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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of new potent inhibitors of CK1 and CDK5, two kinases involved in Alzheimer's disease

Luc Demange · Olivier Lozach · Yoan Ferandin · Nha Thu Hoang · Laurent Meijer · Hervé Galons

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Abstract Cyclin-dependent kinase 5 (CDK5) and Casein kinase 1 (CK1) are both involved in the hyperphosphorylation of the Tau protein and in the amyloid-β production, the two major hallmarks of Alzheimer's disease. In the present paper, we describe the synthesis and biological evaluation of new series of 2,6,9-trisubstituted purines derived from DRF53, a dual specificity inhibitor of the kinase activity of CDK5 ($IC_{50} = 80$ nM) and CK1 ($IC_{50} = 10$ nM), and are able to prevent in a dose-dependent manner the CK1-dependent production of amyloid-β in a cell model. Several molecules (e.g., **6e**, **6g**, **7c**) displayed potent kinase inhibitory activities against CDK5 and CK1 (IC_{50} values ranging from 20 to 50 nM) among which a selective inhibitor of CK1 has been identified (**5a**, $IC_{50} = 60$ nM). In addition, some compounds exhibit submicromolar activities against DYRK1A (dual specificity, tyrosine phosphorylation regulated kinase 1A), a kinase involved in Down syndrome and Alzheimer's disease (**6g**, $IC_{50} = 510$ nM).

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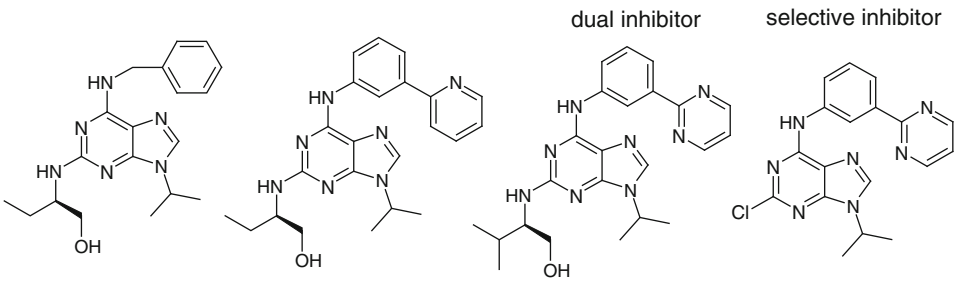
Keywords Alzheimer's disease · Down syndrome · CDK5 · CK1 · Kinase · Roscovitine

Introduction

Alzheimer's disease (AD) is characterized by a progressive and irreversible alteration of memory, a loss of the language skill, and a general deregulation of the cerebral activity. This is the leading cause of dementia among the elderly, and its economical impact is continually growing due to the increase of life expectancy. To date, very few drugs, including inhibitors of acetylcholinesterase and a NMDA receptor antagonist (Gravitz, 2011), are clinically used to temporarily restrain the effects of AD, but there is no treatment to prevent or to cure this pathology at the moment.

The two major hallmarks of AD are the hyperphosphorylation of the Tau protein (responsible for the formation of neurofibrillary tangles of paired helical filaments and for the cellular death) (Götz *et al.*, 2012) and the amyloid-β production (responsible for the formation of extracellular amyloid plaques) (Ittner and Götz, 2011). It has been suggested that the deregulation of several multi-function protein kinases (PKs) including Cyclin-dependent kinases 1 and 5 (CDK1, CDK5) (Cruz and Tsai 2004, 2006; Sadleir and Vassar, 2012; Camins *et al.*, 2006), Casein Kinase (CK1) (Flajolet *et al.*, 2007), Glycogen Synthase Kinase-3 (GSK3) (Huang and Klein, 2006; Martin *et al.*, 2011; Dominguez *et al.*, 2012), and the dual specificity tyrosine phosphorylation kinase 1A (DYRK1A) (Wegiel *et al.*, 2011), might be involved in those hallmarks. Thus, these PKs form a promising new class of potential targets in AD and their inhibition might be promising against this pathology.

We previously reported the synthesis and the biological evaluation of new families of 2,6,9-trisubstituted purines



	(*R*)-roscovitine	DRF 53	**7c**	**5a**
CDK1/cyclinB	0.35 μM	0.22 μM	-	10.0 μM
CDK5/p25	0.20 μM	0.08 μM	0.05 μM	1.3 μM
CK1	2.3 μM	0.01 μM	0.06 μM	0.06 μM

Fig. 1 IC_{50} comparison between previously reported kinase inhibitors (roscovitine and DRF53) and two selected molecules from the present study. See legend of Table 1 for details; – not tested

(Oumata *et al.*, 2008; Bettayeb *et al.*, 2008; Demange *et al.*, 2008, 2012), structurally related to Roscovitine, an advanced CDK inhibitor currently in clinical trials phase 2 or 2b against different kinds of cancers and in phase 1 against renal diseases including glomerulonephritis (Meijer *et al.*, 2006). Among these new series of molecules, DRF53 (Fig. 1), is one of the more potent inhibitors of CDK5 kinase activity ($IC_{50} = 80$ nM) and CK1 kinase activity ($IC_{50} = 10$ nM). Moreover, this molecule is able to prevent, in a dose-dependent manner, the CK1-dependent production of amyloid-β in a cell model (Oumata *et al.*, 2008), and consequently might have applications to prevent the appearance and development of AD's hallmarks. We explored in the present study the potencies of new derivatives of the DRF53 family bearing a 6-aminobiarylic core including a pyrimidine ring in terms of inhibition and selectivity on relevant kinases (CDK1, CDK5, GSK-3, CK1, and DYRK1A).

