3-carboxylic (22 mg, 0.07 mmol) in dioxane (2 mL) was added 4 N HCl in dioxane (1 mL), and the solution was shaken for 18 h at room temperature. The precipitate was filtered and dried, thus affording 2-aminomethyl-5-(pyridin-4-yl)-1H-pyrrole-3-carboxylic acid hydrochloride (**53**; 17 mg, 98% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.41 (s, 2 H), 7.58 (s, 1 H), 8.24 (d, J = 5.86 Hz, 2 H), 8.46 (bs, 2 H), 8.73 (d, J = 6.74 Hz, 2 H), 13.53 (bs, 1 H).

To a solution of **53** (17 mg, 0.066 mmol) in N,N-dimethylformamide/dichloromethane (1:1, 5 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (13 mg, 0.066 mmol), hydroxybenzotriazole (9 mg, 0.066 mmol), and diisopropylethylamine (34 mg, 0.26 mmol). The solution was left shaking overnight at room temperature and was concentrated. The crude product was purified by chromatography (eluant: 98:2 dichloromethane/methanol, then 90:10), thus affording the title compound (13 mg, 45% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.31 (s, 2 H), 6.95 (d, J = 1.95 Hz, 1 H), 7.64 (d, J = 6.22 Hz, 3 H), 8.52 (d, J = 6.22 Hz, 2 H), 12.19 (s, 1 H). HRMS $(M+H)^+$ calcd: 200.0818. Found: 200.0820. Anal. calcd ($C_{11}H_9N_3O$): C, H, N.

2-(Pyridin-4-yl)-5,6,7,8-tetrahydro-1H-pyrrolo[3,2-c]azepin-4-one (20). A mixture of 2-(2-chloropyridin-4-yl)-5,6,7,8-tetrahydro-1H-pyrrolo[3,2-c]azepin-4-one (**51**; 10 mg, 0.04 mmol) and 10% Pd on carbon (20 mg) in methanol (5 mL) was hydrogenated at room temperature and 40 psi of hydrogen for 6 h. After filtration through celite and solvent evaporation, the title compound was obtained (6 mg, 65% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.81−1.63 (m, 2 H), 2.92−2.79 (m, 2 H), 3.14−2.96 (m, 2 H), 6.91 (s, 1 H), 7.60 (d, J = 5.51 Hz, 2 H), 8.54 (d, J = 5.51 Hz, 2 H), 8.64 (t, J = 5.70 Hz, 1 H), 11.89 (s, 1 H). HRMS $(M+H)^+$ calcd: $C_{13}H_{13}N_3O$ + H1, 228.1131. Found: 228.1129. Anal. calcd ($C_{13}H_{13}N_3O$): C, H, N.

4-Oxo-2-(pyridin-4-yl)-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carbaldehyde (21). To a fresh solution of $POCl_3$ (0.06 mL, 0.6 mmol) in N,N-dimethylformamide (3 mL) was added 2-(pyridin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one hydrochloride (**1**; 50 mg, 0.2 mmol), and the mixture was stirred at room temperature for 18 h. After the addition of water, the precipitate was filtered, washed with water, saturated with $NaHCO_3$ (aq), and dried. The title compound was obtained as a white solid (40 mg, 83%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 3.07 (t, J = 6.52 Hz, 2 H), 4.01 (t, J = 6.58 Hz, 2 H), 7.56 (s, 1 H), 7.98 (d, J = 4.76 Hz, 2 H), 8.69 (d, J = 4.63 Hz, 2 H), 9.37 (s, 1 H), 12.66 (s, 1 H). HRMS $(M+H)^+$ calcd: 242.0924. Found: 242.0931. Anal. calcd ($C_{13}H_{11}N_3O_2$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-cyclopropylmethyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (34). A mixture of 1-(2-aminopyrimidin-4-yl)-2-bromoethanone hydrobromide (**64b**; 0.9 g, 3 mmol), cyclopropylmethylamine (1.03 mL, 12 mmol), and piperidin-2,4-dione (0.51 g, 4.5 mmol), dissolved in absolute ethanol (10 mL), was stirred under heating at 70 °C for 3 h in a glass pressure tube. Ethanol was evaporated off, and the crude reaction mixture was purified by flash chromatography (eluant: 4:1 dichloro-

methane/methanol) and then by crystallization from methanol (0.42 g, 49% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.30–0.48 (m, 4 H), 1.12–1.26 (m, 1 H), 2.97 (t, J = 6.77 Hz, 2 H), 3.45 (t, J = 6.46 Hz, 2 H), 4.61 (d, J = 7.07 Hz, 2 H), 7.34–7.44 (d, J = 6.95 Hz, 1 H and one s, 1 H), 7.71 (s, 1 H), 8.15 (d, J = 6.95 Hz, 1 H), 8.31 (s, 1 H). HRMS $(M+H)^+$ calcd: 284.1506. Found: 284.1500. Anal. calcd ($C_{15}H_{17}N_5O$): C, 56.34; H, 5.67; N, 21.906. Found: C, 50.06; H, 5.68; N, 19.64.

