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Synthesis, Protein Kinase Inhibitory Potencies, and in Vitro Antiproliferative Activities of Meridianin Derivatives

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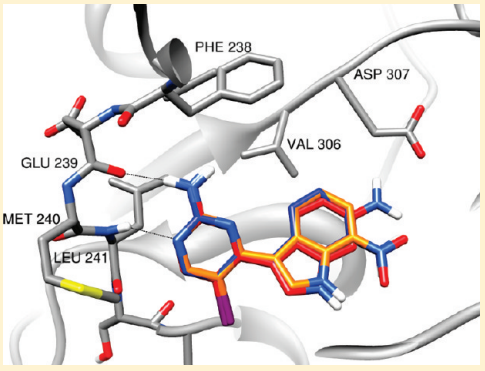
ABSTRACT: The synthesis of new meridianin derivatives is described. The indolic ring system was substituted at the C-4 to C-7 positions either by a bromine atom or by nitro or amino groups. Additionally, an iodine atom or various aryl groups were introduced at the C-5 position of the 2-aminopyrimidine ring. These compounds as well as some of their synthetic intermediates were tested for their kinase inhibitory potencies and for their in vitro antiproliferative activities. We found that this series of compounds is particularly interesting in the development of new inhibitors of DYRK1A and CLK1 kinases. The most effective compounds toward these two kinase families are the 6- and 7-bromo derivatives **30**, **33**, and **34** that showed more than 45-fold selectivity toward DYRK1A/CLK1 kinases over the other kinases tested. Meridianin derivatives could thus be developed toward potent and selective inhibitors of key RNA splicing regulators and potential therapeutic agents.



1. INTRODUCTION

Meridianins are marine alkaloids isolated from the south Atlantic tunicate *Aplidium meridianum*.[1] Meridianins A−G (**1**−**7**)[1−4] (Figure 1) were characterized as indole derivatives substituted in the C-3 position by a 2-aminopyrimidine ring and were found to inhibit various protein kinases[3] and to display antitumor activity.[5] The closely related meriolins, a family of synthetic 7-azaindole meridianin analogues, were also described for their potent kinase inhibitory activities and antiproliferative properties.[6,7]

Our interest in the synthesis of new kinase inhibitors led us to prepare meridianin analogues **8**−**14** (Figure 1) diversely substituted by aryl groups on the 2-aminopyrimidine ring.[8−10]

The meridianin derivatives **8**, **9**, **10**, and **11** were previously tested toward a panel of 9 protein kinases (kinase domain receptor (KDR), insulin growth factor receptor (IGF-1R), c-Met, RET, sarcoma kinase (c-Src), abelson leukemia oncogen cellular homology (c-Abl), cAMP-dependent protein kinase (PKA), cyclin-dependent protein kinase 2 (CDK2)/cyclin A and HER-1 and their in vitro antiproliferative activities were examined toward a human fibroblast primary culture and two human solid cancer cell lines (MCF-7 and PA 1).[9] Despite weak kinase inhibitory potencies, compounds **8**, **9**, **10**, and **11** exhibited high in vitro antiproliferative activities toward PA 1 cells. On the other hand, when compounds **8**−**14** (Figure 1) were tested toward the following kinases: CDK5/p25, casein kinase 1 (CK1)δ/ε, glycogen synthase kinase 3 (GSK-3)α/β, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A (DYRK1A), and extracellular signal regulated kinase (Erk2), they showed submicromolar inhibition potencies toward some of the tested kinases.[10] More particularly, some of them, including compounds **12**−**13**, demonstrated interesting inhibition potencies toward DYRK1A kinase which is known to be involved in Alzheimer's disease[11] and Down syndrome.[12] These promising results led us to expand our effort in the synthesis of new diversely substituted meridianin derivatives as potential kinase inhibitors. More particularly, we focused on analogues of compound **10**, which showed the best antiproliferative activities toward PA1 cells and compound **8**, which was the only one to be active toward the five tested kinases (CK1, GSK-3, DYRK1A, Erk2, CDK5/p25) and to exhibit an interesting antiproliferative activity toward PA1 cells. Moreover, we were also interested in the preparation of analogues of compounds **12** and **14** because they were active toward four of the five tested kinases (CK1, GSK-3, DYRK1A, and Erk2) and

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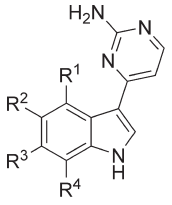
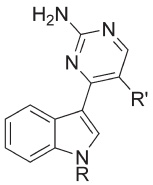


1 Meridianin A R^1 = OH, R^2 = H, R^3 = H, R^4 = H
2 Meridianin B R^1 = OH, R^2 = H, R^3 = Br, R^4 = H
3 Meridianin C R^1 = H, R^2 = Br, R^3 = H, R^4 = H
4 Meridianin D R^1 = H, R^2 = H, R^3 = Br, R^4 = H
5 Meridianin E R^1 = OH, R^2 = H, R^3 = H, R^4 = Br
6 Meridianin F R^1 = H, R^2 = Br, R^3 = Br, R^4 = H
7 Meridianin G R^1 = H, R^2 = H, R^3 = H, R^4 = H



8 R = H, R' = 4-trifluoromethylphenyl
9 R = H, R' = 4-trifluoromethoxyphenyl
10 R = Me, R' = 4-acetylphenyl
11 R = Me, R' = 4-biphenyl
12 R = H, R' = 3-methoxyphenyl
13 R = H, R' = 3-aminophenyl
14 R = H, R' = 4-acetamidophenyl

Figure 1. Meridianins A−G (**1**−**7**) and derivatives **8**−**14** described by our group.

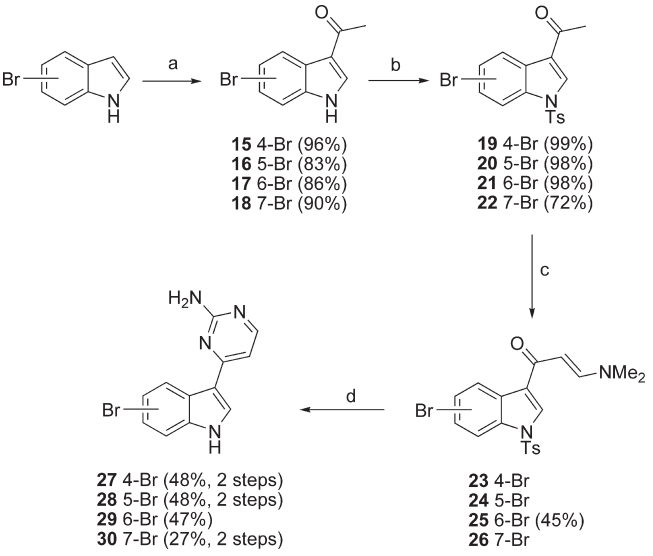
have shown modest antiproliferative activities with IC_{50} values in the 28−38 μM range.

As shown in Figure 1, meridianins A−F (**1**−**6**) are substituted at C-4 to C-7 positions of the indolic ring system either by a hydroxyl and/or a bromine atom. It has been demonstrated that the substitutions on these positions could have a major influence on the biological potencies of meridianins.[3] More particularly, when tested toward CDKs, PKA and CK1, meridianin E, substituted by a hydroxyl group at the C-4 and a bromine atom at the C-7 positions was the most active, whereas the best inhibitory potency against GSK-3 was found for meridianin B bearing a hydroxyl group at the C-4 and a bromine atom at the C-6 positions. For PKG, the best potency was obtained for meridianin C substituted at the C-5 position by a bromine atom. Thus, in this article, we describe the synthesis of brominated analogues of compounds **8**, **10**, **12**, and **14**. The indolic nucleus of these new compounds was substituted by a bromine atom at the C-4, C-5, C-6, or C-7 positions. Moreover, to enlarge the structure−activity relationship study reported in this paper, the C-4 to C-7 positions of the indolic ring were also substituted by nitro or amino groups. These compounds as well as some of their synthetic intermediates were tested for their kinase inhibitory potencies toward five kinases (CDK5/p25, CK1δ/ε, GSK-3α/β, DYRK1A, and cdc2-like kinase 1 (CLK1)) and for their in vitro antiproliferative activities toward a human fibroblast primary culture, a murine hippocampal cell line (HT22) and six human cancer cell lines (PC3, DU145, PA1, SH-SY5Y, IMR-32, and MCF7).

2. SYNTHESIS AND KINASE INHIBITORY ACTIVITY

The construction of the 2-aminopyrimidine ring was based on the well-known Bredereck synthetic approach (Scheme 1).[5,13−15] First, the C-3 position of commercially available 4-, 5-, 6-, and 7-bromoindoles was acetylated using acetyl chloride and tin(IV) chloride in toluene to give derivatives **15**−**18** in 83% to 96% yields. Then, protection of the indolic nitrogen was performed by reaction with tosyl chloride in the presence of 4-(dimethylamino)-pyridine (DMAP) and diisopropylethylamine (DIPEA) in methylene chloride, leading to the formation of compounds **19**−**21** in 98% to 99% yields. The same reaction conditions applied to the 7-bromo analogue **18** gave compound **22** in only 27% yield. Therefore, other reaction conditions were tested and, finally, compound **22** was prepared in 72% yield using tosyl chloride and sodium hydride in *N*,*N*-dimethylformamide (DMF). The enaminone derivative **25** prepared by treatment of **21** with

Scheme 1. Synthesis of Compounds 27−30 (Yields Are Indicated under Brackets)[a]



15 4-Br (96%)
16 5-Br (83%)
17 6-Br (86%)
18 7-Br (90%)

19 4-Br (99%)
20 5-Br (98%)
21 6-Br (98%)
22 7-Br (72%)

27 4-Br (48%, 2 steps)
28 5-Br (48%, 2 steps)
29 6-Br (47%)
30 7-Br (27%, 2 steps)

23 4-Br
24 5-Br
25 6-Br (45%)
26 7-Br

[a] Reagents and conditions: (a) AcCl, $SnCl_4$, toluene; (b) DMAP, DIPEA, TsCl, CH_2Cl_2, or NaH, TsCl, DMF; (c) DMF-DMA, DMF, or DMF/di-*tert*-butylacetal, DMF; (d) guanidine-HCl, K_2CO_3, 2-methoxyethanol.

DMF/dimethylformamide-dimethylacetal (DMF-DMA) in 45% yield was then treated with guanidine hydrochloride in 2-methoxyethanol in the presence of potassium carbonate to give the corresponding 2-aminopyrimidine derivative **29** in 47% yield. The preparation of the corresponding 4-, 5-, and 7-bromo derivatives was undertaken using a similar synthetic pathway but without any purification of the enaminone intermediates **23**, **24**, and **26**. Compounds **27** and **30** were then obtained from **19** and **22** in 48% and 27% yields, respectively, using the same conditions as those described for compound **29**. Compound **28** was obtained in 48% yield from **20** using DMF/di-*tert*-butylacetal instead of DMF-DMA.

Bromo-meridianins **27**−**30** were then iodinated with iodine in dimethylsulfoxide[10] to give the corresponding compounds **31**−**34** in 66% to 86% yield. Suzuki cross-coupling was then performed using various commercially available arylboronic acids, tetrakis(triphenylphosphine)palladium, and sodium carbonate

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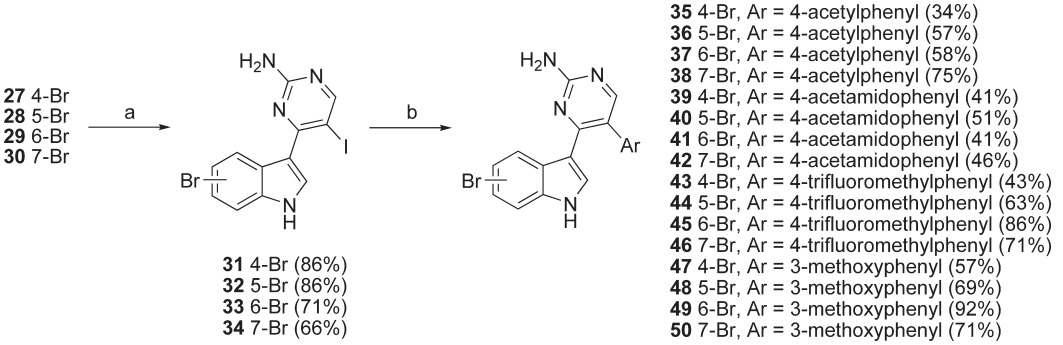
Scheme 2. Synthesis of Compounds 31−50 (Yields Are Indicated under Brackets)[a]



[a] Reagents and conditions: (a) I_2, DMSO; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, EtOH, toluene.

in a H_2O/EtOH/toluene medium.[8] Thus, compounds **35**−**50** were obtained in 34−92% isolated yields (Scheme 2).

The Suzuki cross-coupling products were obtained in either modest or good chemical yields depending on both the position of the bromine atom on the indolic moiety and the nature of the boronic acid used. With all the tested boronic acids, the lowest yields were observed with the 4-bromo derivatives. Moreover, yields were usually better with the 3-methoxyphenylboronic acid. This is probably due to the presence of an electron-donating substituent on the phenyl moiety.

The kinase inhibitory potencies of compounds **31**−**50** as well as those of their synthetic intermediates **27**−**30** were evaluated as IC_{50} values toward five protein kinases (CDK5/p25, CK1δ/ε, GSK-3α/β, DYRK1A, and CLK1) (Table 1).[16]

The best active compounds in this series were bromo-iodo derivatives **33**, **34**, and noniodinated 7-bromo analogue **30**. These 6- and 7-bromo analogues were particularly active toward DYRK1A and CLK1, with IC_{50} values in the nanomolar range (DYRK1A IC_{50}, 0.034−0.068 μM; CLK1, 0.032−0.065 μM). In comparison, the corresponding 4- and 5-bromo analogues **27**, **28**, **31**, and **32** were less active. Moreover, some of these bromo-iodo derivatives **32** and **33** have shown submicromolar inhibition of CDK5/p25 (compound **33**) and CK1δ/ε (compounds **32** and **33**).

Regarding the substitution of the 2-aminopyrimidine ring by aryl groups, the 4-trifluoromethylphenyl series (compounds **43**−**46**) have shown the weakest inhibitory potencies without any submicromolar IC_{50} values toward the tested kinases. 4-Acetylphenyl (**35**−**38**), 4-acetamidophenyl (**39**−**42**), 4-trifluoromethylphenyl (**43**−**46**), and 3-methoxyphenyl (**47**−**50**) derivatives have only shown submicromolar IC_{50} values toward GSK-3α/β and DYRK1A. In all cases, the general pattern was that 7-bromo substituted compounds displayed both the highest inhibitory activity and the strongest selectivity toward DYRK1A and CLK1.

According to these results, this series of compounds is particularly interesting in the development of new inhibitors of DYRK1A and CLK1 kinases, two families of kinases involved in alternative mRNA splicing and neurodegenerative pathologies such as Alzheimer's disease. The most effective compounds are the 6- and 7-bromo derivatives **30**, **33**, and **34** that showed noteworthy inhibitory potencies toward these kinases. Moreover, compounds **30**, **33**, and **34** demonstrated more than 45-fold selectivity toward DYRK1A/CLK1 kinase versus the other kinases tested.