Result and discussion

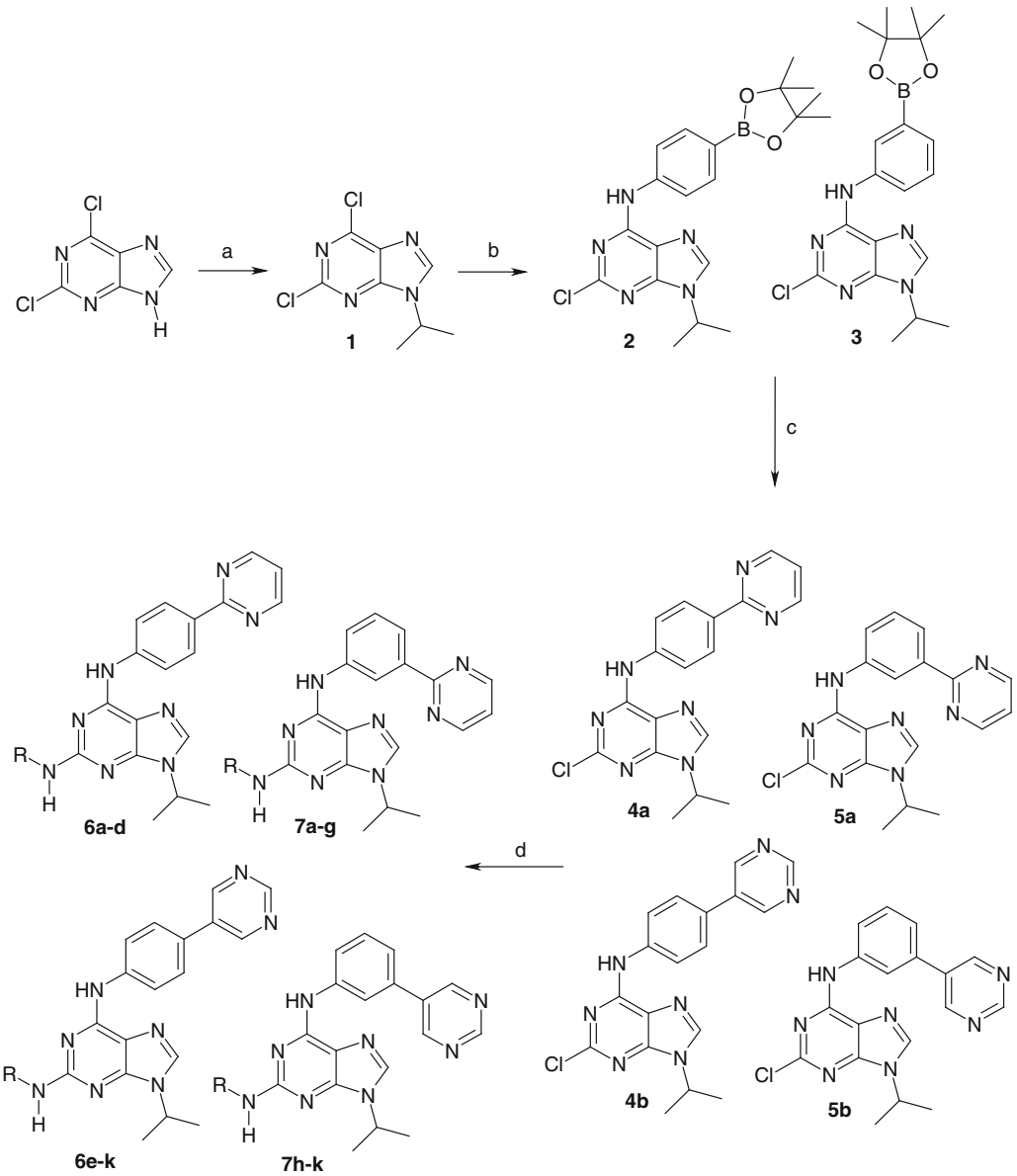
Chemistry

The new families of 2,6,9-trisubstituted purines were easily synthesized from the commercial 2,6-dichloropurine as previously described (Scheme 1) (Oumata *et al.*, 2008). Briefly, the regiospecific 9-alkylation of the starting material was achieved in 70 % yield using isopropylbromide in DMSO at 18 °C (in order to avoid regioselectivity problems, the temperature should be carefully respected), and the resulting 2,6-dichloro-9-isopropylpurine **1** was allowed to react with appropriate boranylaniline to afford in high yield the corresponding stable purines **2** and **3**. These compounds are converted into a biarylic core through a Suzuki cross-coupling reaction using 2-bromopyrimidine or 5-bromopyrimidine to give products **4a–b** and **5a–b** in high yield. Both steps occur with a good regioselectivity, and without the formation of secondary materials. Finally, the substitution of the 2-chlorine of the purine ring by amino alcohols with an excess of triethylamine in DMSO at 150 °C led to an average yield of 35 % depending on the amino alcohol nucleophilicity to the expected 2,6,9-trisubstituted purine families **6a–k** and **7a–k**.