The following compounds were synthesized according to the general procedure detailed above:

1-Methyl-2-(pyridin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (16). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.87 (t, J = 6.89 Hz, 1 H), 3.45 (td, J = 6.95, 2.56 Hz, 2 H), 3.65 (s, 3 H), 6.66 (s, 1 H), 7.06 (m, 1 H), 7.50 (d, J = 6.10 Hz, 2 H), 8.59 (d, J = 6.22 Hz, 2 H). HRMS $(M+H)^+$ calcd: 228.1131. Found: 228.1126. Anal. calcd ($C_{13}H_{13}N_3O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-cyclobutyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (31). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.68–1.89 (m, 2 H), 2.38–2.59 (m, 3 H), 3.11–3.21 (m, 2 H), 3.24–3.63 (m, 2 H), 5.66–5.82 (m, 1 H), 7.30 (d, J = 6.71 Hz, 1 H), 7.42 (s, 1 H), 7.46 (s, 1 H), 8.16 (d, J = 6.83 Hz, 1 H), 8.26 (bs, 2 H). HRMS $(M+H)^+$ calcd: 284.1506. Found: 284.1512. Anal. calcd ($C_{15}H_{17}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-cyclohexyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (37). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15–2.04 (m, 10 H), 2.98–3.07 (m, 2 H), 3.52–3.61 (m, 2 H), 4.04–4.17 (m, 1 H), 7.40–7.49 (m, 2 H), 8.36–8.51 (m, 5 H), 8.58–8.65 (m, 1 H). HRMS $(M+H)^+$ calcd: 312.1819. Found: 312.1816. Anal. calcd ($C_{17}H_{21}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-phenyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (38). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.90–2.99 (m, 2 H), 3.48–3.58 (m, 2 H), 7.54–7.68 (m, 7 H), 8.36–8.75 (m, 4 H). HRMS $(M+H)^+$ calcd: 306.1349. Found: 306.1342. Anal. ($C_{17}H_{15}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-(1-methyl-piperidin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (40). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.07–2.18 (m, 4 H), 2.79 (d, J = 4.63 Hz, 3 H), 3.12–3.20 (m, 2 H), 3.21–3.54 (m, 6 H), 5.66–5.86 (m, 1 H), 7.27 (s, 1 H), 7.41 (s, 1 H), 7.46 (s, 1 H), 8.07 (bs, 2 H), 8.16 (d, J = 6.46 Hz, 1 H), 10.45–10.71 (m, 1H). HRMS $(M+H)^+$ calcd: 327.1928. Found: 327.1937. Anal. calcd ($C_{17}H_{22}N_6O.0.5\ H_2O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-(2-fluoroethyl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (35). A mixture of 2-(2-aminopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (**49**; 100 mg, 0.3 mmol), 1-bromo-2-fluoroethane (0.09 mL, 1.2 mmol), and potassium carbonate (166 mg, 1.2 mmol) in anhydrous N,N-dimethylformamide (2 mL) was stirred under heating at 65 °C for 4 h. After cooling, the reaction mixture was treated with water and ethyl acetate; the organic layer was extracted with brine and then dried over anhydrous sodium sulfate. The crude product was purified by flash chromatography (eluant: 95:5 dichloromethane/ethanol). Pure 2-(2-aminopyrimidin-4-yl)-1-(2-fluoroethyl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester, obtained as a solid (90 mg, 80% yield), was deprotected in dry methanol (5 mL) and 4 N HCl in dioxane (0.6 mL), by stirring the solution at room temperature for 3 h. After concentration to dryness, the material was neutralized with 7 N ammonia in methanol, and the title compound was obtained as a white solid (71 mg, 95% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.91–2.98 (m, 2 H), 3.40–3.50 (m, 2 H), 4.67–4.75 (m, 1 H), 4.80–4.85 (m, 1 H), 4.85–4.90 (m, 1 H), 4.90–4.97 (m, 1 H), 7.40 (d, J = 6.83 Hz, 1 H and 1 singlet, 1H), 7.76 (s, 1 H), 8.14 (d, J = 6.82 Hz, 1 H), 8.17–8.51 (m, 2 H). HRMS $(M+H)^+$ calcd: 276.1255. Found: 276.1244. Anal. calcd ($C_{13}H_{14}FN_5O$): C, H, N.

The following compound was synthesized according to the general procedure detailed above:

2-(2-Aminopyrimidin-4-yl)-1-isopropyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (30). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.50 (d, J = 6.95 Hz, 6 H), 3.05–3.14 (m, 2 H), 3.39–3.48 (m, 2 H), 5.85–5.99 (m, 1 H), 7.27 (d, J = 6.71 Hz, 1 H), 7.36 (s, 1 H), 7.44 (s, 1 H), 7.99 (s, 2 H), 8.14 (d, J = 6.70 Hz, 1 H). HRMS $(M+H)^+$ calcd: 272.1506. Found: 272.1510. Anal. calcd ($C_{14}H_{17}N_5O.0.7\ H_2O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-benzyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (39). To a solution of 2-(2-aminopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (**49**, 300 mg, 0.9 mmol) in anhydrous N,N-dimethylformamide (5 mL) was added 60% NaH (58 mg), and the mixture was stirred for 2 h at room temperature. Benzyl bromide (0.25 mL) was added, and the reaction mixture was stirred overnight. The mixture was poured in water and extracted with ethyl acetate. The organic layers were dried over anhydrous sodium sulfate and concentrated. The residue was purified by flash chromatography (eluant: 10:1 dichloromethane/methanol) to yield 2-(2-aminopyrimidin-4-yl)-1-benzyl-4-oxo-1,4,6,7-tetrahydro-pyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (240 mg, 0.58 mmol) that was subjected to deprotection by dissolution in dry dioxane (200 mL) and methanol (4 mL) followed by treatment with 4 N HCl in dioxane (2 mL). After being stirred for 2 h at room temperature followed by being concentrated to dryness, the material was neutralized with 7 N ammonia in methanol, and the title compound was obtained as a white solid (154 mg, 75% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 2.87–2.94 (m, 2 H), 3.39–3.51 (m, 2 H), 6.01 (s, 2 H), 7.01–7.07 (m, 2 H), 7.19–7.25 (m, 1 H), 7.26–7.34 (m, 3 H), 7.39 (s, 1 H), 7.71 (s, 1 H), 8.00 (s, 2 H), 8.09 (d, J = 6.83 Hz, 1 H). HRMS $(M+H)^+$ calcd: 320.1506. Found: 320.1497. Anal. calcd ($C_{17}H_{22}N_6O$): C, H, N.