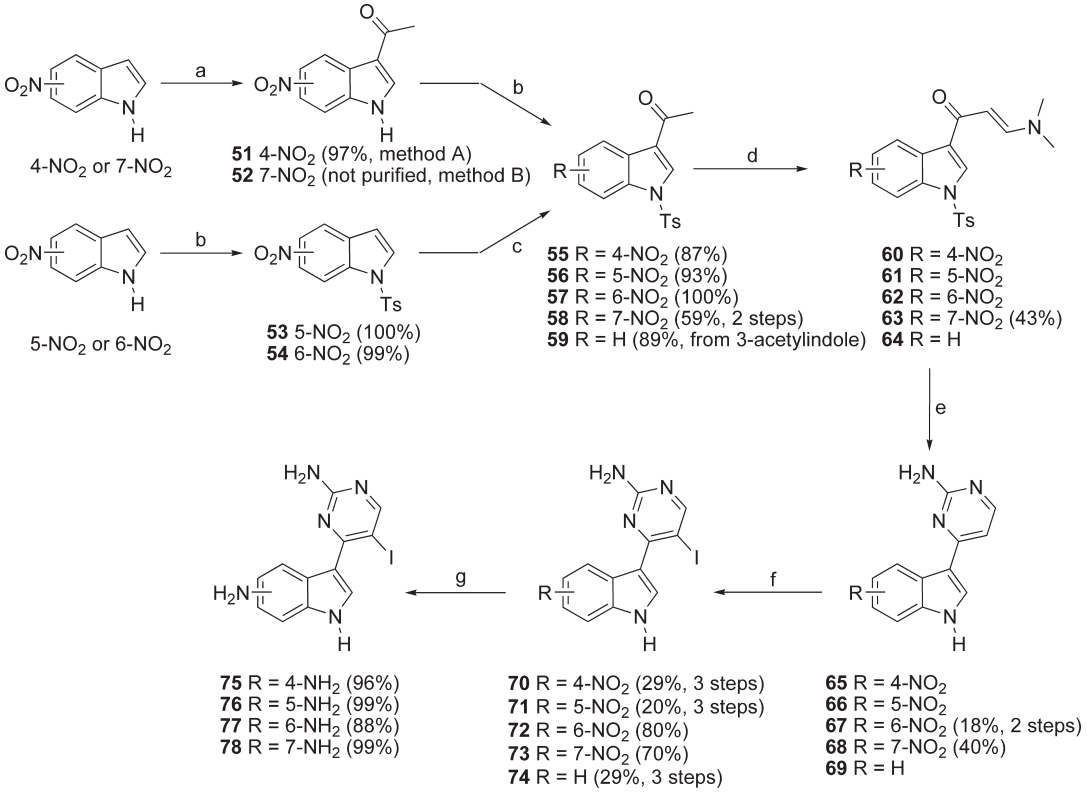
These results prompted us to prepare new analogues bearing an iodine atom at the C-5 position of the aminopyrimidine ring.

Table 1. Kinases Inhibitory Potencies (IC_{50} in μM) for Compounds 27−50

	kinase inhibition, IC_{50} in μM				
compd	CDK5/p25	CK1δ/ε	GSK-3α/β	CLK1	DYRK1A
27	10	5.3	>10	1.4	0.85
28	1.2	7.3	1.3	0.22	0.33
29	1.2	3.8	7.2	0.12	0.13
30	3.1	4.2	>10	0.065	0.068
31	>10	1.3	>10	2.3	1.45
32	1.8	0.77	2.1	0.18	0.33
33	0.68	0.7	1.1	0.032	0.034
34	2.1	1.6	4	0.042	0.039
35	7	>10	1.5	1.5	2.1
36	>10	>10	0.68	1.6	8.45
37	>10	>10	2.0	1.8	>10
38	>10	>10	4.5	0.38	0.29
39	>10	>10	>10	>10	>10
40	6.8	2.1	0.4	0.75	4.15
41	>10	8.1	1.6	0.53	3.1
42	7.5	>10	2.2	0.087	0.19
43	4.7	>10	1	1.3	1.85
44	>10	>10	2	>10	>10
45	>10	>10	6.1	>10	>10
46	>10	>10	8.5	1.5	1.25
47	6	>10	1.4	1.3	0.81
48	7.6	2.2	1.2	2.5	5.65
49	>10	3.2	4.5	2.3	8.2
50	>10	4.2	>10	0.87	0.85

Thus, we synthesized compounds **70**−**73** and **75**−**78** substituted at the C-4 to C-7 positions of the indolic moiety by nitro or amino groups. Nonsubstituted compound **74** was also prepared in order to evaluate the influence of indole substitution on the biological potencies of the tested compounds. The 4- to 7-nitro and amino derivatives (**70**, **73**, **75**, and **78**) as well as the nonsubstituted compound **74** were prepared using a similar synthetic pathway as the one described above for the bromo analogues (Scheme 3). First, the C-3 position of commercially available 4-, and 7-nitroindoles was acylated using acetyl chloride and ionic liquid generated from 1-ethyl-3-methylimidazolium

Scheme 3. Synthesis of Compounds 51−78 (Yields Are Indicated under Brackets)[a]



[a] Reagents and conditions: (a) method A: EmimCl, $AlCl_3$, AcCl; method B: Me_2AlCl, AcCl, CH_2Cl_2; (b) TsCl, NaH, DMF; (c) Ac_2O, $AlCl_3$, CH_2Cl_2; (d) DMF-DMA, DMF; (e) guanidine-HCl, K_2CO_3, 2-methoxyethanol; (f) I_2, DMSO; (g) Fe, NH_4Cl, *i*PrOH, H_2O.

chloride (Emim Cl) and aluminum chloride[17] or dimethylaluminium chloride[18] to give acyl derivative **51** in 97% yield, whereas compound **52** was not purified before the following protection step. The tosylation of the indolic nitrogen of compounds **51**, **52**, and commercially available 3-acetylindole was carried out with tosyl chloride in the presence of NaH in DMF to give compounds **55**, **58**, and **59** in 87%, 59% (2 steps), and 89% yields, respectively.

Regarding the synthesis of 5- and 6-nitro/amino derivatives, the two first steps were inverted. Thus, the 5- and 6-nitroindoles were previously tosylated in the presence of tosyl chloride and sodium hydride before acylation with acetic anhydride and aluminum chloride[19] to give compounds **56** and **57** in good yields.

Using a one-pot protocol, the corresponding enaminone derivatives **60**−**64** prepared by treatment of the previous acylated indoles with DMF-DMA were then treated with guanidine hydrochloride in 2-methoxyethanol in the presence of potassium carbonate to give the corresponding 2-aminopyrimidine derivative **65**−**69**. Finally, treatment of **65**−**69** with iodine in dimethylsulfoxide led to the formation of the corresponding iodinated analogues **70**−**74** in 20% to 80% yields after one step (**72** and **73** from **67** and **68**) or three steps (**70**, **71**, and **74** from **55**, **56**, and **59**). Finally, the reduction of the nitro group of compounds **70**−**73** was performed in mild conditions using Fe powder and NH_4Cl.[20] This method afforded the corresponding 4-, 5-, 6-, and 7-amino analogues **75**−**78** in good yields (88 to 99%).

The kinase inhibitory potencies of compounds **67**−**78** were evaluated as IC_{50} values toward five protein kinases (CDK5/p25, CK1δ/ε, GSK-3α/β, DYRK1A, and CLK1) (Table 2). As in the case of previously discussed brominated analogues, the most sensitive kinases were DYRK1A and CLK1. The most active compounds in this series were derivatives **68**, **72**, and **74** with high inhibitory potencies toward both DYRK1A and CLK1 kinases (IC_{50}, 0.03−0.095 μM). Moreover, compounds **73**, **77**, and **78** that considerably inhibited CLK1 with IC_{50} in the range of 0.026−0.08 μM only showed moderate inhibitory potencies toward DYRK1A (0.11−0.46 μM), indicating that the inhibition

Table 2. Kinases Inhibitory Potencies (IC_{50} in μM) for Compounds 67−78

	kinase inhibition, IC_{50} in μM				
compd	CDK5/p25	CK1δ/ε	GSK-3α/β	CLK1	DYRK1A
67	>10	6.3	>10	0.38	1.1
68	6.3	10	>10	0.07	0.085
69	5	6.2	>10	0.53	0.7
70	>10	>10	>10	7.1	9.1
71	1.6	0.42	0.66	0.12	0.25
72	1.6	1	2.6	0.067	0.095
73	>10	>10	>10	0.041	0.46
74	1.4	0.9	3.7	0.03	0.066
75	>10	>10	>10	1	2.8
76	>10	0.27	>10	0.61	2.3
77	6.2	0.51	4.2	0.08	0.22
78	0.66	1.1	4.4	0.026	0.11

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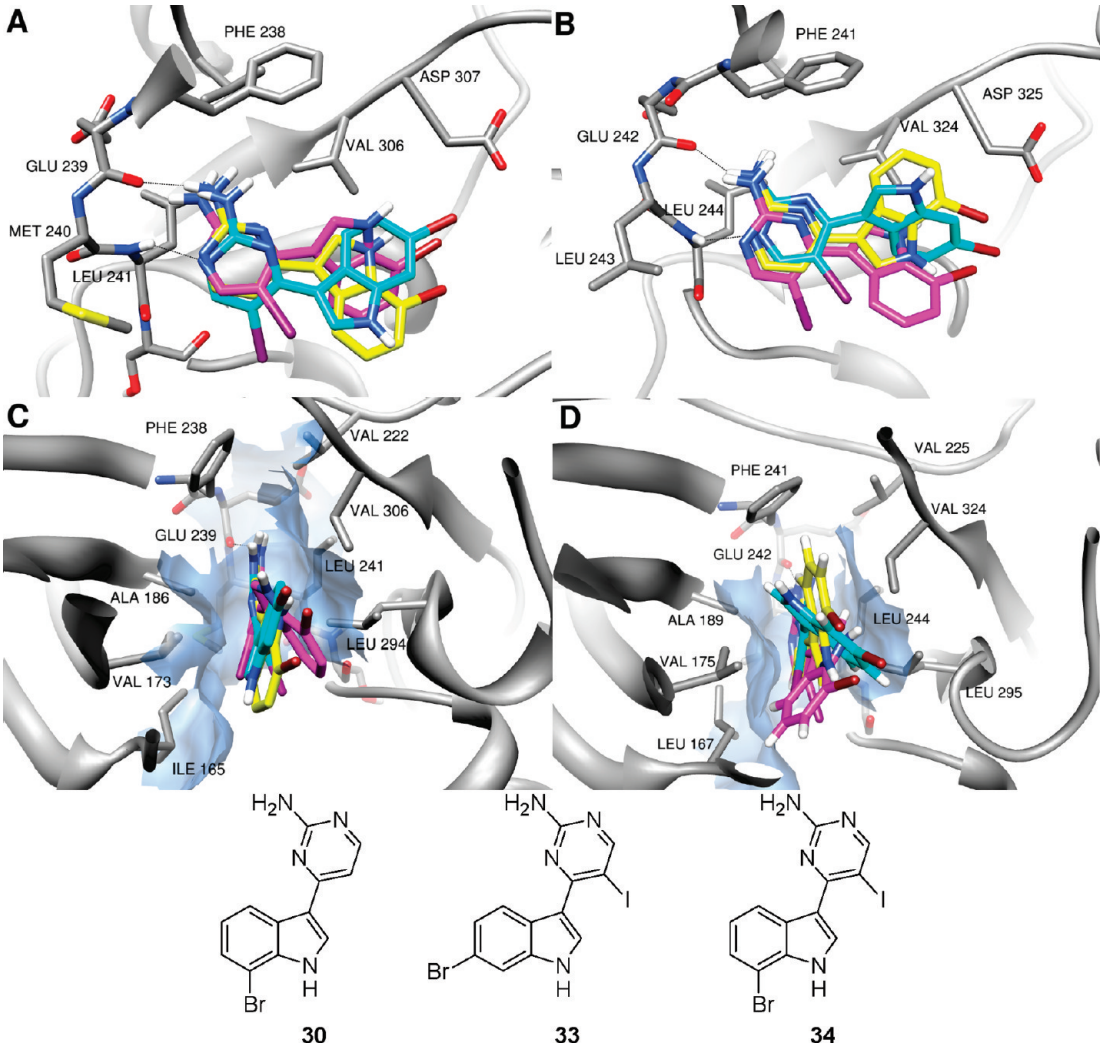

Figure 2. Docking models of compounds **30** (yellow), **33** (cyan), and **34** (magenta) into the ATP binding site of DYRK1A (left) and CLK1 (right).

of these two kinases can be disconnected to some extent. These results indicated that in this series (iodinated or not on the aminopyrimidine ring), the introduction of a nitro/amino group in the 4- or 5- position of the indolic nucleus is detrimental to achieve a potent DYRK1A/CLK1 inhibition. On the contrary, the introduction of a nitro group in the 6- or 7- position has led to potent CLK1 and DYRK1A inhibitors. Moreover, the 6- and 7-amino derivatives have also shown good potencies toward CLK1.

As in the brominated series, the 4- and 5-substitued derivatives were less active than their 6- and 7-counterparts. The fact that compounds **30**, **33**, **34**, **68**, **72**, and **74** highly inhibited CLK1 and DYRK1A prompted us to examine the relationship between these two kinases that are both members of the CMCG branch of the kinome. A sequence comparison between these two kinases has pointed out 32.8% of homology.[21,22] Regarding the ATP binding site, a 70.4% of sequence identity was determined from ATP docking experiments (see below). This was achieved by sequence alignment of protein residues located at a distance ≤5 Å around ATP atoms. Thus molecular modeling experiments were performed to understand the molecular interactions involved between this series of potent inhibitors and CLK1/DYRK1A kinase active sites.

3. MOLECULAR MODELING EXPERIMENTS

The putative binding mode between the ATP binding site of DYRK1A and the most active compounds of these series **30**, **33**, and **34** was examined by molecular modeling experiments using a model based on the 2wo6 DYRK1A structure available in the Protein Data Bank (PDB).[23] Hydrogen atoms were added for all amino acid residues, the preferential position of hydrogen atoms was determined by minimization, and the water molecules were removed from the structure. The results obtained in the docking experiments were sorted out, taking into account the preferred ligand conformation. The selected structures obtained in these docking assays were then minimized. As shown in Figure 2, the meridianin derivatives **30**, **33**, and **34** adopt a similar binding mode. The aminopyrimidine moiety is oriented toward the bottom of the pocket making two hydrogen bonds with the ATP binding site. Thus, the amino group is H-bonded with Glu239 backbone carbonyl and the N-1 atom is H-bonded with Leu241 backbone NH (Figure 2A). Moreover, the heteroaromatic scaffold of compounds **30**, **33**, and **34** is strongly associated to the protein by hydrophobic interactions with Ile165, Val173, Ala186, Leu241, Leu294, and Val306 side chains (Figure 2C). The same approach was also used to propose a probable binding

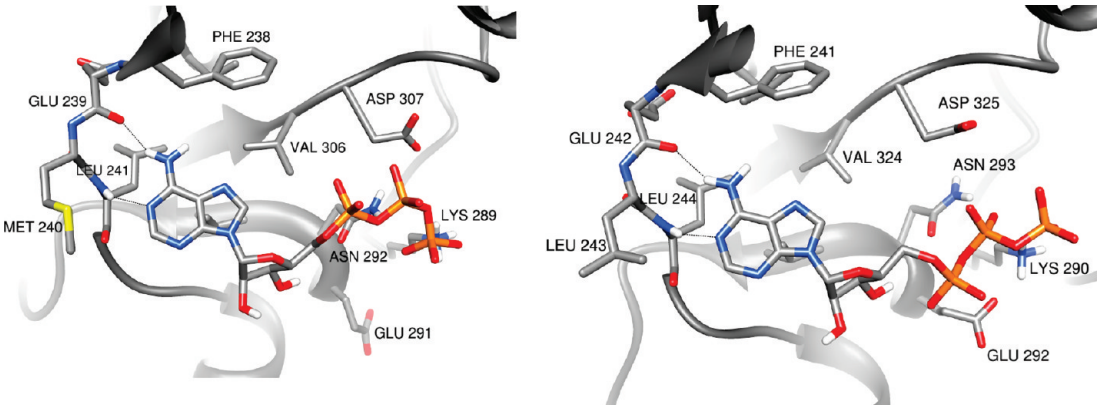

Figure 3. Docking models of ATP into the ATP binding site of DYRK1A (left) and CLK1 (right).

mode of meridianin derivatives **30**, **33**, and **34** with the CLK1 ATP binding site by using a X-ray cocrystal structure of CLK1 (PDB code 1z57).[24] Again, the three meridianin derivatives adopt the same binding mode. In this case, the amino group of the aminopyrimidine moiety is H-bonded with Glu242 backbone carbonyl and the N-1 atom is H-bonded with Leu244 backbone NH (Figure 2B). For this protein kinase, the hydrophobic interactions with compounds **30**, **33**, and **34** involve residue side chains of Leu167, Val175, Ala189, Leu244, Leu295, and Val324 (Figure 2D).