The structures of the newly synthesized trisubstituted purines were confirmed by spectral data: ^{1}H NMR, ^{13}C NMR, and mass spectra analysis. The NMR ^{1}H spectra were recorded in $CDCl_3$; these spectra are divided into two parts. The aliphatic protons correspond to the C^2 amino–alcohol, and to the N^9 isopropyl, that appears as a classical doublet (δ range between 1.47 and 1.63 ppm) coupled with a heptuplet (δ range between 4.21 and 4.67 ppm) with ^{3}J coupling constant range between 6.3 and 8.0 Hz. Among the aromatic protons, the singlet at 7.88 ppm is characteristic from the purine H^8, and taking advantage of multiplicity, integration, and chemical displacement, the other aromatic protons might be easily attributed to the phenyl or pyrimidinyl ring. ^{13}C NMR experiments confirm the scaffold of the structures and the ion peaks of the mass spectrum are in agreement with the molecular formulas of all compounds. Lastly, we determined the melting point for each fully solid new molecule (see "Experimental procedures" section for details).

Biological assay

The inhibition of CDK5/p25, GSK-3α/β, and CK1δ/ε 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



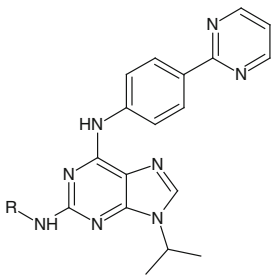
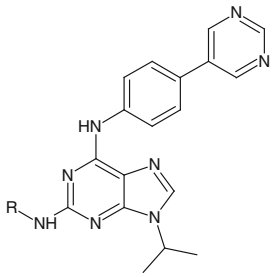
Scheme 1 *Reagents and conditions* (*a*) 2-bromopropane, K_2CO_3, DMSO 15–18 °C, 5 days; (*b*) Ar–NH_2, NEt_3, BuOH, 100 °C, 1 day; (*c*) 2-bromopyrimidine or 5-bromopyrimidine, Na_2CO_3, $Pd[P(C_6H_5)_3]_4$, H_2O, dioxane, 8 h.; (*d*) RNH_2, NEt_3, DMSO, 150 °C, 2–5 days

the "Experimental procedures" section. In order to evaluate the selectivity of the compounds, their inhibition of kinase activity was controlled under the same conditions with other purified kinases (DYRK1A, CDK1/cyclin B, CDK2/cyclin A). IC_{50} values were determined from dose–response curves. Globally, all newly synthesized purines are micromolar or sub-micromolar inhibitors of the catalytic activity of CDK5 and CK1.

The most active compounds against the kinase activity of CDK5 reported in this paper are **6g**, **7c**, **7j,** and **6e** (IC_{50} range between 20 nM and 60 nM). Several newly synthesized purines are more potent than Roscovitine or its optimized derivative Purvalanol A ($IC_{50} = 75$ nM) and exhibit IC_{50} values similar to our reference compound DRF 53 (Table 1) or purine bioisosteres such as pyrazolo[1,5-*a*]-1,3,5-triazine (IC_{50} range between 70 and 320 nM) (Popowycz *et al.*, 2009). Moreover, their activities are very similar to the CDK5-specific inhibitors described by others with different scaffolds including pyrazolopyrimidine ring ($IC_{50} = 30$ nM) (Heathcore *et al.*, 2010), 2,4-diaminothiazoles scaffold (IC_{50} range between 15 nM and 1 μM) (Laha *et al.*, 2011), and a cyclohexyl-thiophene moiety linked with triazole (IC_{50} range between 35 nM and 1 μM) (Shiradkar *et al.*, 2011).

Interestingly, the structure of the biarylic core seems to be very important in terms of selectivity for CK1. In particular, compounds bearing the phenyl-3-(2-pyrimidinyl) moiety (compounds **5a**, **7a–7g**) are very efficient against

Table 1 Biological evaluation of compounds bearing 6-Phenyl-4-(2-pyrimidinyl) and 6-Phenyl-4-(5-pyrimidinyl)