The following compounds were synthesized according to the general procedure detailed above:

1-Ethyl-2-(pyridin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]-pyridin-4-one (22). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.28 (t, J = 7.19 Hz, 3 H), 2.97 (t, J = 6.83 Hz, 2 H), 3.11–3.30 (m, 2 H), 4.21 (q, J = 7.19 Hz, 2 H), 7.18 (s, 1 H), 7.29 (s, 1 H), 8.00 (s, 1 H), 8.01 (s, 1 H), 8.74 (s, 1 H), 8.76 (s, 1 H). HRMS $(M+H)^+$ calcd: 242.1288. Found: 242.1291. Anal. calcd ($C_{14}H_{15}N_3O$): C, H, N.

1-(2-Hydroxyethyl)-2-(pyridin-4-yl)-1,5,6,7-tetrahydropyrrolo-[3,2-c]pyridin-4-one (23). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.97 (t, J = 6.89 Hz, 2 H), 3.28–3.53 (m, 2 H), 3.65 (t, J = 5.43 Hz, 2 H), 4.25 (t, J = 5.49 Hz, 2 H), 7.13–7.18 (m, 1 H), 7.26–7.31 (m, 1 H), 8.12–8.16 (m, J = 6.22 Hz, 2 H), 8.74 (s, 1 H), 8.76 (s, 1 H). HRMS $(M+H)^+$ calcd: 258.1237. Found: 258.1231. Anal. calcd ($C_{14}H_{15}N_3O_2$ 0.6 H_2O): C, H, N.

2-(Pyridin-4-yl)-1-(2,2,2-trifluoroethyl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (24). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.98 (t, J = 6.71 Hz, 2 H), 3.41–3.55 (m, 2 H), 5.22 (q, J = 8.86 Hz, 2 H), 7.04 (s, 1 H), 7.39 (s, 1 H), 7.97 (s, 2 H), 8.77 (s, 1 H), 8.79 (s, 1 H). HRMS $(M+H)^+$ calcd: 296.1005. Found: 296.0998. Anal. calcd ($C_{14}H_{12}F_3N_3O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-methyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (25). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.83–2.89 (m, 2 H), 3.40–3.47 (m, 2 H), 3.96 (s, 3 H), 6.54 (s, 2 H), 6.89 (d, J = 5.37 Hz, 1 H), 7.01 (s, 1 H), 7.09 (s, 1 H), 8.14 (d, J = 5.37 Hz, 1 H). HRMS $(M+H)^+$ calcd: 244.1193. Found: 244.1194. Anal. calcd ($C_{12}H_{13}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-ethyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (26). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.29 (t, J = 7.08 Hz, 3 H), 2.99 (t, J = 6.78 Hz, 2 H), 3.48 (t, J = 6.77 Hz, 2 H), 4.62 (q, J = 7.00 Hz, 2 H), 7.38 (s, 1 H), 7.40 (d, J = 7.00 Hz, 1 H), 7.72 (s, 1 H), 8.16 (d, J = 7.00 Hz, 1 H). HRMS $(M+H)^+$ calcd: 258.1349. Found: 258.1349. Anal. calcd ($C_{13}H_{15}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-(2-hydroxy-ethyl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (27). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.97 (t, J = 6.77 Hz, 2 H), 3.36–3.50 (m, 2 H), 3.66 (t, J = 5.37 Hz, 2 H), 4.58 (t, J = 5.30 Hz, 2 H), 7.33 (d, J = 10.12 Hz, 1 H), 7.35 (d, J = 7.19 Hz, 1 H), 7.69 (s, 1 H), 8.09 (d, J = 6.95 Hz, 1 H). HRMS $(M+H)^+$ calcd: 274.1298. Found: 274.1291. Anal. calcd ($C_{13}H_{15}N_5O_2$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-(2,2,2-trifluoroethyl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (28). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.01 (t, J = 6.70 Hz, 2 H), 3.43–3.51 (m, 2 H), 5.89 (d, J = 7.76 Hz, 2 H), 7.40 (d, J = 6.70 Hz, 1 H), 7.50 (s, 1 H), 7.74 (s, 1 H), 8.23 (d, J = 6.55 Hz, 1 H). HRMS $(M+H)^+$ calcd: 312.1067. Found: 312.1072. Anal. calcd ($C_{13}H_{12}F_3N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-propyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (29). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (t, J = 7.32 Hz, 3 H), 1.57–1.70 (m, 2 H), 2.91–3.00 (m, 2 H), 3.42–3.52 (m, 2 H), 4.56 (t, J = 7.19 Hz, 2 H), 7.31–7.39 (m, 2 H), 7.65 (s, 1 H), 8.12 (d, J = 6.82 Hz, 1 H). HRMS $(M+H)^+$ calcd: 272.1506. Found: 272.1509. Anal. calcd ($C_{14}H_{17}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-butyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (32). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (t, J = 7.44 Hz, 3 H), 1.22–1.34 (m, 2 H), 1.52–1.63 (m, 2 H), 2.91–2.99 (m, 2 H), 3.42–3.51 (m, 2 H), 4.60 (t, J = 7.19 Hz, 2 H), 7.32 (d, J = 6.95 Hz, 1 H), 7.34 (s, 1 H), 7.61 (s, 1 H), 7.97 (s, 2 H), 8.12 (d, J = 6.70 Hz, 1 H). HRMS $(M+H)^+$ calcd: 286.1662. Found: 286.1669. Anal. calcd ($C_{15}H_{19}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-isobutyl-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (33). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.82 (d, J = 6.71 Hz, 6 H), 1.80–1.94 (m, 1 H), 2.88–2.98 (m, 2 H), 3.42–3.54 (m, 2 H), 4.43 (d, J = 7.20 Hz, 2 H), 7.32 (d, J = 6.83 Hz, 1 H), 7.36 (s, 1 H), 7.62 (s, 1 H), 7.97 (s, 2 H), 8.12 (d, J = 6.71 Hz, 1 H). HRMS $(M+H)^+$ calcd: 286.1662. Found: 286.1663. Anal. calcd ($C_{15}H_{19}N_5O$): C, H, N.

