Bioorganic & Medicinal Chemistry Letters 23 (2013) 125–131

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potent inhibitors of CDK5 derived from roscovitine: Synthesis, biological evaluation and molecular modelling

Luc Demange [a,*], Fatma Nait Abdellah [a], Olivier Lozach [b], Yoan Ferandin [b], Nohad Gresh [a], Laurent Meijer [b,c,*], Hervé Galons [c,d]

[a] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques (LCBPT), UMR 8601 CNRS, Université Paris Descartes, Sorbonne Paris Cité, UFR Biomédicale des Saints Pères, 45 rue des Saints Pères, 75270 Paris cedex 06, France
[b] CNRS, USR3151, 'Protein Phosphorylation & Human Disease' group, Station Biologique, 29680 Roscoff, Bretagne, France
[c] ManRos Therapeutics, Hôtel de Recherche, Centre de Perharidy, 29680 Roscoff, France
[d] Laboratoire de Pharmacochimie, Université Paris Descartes, 4, avenue de l'observatoire, 75006 Paris, France

ARTICLE INFO

Article history:
Received 26 September 2012
Revised 29 October 2012
Accepted 31 October 2012
Available online 10 November 2012

Keywords:
CDK5
Kinase
Roscovitine
Buchwald–Hartwig amination

ABSTRACT

Cyclin dependent kinase 5 (CDK5) is a serine/threonine kinase belonging to the cyclin dependent kinase (CDK) family. CDK5 is involved in numerous neuronal diseases (including Alzheimer's or Parkinson's diseases, stroke, traumatic brain injury), pain signaling and cell migration. In the present Letter, we describe syntheses and biological evaluations of new 2,6,9-trisubstituted purines, structurally related to roscovitine, a promising CDK inhibitor currently in clinical trials (CDK1/Cyclin B, IC_{50} = 350 nM; CDK5/p25, IC_{50} = 200 nM). These new molecules were synthesized using an original Buchwald–Hartwig catalytic procedure; several compounds (**3j**, **3k**, **3l**, **3e**, **4k**, **6b**, **6c**) displayed potent kinase inhibitory potencies against CDK5 (IC_{50} values ranging from 17 to 50 nM) and showed significant cell death inducing activities (IC_{50} values ranging from 2 to 9 μM on SH-SY5Y). The docking of the inhibitors into the ATP binding domain of the CDK5 catalytic site highlighted the discriminatory effect of a hydrogen bond involving the CDK5 Lys-89. In addition, the calculated final energy balances for complexation measured for several inhibitors is consistent with the ranking of the IC_{50} values. Lastly, we observed that several compounds exhibit submicromolar activities against DYRK1A (dual specificity, tyrosine phosphorylation regulated kinase 1A), a kinase involved in Down syndrome and Alzheimer's disease (**3g**, **3h**, **4m**; IC_{50} values ranging from 300 to 400 nM).

Cyclin-dependent kinases (CDKs) form a family of 20 ubiquitous serine/threonine kinases (STKs),[1–3] involved in cell cycle,[4,5] apoptosis[6] and gene transcription.[7] Their regulation is maintained by structural modifications,[8–11] but their activation is related to their association with regulatory proteins called cyclins.[1] In the past decade, their deregulation has been observed in multiple pathologies,[5] which has promoted the search for potent and selective inhibitors of their serine/threonine kinase (STK) activity.

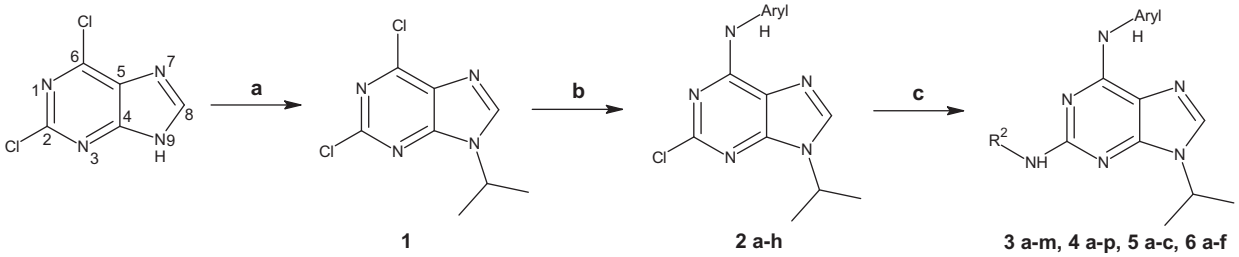
Among the CDKs family, an unusual member, the cyclin-dependent kinase 5 (CDK5), was discovered in 1992.[12–14] CDK5 is activated by proteins identified as p35, its isoform p39, and their respective proteolytic fragments p25 and p29.[14–16] The resulting complexes participate in cell–cell communication and play a key function in neuronal survival, migration and in the development of the cerebral cortex. CDK5 is partially responsible for the hyperphosphorylation of the protein tau, and for the production of the β-amyloïd peptide, the two major hallmarks of the Alzheimer's disease.[17–19] Its implication in other neuronal diseases like Parkinson's disease,[20,21] amyotrophic lateral sclerosis,[22] Huntington's disease,[23] stroke,[24] has been strongly suggested. In addition, CDK5 is involved in the regulation of pain signalling[25,26] and in the pancreatic secretion of insulin.[27] Finally CDK5 is involved in tumor cell metastatic migration, such as prostate cancer cells.[28] Thus, pharmacological inhibitors of CDK5 have a great potential as new drugs against several major pathologies.