Compd.	Scaffold	R	CDK1	CDK2	CDK5	GSK 3α/β	DYRK1A	CK1
6a		(*R*)-1-hydroxy-but-2-yl	0.18		0.10	25	–	1
6b		(*S*)-1-hydroxy-but-2-yl	0.5	0.35	0.27	>10	0.65	1.7
6c		(*R*)-1-hydroxy-3-methylbut-2-yl	–	0.083	0.083	7.1	0.5	1.8
6d		(*S*)-1-hydroxy-3-methylbut-2-yl	–	0.6	1.0	9	0.8	2.3
4b		Cl	–	1.1	2.1	>10	3.2	0.41
6e		(*R*)-1-hydroxy-but-2-yl	0.04	–	0.06	21	–	0.6
6f		(*S*)-1-hydroxy-but-2-yl	0.07	0.2.	0.2	100	1.2	1.3
6g		(*R*)-1-hydroxy-3-methylbut-2-yl	0.017	0.03	0.02	10	0.51	0.34
6h		(*S*)-1-hydroxy-3-methylbut-2-yl	0.27	0.61	0.48	>10	1.1	1.8
6i		(*S*)-1-hydroxy-4-methylpent-2-	–	0.16	0.54	13	1.1	2.2
6j		1,3-dihydroxyprop-2-yl	–	0.33	0.19	35	1.2	0.64
6k		1-hydroxy-2-methylprop-2-yl	–	0.2	0.11	11	2.3	0.5

Purines were tested at various concentrations on CDK1/cyclin B, CDK2/cyclin A, CDK5/p25, GSK-3α/ß, DYRK1A, and CK1δ/ε, as described in the "Experimental procedures" section. IC_{50} values, calculated from the dose–response curves, are reported in μM. IC_{50} values are reported in μM. IC_{50} value reported as >10 indicates that the compound did not display any inhibitory activity at the highest concentration tested (10 μM)

– not tested

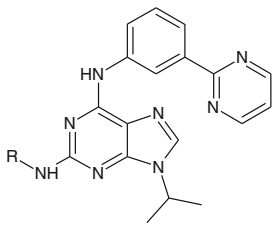
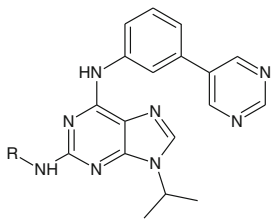
this kinase (Table 2). In this family, compound **7c** is a dual inhibitor of CDK5 and CK1 (CDK 5 : IC_{50} = 50 nM; CK1 : IC_{50} = 60 nM).

Products with a chlorine atom in the 6 position of the purine ring are also highly selective for CK1, and the presence of this halogen is not detrimental in terms of inhibition as shown in the case of compound **5a** (see Table 2, CDK 5 : IC_{50} = 1900 nM; CK1 : IC_{50} = 60 nM) which exhibits an IC_{50} value very close to that of DRF 53. In either case, the substitution of chlorine by an amino alcohol moiety does not improve the potency of the molecule against CK1—moreover, this substitution might be detrimental (compare IC_{50} values for compounds **4b**, and **6e–k**, Table 1). Those results are very surprising because previously reported 2,6,9-trisubstituted purines acting as CK1 inhibitors have an amino alcohol in the 6 position (DRF 53, see structure in Fig. 1, IC_{50} = 10 nM) (Oumata *et al.*, 2008).

We previously studied the docking of DRF53 in the ATP-binding domain of the CK1 catalytic site (Oumata *et al.*, 2008) and suggested the presence of an H-bond between the side chain of Arg 16 and the nitrogen in the 2 position of the pyridyl group belonging to the biarylic moiety. These stabilizing interactions (Fig. 2) might also take place in the case of the strongest CK1 inhibitors reported in this paper (compounds **5a** or **7a–g**, see Table 2).

In the past decade, several CK1 nM inhibitors (isolated from natural sources) such as hymenialdisine (CDK 5:IC_{50} = 30 nM; CK1:IC_{50} = 30 nM), which is an pyrrole-imidazole alkaloid extracted from marine sponge (Meijer *et al.*, 2000), or synthetic compounds such as the potent 2,4-diaryl-imidazole family described by Peifer and coworkers (IC_{50} values against CK1 kinase activity ranging from 4 to 1500 nM) (Peifer *et al.*, 2009) have been reported (for a recent review, see Perez *et al.,* 2011). More recently, few pyrimidinyl pyrrolopyridinones (IC_{50} range between 3 nM and 800 nM) (Huang *et al.*, 2012) and two polyheterocyclic molecules, including both thiazole and benzimidazole rings (IC_{50} range between 40 and 100 nM) (Bischof *et al.*, 2012), have been reported as selective and potent CK1 inhibitors, but their activities against CDKs and precisely against CDK5 are still unknown. Moreover, all these reported inhibitors have generally complex structures and their preparation involves long multi-step syntheses (see Papeo *et al.*, 2005 and Mangu *et al.*, 2010 in

Table 2 Biological evaluation of compounds bearing 6-Phenyl-3-(2-pyrimidinyl) and 6-Phenyl-3-(5-pyrimidinyl)