2-(2-Aminopyrimidin-4-yl)-1-(4,4,4-trifluorobutyl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (36). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.80–1.90 (m, 2 H), 2.35–2.49 (m, 2 H), 2.96 (t, J = 6.77 Hz, 2 H), 3.47 (ddd, J = 6.80, 2.26 Hz, 2 H), 4.64 (t, J = 7.32 Hz, 2 H), 7.35 (d, J = 6.71 Hz 1 H), 7.36 (s, 1 H), 7.68 (s, 1 H), 7.94–8.04 (m, 2 H), 8.13 (d, J = 6.71 Hz, 1 H). HRMS $(M+H)^+$ calcd: 340.1380. Found: 340.1373. Anal. calcd ($C_{15}H_{16}F_3N_5O$): C, H, N.

2-[2-(Cyclohexylmethylamino)pyrimidin-4-yl]-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (45). 2-(2-Aminopyrimidin-4-yl)-1,5,6,7-tetrahydro-pyrrolo[3,2-c]pyridin-4-one (**5**; 140 mg, 0.61 mmol), trifluoroacetic acid (0.56 mL, 7.33 mmol), and cyclohexanecarbaldehyde (0.15 mL, 1.25 mmol) were mixed in N,N-dimethylformamide (10 mL). Sodium triacetoxyborohydride (390 mg, 1.84 mmol) was added and the reaction mixture stirred at room temperature for 20 h. Additional cyclohexanecarbaldehyde (0.15 mL, 1.25 mmol) and sodium triacetoxyborohydride (390 mg, 1.84 mmol) were added, and the reaction mixture was stirred 20 h longer. The reaction was quenched with 0.33 N NaOH (50 mL), and the product was extracted with dichloromethane (50 mL). The dichloromethane extract was dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue was purified by flash chromatography (dichloromethane/methanol, 95:5) to give the product as a beige-colored solid (80 mg, 40% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87–1.29 (m, 5 H), 1.43–1.85 (m, 6 H), 2.87 (t, J = 6.77 Hz, 2 H), 3.18–3.31 (m, 2 H), 3.38–3.49 (m, 2 H), 6.67–6.95 (m, 1 H), 6.85 (d, J = 5.24 Hz, 1 H), 7.03 (d, J = 2.19 Hz, 1 H), 7.06 (t, J = 2.07 Hz, 1 H), 8.15 (d, J = 5.12 Hz, 1 H), 11.65 (s, 1 H). HRMS $(M+H)^+$ calcd: 326.1975. Found: 326.1979. Anal. calcd ($C_{18}H_{23}N_5O$): C, 66.44; H, 7.12; N, 21.52. Found: C, 65.49; H, 7.22; N, 20.64.

The following compounds were synthesized according to the general procedure detailed above:

2-(2-Propylaminopyrimidin-4-yl)-1,5,6,7-tetrahydropyrrolo-[3,2-c]pyridin-4-one (41). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.93 (t, J = 7.38 Hz, 3 H), 1.51–1.64 (m, 2 H), 2.87 (t, J = 6.89 Hz, 2 H), 3.34–3.48 (m, 4 H), 6.81 (s, 1 H), 6.86 (d, J = 5.24 Hz, 1 H), 7.03 (d, J = 2.32 Hz, 1 H), 7.05 (t, J = 2.07 Hz, 1 H), 8.16 (d, J = 5.24 Hz, 1 H), 11.66 (s, 1 H). HRMS $(M+H)^+$ calcd: 272.1506. Found: 272.1509. Anal. calcd ($C_{14}H_{17}N_5O$): C, H, N.