Purines, the scaffold of which is widely used in medicinal chemistry,[29] have provided several CDKs inhibitors. Thus, (*R*)-Roscovitine, 6-benzylaminopurine developed by *Cyclacel Pharmaceuticals* (Chart 1), is one of the most promising molecule.[30–32] Based on this scaffold, we studied new potent CDKs inhibitors, such as DRF 53 (Chart 1).[33] Taking advantage of these structure–activity relationship results, we report here the synthesis of a new library of hybrid structures including 6-aminopyridyl, 6-aminopyrimidinyl, 6-aminopyrazinyl and 6-aminopyridazinyl.

The new trisubstituted purines were synthesized following the efficient three-step convergent procedure outlined in scheme 1. Briefly, the commercially available 2,6-dichloropurine was

* Corresponding authors. Tel.: +33 (0)1 42 86 40 81 (L.D.); tel.: +33 (0)2 98 72 94 92 (L.M.).
E-mail addresses: luc.demange@parisdescartes.fr (L. Demange), meijer@manros-therapeutics.com (L. Meijer).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.10.141



Scheme 1. Reagents and conditions: (a) 2-bromopropane, K_2CO_3, DMSO 15–18 °C, 5 days; (b) aminoaryl, $Pd(OAc)_2$, (±) BINAP, tBuOK, toluene, 100 °C; (c) R^2-NH_2, NEt_3, 110-160 °C.

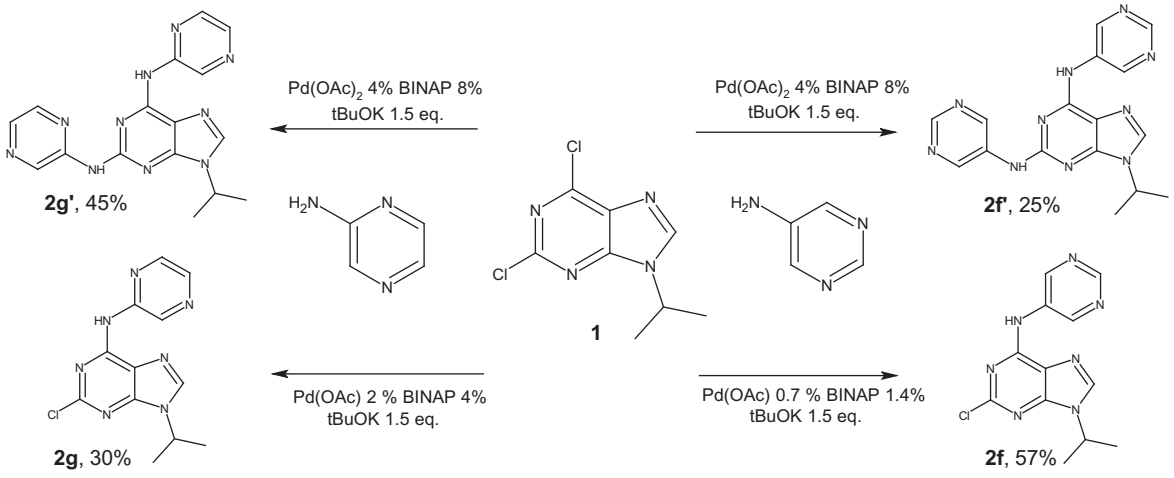
Table 1
Optimization of the Buchwald–Hartwig catalytic conditions for regioselective C-6 amination of compound **1**

Amine	Product	$Pd(OAc)_2$ (%)	BINAP (%)	*t*BuOK (equiv)	Time	Yield (%)
2-Aminopyridine	**2a**	4	8	1.5	6 h	73[35]
3-Aminopyridine	**2b**	4	8	1.5	6 h	76[35]
4-Aminopyridine	**2c**	4	8	1.5	6 h	73[35]
2-Aminopyrimidine	**2d**	8	16	1.5	1 day	22
4-Aminopyrimidine	**2e**	8	16	1.5	1 day	26
5-Aminopyrimidine	**2f**	0.7	1.4	1.5	3 h	57
Aminopyrazine	**2g**	2	4	1.5	1 day	30
4-Aminopyridazine	**2h**	2	4	1.5	5 h	38

All reactions were carried out under argon atmosphere in refluxing dry toluene.

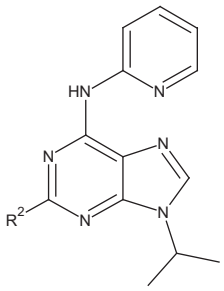
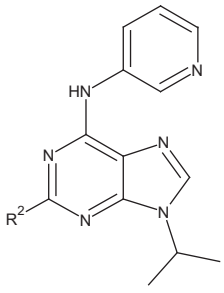
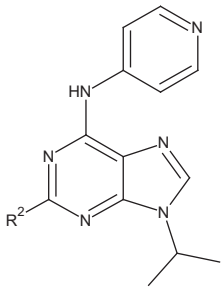
regioselectively alkylated using 2-bromopropane at 15–18 °C in DMSO for 5 days to afford the 2,6-dichloro-9-isopropylpurine **1** in 70% yield.[33] Then, this compound was submitted to the catalytic Buchwald–Hartwig amination we explored previously using functionalized aminopyridines, which led to compounds **2a**–**c** with good yields using 4% of $Pd(OAc)_2$ as catalyst and 8% of BINAP as ligand.[35] In the present study, we extended the scope of this reaction to aminoaryls including two nitrogen atoms (Table 1). The amination of **1** with 2- and 4-aminopyrimidines required double concentrations of catalysts and ligand (8% of $Pd(OAc)_2$, 16% of BINAP) to give products **2d**–**e** with moderate yields (22–26%). Conversely, the use of the previously observed catalytic conditions (4% of $Pd(OAc)_2$ and 8% of BINAP) with 5-aminopyrimidine and aminopyrazine afforded exclusively compounds **2f'** and **2g'** (Scheme 2). This double amination is probably related to the nucleophilicity of the amino group in the aminopyrimidine ring which is lower than those of the 5-aminopyrimidine or those of the aminopyrazine. In our hands, lowering the catalytic amount of $Pd(OAc)_2$ led to the expected purines **2f** and **2g** (Table 1, Scheme 2) with good yields and reduced reaction time in the case of 5-aminopyrimidine. Also, the use of a reduced amount of catalyst and ligand with 4-aminopyridazine afforded exclusively the expected compound **2h** in 38% yield. Lastly, reaction of aminated products **2a**–**h** with appropriate amino-alcohols afforded trisubstituted purines **3a**–**m**, **4a**–**p**, **5a**–**c** and **6a**–**f** in 50–60% yield.[36]