Compd.	Scaffold	R	CDK1	CDK2	CDK5	GSK 3α/β	DYRK1A	CK1
5a		Cl	10	1.9	1.3	>10	>10	0.06
7a		(*R*)-1-hydroxy-but-2-yl	0.3		0.2	11	–	0.06
7b		(*S*)-1-hydroxy-but-2-yl	0.73	0.32	0.5	17	2.1	0.1
7c		(R)-1-hydroxy-3-methylbut-2-yl	–	0.074	0.05	4.3	2	0.06
7d		(S)-1-hydroxy-3-methylbut-2-yl	–	0.7	0.9	11	2	0.18
7e		1-hydroxy-2-methylprop-2-yl	–	–	0.11	9.9	4.7	0.05
7f		1,3-dihydroxyprop-2-yl	–	–	0.62	10	7	0.07
7g		(S)-1-hydroxy-4-methylpent-2-yl	–	–	2	1.5	0.71	0.31
5b		Cl	–	–	1	>10	3	0.38
7h		(R)-1-hydroxy-but-2-yl	0.13	–	0.07	7.3	–	0.3
7i		(S)-1-hydroxy-but-2-yl	0.3	–	0.3	20	–	0.2
7j		(R)-1-hydroxy-3-methylbut-2-yl	–	0.051	0.052	6.9	1	0.41
7k		1-hydroxy-2-methylprop-2-yl	–	0.11	0.11	>10	3	0.32

See legend of Table 1 for details. All values are reported in μM

the case of hymenialdisine derivatives); consequently, the subsequent pharmacomodulations, in order to improve activity or selectivity, might be very challenging. By contrast, the purine scaffold that is described here is chemically accessible, stable, and adjustable. Purines are widely used in medicinal chemistry due to their very favorable biological properties (Legraverend and Grierson, 2006) and their use has been previously suggested in the case of AD, for example to reduce the activity of γ-secretase and therefore the formation of amyloid-*β* peptide and the amyloid plaques (Rivkin *et al.,* 2010).

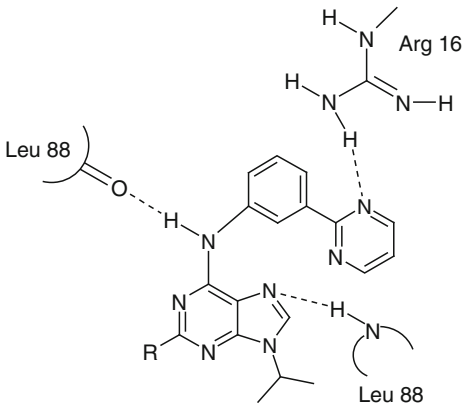


Fig. 2 Suggestion of H-bonding between compounds **7a–g** and key amino acids from the ATP-binding pocket of CK1. This figure takes advantage of our previously reported modeling of DRK53 in ATP domain of the CK1 catalytic site (Oumata *et al.*, 2008)

Lastly, we evaluated the effects of our new purines' derivatives on DYRK1A. This serine/threonine kinase has critical roles in human development and its deregulation is suggested in multiple neurodegenerative pathologies. Thus, its gene is located on chromosome 21, within the Down syndrome (DS) critical region, and its over expression is involved in the phosphorylation abnormalities of protein tau observed in DS and AD (Frost *et al.*, 2011). In addition, DYRK1A has been reported to be involved in the neurofibrillary tangles formation in AD (Wegiel *et al.*, 2008; 2011). Altogether, these results pointed out that this kinase might be a very promising therapeutic target against these pathologies. With the significant exceptions of the analogs of Lamellarin D including in their structure a complex chromeno[3,4-*b*]indole skeleton (Neagoie *et al.*, 2012), the substituted bromo-indolic scaffold of meridianins derivatives (Giraud *et al.*, 2011), and few polysubstituted quinazolines (Rosenthal *et al.*, 2011), there are very few sub-micromolar DYRK1A inhibitors reported in the recent literature (Becker and Sippl, 2011; Wang *et al.*, 2012; Debdab *et al.*, 2011). Moreover, none of these molecules have reached the stage of clinical evaluation. Among our newly synthesized purines, several compounds such as **6b**, **6d**, and **7g** exhibit significant inhibition of DYRK1A (IC_{50} range between 0.5 and 0.7 μM). Moreover, **7g** seems to be quite selective for this kinase (see Table 2). To the best of our knowledge, these molecules are the first purines described as DYRK1A inhibitors. Therefore, they might be

considered as potentially promising for development of new DYRK1A kinase selective inhibitors.

Conclusion

We prepared in good yields new series of 6–aminobiarylic purines structurally related to roscovitine and DRF 53 (Oumata *et al.*, 2008) using a convergent synthesis procedure that includes a Suzuki cross-coupling reaction as the key step. Thus, we identified very potent inhibitors of the kinase activity of CDK5 and CK1 (IC_{50} ranging below 50 nM). The strong binding of CK1 by these inhibitors is reliable to a potential H-bond involving the Arg-16 of the ATP binding site, as previously suggested by molecular modeling in the case of DRF 53. Interestingly, some newly synthesized molecules are promising inhibitors of DYRK1A, another serine/threonine kinase involved in several neuronal pathologies including AD. Although these molecules endowed several atoms considered as H-bond donors and acceptors involved in the inhibitor binding to the ATP kinase pocket, a recent review (Ghose *et al.*, 2012) reveals that such kind of a compound might be considered for the development of new CNS pathology targeted drugs. Thus, further studies will allow us to determine whether the most potent compounds are able to reduce the production of amyloid-β in a cell model in order to identify their potency to be used against Alzheimer's disease.