2-(2-Isobutylaminopyrimidin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (42). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.93 (d, J = 6.71 Hz, 6 H), 1.81–1.95 (m, 1 H), 2.87 (t, J = 6.83 Hz, 2 H), 3.24 (s, 2 H), 3.41 (td, J = 6.98, 2.38 Hz, 2 H), 6.71–6.94 (m, 1 H), 6.87 (d, J = 5.24 Hz, 1 H), 7.04 (d, J = 2.32 Hz, 1 H), 7.06 (t, J = 2.38 Hz, 1 H), 8.16 (d, J = 5.12 Hz, 1 H), 11.66 (s, 1 H). HRMS $(M+H)^+$ calcd: 286.1662. Found: 286.1664. Anal. calcd ($C_{15}H_{19}N_5O$): C, H, N.

2-(2-Benzylaminopyrimidin-4-yl)-1,5,6,7-tetrahydropyrrolo[3,2-c]pyridin-4-one (44). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.87 (t, J = 6.85 Hz, 2 H), 3.42 (td, J = 6.85, 2.44 Hz, 2 H), 4.68 (s, 2 H), 6.92 (d, J = 5.18 Hz, 1 H), 7.07 (d, J = 2.28 Hz, 1 H), 7.08 (t, J = 2.51 Hz, 1 H), 7.21–7.26 (m, 1 H), 7.27–7.48 (m, 1 H), 7.32 (t, J = 7.54 Hz, 2 H), 7.39 (d, J = 7.76 Hz, 2 H), 8.19 (d, J = 5.18 Hz, 1 H), 11.73 (s, 1 H). HRMS $(M+H)^+$ calcd: 320.1506. Found: 320.1511. Anal. calcd ($C_{18}H_{17}N_5O$): C, H, N.

N-[4-(4-oxo-4,5,6,7-tetrahydro-1H-pyrrolo[3,2-c]pyridin-2-yl)pyrimidin-2-yl]benzamide (48). To a mixture of 2-(2-aminopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester (**49**; 0.13 g, 0.39 mmol) and triethylamine (0.22 mL, 1.58 mmol) in dry tetrahydrofuran (5 mL) was added benzoyl chloride (0.09 mL, 0.79 mmol), and the mixture was stirred at room temperature overnight. To this, 1 N NaOH (4 mL) was added; after 0.5 h, the solvent was evaporated, the residue was dissolved in water, and the aqueous phase was extracted with ethyl acetate (20 mL × 2). The organic phase was washed with saturated NH_4Cl (aq) then dried over anhydrous sodium sulfate. After solvent evaporation, the crude product was purified by flash chromatography (97:3 dichloromethane/methanol) to give 2-(2-benzoylaminopyrimidin-4-yl)-4-oxo-1,4,6,7-tetrahydropyrrolo[3,2-c]pyridine-5-carboxylic acid *tert*-butyl ester, as a solid (0.14 g, 83%).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.49 (s, 9 H), 2.98 (t, J = 6.34 z, 2 H), 3.98 (t, J = 6.34 Hz, 2 H), 7.35 (s, 1 H), 7.51–7.66 (m, 4 H), 8.00 (m, 2 H), 10.82 (s, 1 H), 12.11 (bs, 1 H).

To a solution of this product (0.05 g, 0.115 mmol) in tetrahydrofuran (1 mL) was added 4 N HCl in dioxane (0.2 mL), and the reaction was left under stirring at room temperature for 2 h. After concentration to dryness, the material was neutralized with 7 N ammonia in methanol, and the title compound was obtained as a solid (0.036 g, 94%).

^{1}H NMR (DMSO-d_6): δ 2.91 (t, J = 6.77 Hz, 2 H), 3.44 (2 H), 7.22 (bs, 1 H), 7.36 (1 H), 7.57 (m, 2 H), 7.64 (m, 2 H), 8.03 (m, 2 H), 8.59 (d, J = 5.73 Hz, 1 H), 11.26 (bs, 1 H), 12.13 (bs, 1 H). HRMS $(M+H)^+$ calcd: 334.1298. Found: 334.1308. Anal. calcd ($C_{18}H_{15}N_5O_2$): C, H, N.

The following compounds were synthesized according to the general procedure detailed above:

N-[4-(4-oxo-4,5,6,7-tetrahydro-1H-pyrrolo[3,2-c]pyridin-2-yl)pyrimidin-2-yl]acetamide (46). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 3 H), 2.92 (t, J = 6.71, 2 H), 3.51 (m, 2 H), 7.26 (bs, 1 H), 7.37 (1 H, s), 7.60 (d, J = 6.10 Hz, 1 H), 8.50 (d, J = 6.10 Hz, 1 H), 11.11 (bs, 1 H), 12.22 (bs, 1 H). HRMS $(M+H)^+$ calcd: 272.1142. Found: 272.1150. Anal. calcd ($C_{13}H_{13}N_5O_2$): C, H, N.