The inhibition of CDK5/p25, GSK-3αβ and CK1δε serine/threonine kinase (STK) activity was determined in the presence of a range of concentrations of newly synthesized products using an assay with [γ-^{33}P]-ATP as described in the experimental section. In order to evaluate the selectivity of the compounds, their inhibition of STK activity was controlled under the same conditions with other purified kinases (DYRK1A, CDK2/cyclin A). IC_{50} values were determined from dose–response curves. Globally, as shown in Tables 2–4, newly synthesized purines exhibit potent activity against



Scheme 2. C-6 amination of dichloropurine **1** and its side products.

Table 2
Effect of trisubstituted purines on five protein kinases activity

Compd.	Scaffold	R^2	CDK2	CDK5	GSK-3αβ	DYRK1A	CK1
2a		Cl	6.2	2.1	>10	3.8	3
3a		(*R*)-1-Hydroxy-but-2-ylamino	1	2.3	33	3.3	6.2
3b		(*S*)-1-Hydroxy-but-2-ylamino	0.53	4.1	21	2.1	12
3c		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.8	1.1	>10	2.0	6.2
3d		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	1.2	1.9	>10	1.2	11
2b		Cl	0.68	1.1	>10	2.1	2.1
3e		(*R*)-1-Hydroxy-but-2-ylamino	0.05	0.025	10	1.0	1.0
3f		(*S*)-1-Hydroxy-but-2-ylamino	0.014	0.09	>10	1.1	2
3g		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.018	0.022	7.3	0.4	2.2
3h		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	0.12	0.3	>10	0.4	2.7
3i		1,3-Dihydroxyprop-2-ylamino	0.22	0.3	>10	1.2	1.8
2c		Cl	0.7	7.4	18	3.9	2.1
3j		(*R*)-1-Hydroxy-but-2-ylamino	0.038	0.017	>10	4.1	1
3k		(*S*)-1-Hydroxy-but-2-ylamino	0.05	0.05	6	0.8	>1
3l		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.02	0.021	12	0.78	1.8
3m		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	0.13	0.2	7.8	0.9	3.3

Purines were tested at various concentrations on kinases as described in the Experimental Section. IC_{50} values, calculated from the dose–response curves, are reported in μM. -, not tested. IC_{50} value reported as >10 indicates that the compound did not display any inhibitory activity at the highest concentration tested (10 μM).

the STK activity of CDK2 (IC_{50} values ranging from 14 nM to 9 μM except for **4a** and **4c**) and CDK 5 (IC_{50} values ranging from 17 nM to 11 μM except for **4a** and **4c**) and average inhibition on the STK activity of CK1 and DYRK1A (IC_{50} values ranging from 0.5 to 20 μM). Similar to roscovitine, purvalanol and DRF53, these compounds are inactive on GSK-3α/β STK activity, in contrast to molecules belonging to other CDKs inhibitor families, such as indirubins.[37]

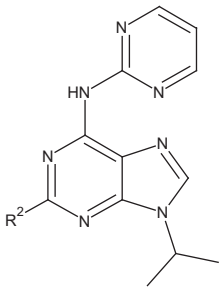
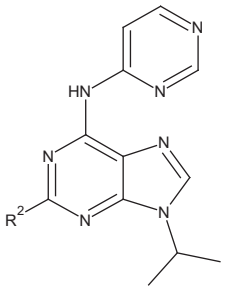
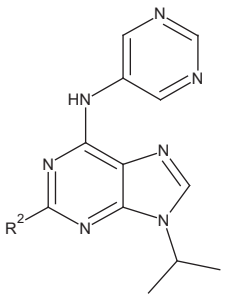
Derivatives bearing an aminopyridine in the C^6 position are endowed with IC_{50} values for CDK5 STK inhibition ranging from 17 nM to 4.1 μM (Table 2). Compounds **3e**, **3g** and **3j**–**l** are more efficient inhibitors for this kinase than roscovitine (IC_{50} = 0.2 μM), purvalanol (IC_{50} = 0.07 μM) and DRF53 (IC_{50} = 0.08 μM) (Chart 1), and showed no selectivity between CDK2 (IC_{50} values ranging from 18 to 50 nM), CDK5 (IC_{50} values ranging from 17 to 50 nM), and CDK1 (data not shown, IC_{50} values ranging from 19 to 120 nM). This result is consistent with the strong structural homology of the ATP binding site for those kinases.[33]

Interestingly, the potency of the CDK STK inhibition depends on the nitrogen position of the aminopyridine core. Thus, compounds **3a**–**d** (on CDK5, IC_{50} values ranging from 1100 to 4100 nM) are globally 10 times less active than compounds **3e**–**i** (on CDK5, IC_{50} values ranging from 22 to 300 nM) which are globally equipotent than compounds **3j**–**l** (on CDK5, IC_{50} values ranging from 17 to 50 nM). Among the latter inhibitors, **3e**, **3g**, **3j** and **3l**, are about tenfold more active than roscovitine.