Experimental procedures

Chemistry

General procedures

Chemical reagents and solvents were purchased from Sigma-Aldrich, Fluka, and Carlo Erba. Reactions were monitored by TLC using Merk silica gel 60F-254 thin layer plates. Column chromatographies were performed on SDS Chromagel 60 ACC 40–63 μM. Melting points were determined on a Köfler hot-stage (Reichert) and were uncorrected. NMR spectra were recorded on Brucker Avance 400 MHz (100 MHz for ^{13}C NMR) at 300 K. Chemical shifts were reported as δ values (ppm) indirectly referenced to the solvent signal or to tetramethylsilane (TMS) as the internal standard. Data are reported in the conventional form. Mass spectra were recorded on a ZQ 2000 Waters using a Z-spray (ESI–MS).

Synthesis and structures of compounds **1**, **2**, and **3** have been previously reported in the literature and are not described in this section.

General procedure for the Suzuki cross-coupling reaction

To a solution of **2** or **3** (0.38 g, 1.0 mmol) in dioxane (5 mL) and Na_2CO_3 1 M (5 mL) under N_2 atmosphere, $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) was added. After 5 min. of stirring, 2-bromopyrimidine or 5-bromopyrimidine (238 mg, 1.5 mmol) was added. The mixture was heated for 1 day at 100 °C, then concentrated in vacuo. The resulting material is diluted in CH_2Cl_2, then washed with water and brine. The organic layer was dried and evaporated. The crude material was purified by chromatography on silica gel, using AcOEt/Cyclohexane as the solvent.

*2-Chloro-6-[4-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine (**4a**)* ^1H NMR($CDCl_3$): δ 1.63 (d, 6H, $J = 6.8$ Hz, $CH(CH_{3)2}$), 4.88 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 7.16 (t, 1H, $J = 4.8$ Hz, $H_{pyrimidyl}$), 7.92 (s, 1H, 8-H), 7.92 (d, 2H, $J = 9.2$ Hz, H_{phenyl}), 8.49 (d, 2H, $J = 8.8$ Hz, H_{phenyl}), 8.79 (d, 2H, $J = 4.8$ Hz, $H_{pyrimidyl}$).

*2-Chloro-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine (**4b**)* m.p. 264–268 °C. ^1H NMR($CDCl_3$): δ 1.63 (d, 6H, $J = 9$ Hz, $(CH_3)_2CHNH$), 4.89 (hept, 1H, $J = 9$ Hz, $(CH_3)_2CHNH$), 7.64 (d, 1H, $J = 9$ Hz, H_{phenyl}), 7.90 (s, 1H, NH), 7.92 (s, 1H, 8-H), 7.98 (d, 1H, $J = 9$ Hz, H_{phenyl}), 8.98 (s, 2H, $H_{pyrimidinyl}$), 9.20 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR (DMSO): δ 22.0, 47.0, 119.3, 121.3, 127.0, 128.1, 132.8, 139.8, 140.6, 150.4, 151.9, 152.1, 154.2, 156.7. MS (ES^+) *m/z* 366 $(M+H)^+$, 388 $(M+Na)^+$.

*2-Chloro-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine (**5a**)* m.p. > 270 °C. ^1H NMR($CDCl_3$): δ 1.62 (d, 6H, $J = 9$ Hz, $(CH_3)_2CHNH$), 4.88 (hept, 1H, $J = 9$ Hz, $(CH_3)_2CHNH$), 5.30 (s, 1H, NH), 7.23 (t, 1H, $J = 6$ Hz, $H_{pyrimidinyl}$), 7.56 (t, 1H, $J = 9$ Hz, H_{phenyl}), 7.89 (s, 1H, 8-H), 8.22 (d, 1H, $J = 8$ Hz, H_{phenyl}), 8.26 (d, 1H, $J = 8$ Hz, H_{phenyl}), 8.60 (bs, 1H, H_{phenyl}), 8.84 (d, 1H, $J = 6$ Hz, $H_{pyrimidinyl}$). ^{13}C NMR(DMSO): δ 22.1, 47.1, 119.2, 120.1, 121.0, 123.0, 123.9, 129.0, 137.8, 139.3, 140.6, 150.5, 152.1, 152.5, 157.8, 163.3. MS (ES^+) *m/z* 366 $(M+H)^+$, 388 $(M+Na)^+$.

*2-Chloro-6-[3-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine (**5b**)* m.p. 188–190 °C. ^1H NMR($CDCl_3$): δ 1.63 (d, 6H, $J = 6.9$ Hz, $(CH_3)_2CHNH$), 4.88 (hept, 1H, $J = 6.9$ Hz, $(CH_3)_2CHNH$), 7.35 (d, 1H, $J = 7.8$ Hz, H_{phenyl}), 7.54 (t, 1H, $J = 7.8$ Hz, H_{phenyl}, 7.77 (d, 1H, $J = 8.6$ Hz, H_{phenyl}), 7.90-7.96 (m, 2H, 8-H + NH), 8.23 (s, 1H, H_{phenyl}), 9.03 (s, 2H, $H_{pyrimidyl}$), 9.24 (s, 1H, $H_{pyrimidyl}$). ^{13}C NMR(DMSO): δ 22.0, 47.0, 119.1, 119.4, 121.3, 121.6, 129.5, 133.2, 133.8, 139.8, 140.5, 150.4, 151.9, 152.2, 154.5, 157.3. MS (ES^+) *m/z* 366 $(M+H)^+$, 388 $(M+Na)^+$.