In both cases, the interaction between the meridianin derivatives **30**, **33**, and **34** and the ATP binding site of the kinase is driven by the H-bonding of the aminopyrimidine moiety and the enzyme as described above.

On the other hand, the orientation of the indolic ring system differs from a complex model to another. The preferential positioning of the indole moiety is directed by hydrophobic interactions (see above) and steric effects due to the presence of a bromine atom in the 6- or 7-position and/or the one of an iodine atom at the 5-position of the aminopyrimidine ring.

To compare the ATP pocket binding mode of meridianin derivatives **30**, **33**, and **34** with the one of ATP, docking experiments were performed with ATP. As shown in Figure 3, the adenine moiety of ATP interacted with DYRK1A and CLK1 in a similar manner to that observed for meridianin derivatives. Two hydrogen bonds were formed between the adenine moiety and DYRK1A or CLK1. Specifically, the amino group was H-bonded to the backbone carbonyl of Glu239 (for DYRK1A) or Glu242 (for CLK1) and the N1 atom was H-bonded with the backbone NH of Leu241 (for DYRK1A) or Leu244 (for CLK1).

The morphological differences between both ATP binding sites were examined in more detail in order to explain the higher activity toward CLK1 over DYRK1A observed for compounds **73**, **77**, and **78**. As shown in Figure 4, the aminopyrimidine moiety of meridianin derivatives **73**, **77**, and **78** adopt the same binding mode with the ATP binding site of DYRK1A/CLK1 than the one described above for compounds **30**, **33**, and **34**. Unfortunately, taking into account the result of our molecular modeling studies, it was not possible to explain the best inhibitory potencies of compounds **73**, **77**, and **78** toward CLK1. This could be due to diffences existing between the water molecule networks in the ATP binding site of these two kinases. However, to validate this hypothesis, the resolution of cocrystal structures of this series of compounds in complex with CLK1/DYRK1A kinases will be needed.

4. IN VITRO ANTIPROLIFERATIVE ACTIVITY

As some of the kinases inhibited by compounds **27**−**50** and **67**−**78** are involved in the cellular proliferation, the antiproliferative activities of these compounds were also evaluated (Table 3). In vitro antiproliferative activities of compounds **27**−**50** and **67**−**78** were tested toward a human fibroblast primary culture, a murine hippocampal cell line (HT22), and six cancer cell lines: PC3, DU145 (human prostate cancer), PA1 (human ovarian teratocarcinoma), MCF7 (human breast cancer), and SH-SY5Y, IMR-32 (human neuroblastoma). The antiproliferative effect of the tested drug was assessed by the resazurin reduction test[25] for fibroblast, PA1, PC3, and DU145 cell lines. The MTS reduction assay was used for SH-SY5Y, IMR-32, and HT22 cells. Under the conditions used, the most active compounds were the 6- and 7-bromoderivatives **33**, **37**, and **38** with submicromolar IC_{50} values for some of the cells line tested. Moreover, compounds **34**, **42**, **70**, **72**, **73**, **74**, and **78** also showed modest antiproliferative activities, with IC_{50} values in the micromolar range toward some of the cell lines tested.

5. CONCLUSION

In conclusion, this work reports a structure−activity relationship study of new meridianin derivatives as kinase inhibitors and/or antiproliferative agents. The synthetic approach reported in this study allowed the substitution of the 4-, 5-, 6-, or 7-position of the indolic moiety by a bromine atom or nitro or amino groups. For this series of compounds, the most interesting inhibitory potencies were observed toward DYRK1A and CLK1 kinases. The results of this structure−activity relationship study have demonstrated that potent DYRK1A/CLK1 inhibitions were observed for compounds substituted at the 6- or 7-position of the indolic moiety by a bromine atom or a nitro or an amino group. In addition, compounds **27**−**50** and **67**−**78** were evaluated for their in vitro antiproliferative potencies toward six different cancer cell lines, a fibroblast primary culture, and a murine hippocampal cell line. IC_{50} values in the micromolar/submicromolar range were determined for compounds **33**, **34**, **37**, **38**, **42**, **70**, **72**−**74**, and **78** toward the cell line tested. As there is no direct correlation between the measured kinase inhibitory potencies and the antiproliferative activities of these compounds, there must be other biological target(s) involved in the antiproliferative effects of this series. As is almost always seen with kinase inhibitors (and probably also with any enzyme inhibitor), the efficacy on isolated enzyme is higher than that on cells. This is likely the consequence

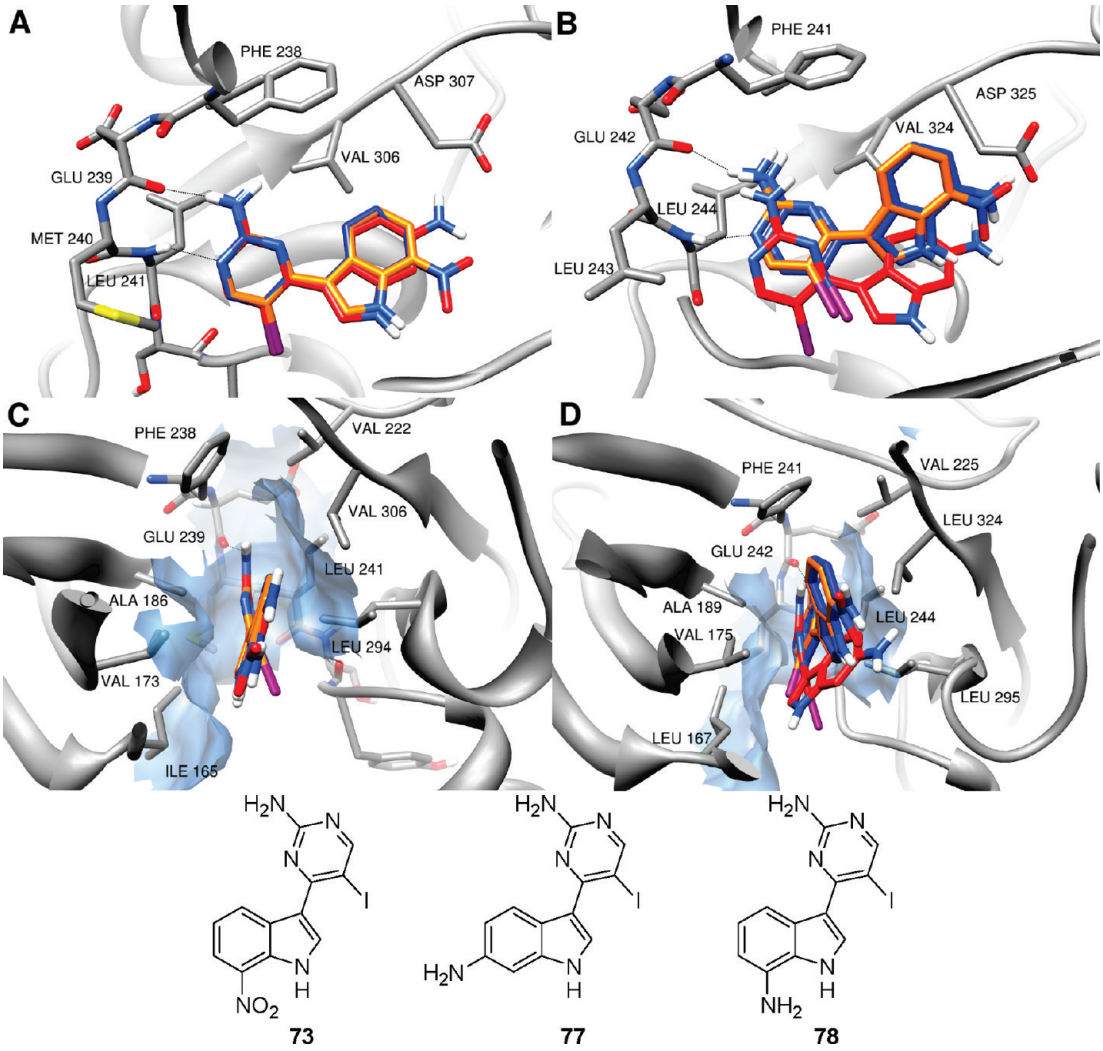

Figure 4. Docking models of compounds **73** (orange), **77** (red), and **78** (blue) into the ATP binding site of DYRK1A (left) and CLK1 (right).

of the combination of several factors including interaction with other targets within the cell (multitarget effects), nonspecific binding to cellular proteins (ionic or hydrophobic interactions), intracellular metabolism and possible inactivation, limitations in the passage across biological membranes (plasma membrane, nuclear envelope), and high intracellular ATP concentration. A more precise evaluation of the effects of CLK/DYRK inhibition by our inhibitors would require the generation of phospho-specific antibodies that cross-react with sites that are specifically and uniquely phosphorylated by CLK/DYRK. We are currently attempting various approaches to design, generate, and validate these tools. Co-crystallization assays between compounds **30**, **33**, and **34** and DYRK1A and CLK1 will be undertaken to investigate the mode of interaction of these compounds within the ATP binding site of these kinases. This approach would provide valuable information and facilitate further development of the structure based design of new DYRK1A and/or CLK1 kinase inhibitors in this series.

6. EXPERIMENTAL SECTION

6.1. In Vitro Kinase Inhibition Assays. *Buffers. Buffer A.* $MgCl_2$ (10 mM), 1 mM ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 25 mM Tris-HCl pH 7.5, 50 μg heparin/mL.

Buffer B. β-Glycerophosphate (60 mM), 15 mM *p*-nitrophenylphosphate, 25 mM 3-(*N*-morpholino)propanesulfonic acid (Mops) (pH 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenylphosphate.

Kinase Preparations and Assays. Kinase activities were assayed in buffer A or B, at 30 °C, at a final adenosine triphosphate (ATP) concentration of 15 μM. Blank values were subtracted and activities expressed in % of the maximal activity, i.e., in the absence of inhibitors. Controls were performed with appropriate dilutions of dimethylsulfoxide (DMSO).

CDK5/p25. (Human, recombinant) was prepared as previously described.[26,27] Its kinase activity was assayed in buffer B, with 1 mg of histone H1/mL, in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 μL. After 30 min of incubation at 30 °C, 25 μL aliquots of supernatant were spotted onto 2.5 cm × 3 cm pieces of Whatman P81 phosphocellulose paper, and 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL of phosphoric acid/L of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid.

GSK-3α/β. (Porcine brain, native) was assayed, as described for CDK5/p25 but in buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine).[28] GS-1 was synthesized by Millegen (Labege, France).

Table 3. Antiproliferative Activity (IC_{50} in μM) of Compounds 27−50 and 67−78 (nd: Not Determined)

	antiproliferative activity, IC_{50} in μM							
compd	SHSY-5Y	IMR-32	MCF7	HT22	PA 1	PC3	DU145	fibro
27	>25	>25	>25	>25	nd	nd	nd	nd
28	>25	>25	24	9.3	nd	nd	nd	nd
29	>25	>25	>25	>25	nd	nd	nd	nd
30	>25	25	>25	>25	nd	nd	nd	nd
31	>10	>10	>10	>10	>10	>10	nd	nd
32	23	13	>25	8	>10	>10	nd	nd
33	3.2	3.8	5.2	5.1	<1	2.9 ± 0.3	<1	3.5 ± 0.3
34	8	7.3	17	12	2.0 ± 0.1	15 ± 1	4.2 ± 0.3	10 ± 2
35	>10	>10	>10	>10	>10	>10	nd	nd
36	>25	13	4.2	10	>10	>10	nd	nd
37	0.4	0.33	1	2.5	<0.6	<0.6	<0.6	<0.6
38	>10	>10	>10	>10	0.8 ± 0.2	3.6 ± 0.4	5.7 ± 0.8	18.4 ± 0.8
39	>10	>10	>10	>10	>10	>10	nd	nd
40	>10	>10	>10	>10	>10	>10	nd	nd
41	>10	>10	>10	>10	12 ± 2	14 ± 2	20 ± 1	24 ± 2
42	25	25	>25	>25	3.0 ± 0.3	5 ± 3	13 ± 1	8 ± 1
43	>10	>10	>10	>10	>10	>10	nd	nd
44	>10	>10	>10	>10	>10	>10	nd	nd
45	>10	>10	>10	>10	>10	>10	nd	nd
46	>10	>10	>10	>10	17 ± 3	8 ± 2	18 ± 1	14 ± 2
47	>10	>10	>10	>10	>10	>10	nd	nd
48	>25	20	>25	18	>10	>10	nd	nd
49	>25	18	>25	16	>10	>10	nd	nd
50	>10	>10	>10	>10	13 ± 3	29 ± 3	33 ± 4	31 ± 3
67	>25	>25	>25	>25	nd	nd	nd	nd
68	>25	>25	>25	>25	nd	nd	nd	nd
69	>25	>25	>25	>25	nd	nd	nd	nd
70	>10	>10	>10	>10	3.3 ± 0.7	7 ± 0	5.4 ± 0.2	5 ± 2
71	>25	10	>25	20	8.4 ± 0.1	26 ± 3	13.3 ± 0.4	19 ± 6
72	12	10	>25	18	2.0 ± 0.5	11 ± 3	6.3 ± 0.9	13 ± 2
73	>10	>10	>10	>10	3.2 ± 0.6	6 ± 2	5.2 ± 0.4	4.7 ± 0.6
74	7.5	10	17	7.3	3.3 ± 0.1	10.6 ± 0.8	5 ± 2	8 ± 4
75	>10	>10	>10	>10	18 ± 0	>50	>50	>50
76	>10	>10	>10	>10	11 ± 1	48 ± 6	40 ± 6	44 ± 3
77	>10	>10	>10	>10	13 ± 4	47 ± 15	26 ± 6	36 ± 8
78	9	4	20	12	2.5 ± 0.2	14 ± 3	6.1 ± 0.3	10.6 ± 0.6

CK1δ/ε. (Porcine brain, native) was assayed as described for CDK5/p25 but using the CK1-specific peptide substrate RRKHAAIGpSAYSITA,[29] obtained from Millegen (Labege, France).