N-[4-(4-Oxo-4,5,6,7-tetrahydro-1H-pyrrolo[3,2-c]pyridin-2-yl)-pyrimidin-2-yl]isobutyramide (47). ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 (s, 3 H), 1.16 (s, 3 H), 2.92 (m, 2 H), 3.44 (m, 2 H), 7.25 (bs, 1 H), 7.36 (s, 1 H), 7.58 (d, J = 5.85 Hz, 1 H), 8.50 (d, J = 5.85 Hz, 1 H), 11.03 (bs, 1 H), 12.16 (bs, 1 H). HRMS $(M+H)^+$ calcd: 300.1455. Found: 300.1469. Anal. calcd ($C_{15}H_{17}N_5O_2$): C, H, N.

2,4-Dioxopiperidine-1-carboxylic acid tert-butyl ester (56) and 2,4-dioxopiperidine (57). To a solution of N-Boc-β-alanine (25 g, 132 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (20.9 g, 145 mmol), and 4-dimethylaminopyridine (24.2 g, 198 mmol) in anhydrous dichloromethane (700 mL) at 0 °C was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (30.4 g, 158 mmol), and the resulting solution was stirred overnight at room temperature. The reaction mixture was washed (500 mL × 4) with 5% potassium bisulfate (aq). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated, thereby affording crude [3-(2,2-dimethyl-4,6-dioxo-[1,3]-dioxan-5-yl)-3-

oxo-propyl]-carbamic acid *tert*-butyl ester (**55**) that was dissolved in 600 mL of ethyl acetate and refluxed for 4 h. The volume was reduced to 150 mL, and the resulting solution was allowed to crystallize at 4 °C overnight. The solid was filtered and washed with cold ethyl acetate, affording **56** (18.4 g, 65% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ 1.44 (s, 9 H), 2.44 (m, 2 H), 3.71 (m, 2 H), 4.95 (s, 1 H), 11.2 (bs, 1 H). ESI (+) MS: *m*/*z* 214 (MH+). Optionally, the *tert*-butoxycarbonyl group can be removed by acidic treatment (4 N HCl in dioxane or tetrahydrofuran) at room temperature to yield **57**.

2-Bromo-1-(3-fluoropyridin-4-yl)ethanone hydrobromide (64d). To a stirred solution of commercially available 3-fluoro-4-acetylpyridine (**63d**; 5.3 g, 38.1 mmol) in glacial acetic acid (14 mL) and 48% hydrobromic acid (5.3 mL) was added bromine (2 mL, 38 mmol) in glacial acetic acid (5.3 mL) dropwise. After the addition was completed, the solution was stirred at 60 °C for 2.5 h and cooled to room temperature, and ethyl acetate (70 mL) was added. After 0.5 h of stirring, the mixture was filtered, and the solid was washed thoroughly with ethyl acetate and dried. After evaporation of the solvent, the title compound was obtained in 82% yield (9.3 g).

^{1}H NMR (400 MHz, DMSO-d_6): δ 4.88 (s, 2 H), 7.75–7.79 (m, 1 H), 8.62 (dd, J = 4.94, 1.52 Hz, 1 H), 8.79 (d, J = 2.32 Hz, 1 H). ESI (+) MS: *m*/*z* 219 (MH+).

The following compound was synthesized according to the general procedure detailed above:

2-Bromo-1-(pyrimidin-4-yl)ethanone hydrobromide (64e). ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.0 (s, 2 H), 7.98 (d, J = 5 Hz, 1 H), 9.12 (d, J = 5 Hz, 1 H), 9.42 (s, 1 H). ESI (+) MS: *m*/*z* 202 (MH+).

4-(2-Bromo-acetyl)pyrrolo[2,3-b]pyridine-1-carboxylic acid tert-butyl ester (64f). To a suspension of commercially available 4-chloro-1H-pyrrolo[2,3-b]pyridine (**58**; 0.5 g, 3.2 mmol) and dimethylaminopyridine (0.04 g, 0.32 mmol) in acetonitrile (10 mL) was added a solution of di-*tert*-butyl-dicarbonate (0.86 g, 3.9 mmol) in acetonitrile (3 mL) dropwise. After stirring for 1 h at room temperature, the solution was diluted with ice water and extracted with ethyl acetate. The organic layer was washed with 5% potassium bisulfate (aq), then with brine, and dried over anhydrous sodium sulfate. After solvent removal, 4-chloro-pyrrolo[2,3-b]pyridine-1-carboxylic acid *tert*-butyl ester was obtained (**59**; 0.8 g, 96% yield). Part of this crude product (0.5 g, 1.98 mmol) was dissolved in anhydrous N,N-dimethylformamide (5 mL), and to the solution, thoroughly degassed with argon, were added palladium-tetrakis-(triphenylphosphine) (0.1 g + 0.04 g after 5 h + 0.04 g after 8 h) and 1-ethoxyvinyltri-*n*-butyltin (0.96 mL, 2.83 mmol); the solution was thoroughly degassed with argon and heated with stirring at 100 °C for 10 h. Water was added, and the reaction mixture was extracted with ethyl acetate and dried over anhydrous sodium sulfate. After concentration, the crude oil was purified by chromatography (eluant: 5:1 *n*-hexane/ethyl acetate), and 4-(1-ethoxyvinyl)pyrrolo[2,3-b]pyridine-1-carboxylic acid *tert*-butyl ester (**63f**, 0.38 g, 67% yield) was obtained as a yellow oil. To part of this product (0.37 g, 1.28 mmol), dissolved in tetrahydrofuran (16 mL) and water (1 mL), was added N-bromosuccinimide (0.23 g, 1.28 mmol), and the solution was stirred at room temperature for 3 h. After solvent removal, the crude reaction product was purified by chromatography (eluant: 3:1 *n*-hexane/ethyl acetate), yielding the title product (0.2 g, 46% yield).