Docking of **3j** in the CDK5 active site (IC_{50} = 17 nM) confirms the basic interactions between the purine scaffold and the kinase ATP binding site (Fig. 2),[33] but the most important observation relates to the onset of an H-bond between Lys 89 Nε and the aromatic nitrogen of pyridine. Such a bond could explain the potent inhibition of CDK5 by compounds bearing 3- and 4-aminopyridine. We also observed that the H-bond between Cys 83 and the N^6–H is preserved and the onset of two additional H-bond which involve on the one hand Cys 83 and purine N^7 and on the other hand Gln 130 and the purine amino alcohol hydroxyl group of the R^2 substituent. Docking furthermore confirms the presence of a hydrophobic pocket which involves Ala 30, Phe 80 and Val 64.

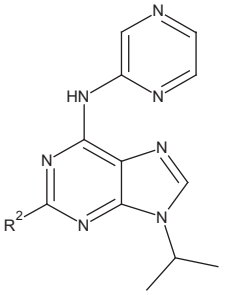
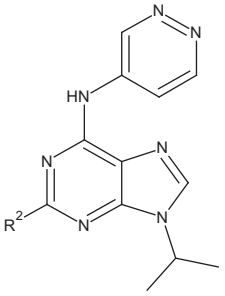
Studies on purines substituted at C^6 by aminopyrimidines, aminopyrazines or aminopyridazines revealed that nitrogen effects are cumulative for STK inhibition (Tables 3 and 4). Thus, compounds **4k**–**m** and **6a**–**c** belonging, respectively to the 5-aminopyrimidine serial and the 4-pyridazinyl series, are potent CDK2 and CDK5 inhibitors including IC_{50} values in the 18-76 nM range. This result is consistent with the docking of these inhibitors in the CDK5 ATP binding site which highlights the persistence of an H-bond between Lys89 and, respectively N^3 and N^4 of the aminoarylic structure of **4k** and **6a** (Supplementary data Fig. 1A and 1B). In marked contrast, compounds **4a**–**d** belonging to the 2-aminopyrimidine serial are inactive, owing to the unfavorable position of their two nitrogens; their IC_{50} values are close or superior to 10,000 nM.

Table 3
Effect of trisubstituted purines bearing an N^6 aminopyrimidinyl on five protein kinases activity

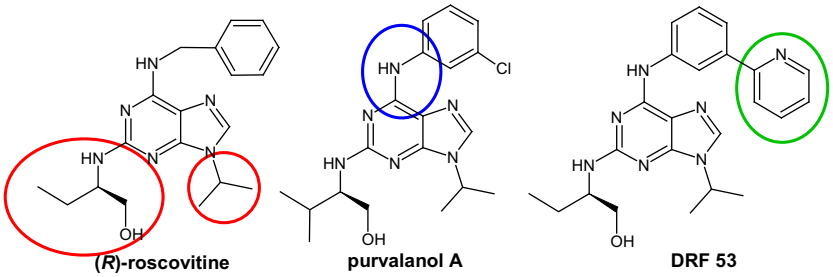
Compd.	Scaffold	R^2	CDK2	CDK5	GSK-3αβ	DYRK1A	CK1
2d		Cl	>10	>10	>10	>10	8
4a		(*R*)-1-Hydroxy-but-2-ylamino	>10	>10	>10	>10	>10
4b		(*S*)-1-Hydroxy-but-2-ylamino	7.3	11	>10	2.0	>10
4c		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	>10	>10	>10	>10	>10
4d		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	9	10	>10	7	>10
2e		Cl	13	>10	>10	>10	8
4e		(*R*)-1-Hydroxy-but-2-ylamino	1.2	1.8	>10	3.2	5.8
4f		(*S*)-1-Hydroxy-but-2-ylamino	2.0	4.8	>10	2.8	8.3
4g		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.33	0.73	>10	1	2.8
4h		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	0.79	3.2	22	1.1	4.9
4i		1,3-Dihydroxyprop-2-ylamino	6	8.5	>10	5.8	7
4j		1-Hydroxy-2-methylprop-2-ylamino	0.73	3.2	>10	16	9
2f		Cl	0.18	2.8	>10	5.3	2.3
2f'		5-Aminopyrimidinyl	0.17	0.36	>10	0.48	0.22
4k		(*R*)-1-Hydroxy-but-2-ylamino	0.018	0.042	>10	0.28	0.6
4l		(*S*)-1-Hydroxy-but-2-ylamino	0.023	0.076	>10	0.27	0.93
4m		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.02	0.038	5	0.3	1.3
4n		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	0.11	0.37	16	0.49	2
4o		1-Hydroxy-2-methylprop-2-ylamino	0.10	0.18	>10	2.5	0.82
4p		1,3-Dihydroxyprop-2-ylamino	0.26	0.3	>10	1.9	2.1

See legend of Table 2 for details. All values are reported in μM.