DYRK1A. (Rat, recombinant, expressed in *E. coli* as a glutathione transferase (GST) fusion protein) was purified by affinity chromatography on glutathione-agarose and assayed as described for CDK5/p25 using myelin basic protein (1 mg/mL) as a substrate.

CLK1. (Human, recombinant, expressed in *E. coli* as GST fusion protein) was assayed in buffer A (+ 0.15 mg BSA/ml) with RS peptide (GRSRSRSRSRSR) (1 μg/assay).

6.2. In Vitro Antiproliferative Assays. *Cell Cultures.* Stock cell cultures were maintained as monolayers in 75 cm^2 culture flasks in Glutamax Eagle's minimum essential medium (MEM) with Earle's salts supplemented with 10% fetal calf serum, 5 mL of 100 mmol/L sodium pyruvate, 5 mL of 100× nonessential amino acids and 2 mg of gentamicin base. Cells were grown at 37 °C in a humidified incubator under an atmosphere containing 5% CO_2.

Survival Assays. Cells were plated at a density of 5×10^3 cells in 150 μL culture medium in each well of 96-well microplates and were allowed to adhere for 16 h before treatment with tested drug. A stock solution 20 mmol/L of each tested drug was prepared in dimethylsulfoxide (DMSO) and kept at −20 °C until use. Then, 50 μL of each tested solution were added to the cultures. A 48 h, continuous drug exposure protocol was used. The antiproliferative effect of the tested drug was assessed by the resazurin reduction test.

Resazurin Reduction Test. Plates were rinsed with 200 μL of PBS at 37 °C and emptied by overturning on absorbent toweling. Then, 150 μL of a 25 μg/mL solution of resazurin in MEM without phenol red was added to each well. Plates were incubated for 1 h at 37 °C in a humidified atmosphere containing 5% CO_2. Fluorescence was then measured on an automated 96-well plate reader (Fluoroscan Ascent FL, Labsystem) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Under the conditions used, fluorescence was proportional to

the number of living cells in the well. The IC_{50}, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage, defined as fluorescence in experimental wells compared with fluorescence in control wells, after subtraction of the blank values.

Cell Lines and Culture Conditions. Cell Survival Quantification. SH-SY5Y and HT22 cells were grown in DMEM medium from Invitrogen (Cergy Pontoise, France), while IMR 32 was grown in RPMI (Invitrogen). All media were supplemented with antibiotics (penicillin−streptomycin) from Lonza and 10% volume of fetal calf serum from Invitrogen. Cells were cultured at 37 °C with 5% CO_2. Compound treatments were performed on exponentially growing cultures at the indicated time and concentrations. Control experiments were carried out also using appropriate dilutions of DMSO (maximum 1% DMSO). Cell viability was determined by measuring the reduction of MTS as described in ref 30.

6.3. Chemistry. IR spectra were recorded on a Shimazu FTIR 84000S spectrometer; only structurally important peaks ($\bar{\nu}$) are presented in cm^{-1}. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained on a Bruker AC 400 spectrometer. Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively, using residual solvent signals as reference. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quadruplet (q), doubled doublet (dd), broad signal (br s), multiplet (m). HRMS spectra were recorded in electrospray ionization mode (ESI+) on a micro q-tof Micromass (3000 V) with an internal lock mass (H_3PO_4) and an external lock mass (Leu-enkephalin).

Melting points were determined with a Reichert microscope apparatus and are uncorrected.

Reactions were monitored by TLC using commercial plates of 0.25 mm thickness silica gel on an aluminum support (60 F254 from Merck); plates were visualized by UV fluorescence at 254 nm then revealed with a vanillin solution. Retention factors (R_f) are given for such analyses. Chromatographic purifications were performed on flash silica gel Geduran SI 60 (Merck) 0.040−0.063 mm column chromatography.

Purifications by preparative high performance liquid chromatography (HPLC) were performed if necessary for tested compounds (compounds **34**, **46**, and **50**) on a small amount using a Varian liquid chromatograph (Varian Prep Star 218 equipped with a Varian Pro Star 335 photodiode array detector) and a C18 Varian Dynamax Microsorb 60−8 column (250 mm × 41.4 mm, 8 μm). Detection wavelength and flow rate was indicated for each compound. Solvents were (A) water, 0.1% trifluoroacetic acid (TFA); (B) acetonitrile and experiments were performed in an isocratic mode.

The purity of tested compounds was established to be ≥95% by HPLC analysis using a Dionex liquid chromatograph (TTC-100, 25 °C; P580; UVD340U) and a C18 Dionex Acclaim 120 column (4.6 mm × 250 mm, 5 μm, 120 Å). Detection wavelength was indicated for each compound. Solvents were (A) water, 0.1% TFA; (B) acetonitrile; two methods were used: method I, 95:5 A/B for 5 min then 95:5 A/B to 5:95 A/B in 20 min and then 5:95 A/B for 5 min; 0.5 mL/min; method II, 70:30 A/B in an isocratic mode.

Compounds **15**−**18** were prepared according to the procedure published by MacKay et al.[31] or minor modifications thereof in 96, 83, 86, and 90% yield respectively.

The spectroscopic data for compounds **15**−**17** were identical to those described in literature.[14,31]

Compounds **19**−**21** were prepared according to procedure described by MacKay et al.[31] or minor modifications thereof in 99, 98, and 98% yield, respectively, and following the procedure reported by Fresneda et al.[14] for compound **22** which was obtained in 72% yield.

The spectroscopic data obtained for compounds **19**−**21** were identical to those described in literature.[14,31,32]

Compounds **23**, **25**, and **26** were prepared according to a procedure described by Fresneda et al.[14] (DMF-DMA in DMF) or by Rossignol et al.[8] (DMF/di-*tert*-butylacetal in DMF) for **24**. Compound **25** was obtained in 45% yield. Compounds **23**, **24**, and **26** were not purified before the following cyclization step.

The spectroscopic data obtained for compound **25** were identical to those described in literature.[14]

Compounds **27**, **28**, **29**, and **30** were prepared according to a procedure described by Fresneda et al.[14] or minor modifications thereof and were obtained in 48% (from **19**), 48% (from **20**), 47% and 27% (from **22**) yield, respectively.

The spectroscopic data obtained for compounds **27**, **28**, and **29** were identical to those described in literature.[1,14,19,33]

Compounds **31**−**34** were prepared according to a procedure described by Akue-Gedu et al.[10] and Jovanovic et al.[34] or minor modifications thereof.

Compounds **35**−**50** were prepared according to a procedure described by Rossignol et al.[8] or minor modifications thereof.

Compounds **51**−**52** were prepared according to a procedure described by Yeung et al.[17] and Okauchi et al.[18] or minor modifications thereof.

Compounds **53**−**55** and **58**−**59** were prepared according to procedure described by Fresneda et al.[14] or minor modifications thereof in 100%, 99%, 87%, 59% (2 steps), and 89% yield, respectively.

The spectroscopic data obtained for compound **59** were identical to those described in literature.[10]

Compounds **56**−**57** were prepared according to procedure described by Simon et al.[19] or minor modifications thereof in 93% and 100% yield, respectively.

Compounds **60**−**64** were prepared according to a procedure described by Fresneda et al.[14] (DMF-DMA in DMF). Compound **63** was obtained in 43% yield. Compounds **60**, **61**, **62**, and **64** were not purified before the following cyclization step.

The spectroscopic data obtained for compound **64** were identical to those described in literature.[10]

Compounds **65**−**69** were prepared according to a procedure described by Fresneda et al.[14] Compound **67** was obtained in 18% yield (from **57**). Compound **68** was obtained in 40% yield. Compounds **65**, **66**, and **69** were not purified before the following cyclization step.

The spectroscopic data obtained for compound **69** were identical to those described in literature.[10]

Compounds **70**−**74** were prepared according to a procedure described by Akue-Gedu et al.[10] and Jovanovic et al.[34] or minor modifications thereof. Compounds **70**, **71**, and **74** were obtained in 29%, 20%, and 29% yield from **55**, **56**, and **59**, respectively. Compounds **72** and **73** were obtained in 80% and 70% yield.

Compounds **75**−**78** were prepared according to a procedure described by Owa et al.[20] or minor modifications thereof in 88% to 99% yield.

*3-Acetyl-7-bromo-1H-indole (**18**).* Compound **18** was obtained as a white powder in 90% yield; mp 190−191 °C; R_f 0.50 (cyclohexane/EtOAc 50:50). ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H, CH_3), 7.12 (t, 1H, J = 8.0 Hz), 7.43 (d, 1H, J = 8.0 Hz), 8.19 (d, 1H, J = 8.0 Hz), 8.35 (s, 1H), 12.15 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 27.3 (CH_3), 104.5 (2C), 117.5 (C), 120.6 (CH), 123.0 (CH), 125.3 (CH), 126.9 (C), 135.1 (CH), 192.8 (CO). IR (ATR): 3200, 1631, 1432. HRMS: m/z $[M + Na]^+$ calcd for $C_{10}H_8\,^{79}BrNNaO$, 259.9687; found, 259.9691.

*N-[4-(Methylphenyl)sulfonyl]-3-acetyl-7-bromo-1H-indole (**22**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 85:15) provided **22** as a white powder in 72% yield; mp 159−160 °C; R_f 0.50 (cyclohexane/EtOAc 75:25). ^1H NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 2.64 (s, 3H, $COCH_3$), 7.27 (t, 1H, J = 8.0 Hz), 7.46 (d, 2H, J = 8.0 Hz), 7.58 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 7.89 (d, 2H, J = 8.0 Hz), 8.35 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 8.91 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.0 (CH_3), 27.9 ($COCH_3$), 118.7 (C), 131.6 (CH), 126.2 (CH), 127.3 (2CH), 130.1 (2CH), 131.1 (C), 131.4 (CH), 132.9 (C), 135.6 (C), 138.3 (CH), 145.6 (2C), 193.6 (CO). IR (ATR):

1672, 1365, 1169. HRMS: m/z $[M + Na]^+$ calcd for $C_{17}H_{14}{}^{79}BrNNaO_3S$, 413.9775; found, 413.9785.

*4-(4-Bromo-1H-indol-3-yl)pyrimidin-2-amine (**27**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH from 98:1 to 98:2) provided **27** as a pale-yellow powder in 48% yield from compound **19**; mp 218−221 °C; R_f 0.50 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.43 (br s, 2H, NH_2), 6.74 (d, 1H, J = 4.8 Hz), 7.07 (t, 1H, J = 8.0 Hz), 7.29 (d, 1H, J = 7.2 Hz), 7.49 (d, 1H, J = 8.0 Hz), 7.70 (s, 1H), 8.16 (d, 1H, J = 4.8 Hz), 11.84 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 111.6 (CH), 112.0 (CH), 113.0 (C), 116.1 (C), 122.7 (CH), 123.7 (C), 124.5 (CH), 128.6 (CH), 137.8 (C), 156.4 (CH), 161.9 (C), 163.0 (C). IR (ATR): 3408, 3306, 3161, 1647, 1577, 1527, 1465. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{10}{}^{79}BrN_4$, 289.0089; found, 289.0087. HPLC (method I): purity >99%, λ = 228 nm, t_R = 20.4 min.

*4-(5-Bromo-1H-indol-3-yl)pyrimidin-2-amine (**28**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH from 98:2 to 90:10) provided **28** as a pale-yellow powder in 48% yield from compound **20**; mp 100−102 °C; R_f 0.50 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.49 (br s, 2H, NH_2), 7.00 (d, 1H, J = 5.2 Hz), 7.28 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.40 (d, 1H, J = 8.8 Hz), 8.10 (d, 1H, J = 5.2 Hz), 8.25 (d, 1H, J = 2.4 Hz), 8.75 (d, 1H, J = 1.6 Hz), 11.85 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 105.3 (CH), 113.1 (C), 113.5 (C), 114.0 (CH), 124.7 (C), 124.9 (C), 126.9 (CH), 130.8 (C), 135.7 (CH), 155.4 (C), 162.3 (CH), 163.0 (CH). IR (ATR): 3383, 3371, 3120, 1575, 1452, 1419. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{10}{}^{79}BrN_4$, 289.0089; found, 289.0092. HPLC (method I): purity >99%, λ = 228 nm, t_R = 20.9 min.

*4-(6-Bromo-1H-indol-3-yl)pyrimidin-2-amine (**29**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH from 98:1 to 98:2) provided **29** as a pale-yellow powder in 47% yield from compound **25**; mp 219−222 °C; R_f 0.50 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.46 (br s, 2H, NH_2), 7.01 (d, 1H, J = 5.2 Hz), 7.24 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.6 Hz), 7.63 (d, 1H, J = 1.6 Hz), 8.12 (d, 1H, J = 5.2 Hz), 8.23 (d, 1H, J = 2.8 Hz), 8.56 (d, 1H, J = 8.8 Hz), 11.78 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 105.5 (CH), 114.0 (C), 114.6 (CH), 114.9 (C), 123.2 (CH), 124.4 (CH), 124.6 (C), 129.5 (CH), 138.1 (C), 157.5 (CH), 162.4 (CH), 163.7 (C). IR (ATR): 3433, 3321, 3171, 1660, 1570, 1516, 1448. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{10}{}^{79}BrN_4$, 289.0089; found, 289.0091. HPLC (method I): purity >96%, λ = 228 nm, t_R = 21.6 min.

*4-(7-Bromo-1H-indol-3-yl)pyrimidin-2-amine (**30**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH from 98:1 to 98:2) provided **30** as a yellow powder in 27% yield from compound **22**; mp 218−219 °C; R_f 0.10 (CH_2Cl_2/MeOH 98:2). ^{1}H NMR (DMSO-d_6): δ 6.47 (br s, 2H, NH_2), 7.05−7.09 (m, 2H), 7.40 (d, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 8.0 Hz), 8.25 (d, 1H, J = 8.0 Hz), 8.64 (d, 1H, J = 8.0 Hz), 11.87 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 104.3 (C), 105.4 (CH), 114.8 (C), 121.5 (CH), 121.9 (CH), 124.5 (CH), 127.0 (C), 129.1 (CH), 135.2 (C), 157.1 (CH), 162.0 (C), 163.4 (C). IR (ATR): 1575, 1516, 1426, 1166. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{10}{}^{79}BrN_4$, 289.0089; found, 289.0087.

*5-Iodo-4-(4-bromo-1H-indol-3-yl)pyrimidin-2-amine (**31**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 40:60) provided **31** as a yellow powder in 86% yield; mp 245−248 °C; R_f 0.45 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 6.75 (br s, 2H, NH_2), 7.05 (t, 1H, J =8.0 Hz), 7.21 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 7.46 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 7.53 (d, 1H, J = 2.8 Hz), 8.45 (s, 1H), 11.67 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 83.9 (C), 111.3 (CH), 113.0 (C), 117.1 (C), 122.6 (CH), 123.4 (CH), 124.3 (C), 126.6 (CH), 136.5 (C), 162.0 (C), 163.1 (CH), 165.4 (C). IR (ATR): 3399, 3308, 3173, 1645, 1558, 1548, 1512. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9{}^{79}BrI\,N_4$, 414.9055; found, 414.9070. HPLC (method I): purity >99%, λ = 250 nm, t_R = 23.3 min.