^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (s, 9 H), 4.72 (s, 2 H), 7.12 (d, J = 3.9 Hz, 1 H), 7.59 (d, J = 4.5 Hz, 1 H), 7.83 (d, J = 3.9 Hz, 1 H), 8.89 (d, J = 4.5 Hz, 1 H). ESI (+) MS: *m*/*z* 340 (MH+).

2-Bromo-1-(9H-purin-6-yl)ethanone (64g). To a solution of commercially available 6-chloro-9-(tetrahydropyran-2-yl)-9H-purine (**60**; 0.5 g, 2.1 mmol) in anhydrous N,N-dimethylformamide (5 mL), thoroughly degassed with argon, were added palladium-tetrakis-(triphenylphosphine) (0.12 g, 0.1 mmol + 0.06 g after 5 h) and 1-ethoxyvinyltri-*n*-butyltin (1 mL, 3 mmol); the solution was thoroughly degassed with argon and warmed at 100 °C under stirring for 8 h. Water was added, and the reaction mixture was extracted with ethyl acetate and dried over anhydrous sodium sulfate. After concentration, the crude oil was purified by chromatography (eluant: ethyl acetate). 6-(1-Ethoxy-vinyl)-9-(tetrahydropyran-2-yl)-9H-purine was obtained pure (**63g**; 0.54 g) in 94% yield. A portion of the compound (0.43 g, 1.57 mmol), dissolved in tetrahydrofuran (24 mL) and water (1.5 mL), was treated with N-bromosuccinimide (0.28 g, 1.57 mmol) and stirred at room temperature for 0.25 h. The solution was evaporated under reduced pressure, taken up with water, and filtered to obtain the title compound (0.3 g, 81% yield) as a yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 5.12 (s, 2 H), 8.85 (s, 1 H), 9.14 (s, 1 H). ESI (+) MS: *m*/*z* 242 (MH+).

2-Bromo-1-(2-phenylamino-pyrimidin-4-yl)-ethanone (64i). To a solution of sodium (614 mg, 26.7 mmol) in absolute ethanol (70 mL) was added phenylguanidine carbonate (4.43 g, 13.35 mmol), followed by a solution in absolute ethanol (20 mL) of 1-dimethylamino-4,4-dimethoxypent-1-en-3-one (**61**; 5 g, 26.7 mmol), obtained as described in the literature.[22] The suspension was refluxed for 20 h; then, 2/3 of the solvent was removed, and water (250 mL) was added. The precipitate was extracted with ethyl acetate, and the organic phase was washed with saturated sodium dihydrogenphosphate (aq) and with brine, dried over anhydrous sodium sulfate, and concentrated to yield [4-(1,1-dimethoxyethyl)pyrimidin-2-yl]phenylamine (**62**; 4.3 g, 62%). This ketal (4.2 g, 16.19 mmol) was dissolved in 88% formic acid (25 mL) and stirred at room temperature for 2.5 h. The reaction mixture was diluted with water (200 mL); the precipitate was filtered and washed copiously with water, yielding 1-(2-phenylaminopyrimidin-4-yl)-ethanone (**63i**) as a yellow solid (3.1 g, 90%). To a solution of this ketone (1.3 g, 6.1 mmol) in dichloromethane (40 mL) were added triethylamine (5.1 mL, 36.6 mmol) and *tert*-butyldimethylsilyl trifluoromethansulphonate (4.2 mL, 18.3 mmol). The orange solution was stirred overnight at room temperature; diluted with dichloromethane (150 mL); washed with 5% sodium bicarbonate (aq, ×2), with water, and with brine; dried over anhydrous sodium sulfate; and concentrated to give (*tert*-butyldimethylsilanyl)-{4-[1-(*tert*-butyldimethylsilanyloxy)-vinyl]pyrimidin-2-yl}phenylamine (**63m**; 2.64 g, 96%). To derivative **63m** (1.32 g, 2.94 mmol), dissolved in tetrahydrofuran (25 mL) and water (3 mL) at room temperature, was added solid N-bromosuccinimide (0.549 g, 3.09 mmol), and the reaction mixture was stirred for 20 h at room temperature. After concentration and aqueous workup with ethyl acetate, the crude material was purified by flash chromatography (eluant: 4:1 *n*-hexane/ethyl acetate) to yield the title compound (0.66 g, 46%).

^{1}H NMR (300 MHz, DMSO-d_6): δ 4.65 (s, 2 H), 6.7 (m, 1 H), 6.9 (d, J = 5 Hz, 1 H), 7.0 (m, 2 H), 7.4 (d, J = 7.7 Hz, 2 H), 8.4 (d, J = 5 Hz, 1 H), 9.6 (s, 1 H). ESI (+) MS: *m*/*z* 293 (MH+).