Table 4
Effect of trisubstituted purines bearing an N^6 aminopyrazinyl or aminopyridazinyl on five protein kinases activity

Compd.	Scaffold	R^2	CDK2	CDK5	GSK-3αβ	DYRK1A	CK1
2g		Cl	3.1	12	>10	9	7
5a		(*R*)-1-Hydroxy-but-2-ylamino	0.21	0.9	>10	0.4	2.8
5b		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.088	0.3	>10	0.3	4
5c		1-Hydroxy-2-methylprop-2-ylamino	1.0	3	>10	4	4
2g'		Aminopyrazinyl	2.8	13	10	0.5	1.1
2h		Cl	1.6	3	>10	3.4	1.1
6a		(*R*)-1-Hydroxy-but-2-ylamino	0.072	0.06	13	0.45	0.5
6b		(*S*)-1-Hydroxy-but-2-ylamino	0.029	0.042	7.3	0.3	1
6c		(*R*)-1-Hydroxy-3-methylbut-2-ylamino	0.028	0.041	6	0.28	0.8
6d		(*S*)-1-Hydroxy-3-methylbut-2-ylamino	0.12	0.23	4.3	0.48	1.9
6e		1,3-Dihydroxyprop-2-ylamino	0.11	0.2	>10	1.0	0.7
6f		1-Hydroxy-2-methylprop-2-ylamino	0.046	0.1	13	3	0.6

See legend of Table 2 for details. All values are reported in μM.



	(R)-roscovitine	purvalanol A	DRF 53
CDK1/cyclin B	0.35 μM	0.004 μM[34]	0.22 μM
CDK2/cyclin A	0.70 μM	0.070 μM[34]	-
CDK5/p25	0.20 μM	0.075 μM[34]	0.08 μM
CK1	2.30 μM	100% inhibition at 0.1 μM	0.014 μM
SH-SY5Y	17.4 μM	-	17.2 μM

Chart 1. Structure of (*R*)-roscovitine, purvalanol A and DRF 53, and their activities on relevant kinases and in cell survival assays. IC_{50} values are reported in μM. (See above-mentioned reference for further information.)

For each series, the influence of the C-2 amino alcohol appears to be limited. Nevertheless, as reported in the case of roscovitine, the (*R*) stereoisomer appears to have a higher affinity for CDK5 than the (*S*) one. This is particularly highlighted upon comparing **3l** (CDK5:IC_{50} = 21 nM) and **3m** (CDK5:IC_{50} = 200 nM), **4m** (CDK5:IC_{50} = 38 nM) and **4n** (CDK5:IC_{50} = 370 nM) and lastly **3g** (CDK5:IC_{50} = 22 nM) and **3h** (CDK5:IC_{50} = 300 nM). Finally, the substitution of an amino alcohol by a halogen is very detrimental in term of CDK5 STK inhibition (IC_{50} >2000 nM for **2a**, **2c**–**h**). Surprisingly, this effect is less detrimental on CDK2 inhibition (**2b** IC_{50} = 680 nM; **2c** IC_{50} = 700 nM and **2f** IC_{50} = 180 nM). Lastly, compound **2f′** (CDK5:IC_{50} = 360 nM), which bears on C-2 a 5-aminopyrimidinyl group retains and average activity (Table 3).

We calculated δE_s and δE, the final energy balances for complexation in presence or in absence of solvation. It is the difference between, on the one hand, the inhibitor-CDK5 intermolecular interaction energy plus the continuum solvation energy of the complex; and on the other hand, the sum of the separately energy-minimized ligand and protein conformational energies plus their continuum solvation energies at the conformational energy minima. The ranking of energy balances of the outcome of energy minimization is globally consistent with that of the IC_{50} values (Table 5). However while the δE ranking of the structurally related compounds **4a**, **2f′**, **6a** and **3j** is the same as that of the IC_{50} values observed for CDK5, the affinity of DRF 53 which belongs to a different family of inhibitor appears overestimated with respect to this series. This indicates the possibilities and limitations of the computational approach used in this study.

Globally, our highest-affinity compounds exhibits activities against CDK5 similar or better than those previously reported by our group (DRF 53, Chart 1)[33,38] and by others, including specific CDK5 inhibitors with scaffolds such as pyrazolopyrimidine ring (IC_{50} = 30 nM),[39] 2,4-diaminothiazoles (IC_{50} range between 15 nM and 1 μM)[40] or cyclohexyl-thiophene moiety linked with triazole (IC_{50} range between 35 nM and 1 μM).[41] The latter compounds have complex chemical structures and their preparation involves multi-step syntheses; consequently, large-scale syntheses are more difficult, and subsequent pharmacomodulations, in order to improve activity or selectivity, might be very challenging. By contrast, the purine scaffold we used here is chemically accessible, stable and adjustable fur further optimizations.

Interestingly, DYRK1A, a Serine Threonine kinase involved in neurodegenerative pathologies, such as the Down syndrome,[42] is targeted by several compounds including a C^6 5-aminopyrimidinyl (Table 3), aminopyrazinyl or 4-aminopyridazinyl (Table 4). With the significant exception of the analogs of Lamellarin D which include in their structure a complex chromeno[3,4-*b*]indole skeleton (IC_{50} = 0.07 μM),[43] there are few sub-micromolar DYRK1A inhibitors reported in the recent literature, and none of them has reached the stage of clinical evaluation.[44,45] Thus, products **4k**–**n**, **5a**, **5b** and **6a**–**d** are the first purine-like sub-micromolar DYRK1A inhibitors (IC_{50} values ranging from 280 to 480 nM), in contrast to roscovitine (87% of remaining STK activity for DYRK1A at 1 μM) or purvalanol (88% of remaining STK activity for DYRK1A at 0.1 μM).[32] Thus, these molecules might pave the way for the design of novel families of DYRK1A inhibitors.