*5-Iodo-4-(5-bromo-1H-indol-3-yl)pyrimidin-2-amine (**32**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **32** as a yellow powder in 86% yield; mp 203−205 °C; R_f 0.55 (cyclohexane/EtOAc 50:50). ^{1}H NMR (DMSO-d_6): δ 6.80 (br s, 2H, NH_2), 7.29 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.43 (d, 1H, J = 8.4 Hz), 8.38 (d, 1H, J = 2.0 Hz), 8.45 (s, 1H), 8.51 (s, 1H), 11.84 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 74.8 (C), 113.1 (C), 113.3 (C), 113.8 (CH), 124.3 (CH), 124.7 (CH), 127.8 (C), 130.7 (CH), 134.6 (C), 161.7 (C), 162.1 (C), 166.1 (CH). IR (ATR): 3365, 3319, 3174, 1645, 1550, 1537, 1518, 1458. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9{}^{79}BrI\,N_4$, 414.9055; found, 414.9070. HPLC (method I): purity >99%, λ = 250 nm, t_R = 22.9 min.

*5-Iodo-4-(6-bromo-1H-indol-3-yl)pyrimidin-2-amine (**33**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **33** as a yellow powder in 71% yield; mp 209−210 °C; R_f 0.70 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 6.75 (br s, 2H, NH_2), 7.22 (dd, 1H, J_1 = 8.6 Hz, J_2 = 1.6 Hz), 7.65 (d, 1H, J = 1.6 Hz), 8.25 (d, 1H, J = 8.8 Hz), 8.45 (d, 1H, J = 2.8 Hz), 8.50 (s, 1H), 11.76 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 74.8 (C), 113.9 (C), 114.4 (CH), 114.7 (C), 123.0 (CH), 124.3 (CH), 125.2 (C), 130.3 (CH), 136.8 (C), 161.7 (C), 162.0 (C), 165.8 (CH). IR (ATR): 3396, 3306, 3157, 1552, 1539, 1516, 1450. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9{}^{79}BrI\,N_4$, 414.9055; found, 414.9069. HPLC (method I): purity >99%, λ = 250 nm, t_R = 23.2 min.

*5-Iodo-4-(7-bromo-1H-indol-3-yl)pyrimidin-2-amine (**34**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 60:40) provided **34** as a yellow powder in 66% yield. Purification of a small amount was performed by preparative HPLC (system: 25:75 A/B, flow 40 mL/min, λ = 254 nm, t_R = 5.6 min); mp 236−237 °C; R_f 0.50 (cyclohexane/EtOAc 60:40). ^{1}H NMR (DMSO-d_6): δ 6.78 (br s, 2H, NH_2), 7.07 (t, 1H, J = 8.0 Hz), 7.41 (d, 1H, J = 7.2 Hz), 8.28 (d, 1H, J = 8.0 Hz), 8.40 (d, 1H, J = 3.2 Hz), 8.52 (s, 1H), 11.89 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 75.1 (C), 104.3 (C), 114.9 (C), 121.6 (CH), 121.9 (CH), 124.7 (CH), 127.8 (C), 130.1 (CH), 134.2 (C), 161.7 (C), 162.0 (C), 165.9 (CH). IR (ATR): 3485, 3300, 3171, 1551, 1539, 1515, 1176. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9{}^{79}BrI\,N_4$, 414.9055; found, 414.9075. HPLC (method I): purity >99%, λ = 250 nm, t_R = 23.4 min.

*4-(4-Bromo-1H-indol-3-yl)-5-(4-acetylphenyl)pyrimidin-2-amine (**35**).* Purification of the crude residue by flash chromatography (EtOAc then EtOAc/MeOH 95:5) provided **35** as a yellow powder in 34% yield; mp 245−246 °C; R_f 0.25 (cyclohexane/EtOAc 20:80). ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 6.77 (br s, 2H, NH_2), 6.98 (t, 1H, J = 7.6 Hz), 7.14 (d, 1H, J = 7.6 Hz), 7.28 (d, 2H, J = 8.4 Hz), 7.31 (d, 1H, J = 2.8 Hz), 7.39 (d, 1H, J = 8.0 Hz), 7.72 (d, 2H, J = 8.4 Hz), 8.29 (s, 1H), 11.51 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 26.5 (CH_3), 111.3 (CH), 112.9 (C), 114.3 (C), 122.6 (CH), 123.4 (CH), 123.9 (C), 124.9 (C), 127.8 (2CH), 128.9 (2CH), 134.4 (C), 136.7 (3C), 141.8 (CH), 145.5 (CH), 148.4 (C), 197.3 (CO). IR (ATR): 1652, 1601, 1569, 1464, 1183. HRMS: m/z $[M + H]^+$ calcd for $C_{20}H_{16}{}^{79}BrN_4O$, 407.0507; found, 407.0507. HPLC (method I): purity >96%, λ = 230 nm, t_R = 21.8 min.

*4-(5-Bromo-1H-indol-3-yl)-5-(4-acetylphenyl)pyrimidin-2-amine (**36**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 30:70) provided **36** as a yellow powder in 57% yield; mp 186−187 °C; R_f 0.45 (cyclohexane/EtOAc 20:80). ^{1}H NMR (DMSO-d_6): δ 2.59 (s, 3H, CH_3), 6.77−6.79 (m, 3H), 7.24 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.6 Hz), 7.33 (d, 1H, J = 8.4 Hz), 7.42 (d, 2H, J = 8.4 Hz), 7.95 (d, 2H, J = 8.4 Hz), 8.09 (s, 1H), 8.38 (d, 1H, J = 1.6 Hz), 11.47 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 26.6 (CH_3), 112.2 (C), 112.9 (C), 113.5 (CH), 120.0 (C), 124.2 (CH), 124.5 (CH), 127.6 (C), 128.6 (2CH), 129.5 (2CH), 130.1 (CH), 134.5 (C), 135.2 (C), 143.6 (C), 158.3 (C), 159.2 (CH), 162.6 (C), 197.3 (CO). IR (ATR): 1699, 1600, 1538, 1524, 1458. HRMS: m/z $[M + H]^+$ calcd for $C_{20}H_{16}{}^{79}BrN_4O$, 407.0507; found, 407.0500. HPLC (method I): purity >98%, λ = 230 nm, t_R = 22.7 min.

*4-(6-Bromo-1H-indol-3-yl)-5-(4-acetylphenyl)pyrimidin-2-amine (**37**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50 then 40:60) provided **37** as a yellow powder in 58% yield; mp 249−250 °C; R_f 0.30 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR (DMSO-d_6): δ 2.59 (s, 3H, CH_3), 6.74 (br s, 2H, NH_2), 6.76 (d, 1H, J = 2.8 Hz), 7.16 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.0 Hz), 7.43 (d, 2H, J = 8.4 Hz), 7.55 (d, 1H, J = 2.0 Hz), 7.96 (d, 2H, J = 8.4 Hz), 8.09 (s, 1H), 8.26 (d, 1H, J = 8.8 Hz), 11.38 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 26.6 (CH_3), 113.6 (C), 113.9 (C), 116.8 (CH), 117.0 (C), 118.1 (CH), 120.8 (CH), 121.2 (C), 123.0 (C), 128.0 (C), 128.7 (CH), 129.6 (2CH), 131.3 (2CH), 132.5 (C), 132.6 (CH), 133.6 (C), 148.4 (C), 197.3 (CO). IR (ATR): 1677, 1598, 1532, 1457, 1136. HRMS: m/z $[M + H]^+$ calcd for $C_{20}H_{16}{}^{79}BrN_4O$, 407.0507; found, 407.0502. HPLC (method I): purity >95%, λ = 228 nm, t_R = 22.8 min.

*4-(7-Bromo-1H-indol-3-yl)-5-(4-acetylphenyl)pyrimidin-2-amine (**38**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **38** as a yellow powder in 75% yield; mp 220−221 °C; R_f 0.30 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 2.59 (s, 3H, CH_3), 6.74 (d, 1H, J = 2.8 Hz), 6.77 (br s, 2H, NH_2), 7.02 (t, 1H, J = 8.0 Hz), 7.36 (d, 1H, J = 8.0 Hz), 7.44 (d, 2H, J = 8.0 Hz), 7.96 (d, 2H, J = 8.0 Hz), 8.12 (s, 1H), 8.33 (d, 1H, J = 8.0 Hz), 11.51 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 26.7 (CH_3), 104.2 (C), 113.8 (C), 117.4 (CH), 120.3 (C), 121.5 (CH), 121.9 (CH), 124.6 (CH), 127.7 (C), 128.7 (2CH), 129.6 (2CH), 130.6 (CH), 132.7 (C), 133.7 (C), 134.2 (C), 135.3 (C), 143.5 (C), 197.4 (CO). IR (ATR): 1682, 1578, 1536, 1455, 1141. HRMS: m/z $[M + H]^+$ calcd for $C_{20}H_{16}{}^{79}BrN_4O$, 407.0507; found, 407.0508. HPLC (method I): purity >96%, λ = 228 nm, t_R = 22.8 min.

*4-[2-Amino-4-(4-bromo-1H-indol-3-yl)pyrimidin-5-yl]benzamide (**39**).* Purification of the crude residue by flash chromatography (EtOAc/MeOH 95:5) provided **39** as a powder yellow in 41% yield; mp > 250 °C; R_f 0.15 (EtOAc/MeOH 95:5). ^{1}H NMR (DMSO-d_6): δ 6.73 (br s, 2H, NH_2), 6.98 (t, 1H, J = 8.0 Hz), 7.13 (d, 1H, J = 8.0 Hz), 7.19 (d, 2H, J = 8.0 Hz), 7.25 (br s, 1H, $CONH_2$), 7.30 (d, 1H, J = 2.8 Hz), 7.38 (d, 1H, J = 8.0 Hz), 7.62 (d, 2H, J = 8.0 Hz), 7.81 (br s, 1H, $CONH_2$), 8.28 (s, 1H), 11.51 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 111.2 (CH), 112.9 (C), 114.5 (C), 122.4 (CH), 123.2 (CH), 124.1 (C), 125.0 (C), 126.8 (CH), 126.9 (2CH), 128.5 (2CH), 131.7 (C), 136.6 (C), 140.7 (C), 157.5 (CH), 160.9 (C), 162.1 (C), 167.4 (CO). IR (ATR): 3400−3100, 1670, 1579, 1549, 1513, 1476, 1194. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}{}^{79}BrN_5O$, 408.0460; found, 408.0467. HPLC (method I): purity >99%, λ = 230 nm, t_R = 19.6 min.

*4-[2-Amino-4-(5-bromo-1H-indol-3-yl)pyrimidin-5-yl]benzamide (**40**).* Purification of the crude residue by flash chromatography (EtOAc/MeOH 95:5) provided **40** as a powder in 51% yield; mp 174−175 °C; R_f 0.55 (EtOAc/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.70−6.72 (m, 3H), 7.24 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.32−7.36 (m, 4H), 7.89 (d, 2H, J = 8.4 Hz), 7.99 (br s, 1H, $CONH_2$), 8.07 (s, 1H), 8.46 (d, 1H, J = 2.0 Hz), 11.46 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 112.2 (C), 112.9 (C), 113.5 (CH), 120.3 (C), 124.4 (CH), 124.5 (CH), 127.7 (C), 127.9 (2CH), 129.1 (2CH), 130.1 (CH), 132.6 (C), 134.5 (C), 141.6 (C), 158.2 (CH), 159.2 (C), 162.6 (C), 167.3 (CO). IR (ATR): 3410−3175, 1680, 1608, 1585, 1533, 1456, 1149. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}{}^{79}BrN_5O$, 408.0460; found, 408.0475. HPLC (method I): purity >99%, λ = 230 nm, t_R = 20.8 min.

*4-[2-Amino-4-(6-bromo-1H-indol-3-yl)pyrimidin-5-yl]benzamide (**41**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 90:10) provided **41** as a yellow powder in 41% yield; mp 176−177 °C; R_f 0.35 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.69−6.72 (m, 3H), 7.16 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.6 Hz), 7.35 (d, 2H, J = 8.0 Hz), 7.38 (br s, 1H, $CONH_2$), 7.55 (d, 1H, J = 1.6 Hz), 7.89 (d, 2H, J = 8.0 Hz), 8.00 (br s, 1H, $CONH_2$), 8.06 (s, 1H), 8.31 (d, 1H, J = 8.4 Hz), 11.39 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 112.8 (C), 114.1 (CH), 114.5 (C), 120.3 (C), 124.7 (CH), 124.5 (CH), 125.1 (C), 127.9 (2CH), 129.1 (2CH), 129.6 (CH), 132.6 (C), 136.7 (C), 141.6 (C), 158.1 (CH), 159.2 (C), 162.6 (C), 167.4 (CO). IR (ATR): 1665, 1610, 1582, 1536, 1462. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}{}^{79}BrN_5O$, 408.0460; found, 408.0477. HPLC (method I): purity >99%, λ = 230 nm, t_R = 20.9 min.

*4-[2-Amino-4-(7-bromo-1H-indol-3-yl)pyrimidin-5-yl]benzamide (**42**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 95:5 to 90:10) provided **42** as a yellow powder in 46% yield; mp 173−174 °C; R_f 0.15 (CH_2Cl_2/MeOH 95:5). ^{1}H NMR (DMSO-d_6): δ 6.68 (d, 1H, J = 3.2 Hz), 6.72 (br s, 2H, NH_2), 7.02 (t, 1H, J = 7.6 Hz), 7.35−7.38 (m, 4H), 7.90 (d, 2H, J = 8.0 Hz), 8.01 (br s, 1H, $CONH_2$), 8.09 (s, 1H), 8.39 (d, 1H, J = 8.0 Hz), 11.50 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 104.0 (C), 113.8 (C), 120.5 (C), 121.4 (CH), 122.0 (CH), 124.4 (CH), 127.8 (C), 127.9 (2CH), 129.2 (2CH), 129.6 (CH), 132.7 (C), 134.1 (C), 141.6 (C), 158.2 (CH), 159.2 (C), 162.6 (C), 167.4 (CO). IR (ATR): 3400−3110, 1657, 1608, 1582, 1531, 1459, 1132. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{15}{}^{79}BrN_5O$, 408.0460; found, 408.0475. HPLC (method I): purity >98%, λ = 230 nm, t_R = 20.7 min.

*4-(4-Bromo-1H-indol-3-yl)-5-(4-trifluoromethylphenyl)pyrimidin-2-amine (**43**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 95:5) provided **43** as a white powder in 43% yield; mp > 250 °C; R_f 0.30 (cyclohexane/EtOAc 20:80). ^{1}H NMR (DMSO-d_6): δ 6.79 (br s, 2H, NH_2), 6.99 (t, 1H, J = 8.0 Hz), 7.14 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 7.31 (d, 1H, J = 8.0 Hz), 7.34 (d, 2H, J = 8.0 Hz), 7.39 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 7.51 (d, 2H, J = 8.0 Hz), 8.29 (s, 1H), 11.53 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 111.2 (CH), 112.8 (C), 114.2 (C), 122.5 (CH), 123.3 (CH), 123.4 (C), 124.6 (2CH, q, J_{CF} = 3,8 Hz), 125.1 (C, q, J_{CF} = 270 Hz), 125.6 (C), 126.4 (C, q, J_{CF} = 32 Hz), 126.9 (2CH), 129.4 (CH), 136.6 (C), 141.9 (C), 157.6 (CH), 161.0 (C), 162.2 (C). IR (ATR): 1575, 1515, 1472, 1319, 1110. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{13}{}^{79}BrF_3N_4$, 433.0276; found, 433.0290. HPLC (method I): purity >99%, λ = 230 nm, t_R = 24.5 min.