General procedure for the N^2 amination

To a solution of **4** or **5** (0.2 g, 0.5 mmol) and the selected amino alcohol (2.5 mmol) in DMSO (1 mL), NEt_3 (0.5 mL) was added. The mixture was heated at 150 °C for 1–5 days. After cooling to r.t., 20 mL of CH_2Cl_2 was added, and the resulting solution was extracted three times with water and brine. The organic layer was dried and evaporated. The crude material was purified by chromatography on silica gel, using AcOEt/Cyclohexane/NEt_3 as the solvent.

(R)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6a)*** Yield 39 %. ^{1}H NMR($CDCl_3$): δ 0.99 (t, 3H, J = 8 Hz, CH_3CH$_2$), 1.48 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 1.52–1.67 (m, 2H, CH$_3$CH_2), 3.57-3.66 (m, 1H, CH_2OH), 3.77–3.85 (m, 1H, CH_2OH), 3.90-3.99 (m, 1H, C*H*NH), 4.55 (hept, 1H, J = 6.8 Hz, NC*H*(CH$_3$)$_2$), 4.97–5.03 (m, 1H, N*H*), 7.07 (t, 1H, J = 5.0 Hz, H$_{pyrimidinyl}$), 7.53 (s,1H, 8-H), 7.84 (d, 2H, J = 8.5 Hz, H$_{phenyl}$), 8.36 (d, 2H, J = 8.5 Hz, H$_{phenyl}$), 8.71 (d, 2H, J = 5.0 Hz, H$_{pyrimidinyl}$). ^{13}C NMR: δ 10.9, 22.4, 22.5, 24.9, 46.6, 56.1, 63.7, 115.2, 118.5, 119.4, 129.0, 131.9, 135.3, 141.6, 152.0, 157.1, 164.4. MS (ES$^+$) m/z 419 (M+H)$^+$, 441 (M+Na)$^+$.

(S)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6b)*** Yield 43 %. ^{1}H NMR($CDCl_3$): δ 1.07 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.59 (d, 6H, J = 6.8 Hz, (CH_3)$_2$CHN), 1.63-1.67 (m, 2H, CH$_3$CH_2), 3.70–3.85 (m, 2H, CH_2OH), 3.96–4.02 (m, 1H, C*H*NH), 4.21 (hept, 1H, J = 6.8 Hz, (CH$_3$)$_2$C*H*N), 7.15 (t, 1H, J = 4.8 Hz, H$_{pyrimidinyl}$), 7.62 (s,1H, 8-H), 7.92 (d, 2H, J = 8.4 Hz, H$_{phenyl}$), 8.45 (d, 2H, J = 8.4 Hz, H$_{phenyl}$), 8.78 (d, 2H, J = 4.8 Hz, H$_{pyrimidinyl}$). ^{13}C NMR: δ 10.8, 22.3, 22.5, 24.9, 46.5, 56.1, 63.6, 115.2, 118.5, 119.3, 129.0, 131.8, 135.3, 141.6, 152.0, 157.2, 164.4. MS (ES$^+$) m/z 419 (M+H)$^+$, 441 (M+Na)$^+$.

(R)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6c)*** Yield 38 %, m.p. 128–133 °C. ^{1}H NMR($CDCl_3$): δ 0.99 (d, 6H, J = 6.5 Hz, (CH_3)$_2$CHCH), 1.47 (d, 6H, J = 6.6 Hz, NCH(C$H_{3)2}$), 1.91–2.02 (m, 1H, CHC*H*(CH$_3$)$_2$), 3.69 (dd, 1H, J = 10.8 Hz and J' = 8.0 Hz, CH_2OH), 3.78–3.92 (m, 2H, CH_2OH + C*H*NH), 4.52 (hept, 1H, J = 6.4 Hz, (CH$_3$)$_2$C*H*NH), 5.00 (1H, bd, J = 7.1 Hz, N*H*), 7.07 (t, 1H, J = 4.5 Hz, H$_{pyrimidinyl}$), 7.52 (s,1H, 8-H), 7.82 (d, 2H, J = 7.8 Hz, H$_{phenyl}$), 7.91 (s, 1H, N*H*), 8.36 (d, 2H, J = 7.8 Hz, H$_{phenyl}$), 8.71 (d, 2H, J = 4.5 Hz, H$_{pyrimidinyl}$). ^{13}C NMR: δ 18.9, 19.4, 22.4, 30.0, 46.4, 59.5, 65.1, 115.1, 118.4, 119.2, 128.8, 131.7, 135.1, 141.9, 150.0, 151.8, 157.1, 159.8, 164.4. MS (ES$^+$) m/z 433 (M+H)$^+$, 455 (M+Na)$^+$.