Lastly, we also studied our compounds as inhibitors of CK1, a kinase involved in multiple physiological events and responsible

Table 5
Comparison between the inhibition of CDK5 (IC_{50} values) and the energy minimization (calculated without and with energy of solvation) for relevant compounds

Compd.	IC_{50} (μM)	δE Energy minimization (without solvation) (kcal/mol)	δE_s Energy minimization (with solvation) (kcal/mol)
Roscovitine	0.2	−41	−32
Purvalanol	0.07	−49.5	−30.7
DRF 53	0.08	−52.5	−34.0
4a	10.0	−25.7	−15.9
2f′	0.36	−30.8	−21
6a	0.06	−38.2	−26.1
3j	0.01	−41.9	−28.9

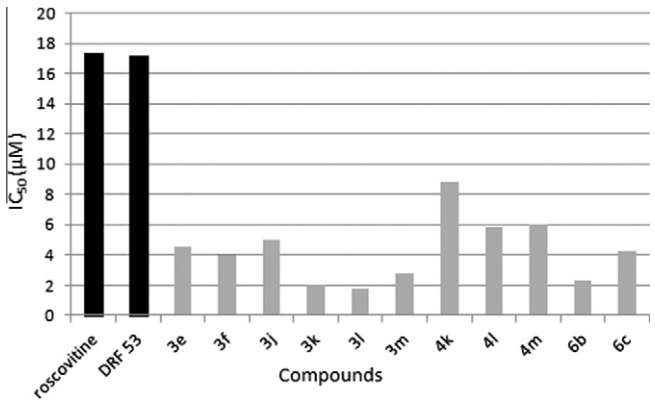


Figure 1. Effect of prepared purines on the survival of SH-SY5Y cells. The compounds were tested at various concentrations for their effects on SH-SY5Y cell survival after 48 h incubation estimated using the MTS reduction assay as described in experimental section. IC_{50} values, calculated from the dose–response curves, are reported in μM.

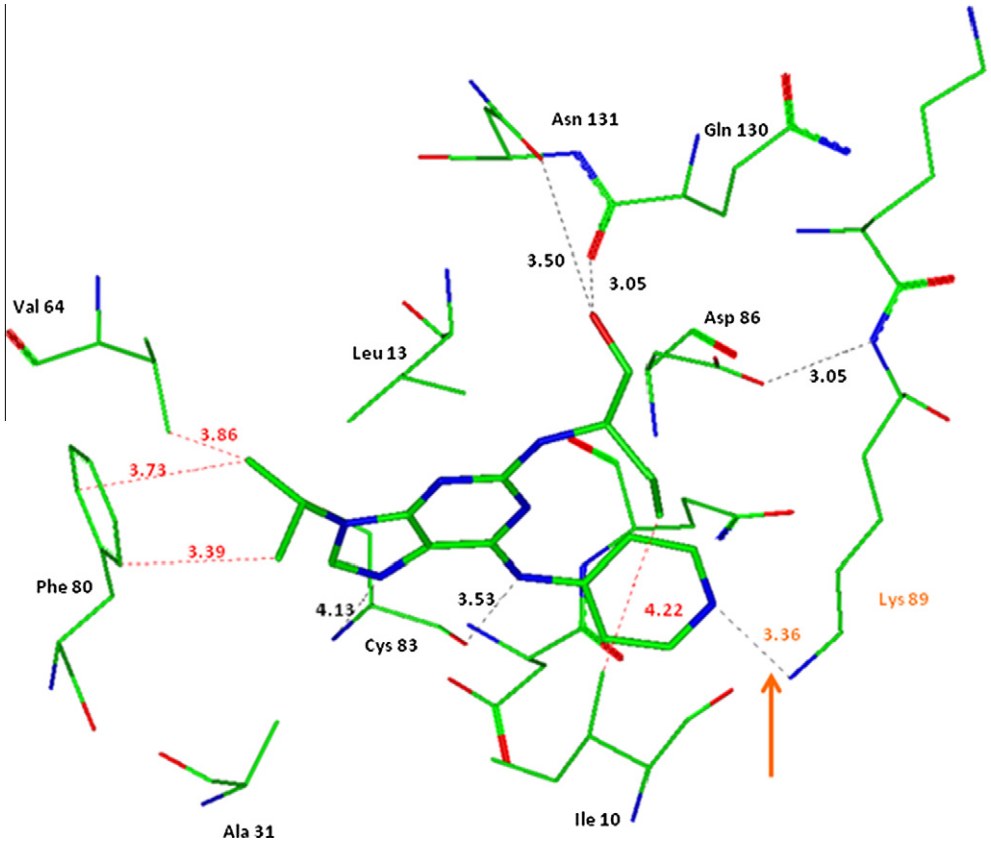


Figure 2. Compound **3j** docked in the ATP binding pocket of CDK5. The classical hydrogen bonds network between the inhibitor and the kinase, and the interaction between Lys 89 and the relevant nitrogen of the aminoarylic core (orange arrow) are outlined as black dashed lines. H-Bond lengths are also specified. Hydrophobic interactions are outlined as red dashed lines.

for amyloid-β formation.[32] To date, one of the most potent inhibitors is DRF 53 (IC_{50} = 80 nM, Chart 1), which encompasses a C^6 aminobiaryl including a terminal pyridine ring involved in a H-bond with the ATP binding pocket. By contrast, compounds synthesized in the present study lacking such an entity, are less potent against CK1 STK activity, and exhibits IC_{50} values in the 0.800–10 μM range.

In addition to these STK inhibition studies on purified kinases, the most active purines were studied for their cellular effects on the survival of human neuroblastoma SH-SY5Y cells (Fig. 1). Thus, SH-SY5Y cells were exposed for 48 h to various concentrations of purines and cell viability was estimated by the MTS reduction assay as described in the Experimental Section. All selected compounds displayed significant cell death inducing activity, with IC_{50} values ranging from 1.8 to 8.8 μM. Moreover, these molecules are globally 5 to 10-fold more potent than roscovitine and DRF 53. This result suggests a good correlation between CDKs inhibition and cell death inducing activity.