*4-(5-Bromo-1H-indol-3-yl)-5-(4-trifluoromethylphenyl)pyrimidin-2-amine (**44**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **44** as a white powder in 63% yield; mp 108−109 °C; R_f 0.40 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 6.78−6.79 (m, 3H), 7.24 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.0 Hz), 7.33 (d, 1H, J = 8.8 Hz), 7.50 (d, 2H, J = 8.0 Hz), 7.72 (d, 2H, J = 8.0 Hz), 8.12 (s, 1H), 8.32 (d, 1H, J = 2.0 Hz), 11.51 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 112.3 (C), 113.1 (C), 113.6 (CH), 119.8 (C), 124.0 (C, q, J_{CF} = 270 Hz), 124.2 (CH), 124.6 (CH), 125.5 (CH), 125.7 (2CH, q, J_{CF} = 4.0 Hz), 127.7 (C), 129.5 (C, q, J_{CF} = 32 Hz), 130.2 (2CH), 134.5 (C), 143.2 (C), 158.5 (CH), 159.3 (C), 162.8 (C). IR (ATR): 3428−3199, 1579, 1530, 1457, 1323, 1123. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{13}{}^{79}BrF_3N_4$, 433.0276; found, 433.0269. HPLC (method I): purity >95%, λ = 230 nm, t_R = 24.7 min.

*4-(6-Bromo-1H-indol-3-yl)-5-(4-trifluoromethylphenyl)pyrimidin-2-amine (**45**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **45** as a yellow powder in 86% yield; mp 201−202 °C; R_f 0.30 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 6.73 (d, 1H, J = 2.8 Hz), 6.75 (br s, 2H, NH_2), 7.16 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.50 (d, 2H, J = 8.0 Hz), 7.55 (d, 1H, J = 1.2 Hz), 7.73 (d, 2H, J = 8.0 Hz), 8.10 (s, 1H), 8.25 (d, 1H, J = 8.8 Hz), 11.42 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 112.6 (C), 114.2 (CH), 114.6 (C), 119.6 (C), 122.8 (CH), 124.1 (CH), 124.9 (C), 125.5 (2CH, q, J_{CF} = 3.8 Hz), 127.0 (C, q, J_{CF} = 271 Hz), 127.4 (C, q, J_{CF} = 31 Hz), 129.8 (CH), 130.1 (3CH), 131.8 (C), 136.7 (C), 159.5 (C), 162.8 (C). IR (ATR): 3329−3224, 1579, 1532, 1458, 1324, 1115. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{13}{}^{79}BrF_3N_4$, 433.0276; found, 433.0259. HPLC (method I): purity >99%, λ = 230 nm, t_R = 24.8 min.

*4-(7-Bromo-1H-indol-3-yl)-5-(4-trifluoromethylphenyl)pyrimidin-2-amine (**46**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 99:1 to 97:3) provided **46** as a yellow powder in 71% yield. Purification of a small amount was performed by preparative

HPLC (system: 30:70 A/B, flow 38 mL/min, λ = 228 nm, t_R = 5.8 min); mp 104−105 °C; R_f 0.45 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 6.73 (d, 1H, J = 2.8 Hz), 6.78 (br s, 2H, NH_2), 7.01 (t, 1H, J = 7.6 Hz), 7.36 (d, 1H, J = 7.2 Hz), 7.52 (d, 2H, J = 8.4 Hz), 7.74 (d, 2H, J = 8.4 Hz), 8.13 (s, 1H), 8.31 (d, 1H, J = 8.0 Hz), 11.54 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 113.7 (C), 119.8 (C), 120.6 (CH), 122.2 (CH), 124.2 (C, q, J_{CF} = 271 Hz), 124.5 (CH), 125.5 (2CH, q, J_{CF} = 4.0 Hz), 127.6 (C), 127.8 (C, q, J_{CF} = 30 Hz), 128.5 (C), 129.6 (CH), 130.2 (CH), 134.2 (C), 138.7 (2CH), 142.9 (C), 164.5 (C), 179.5 (C). IR (ATR): 1576, 1538, 1456, 1322, 1123. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{13}{}^{79}BrF_3N_4$, 433.0276; found, 433.0295. HPLC (method I): purity >99%, λ = 230 nm, t_R = 25.21 min.

*4-(4-Bromo-1H-indol-3-yl)-5-(3-methoxyphenyl)pyrimidin-2-amine (**47**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 40:60) provided **47** as a yellow powder in 57% yield; mp 224−225 °C; R_f 0.25 (cyclohexane/EtOAc 20:80). ^{1}H NMR (DMSO-d_6): δ 3.32 (s, 3H, OCH_3), 6.63−6.68 (m, 4H), 6.74 (dd, 1H, J_1 = 7.6 Hz, J_2 = 1.2 Hz), 6.99 (t, 1H, J = 8.0 Hz), 7.06 (t, 1H, J = 8.0 Hz), 7.14 (d, 1H, J = 8.0 Hz), 7.27 (d, 1H, J = 2.8 Hz), 7.39 (dd, 1H, J_1 = 8.0 Hz, J_2 = 0.8 Hz), 8.25 (s, 1H), 11.45 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 54.5 (OCH_3), 111.2 (CH), 111.9 (CH), 112.9 (2C), 114.3 (CH), 121.1 (2CH), 122.4 (CH), 123.2 (CH), 124.8 (C), 125.2 (C), 128.8 (2CH), 136.6 (2C), 138.7 (C), 158.5 (2C). IR (ATR): 1599, 1576, 1467, 1196. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{16}{}^{79}BrN_4O$, 395.0507; found, 395.0510. HPLC (method I): purity >98%, λ = 230 nm, t_R = 22.8 min.

*4-(5-Bromo-1H-indol-3-yl)-5-(3-methoxyphenyl)pyrimidin-2-amine (**48**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **48** as a yellow powder in 69% yield; mp 166−167 °C; R_f 0.40 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 6.65 (br s, 2H, NH_2), 6.74 (d, 1H, J = 2.8 Hz), 6.82−6.85 (m, 2H), 6.93 (dd, 1H, J_1 = 7.6 Hz, J_2 = 2.0 Hz), 7.24 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.29−7.34 (m, 2H), 8.03 (s, 1H), 8.52 (d, 1H, J = 2.0 Hz), 11.46 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 55.0 (OCH_3), 112.3 (C), 112.7 (CH), 113.1 (C), 113.5 (CH), 114.9 (CH), 120.8 (C), 121.7 (CH), 124.5 (CH), 124.6 (CH), 127.8 (C), 129.8 (CH), 130.2 (CH), 134.5 (C), 139.9 (C), 157.6 (CH), 159.4 (C), 159.5 (C), 162.2 (C). IR (ATR): 3339−3210, 1587, 1528, 1471. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{16}{}^{79}BrN_4O$, 395.0507; found, 395.0500. HPLC (method I): purity >99%, λ = 230 nm, t_R = 23.5 min.

*4-(6-Bromo-1H-indol-3-yl)-5-(3-methoxyphenyl)pyrimidin-2-amine (**49**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **49** as a yellow powder in 92% yield; mp 156−157 °C; R_f 0.30 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 6.62 (br s, 2H, NH_2), 6.71 (d, 1H, J = 2.8 Hz), 6.82−6.85 (m, 2H), 6.93 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.6 Hz), 7.17 (dd, 1H, J_1 = 8.8 Hz, J_2 = 1.6 Hz), 7.31 (t, 1H, J = 8.0 Hz), 7.54 (d, 1H, J = 1.6 Hz), 8.02 (s, 1H), 8.40 (d, 1H, J = 8.8 Hz), 11.38 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 55.0 (OCH_3), 112.7 (CH), 112.8 (C), 114.0 (CH), 114.5 (C), 114.8 (CH), 120.8 (C), 121.7 (CH), 122.7 (CH), 124.5 (CH), 125.2 (C), 129.6 (CH), 129.8 (CH), 136.6 (C), 139.9 (C), 157.9 (CH), 159.2 (C), 159.5 (C), 162.3 (C). IR (ATR): 1577, 1538, 1457, 1133. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{16}{}^{79}BrN_4O$, 395.0507; found, 395.0507. HPLC (method I): purity >96%, λ = 230 nm, t_R = 23.6 min.

*4-(7-Bromo-1H-indol-3-yl)-5-(3-methoxyphenyl)pyrimidin-2-amine (**50**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 60:40 then 50:50) provided **50** as a yellow powder in 71% yield. Purification of a small amount was performed by preparative HPLC (system: 30:70 A/B, flow 38 mL/min, λ = 228 nm, t_R = 4.8 min); mp 163−164 °C; R_f 0.30 (cyclohexane/EtOAc 40:60). ^{1}H NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 6.64 (br s, 2H, NH_2), 6.73 (d, 1H, J = 2.8 Hz), 6.83−6,87 (m, 2H), 6.94 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.8 Hz), 7.02 (t, 1H, J = 7.6 Hz), 7.32 (t, 1H, J = 8.0 Hz), 7.36 (d, 1H, J = 7.6 Hz), 8.04 (s, 1H), 8.49 (d, 1H, J = 8.0 Hz), 11.48 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 55.0 (OCH_3), 103.9 (C), 112.7 (CH), 113.8 (C), 114.8 (CH), 120.9 (C), 121.4 (CH), 121.7 (CH), 122.3 (CH), 124.4 (CH), 127.9 (C), 129.6 (CH), 129.8 (CH), 134.1 (C), 139.9 (C), 158.0 (CH), 159.1 (C), 159.5 (C), 162.4 (C). IR (ATR): 1595, 1578, 1464, 1163. HRMS: m/z $[M + H]^+$ calcd for $C_{19}H_{16}{}^{79}BrN_4O$, 395.0507; found, 395.0501. HPLC (method I): purity >98%, λ = 230 nm, t_R = 23.7 min.

*3-Acetyl-4-nitro-1H-indole (**51**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 20:80) provided **51** as a yellow powder in 97% yield; mp 218−219 °C; R_f 0.30 (cyclohexane/EtOAc 20:80). ^{1}H NMR (DMSO-d_6): δ 2.47 (s, 3H, CH_3), 7.38 (t, 1H, J = 8.0 Hz), 7.59 (d, 1H, J = 8.0 Hz), 7.80 (d, 1H, J = 8.0 Hz), 8.56 (s, 1H), 12.57 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 27.5 ($COCH_3$), 115.2 (C), 116.3 (C), 116.6 (2CH), 122.5 (CH), 136.4 (CH), 138.7 (C), 144.1 (C), 190.9 (CO). IR (ATR): 3231, 1638, 1623, 1514, 1313, 1167. HRMS: m/z $[M + Na]^+$ calcd for $C_{10}H_8N_2$ NaO_3, 227.0433; found, 227.0421.

*3-Acetyl-7-nitro-1H-indole (**52**).* Compound **52** was obtained as a sufficiently pure brown powder and directly used in the next step; mp 228−229 °C; R_f 0.40 (cyclohexane/EtOAc 50:50). ^{1}H NMR (DMSO-d_6): δ 2.53 (s, 3H, CH_3), 7.42 (t, 1H, J = 8.0 Hz), 8.19 (d, 1H, J = 8.0 Hz), 8.44 (s, 1H), 8.66 (d, 1H, J = 8.0 Hz), 12.56 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 27.5 (CH_3), 117.3 (2C), 119.7 (CH), 121.6 (CH), 128.9 (C), 129.5 (CH), 136.6 (CH), 136.7 (C), 193.2 (CO). IR (ATR): 3284, 1640, 1629, 1534, 1357, 1109. HRMS: m/z $[M + H]^+$ calcd for $C_{10}H_9N_2O_3$, 205.0613; found, 205.0614.

*N-[4-(Methylphenyl)sulfonyl]-5-nitro-1H-indole (**53**).* Purification of the crude residue by flash chromatography (CH_2Cl_2) provided **53** as a white powder in 100% yield; mp 150−151 °C; R_f 0.40 (cyclohexane/EtOAc 80:20). ^{1}H NMR (DMSO-d_6): δ 2.37 (s, 3H, CH_3), 6.82 (d, 1H, J = 3.6 Hz), 7.29 (d, 2H, J = 7.6 Hz), 7.75 (d, 1H, J = 3.6 Hz), 7.81 (d, 2H, J = 8.4 Hz), 8.09 (d, 1H, J = 9.2 Hz), 8.19 (dd, 1H, J_1 = 9.2 Hz, J_2 = 2.0 Hz), 8.46 (d, 1H, J = 2.0 Hz). ^{13}C NMR (DMSO-d_6): δ 21.7 (CH_3), 109.5 (CH), 113.6 (CH), 117.8 (CH), 119.8 (CH), 127.0 (CH), 129.3 (CH), 130.3 (CH), 130.6 (C), 134.7 (C), 137.7 (C), 144.3 (C), 146.0 (C). IR (ATR): 1518, 1441, 1373, 1348, 1172, 1121. HRMS: m/z $[M + Na]^+$ calcd for $C_{15}H_{12}N_2NaO_4S$, 339.0415; found, 339.0417.

*N-[4-(Methylphenyl)sulfonyl]-6-nitro-1H-indole (**54**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 70:30 then 50:50) provided **54** as a yellow powder in 99% yield; mp 196−197 °C; R_f 0.45 (cyclohexane/EtOAc 70:30). ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3), 7.05 (d, 1H, J = 3.6 Hz), 7.41 (d, 2H, J = 8.0 Hz), 7.85 (d, 1H, J = 8.8 Hz), 7.92 (d, 2H, J = 8.0 Hz), 8.13 (dd, 1H, J_1 = 8.8 Hz, J_2 = 1.6 Hz), 8.22 (d, 1H, J = 3.6 Hz), 8.74 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 20.9 (CH_3), 108.8 (CH), 109.5 (CH), 118.5 (CH), 122.4 (CH), 126.7 (2CH), 130.5 (2CH), 132.5 (CH), 132.8 (C), 133.7 (C), 135.4 (C), 144.4 (C), 146.1 (C). IR (ATR): 1527, 1508, 1430, 1364, 1341, 1169, 1099. HRMS: m/z $[M + Na]^+$ calcd for $C_{15}H_{12}N_2Na$-O_4S, 339.0415; found, 339.0410.

*3-Acetyl-N-[4-(methylphenyl)sulfonyl]-4-nitro-1H-indole (**55**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 70:30 then 50:50) provided **55** as a yellow powder in 87% yield; mp 189−190 °C; R_f 0.50 (cyclohexane/EtOAc 70:30). ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 2.61 (s, 3H, $COCH_3$), 7.47 (d, 2H, J = 8.4 Hz), 7.61 (t, 1H, J = 8.0 Hz), 7.84 (d, 1H, J = 8.0 Hz), 8.09 (d, 2H, J = 8.4 Hz), 8.31 (d, 1H, J = 8.0 Hz), 9.00 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.1 (CH_3), 28.4 ($COCH_3$), 117.4 (CH), 117.7 (C), 119.7 (CH), 120.4 (C), 126.1 (CH), 127.1 (2CH), 130.6 (2CH), 132.9 (C), 135.5 (CH), 143.9 (C), 146.9 (2C), 192.3 (CO). IR (ATR): 1672, 1530, 1381, 1173, 1089. HRMS: m/z $[M + Na]^+$ calcd for $C_{17}H_{14}N_2NaO_5S$, 381.0521; found 381.0516.