II. Registry Numbers (RN). 2-Bromo-1-(4-pyridinyl)-ethanone hydrobromide (RN, 5349-17-7), 2-bromo-1-(3-fluoro-4-pyridinyl)-ethanone hydrobromide (RN, 845538-59-2), 2-bromo-1-(pyridin-4-yl)propan-1-one (RN, 780692-61-7), 2-bromo-1-(3-pyridinyl)-ethanone (RN, 6221-12-1), α-bromoacetophenone (RN, 70-11-1), 1-(2-amino-4-pyrimidinyl)-2-bromo-ethanone (RN, 106157-91-9), 4-acetylpyrimidine (RN, 39870-05-8), 3-fluoro-4-acetylpyridine (RN, 87674-21-3), 4-chloro-1H-pyrrolo[2,3-b]pyridine (RN, 55052-28-3), 6-chloro-9-(tetrahydro-2H-pyran-2-yl)-9H-purine (RN, 7306-68-5), 1-(dimethylamino)-4,4-dimethoxy-1-penten-3-one (RN, 106157-94-2).

III. Molecular Modeling. Molecular modeling activities were performed with the Insight II and Cerius 2 programs (Accelrys). Docking was also performed with the QXP program. The homology model of Cdc7 was built with the "Homology" module of Insight II (Accelrys).

Sequence similarity searches were performed using BLAST[23] and visualized by Blixem.[24] The alignment was performed using Clustal W[25] and visualized using two different programs: Belvu (http://www.cgb.ki.se/cgb/groups/sonnhammer/Belvu.html) and Vector NTI AlignX (Invitrogen).

IV. *In Vitro* Kinase Assays. Inhibition of kinase activity was assessed using a Dowex resin capture technique. In this assay, 3

μM MCM2 (10-294) is phosphorylated by 2 nM Cdc7/Dbf4 in the presence of ATP (1.4 μM) traced with P^{33}-ATP in a kinase buffer (50 mM Hepes, pH 7.9, 2 mM β-glycerylphosphate, 2 mM DTT, 3 μM $NaVO_3$, 15 mM $MgCl_2$, 0.2 mg/ml BSA) for 60 min. By the addition of an acidic suspension of Dowex resin (SIGMA, custom-prepared resin Dowex 1 × 8 200–400 mesh equilibrated in 150 mM sodium formate, pH 3.00), the unreacted ATP is captured and separated from the supernatant which contains the phosphorylated substrate: this is then transferred onto a new plate for radioactivity counting. The assay is run in a robotized format on 384-well plates.

The kinase activity of Cdc7/Dbf4 was determined by measuring the rate of phosphorylation of Mcm2 (aa 10-294) in the presence of ATP traced with radiolabeled ATP (Revidue [γ^{33}P] ATP, Amersham Pharmacia Biotech, U.K.). The buffer composition was determined by measuring the rate of substrate transphosphorylation in experiments where the buffer pH and concentrations of Mg^{2+} and Mn^{2+} were varied from 7 to 8, 1 to 20 mM, and 0 to 5 mM, respectively. Final concentrations of 2 mM β-glycerylphosphate, 1 mM DTT, 3 μM M Na_3VO_4, and 0.2 mg/ml BSA were kept constant. The choice of the buffer providing the highest velocity was made by using a statistical approach (DOE) and resulted in the choice of HEPES 50 mM, pH 7.9, containing 15 mM Mg^{2+}. The rate of transphosphorylation was measured through the selective capture of unreacted ATP by a strong anion exchange resin (Dowex 1 × 8, formate form, Supelco, PA), essentially according to the literature.[26] Briefly, to 15 μL of reaction mixture was added 60 μL of a 1:3 (v/v) suspension of resin equilibrated with 150 mM Na-formate in water, pH 3.0, which also served to stop the reaction. After careful mixing and then allowing the resin to settle for 1 h, 20 μL of supernatant was carefully withdrawn using a Biomek FX pipet (Beckman Coulter inc., CA) and added to a 384-well plate (Optiplate, Packard) containing 60 μL of Microscint 40 (Perkin-Elmer). The radioactivity remaining in the supernatant that was incorporated into the positively charged substrate was then measured using a Top Count NXT (Packard). The assay was run in a robotized format on 384-well plates. Inhibitory potency evaluation was performed at 25 °C using a 60 min end-point assay where the concentrations of ATP and Mcm2 were kept equal to 2xαKm and saturated (>5xαKm), respectively. The Cdc7/Dbf4 concentration was kept sufficiently low to avoid substrate depletion within the duration of the end-point assay and to reduce the limit for tight-binding inhibitors.

V. Cell Culture. A2780 (European collection of cell culture) was grown in an I1640 medium supplemented with heat-inactivated 10% fetal calf serum.

VI. Inhibition of Cell Proliferation. Cells were seeded at different densities ranging from 2500 to 5000 cells/well in black 96-well plates with the appropriate complete medium. After 24 h, the plates were treated with the compounds to be tested and incubated for 72 h at 37 °C under a 5% CO_2 atmosphere. At the end of incubation, cells were lysed, and the ATP content in the well was used as a measure of viable cells. This was determined using a thermostable firefly luciferase based assay (CellTiter-Glo) from Promega. IC50 values were calculated using the rate of percent of growth versus the untreated control.

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