In conclusion, we have described procedures to introduce a new aminoaryl core in the 6 position of the purine scaffold by resorting to an innovative variant of the Buchwald–Hartwig amination procedure. Several compounds exhibit a strong potency to inhibit CDK5 with IC_{50} values in the 20 nM range and, by contrast to DRF53, are selective for CDK5 compared to CK1. The docking of these compounds in the CDK5 ATP binding site highlights an essential hydrogen bond involving the Lys 89 N ε and a nitrogen on the 6-aminoaryl which impacts the structure–activity relationship. For several inhibitors, we have shown a significant correlation between the ranking of IC_{50} values and that of the energy balances at the outcome of energy-minimization. Moreover, the most potent molecules exhibit a significant cell death inducing activity. In conclusion we suggest that this new series of molecules constitutes an incentive for the development of novel therapeutic agents against several diseases involving CDKs deregulation.

Acknowledgments

This research was supported by Grants from the 'Cancéropole Grand-Ouest' Grant (L.M.), the 'Institut National du Cancer' (INCa « Cancer Détection d'innovations 2006 ») (L.M.), the 'Association France-Alzheimer Finistère' (L.M.) and the 'Ligue Nationale contre le Cancer (Grand-Ouest)' (L.M.).

Professor Christiane Garbay is gratefully acknowledged for critical reading of the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.141.

References and notes

1. Malumbres, M.; Barbacid, M. *Trends Pharmacol. Sci.* **2005**, *30*, 630.
2. Malumbres, M.; Harlow, E.; Hunt, T.; Hunter, T.; Lahti, J. M.; Manning, G.; Morgan, D. O.; Tsai, L.-H.; Wolgemuth, D. J. *Nat. Cell Biol.* **2009**, *11*, 1275.
3. Carassou, P.; Meijer, L.; Le Moulec, S.; Aoun, J.; Bengrine-Lefèvre, L. *Bull. Cancer* **2012**, *99*, 163.
4. Malumbres, M.; Barbacid, M. *Nat. Rev. Cancer* **2001**, *1*, 222.
5. Meijer, L. *Bull. Cancer* **2006**, *93*, 41.
6. Borgne, A.; Golsteyn, R. M. *Prog. Cell Cycle Res.* **2003**, *5*, 453.
7. Garriga, J.; Grana, X. *Gene* **2004**, *337*, 15.