*3-Acetyl-N-[4-(methylphenyl)sulfonyl]-5-nitro-1H-indole (**56**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 70:30 then 50:50 and finally pure CH_2Cl_2) provided **56** as a white powder in 93% yield; mp 231−232 °C; R_f 0.70 (cyclohexane/EtOAc 60:40). ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.63 (s, 3H,

$COCH_3$), 7.48 (d, 2H, J = 8.4 Hz), 8.10 (d, 2H, J = 8.4 Hz), 8.19 (d, 1H, J = 9.6 Hz), 8.27 (dd, 1H, J_1 = 9.6 Hz, J_2 = 2.0 Hz), 8.98 (d, 1H, J = 2.0 Hz), 9.06 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.0 (CH_3), 27.7 ($COCH_3$), 113.9 (CH), 117.9 (CH), 120.6 (C), 120.7 (CH), 126.9 (C), 127.3 (2CH), 130.6 (2CH), 132.9 (C), 136.8 (C), 136.9 (CH), 144.6 (C), 146.9 (C), 193.7 (CO). IR (ATR): 1680, 1520, 1386, 1343, 1165, 1079. HRMS: m/z $[M + Na]^+$ calcd for $C_{17}H_{14}N_2NaO_5S$, 381.0521; found, 381.0526.

*3-Acetyl-N-[4-(methylphenyl)sulfonyl]-6-nitro-1H-indole (**57**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 70:30 then 50:50) provided **57** as a white powder in 100% yield; mp 200−201 °C; R_f 0.10 (cyclohexane/EtOAc 80:20). ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.63 (s, 3H, $COCH_3$), 7.48 (d, 2H, J = 8.0 Hz), 8.09 (d, 2H, J = 8.0 Hz), 8.25 (dd, 1H, J_1 = 8.8 Hz, J_2 = 0.8 Hz), 8.39 (d, 1H, J = 8.8 Hz), 8.72 (d, 1H, J = 0.8 Hz), 9.17 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.9 (CH_3), 27.9 ($COCH_3$), 108.7 (CH), 119.9 (CH), 120.3 (C), 123.0 (CH), 127.3 (2CH), 130.7 (2CH), 131.8 (C), 132.9 (C), 133.1 (C), 138.5 (CH), 144.9 (C), 146.9 (C), 193.6 (CO). IR (ATR): 1675, 1517, 1385, 1338, 1168, 1088. HRMS: m/z $[M + H]^+$ calcd for $C_{17}H_{15}N_2O_5S$, 359.0702; found, 359.0696.

*3-Acetyl-N-[4-(Methylphenyl)sulfonyl]-7-nitro-1H-indole (**58**).* Purification of the crude residue by flash chromatography (CH_2Cl_2) provided **58** as a yellow powder in 59% yield (2 steps); mp 213-214 °C; R_f 0.75 (CH_2Cl_2). ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 2.64 (s, 3H, $COCH_3$), 7.54 (d, 2H, J = 8.0 Hz), 7.59 (t, 1H, J = 8.0 Hz), 7.93 (d, 1H, J = 8.0 Hz), 8.02 (d, 2H, J = 8.0 Hz), 8.58 (d, 1H, J = 8.0 Hz), 9.13 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 21.1 (CH_3), 28.0 ($COCH_3$), 120.9 (C), 121.5 (CH), 124.5 (C), 125.4 (CH), 127.1 (2CH), 127.2 (CH), 129.9 (2CH), 130.1 (C), 134.0 (C), 138.7 (CH), 146.1 (2C), 193.7 (CO). IR (ATR): 1676, 1535, 1388, 1364, 1169, 1090. HRMS: m/z $[M + Na]^+$ calcd for $C_{17}H_{14}N_2O_5NaS$, 381.0521; found, 381.0533.

*(2E)-3-(Dimethylamino)-1-{7-nitro-1-[4-(methylphenyl)sulfonyl]-1H-indol-3-yl}-2-propen-1-one (**63**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 25:75 then pure EtOAc) provided **63** as a yellow powder in 43% yield; mp 218−219 °C; R_f 0.15 (cyclohexane/EtOAc 25:75). ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 2.97 (br s, 3H, NCH_3), 3.15 (br s, 3H, NCH_3), 5.96 (d, 1H, J = 12.4 Hz), 7.49−7.54 (m, 3H), 7.70 (d, 1H, J = 12.4 Hz), 7.86 (d, 1H, J = 8.0 Hz), 7.91 (d, 2H, J = 8.4 Hz), 8.71 (d, 1H, J = 8.0 Hz), 8.89 (s, 1H). ^{13}C NMR (DMSO-d_6): 21.1 (CH_3), 92.6 (CH), 120.9 (CH), 123.3 (C), 124.6 (CH), 124.8 (C), 126.8 (2CH), 127.9 (CH), 129.9 (2CH), 131.9 (C), 133.9 (CH), 134.3 (C), 138.3 (C), 145.8 (C), 153.3 (CH), 181.3 (CO). IR (ATR): 1644, 1547, 1379, 1367, 1173, 1039. HRMS: m/z $[M + Na]^+$ calcd for $C_{20}H_{19}N_3NaO_5S$, 436.0943; found, 436.0959.

*4-(6-Nitro-1H-indol-3-yl)pyrimidin-2-amine (**67**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 97:3 then 95:5) provided **67** as a yellow powder in 18% yield (2 steps); mp > 250 °C; R_f 0.50 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.46 (br s, 2H, NH_2), 7.07 (d, 1H, J = 5.2 Hz), 7.97 (d, 1H, J = 8.8 Hz), 8.17 (d, 1H, J = 5.2 Hz), 8.38 (s, 1H), 8.60 (s, 1H), 8.80 (d, 1H, J = 8.8 Hz), 12.37 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 105.3 (CH), 108.5 (CH), 114.5 (C), 114.9 (CH), 122.6 (CH), 130.0 (C), 134.0 (CH), 135.5 (C), 142.3 (C), 157.5 (CH), 161.4 (C), 163.5 (C). IR (ATR): 3481−3347, 1582, 1557, 1498, 1456, 1336, 1061. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{10}N_5O_2$, 256.0834; found, 256.0822. HPLC (method I): purity >96%, λ = 228 nm, t_R = 20.2 min.

*4-(7-Nitro-1H-indol-3-yl)pyrimidin-2-amine (**68**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 97:3 then 95:5 and finally 90:10) provided **68** as a orange−yellow powder in 40% yield; mp decomposition; R_f 0.50 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.58 (br s, 2H, NH_2), 7.15 (d, 1H, J = 5.2 Hz), 7.36 (t, 1H, J = 8.0 Hz), 8.17 (d, 1H, J = 5.2 Hz), 8.18 (d, 1H, J = 8.0 Hz), 8.35 (d, 1H, J = 2.0 Hz), 9.20 (d, 1H, J = 8.0 Hz), 12.36 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 105.6 (CH), 115.0 (C), 119.3 (CH), 120.0 (CH), 129.1 (C), 129.3 (C), 130.9 (CH), 131.2 (CH), 132.8 (C), 157.6 (CH), 161.4 (C), 163.4 (C). IR (ATR): 3464−3218, 1568, 1526, 1483, 1365, 1122. HRMS: m/z $[M + H]^+$calcd for $C_{12}H_{10}N_5O_2$, 256.0834; found, 256.0826. HPLC (method I): purity >96%, λ = 228 nm, t_R = 20.4 min.

*5-Iodo-4-(4-nitro-1H-indol-3-yl)pyrimidin-2-amine (**70**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 95:5) provided **70** as a black powder in 29% yield (3 steps); mp 196−197 °C; R_f 0.45 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.67 (br s, 2H, NH_2), 7.36 (t, 1H, J = 8.0 Hz), 7.91−7.96 (m, 3H), 8.44 (s, 1H), 12.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 115.8 (C), 117.2 (C), 117.3 (CH), 118.8 (CH), 120.8 (CH), 131.7 (CH), 138.1 (2C), 141.6 (C), 161.8 (C), 163.4 (CH), 164.8 (C). IR (ATR): 3500−3350, 1647, 1557, 1546, 1509, 1335, 1109. HRMS: m/z $[M + H]^+$calcd for $C_{12}H_9N_5O_2I$, 381.9801; found, 381.9814. HPLC (method I): purity >95%, λ = 230 nm, t_R = 21.8 min.

*5-Iodo-4-(5-nitro-1H-indol-3-yl)pyrimidin-2-amine (**71**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **71** as a yellow powder in 20% yield (3 steps); mp 244−245 °C; R_f 0.50 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.86 (br s, 2H, NH_2), 7.66 (d, 1H, J = 8.8 Hz), 8.07 (d, 1H, J = 8.8 Hz), 8.53 (s, 1H), 8.57 (s, 1H), 8.99 (s, 1H), 12.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 75.5 (C), 112.5 (CH), 115.9 (C), 117.3 (CH), 118.7 (CH), 125.3 (C), 132.5 (CH), 139.0 (C), 141.6 (C), 161.3 (C), 162.2 (C), 166.1 (CH). IR (ATR): 3509, 3324, 1639, 1532, 1506, 1452, 1331, 1121. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9N_5O_2I$, 381.9801; found, 381.9805. HPLC (method I): purity >96%, λ = 230 nm, t_R = 22.4 min.

*5-Iodo-4-(6-nitro-1H-indol-3-yl)pyrimidin-2-amine (**72**).* Purification of the crude residue by flash chromatography (CH_2Cl_2/MeOH 95:5) provided **72** as a orange powder in 80% yield; mp 259−260 °C; R_f 0.55 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.85 (br s, 2H, NH_2), 7.96 (dd, 1H, J = 8.4 Hz, J = 2.4 Hz), 8.39 (d, 1H, J = 8.4 Hz), 8.42 (d, 1H, J = 2.4 Hz), 8.55 (s, 1H), 8.74 (d, 1H, J = 2.4 Hz), 12.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 75.0 (C), 108.9 (CH), 114.7 (C), 114.9 (CH), 122.4 (CH), 130.8 (C), 134.4 (C), 134.9 (CH), 142.4 (C), 161.2 (C), 162.1 (C), 166.1 (CH). IR (ATR): 3350, 3180, 1652, 1553, 1501, 1460, 1343, 1073. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9N_5O_2I$, 381.9801; found, 381.9820. HPLC (method I): purity >99%, λ = 230 nm, t_R = 22.7 min.

*5-Iodo-4-(7-nitro-1H-indol-3-yl)pyrimidin-2-amine (**73**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **73** as a yellow powder in 70% yield; mp 246−247 °C; R_f 0.50 (cyclohexane/EtOAc 50:50). ^{1}H NMR (DMSO-d_6): δ 6.86 (br s, 2H, NH_2), 7.36 (t, 1H, J = 8.0 Hz), 8.19 (d, 1H, J = 8.0 Hz), 8.50 (d, 1H, J = 2.4 Hz), 8.56 (s, 1H), 8.77 (d, 1H, J = 8.0 Hz), 12.36 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 111.3 (C), 115.3 (C), 119.5 (CH), 120.1 (CH), 128.2 (C), 130.1 (C), 131.0 (CH), 131.7 (CH), 132.9 (C), 161.0 (C), 162.2 (C), 166.2 (CH). IR (ATR): 3512−3392, 1611, 1535, 1516, 1480, 1361, 1134. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_9N_5O_2I$, 381.9801; found, 381.9784. HPLC (method II): purity >98%, λ = 230 nm, t_R = 40.7 min.

*5-Iodo-4-(1H-indol-3-yl)pyrimidin-2-amine (**74**).* Purification of the crude residue by flash chromatography (cyclohexane/EtOAc 50:50) provided **74** as a yellow powder in 29% yield (3 steps); mp 167−168 °C; R_f 0.70 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 6.71 (br s, 2H, NH_2), 7.10 (t, 1H, J = 7.2 Hz), 7.18 (t, 1H, J = 7.2 Hz), 7.46 (d, 1H, J = 7.8 Hz), 8.30 (d, 1H, J = 7.8 Hz), 8.42 (d, 1H, J = 2.8 Hz), 8.49 (s, 1H), 11.67 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 74.8 (C), 111.6 (CH), 113.7 (C), 120.1 (CH), 121.9 (CH), 122.4 (CH), 126.1 (C), 129.4 (CH), 135.9 (C), 162.0 (2C), 165.8 (CH). IR (ATR): 3490−3280, 1554, 1515, 1456, 1121. HRMS: m/z $[M + H]^+$calcd for $C_{12}H_{10}N_4I$, 336.9950; found, 336.9964. HPLC (method II): purity >99%, λ = 230 nm, t_R = 16.5 min.

*4-(4-Amino-1H-indol-3-yl)-5-iodo-pyrimidin-2-amine (**75**).* Purification of the crude residue by flash chromatography (EtOAc) provided

75 as a yellow powder in 96% yield; mp > 250 °C; R_f 0.30 (cyclohexane/EtOAc 30:70). ^{1}H NMR (DMSO-d_6): δ 5.47−5.49 (br s, 2H, $NH_{2\ indole}$), 6.33 (d, 1H, J = 8.0 Hz), 6.69 (d, 1H, J = 8.0 Hz), 6.85 (br s, 2H, $NH_{2\ pyrimidine}$), 6.89 (t, 1H, J = 8.0 Hz), 7.88 (s, 1H), 8.51 (s, 1H), 11.42 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 76.7 (C), 100.6 (CH), 105.0 (CH), 113.6 (C), 114.6 (C), 123.3 (CH), 128.6 (CH), 137.6 (C), 142.7 (C), 161.8 (C), 163.0 (C), 165.7 (CH). IR (ATR): 3470−3280, 1546, 1525, 1456, 1101. HRMS: m/z $[M + H]^+$calcd for $C_{12}H_{11}N_5I$, 352.0059; found, 352.0054. HPLC (method I): purity >95%, λ = 230 nm, t_R = 18.4 min.

*4-(5-Amino-1H-indol-3-yl)-5-iodo-pyrimidin-2-amine (**76**).* Purification of the crude residue by flash chromatography (EtOAc) provided **76** as a yellow powder in 99% yield; mp 197−198 °C; R_f 0.45 (EtOAc). ^{1}H NMR (DMSO-d_6): δ 4.51−4.72 (br s, 2H, $NH_{2\ indole}$), 6.56 (br s, 3H, $NH_{2\ pyrimidine}$ + 1H), 7.13 (d, 1H, J = 8.4 Hz), 7.55 (s, 1H), 8.24 (s, 1H), 8.45 (s, 1H), 11.25 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 74.8 (C), 106.0 (CH), 111.6 (CH), 112.3 (CH), 112.4 (C), 127.3 (C), 128.8 (CH), 129.5 (C), 142.3 (C), 161.9 (C), 162.6 (C), 165.5 (CH). IR (ATR): 3500−3200, 1553, 1514, 1470, 1158. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{11}N_5I$, 352.0059; found, 352.0047. HPLC (method II): purity >99%, λ = 230 nm, t_R = 5.9 min.

*4-(6-Amino-1H-indol-3-yl)-5-iodo-pyrimidin-2-amine (**77**).* Purification of the crude residue by flash chromatography (EtOAc) provided **77** as a yellow powder in 88% yield; mp 137−138 °C; R_f 0.30 (CH_2Cl_2/MeOH 90:10). ^{1}H NMR (DMSO-d_6): δ 4.84−4.89 (br s, 2H, $NH_{2\ indole}$), 6.49 (d, 1H, J = 8.0 Hz), 6.58−6.62 (m, 3H), 8.04 (d, 1H, J = 8.0 Hz), 8.21 (d, 1H, J = 2.0 Hz), 8.43 (s, 1H), 11.12 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 74.2 (C), 94.9 (CH), 110.8 (CH), 113.6 (C), 117.7 (C), 123.0 (CH), 126.9 (CH), 137.6 (C), 144.6 (C), 161.9 (C), 162.1 (C), 165.6 (CH). IR (ATR): 3424−3280, 1542, 1533, 1446, 1124. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{11}N_5I$, 352.0059; found, 352.0062. HPLC (method I): purity >99%, λ = 230 nm, t_R = 17.3 min.

*4-(7-Amino-1H-indol-3-yl)-5-iodo-pyrimidin-2-amine (**78**).* Purification of the crude residue by flash chromatography (EtOAc) provided **78** as a yellow powder in 99% yield; mp 193−194 °C; R_f 0.10 (cyclohexane/EtOAc 30:70). ^{1}H NMR (DMSO-d_6): δ 5.10−5.11 (br s, 2H, $NH_{2\ indole}$), 6.39 (d, 1H, J = 8.2 Hz), 6.65 (br s, 2H, $NH_{2\ pyrimidine}$), 6.82 (t, 1H, J = 8.2 Hz), 7.49 (d, 1H, J = 8.2 Hz), 8.29 (d, 1H, J = 2.0 Hz), 8.47 (s, 1H), 11.23 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 105.7 (CH), 110.7 (CH), 114.2 (C), 121.2 (CH), 125.4 (C), 126.9 (C), 127.9 (CH), 133.7 (2C), 162.0 (C), 162.6 (C), 165.6 (CH). IR (ATR): 3520−3180, 1564, 1511, 1438, 1167. HRMS: m/z $[M + H]^+$ calcd for $C_{12}H_{11}N_5I$, 352.0059; found, 352.0055. HPLC (method II): purity >99%, λ = 230 nm, t_R = 5.8 min.

6.4. Molecular Modeling Experiments. For docking experiments, all protein structures were prepared using chimera[35] and Sybyl-X1.1[36] from 2wo6 PBD file for DYRK1A[23] and 1z57[24] for CLK1. All the structures of ligands used were first designed with Sybyl-X1.1 and then optimized with Gaussian03[37] (DFT/B3LYP/6-31 g).

Docking experiments were performed with the surflex module of Sybyl-X1.1. The GeomX docking option was used with Threshold set to 0.20 and Bloat set to 3. Docking performed in these conditions considered potential hydrogen bonding without taking into account electrostatic charges.

The results obtained in the docking experiments were analyzed taking into account the preferred ligand conformation calculated with Gaussian considering the π delocalization between the aminopyrimidine moiety and the indolic ring and/or the steric effect due to the presence of the iodine atom.

Then, the selected docking results were minimized with constraint on the H-bonding network established between the aminopyrimidine moiety and the ATP binding site. To check the stability of the protein−ligand interactions, the result of this minimization was minimized again without any constraint. The same results were obtained with and without constraints. These minimizations were carried out with the Tripos force field, Gasteiger-Hückel charges, dielectric constants set to 78 and conjugate gradient method.

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ABBREVIATIONS USED

c-Abl, Abelson leukemia oncogen cellular homologue; ATP, adenosine triphosphate; BSA, bovin serum albumin; CDK, cyclin-dependent protein kinase; CK, casein kinase; CLK, cdc2-like kinase; DIPEA, diisopropylethylamine; DMA, dimethylacetal; DMAP, 4-(dimethylamino)pyridine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DTT, dithiothreitol; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; DYRK, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase; Emim, 1-ethyl-3-methyl imidazolium; Erk, extracellular signal regulated kinase; GSK-3, glycogen synthase kinase 3; GST, glutathione transferase; His_6, hexahistidine; IGF-1R, insulin growth factor receptor-1; KDR, kinase domain receptor; MEM, minimum essential medium; Mops, 3-(*N*-morpholino)propanesulfonic acid; PKA, cAMP-dependent protein kinase; PKG, protein kinase G; Src, sarcoma kinase; TFA, trifluoroacetic acid

REFERENCES

(1) Franco, L. H.; Bal de Kier Joffé, E.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. Indole alkaloids from the tunicate *Aplidium meridianum*. *J. Nat. Prod.* **1998**, *61*, 1130–1132.

(2) Franco, L. H.; Palermo, J. A. Synthesis of 2-(pyrimidin-4-yl)indoles. *Chem. Pharm. Bull.* **2003**, *51*, 975–977.

(3) Gompel, M.; Leost, M.; Bal de Kier Joffé, E.; Puricelli, L.; Franco, L. H.; Palermo, J.; Meijer, L. Meridianins, a new family of protein kinase inhibitors isolated from the Ascidian *Aplidium meridianum*. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1703–1707.

(4) Seldes, A. M.; Rodriguez Brasco, M. F.; Hernandez Franco, L.; Palermo, J. A. Identification of two meridianins from the crude extract of the tunicate *Aplidium meridianum* by tandem mass spectrometry. *Nat. Prod. Res., Part A* **2007**, *21*, 555–563.

(5) Radwan, M. A. A.; El-Sherbiny, M. Synthesis and antitumor activity of indolylpyrimidines: marine natural product meridianin D analogues. *Bioorg. Med. Chem.* **2007**, *14*, 1206–1211.

(6) Bettayeb, K.; Tirado, O. M.; Marionneau-Lambot, S.; Ferandin, Y.; Lozach, O.; Morris, J. C.; Mateo-Lozano, S.; Drueckes, P.; Schächtele, C.; Kubbutat, M. H. G.; Liger, F.; Marquet, B.; Joseph, B.; Echalier, A.; Endicott, J. A.; Notario, V.; Meijer, L. Meriolins, a new class

of cell death inducing kinase inhibitors with enhanced selectivity for cyclin-dependent kinases. *Cancer Res.* **2007**, *67*, 8325–8334.

(7) Echalier, A.; Bettayeb, K.; Ferandin, Y.; Lozach, O.; Clément, M.; Valette, A.; Liger, F.; Marquet, B.; Morris, J. C.; Endicott, J. A.; Joseph, B.; Meijer, L. Meriolins (3-(pyrimidin-4-yl)-7-azaindoles): synthesis, kinase inhibitory activity, cellular effects, and structure of a CDK2/cyclin A/meriolin complex. *J. Med. Chem.* **2008**, *51*, 737–751.

(8) Rossignol, E.; Youssef, A.; Moreau, P.; Prudhomme, M.; Anizon, F. Synthesis of aminopyrimidylindoles structurally related to meridianins. *Tetrahedron* **2007**, *63*, 10169–10176.

(9) Rossignol, E.; Debiton, E.; Fabbro, D.; Moreau, P.; Prudhomme, M.; Anizon, F. In vitro antiproliferative activities and kinase inhibitory potencies of meridianin derivatives. *Anti-Cancer Drugs* **2008**, *19*, 789–792.

(10) Akué-Gédu, R.; Debiton, E; Ferandin, Y.; Meijer, L.; Prudhomme, M.; Anizon, F.; Moreau, P. Synthesis and biological activities of aminopyrimidyl-indoles structurally related to meridianins. *Bioorg. Med. Chem.* **2009**, *17*, 4420–4424.

(11) Kimura, R.; Kamino, K.; Yamamoto, M.; Nuripa, A.; Kida, T.; Kazui, H.; Hashimoto, R.; Tanaka, T.; Kudo, T.; Yamagata, H.; Tabara, Y.; Miki, T.; Akatsu, H.; Kosaka, K.; Funakoshi, E.; Nishitomi, K.; Sakaguchi, G.; Kato, A.; Hattori, H.; Uema, T.; Takeda, M. The DYRK1A gene, encoded in chromosome 21 Down syndrome critical region, bridges between β-amyloid production and tau phosphorylation in Alzheimer disease. *Hum. Mol. Genet.* **2007**, *16*, 15–23.

(12) Branchi, I.; Bichler, Z.; Minghetti, L.; Delabar, J. M.; Malchiodi-Albedi, F.; Gonzalez, M. C.; Chettouh, Z.; Nicolini, A.; Chabert, C.; Smith, D. J.; Rubin, E. M.; Migliore-Samour, D.; Alleva, E. Transgenic mouse in vivo library of human Down syndrome critical region 1: association between DYRK1A overexpression, brain development abnormalities, and cell cycle protein alteration. *J. Neuropathol. Exp. Neurol.* **2004**, *63*, 429–440.

(13) Fresneda, P. M.; Molina, P.; Delgado, S.; Bleda, J. A. Synthetic studies towards the 2-aminopyrimidine alkaloids variolins and meridianins from marine origin. *Tetrahedron Lett.* **2000**, *41*, 4777–4780.

(14) Fresneda, P. M.; Molina, P.; Bleda, J. A. Synthesis of the indole alkaloids meridianins from the tunicate *Aplidium meridianum*. *Tetrahedron* **2001**, *57*, 2355–2363.

(15) Fresneda, P. M.; Delgado, S.; Francesch, A.; Manzanares, I.; Cuevas, C.; Molina, P. Synthesis and cytotoxic evaluation of new derivatives of the marine alkaloid variolin B. *J. Med. Chem.* **2006**, *49*, 1217–1221.

(16) The only precise comparisons that can be made between efficacy of an inhibitor on various kinases would require determination of a K_d at the optimal Km, ATP for each kinase. Comparisons between various kinases on the basis of IC_{50} values has thus limited value. Furthermore, recombinant kinases always contain a variable (and undetermined) proportion of unfolded or misfolded and inactive proteins that still interact with the inhibitor and thus biases the determination of both IC_{50} and K_d values. During the course of large scale screening and SAR studies, determination of each K_d is unpractical and determination of IC_{50} values provides sufficient precision to guide the synthesis of more active analogs. We have chosen a 15 μM ATP concentration that favours the identification of ATP-competitive compounds.

(17) Yeung, K. S.; Farkas, M. E.; Qiu, Z.; Yang, Z. Friedel−Crafts acylation of indoles in acidic imidazolium chloroaluminate ionic liquid at room temperature. *Tetrahedron Lett.* **2002**, *43*, 5793–5795.

(18) Okauchi, T.; Itonaga, M.; Minami, T.; Owa, T.; Kitoh, K.; Yoshino, H. A general method for acylation of indoles at the 3-position with acyl chlorides in the presence of dialkylaluminium chloride. *Org. Lett.* **2000**, *2*, 1485–1487.

(19) Simon, G.; Couthon-Gourves, H.; Haelters, J.-P.; Corbel, B.; Kervarec, N.; Michaud, F.; Meijer, L. Towards the syntheses of *N*-H and *N*-alkylated derivatives of meridianins. *J. Heterocycl. Chem.* **2007**, *44*, 793–801.

(20) Owa, T.; Yoshino, H.; Okauchi, T.; Yoshimatsu, K.; Ozawa, Y.; Sugi, N. H.; Nagasu, T.; Koyanagi, N.; Kitoh, K. Discovery of novel antitumor sulfonamides targeting G1 phase of the cell cycle. *J. Med. Chem.* **1999**, *42*, 3789–3799.

(21) Mott, B. T.; Tanega, C.; Shen, M.; Maloney, D. J.; Shinn, P.; Leister, W.; Marugan, J. J.; Inglese, J.; Austin, C. P.; Misteli, T.; Auld, D. S.; Thomas, C. J. Evaluation of substituted 6-arylquinazolin-4-amines as potent and selective inhibitors of cdc2-like kinases (Clk). *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6700–6705.

(22) Eswaran, J.; Roos, A. K.; Soundararajan, M.; Pike, A. C. W.; Fedorov, O.; King, O.; Burgess-Brown, N.; Philips, C.; Filippakopoulos, P.; Von Delf, F.; Muller-Knapp, S.; Lee, W.-H.; Marsden, B. D.; Boundra, C.; Knapp, S. DYRK1A: Human dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A in complex with K00611a. PDB: 2vx3. DOI: 10.2210/pdb2vx3/pdb.

(23) Soundararajan, M.; Knapp, S. The crystal structure of the human dual specificity tyrosine-phosphorylation-regulated kinase 1A. DOI: 10.2210/pdb2wo6/pdb.

(24) Debreczeni, J.; Das, S.; Knapp, S.; Bullock, A.; Guo, K.; Amos, A.; Fedorov, O.; Edwards, A.; Sundstrom, M.; von Delft, F.; Niesen, F. H.; Ball, L.; Sobott, F.; Arrowsmith, C. Crystal structure of human CLK1A in complex with 10*Z*-Hymenialdisine. DOI: 10.2210/pdb1z57/pdb

(25) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. Investigation of the alamar blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem.* **2000**, *267*, 5421–5426.

(26) Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Biernat, J.; Mandelkow, E.-M.; Eisenbrand, G.; Meijer, L. Indirubins inhibit glycogen synthase kinase-3β and CDK5/P25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease: a property common to most cyclin-dependent kinase inhibitors? *J. Biol. Chem.* **2001**, *276*, 251–260.

(27) Bach, S.; Knockaert, M.; Reinhardt, J.; Lozach, O.; Schmitt, S.; Baratte, B.; Koken, M.; Coburn, S. P.; Tang, L.; Jiang., T.; Liang, D. C.; Galons, H.; Dierick, J. F.; Pina, L. A.; Meggio, F.; Totzke, F.; Schächtele, C.; Lerman, A. S.; Carnero, A.; Wan, Y.; Gray, N.; Meijer, L. Roscovitine targets, protein kinases and pyridoxal kinase. *J. Biol. Chem.* **2005**, *280*, 31208–31219.

(28) Primot, A.; Baratte, B.; Gompel, M.; Borgne, A.; Liabeuf, S.; Romette, J. L.; Jho, E. H.; Costantini, F.; Meijer, L. Purification of GSK-3 by affinity chromatography on immobilized axin. *Protein Expression Purif.* **2000**, *20*, 394–404.

(29) Reinhardt, J.; Ferandin, Y.; Meijer, L. Purification of CK1 by affinity chromatography on immobilized axin. *Protein Expression Purif.* **2007**, *54*, 101–109.

(30) Ribas, J.; Boix, J. Cell differentiation caspase inhibition and macromolecular synthesis blockage but not BCL-2 or BCL-XL proteins protect SH-SY5Y cells from apoptosis triggered by two CDK inhibitory drugs. *Exp. Cell. Res.* **2004**, *295*, 9–24.

(31) MacKay, J. A.; Bishop, R. L.; Rawal, V. H. Rapid synthesis of the *N*-methylwelwitindolinone skeleton. *Org. Lett.* **2005**, *7*, 3421–3424.

(32) Jiang, B.; Gu, X.-H. Synthesis and cytotoxicity evaluation of bis(indolyl)thiazole, bis(indolyl)pyrazinone and bis(indolyl)pyrazine: analogues of the cytotoxic marine bis(indole) alkaloid. *Bioorg. Med. Chem.* **2000**, *8*, 363–371.

(33) Jiang, B.; Yang, C. Synthesis of indolylpyrimidines *via* cross-coupling of indolylboronic acid with chloropyrimidines: facile synthesis of meridianin D. *Heterocycles* **2000**, *53*, 1489–1498.

(34) Jovanovic, M. V. Iodination of some diazines and diazine *N*-oxides. *Heterocycles* **1984**, *22*, 1195–1210.

(35) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.* **2004**, *25*, 1605–1612.

(36) *Sybyl 7.3*; Tripos International: 1699 South Hanley Rd. St. Louis, Missouri, 63144.

(37) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson,

G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A. *Gaussian 03, Revision D.01*; Gaussian, Inc.: Wallingford, CT, 2004.