8. Abbas, T.; Dutta, A. *Cell Cycle* **2006**, *5*, 1123.
9. Lolli, G.; Johnson, L. N. *Cell Cycle* **2005**, *4*, 572.
10. Lee, M. H.; Yang, H. Y. *Cell. Mol. Life Sci.* **1907**, *2001*, 58.
11. Coqueret, O. *Trends Cell Biol.* **2003**, *13*, 41.
12. Hellmich, M. R.; Pant, H. C.; Wada, E.; Battey, J. F. *Proc. Natl. Acad. Sci.* **1992**, *89*, 10867.
13. Lew, J.; Beaudette, K.; Litwin, C. M.; Wang, J. H. *J. Biol. Chem.* **1992**, *267*, 13383.
14. Dhariwala, F. A.; Rajadhyaksha, M. S. *Cell Mol. Neurobiol.* **2008**, *28*, 351.
15. Brown, N. R.; Noble, M. E.; Endicott, J. A.; Johnson, L. N. *Nat. Cell Biol.* **1999**, *1*, 438.
16. Ko, J.; Humbert, S.; Bronson, R. T.; Takahashi, S.; Kulkarni, A. B.; Li, E.; Tsai, L. H. *J. Neurosci.* **2001**, *21*, 6758.
17. Cruz, J.; Tsai, L. H. *Trends Mol. Med.* **2004**, *10*, 452.
18. Cruz, J. C.; Kim, D.; Moy, L. Y.; Dobbin, M. M.; Sun, X.; Bronson, R. T.; Tsai, L. H. *J. Neurosci.* **2006**, *26*, 10536.
19. Sadleir, K. R.; Vassar, R. *J. Biol. Chem.* **2012**, *287*, 7224.
20. Wong, A. S. L.; Lee, R. H. K.; Cheung, A. Y.; Yeung, P. K.; Chung, S. K.; Cheung, Z. H.; Ip, N. Y. *Nat. Cell Biol.* **2011**, *13*, 568.
21. Zelda, H.; Ip, C.; Ip, H. Y. *Trends Cell Biol.* **2012**, *22*, 169.
22. Nguyen, M. D.; Lariviere, R. C.; Julien, J.-P. *Neuron* **2001**, *30*, 135.
23. Anne, S. L.; Saudou, F.; Humbert, S. *J. Neurosci.* **2007**, *27*, 7318.
24. Menn, B.; Bach, S.; Blevins, T. L.; Campbell, M.; Meijer, L.; Timsit, S. *PLoS ONE* **2010**, 5.
25. Pareek, T. K.; Kulkarni, A. B. *Cell Cycle* **2006**, *5*, 585.
26. Utreras, E.; Futatsugi, A.; Rudrabhatla, P.; Keller, J.; Iadarola, M. J.; Pant, H. C.; Kulkarni, A. B. *J. Biol. Chem.* **2009**, *284*, 2275.
27. Wei, F.-Y.; Nagashima, K.; Ohshima, T.; Saheki, Y.; Lu, Y.-F.; Matsushita, M.; Yamada, Y.; Mikoshiba, K.; Seino, Y.; Matsui, H.; Tomizawa, K. *Nat. Med.* **2005**, *11*, 1104.
28. Strock, C. J.; Park, J. I.; Nakakura, E. K.; Bova, G. S.; Isaacs, J. T.; Ball, D. W.; Nelkin, B. D. *Cancer Res.* **2006**, *66*, 7509.
29. Legraverend, M.; Grierson, D. S. *Bioorg. Med. Chem.* **2006**, *14*, 3987.
30. Meijer, L.; Raymond, E. *Acc. Chem. Res.* **2003**, *36*, 417.
31. Meijer, L.; Bettayeb, K.; Galons, H. Roscovitine (CYC202, Seliciclib) In *Monographs on enzyme inhibitors. CDK inhibitors and their potential as anti-tumor agents*; Smith, P. J., Yue, E., Eds.; CRC Press: Taylor & Francis: Boca Raton, Fl.;, 2006; Vol. 2, pp 187–226. chapter 9.
32. Bain, J.; Plater, L.; Elliott, M.; Shpiro, N.; Hastie, C. J.; McLauchlan, H.; Klevernic, I.; Arthur, J. S.; Alessi, D. R.; Cohen, P. *Biochem. J.* **2007**, *408*, 297.
33. Oumata, N.; Bettayeb, K.; Ferandin, Y.; Demange, L.; Lopez-Giral, A.; Goddard, M.-L.; Myrianthopoulos, V.; Mikros, V.; Flajolet, M.; Greengard, P.; Meijer, L.; Galons, H. *J. Med. Chem.* **2008**, *51*, 5229.
34. Popowycz, F.; Fournet, G.; Schneider, C.; Bettayeb, K.; Ferandin, Y.; Lamigeon, C.; Tirado, O. M.; Mateo-Lozano, S.; Notario, V.; Colas, P.; Bernard, P.; Meijer, L.; Joseph, B. *J. Med. Chem.* **2009**, *52*, 655.
35. Demange, L.; Oumata, N.; Quinton, J.; Bouaziz, S.; Lozach, O.; Meijer, L.; Galons, H. *Heterocycles* **2008**, *75*, 1735. General procedure for Buchwald Hartwig amination: a solution of $Pd(OAc)_2$ and BINAP in 10 mL of dry toluene was warmed at 45 °C for 5 min. 2,6-Dichloro-9-iso-propylpurine was then added under N_2 bubbling. The mixture was kept at 45 °C for 10 min and *tert*-BuOK was added. After 10 min, the required aminoaryl was added. The mixture was heated under N_2 until the reaction was completed (3 h to 2 days depending on the amine) at 100 °C. The proportion for each reactant, and the reaction time depend on the nature of the aminoaryl and are summarized in Table 1 After cooling at room temperature, the mixture was filtrated through celite, and toluene was evaporated. The residue was diluted in CH_2Cl_2 (75 mL) and washed (1×10 mL) with water and brine (2×10 mL). The organic layer was dried and concentrated under vacuum. The residue was purified by chromatography on silica gel using various amount of AcOEt/cyclohexane/ethanol as eluent.
36. *General procedure for 2 chlorine nucleophilic substitution*: A mixture of 2-chloro-6-arylamino-9-iso-propylpurine (0.3 mmol), NEt_3 (0.45 mmol) and amino-alcohol (1.5 mmol) in DMSO (from 0 to 1 mL, depending on the aminoaryl stability) was heated at 120 °C until the reaction was completed (3 h to 5 days). After cooling at room temperature, the mixture was diluted with CH_2Cl_2 (35 mL) and washed with water (5 mL) and brine (5 mL). The organic layer was dried and concentrated under vacuum. The residue was purified by chromatography on silica gel using various amount of AcOEt/ethanol/NEt_3 as eluent.
37. Leclerc, S.; Garnier, M.; Hoessel, R.; Marko, D.; Bibb, J. A.; Snyder, G. L.; Greengard, P.; Biernat, J.; Wu, Y. Z.; Mandelkow, E.-M.; Eisenbrand, G.; Meijer, L. *J. Biol. Chem.* **2001**, *276*, 251.
38. Demange, L.; Lozach, O.; Ferandin, Y.; Hoang, N.-T.; Meijer, L.; Galons, H. *Med. Chem. Res.* **2012**. http://dx.doi.org/10.1007/s00044-012-0334-1.
39. Heathcore, D. A.; Patel, H.; Kroll, S. H. B.; Hazel, P.; Periyasamy, M.; Alikian, M.; Kanneganti, S. K.; Jogalekar, A. S.; Scheiper, B.; Barbazanges, M.; Blum, A.; Brackow, J.; Siwicka, A.; Pace, R. D. M.; Fuchter, M. J.; Snyder, J. P.; Liotta, D. C.; Freemont, P. S.; Aboagye, E. O.; Coombes, R. C.; Barrett, A. G. M.; Ali, S. *J. Med. Chem.* **2010**, *53*, 85082.
40. Laha, J. K.; Zhang, X.; Qiao, L.; Liu, M.; Chatterjee, S.; Robinson, S.; Kosic, K. S.; Cuny, G. D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2098.
41. Shiradkar, M.; Thomas, J.; Kanase, V.; Dighe, R. *Eur. J. Med. Chem.* **2011**, *46*, 2066.
42. Wegiel, J.; Gong, C. X.; Hwang, Y. W. *FEBS J.* **2011**, *278*, 239.
43. Neagoie, C.; Vedrenne, E.; Buron, F.; Mérour, J. Y.; Rosca, S.; Bourg, S.; Lozach, O.; Meijer, L.; Baldeytou, B.; Lansiaux, A.; Routier, S. *Eur. J. Med. Chem.* **2012**, *49*, 379.
44. Becker, W.; Sippli, W. *FEBS Lett.* **2011**, *278*, 246.
45. Wang, D.; Wang, F.; Tan, Y.; Dong, L.; Chen, L.; Zhu, W.; Wang, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 168.