(S)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6d)*** Yield 47 %, m.p. 129–135 °C. ^{1}H NMR($CDCl_3$): δ 0.98 (d, 6H, J = 6.9 Hz, (CH_3)$_2$CHCH), 1.47 (d, 6H, J = 6.6 Hz, NCH(C$H_{3)2}$), 1.91–2.02 (m, 1H, CHC*H*(CH$_3$)$_2$), 3.68 (dd, 1H, J = 10.3 Hz and J' = 7.8 Hz, CH_2OH), 3.79–3.93 (m, 2H, CH_2OH + C*H*NH), 4.52 (hept, 1H, J = 6.6 Hz, (CH$_3$)$_2$C*H*NH), 5.00 (1H, bd, J = 6.4 Hz, N*H*), 7.07 (t, 1H, J = 4.8 Hz, H$_{pyrimidinyl}$), 7.52 (s,1H, 8-H), 7.82 (d, 2H, J = 8.6 Hz, H$_{phenyl}$), 7.91 (s, 1H, N*H*), 8.35 (d, 2H, J = 8.6 Hz, H$_{phenyl}$), 8.70 (d, 2H, J = 4.8 Hz, H$_{pyrimidinyl}$). ^{13}C NMR: δ 18.9, 19.4, 22.4, 30.0, 46.4, 59.5, 65.0, 115.0, 118.4, 119.2, 128.8, 131.5, 135.0, 141.9, 150.7, 151.8, 157.1, 159.8, 164.4. MS (ES$^+$) m/z 433 (M+H)$^+$, 455 (M+Na)$^+$.

(R)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6e)*** Yield 40 %, m.p. 112–118 °C. ^{1}H NMR($CDCl_3$): δ 1.06 (t, 3H, J = 7,2 Hz, CH_3CH$_2$), 1.57 (d, 6H, J = 6,8 Hz, NCH(CH_3)$_2$), 1.60-1.70 (m, 2H, CH$_3$CH_2), 3.70 (dd, 1H, J = 6,8 Hz and J' = 11 Hz, CH_2OH), 3.88 (d, 1H, J = 11 Hz, CH_2OH), 3.96-4.02 (m, 1H, C*H*NH), 4.65 (hept, 1H, J = 6,8 Hz, C*H*(CH$_3$)$_2$), 7.64 (d, 1H, J = 8.8 Hz, H$_{phenyl}$), 7.86 (s, 1H, 8-H), 7.99 (d, 2H, J = 8.8 Hz, H$_{phenyl}$), 8.97 (s, 2H, H$_{pyrimidinyl}$), 9.2 (s, 1H, $_{pyrimidinyl}$). ^{13}C NMR: δ 10.9, 22.4, 22.5, 24.9, 46.7, 55.9, 67.1, 115.1, 120.4, 127.3, 128.0, 133.9, 135.3, 140.2, 150.8, 152.0, 154.4, 156.9, 159.6, MS (ES$^+$) m/z 419 (M+H)$^+$, 441 (M+Na)$^+$, 457 (M+K)$^+$.

(S)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6f)*** Yield 29 %, m.p. 110–115 °C. ^{1}H NMR($CDCl_3$): δ 0.96 (t, 3H, J = 7,2 Hz, CH_3CH$_2$),1.63 (d, 6H, J = 6.8 Hz, NCH(C$H_{3)2}$), 1.63–1.67 (m, 2H, CH$_3$CH_2), 3.70–3.85 (m, 2H, CH_2OH), 3.96-4.02 (m, 1H, C*H*NH), 4.82 (hept, 1H, J = 6.8 Hz, C*H*(CH$_3$)$_2$), 7.64 (d, 1H, J = 8.4 Hz, H$_{phenyl}$), 7.86 (s, 1H, 8-H), 7.99 (d, 2H, J = 8.4 Hz, H$_{phenyl}$), 8.97 (s, 2H, H$_{pyrimidinyl}$), 9.2 (s, 1H, H$_{pyrimidinyl}$). ^{13}C NMR: δ 10.9, 22.3, 22.4, 24.8, 46.7, 55.8, 66.6, 115.2, 120.4, 127.1, 127.8, 133.8, 135.2, 151.9, 154.3, 156.7, 159.5. MS (ES$^+$) m/z 419 (M+H)$^+$, 441 (M+Na)$^+$, 457 (M+K)$^+$.

(R)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6g)*** Yield 39 %. ^{1}H NMR($CDCl_3$): δ 0.99 (d, 6H, J = 6.8 Hz, CH_3CH),1.50 (d, 6H, J = 6.6 Hz, NCH(C$H_{3)2}$), 1.93–2.03 (m, 1H, (CH$_3$)$_2$C*H*), 3.68 (dd, 1H, J = 10.8 Hz and J' = 7.7 Hz, CH_2OH), 3.82–3.90 (m, 2H, CH_2OH + C*H*NH), 4.57 (hept, 1H, J = 6.6 Hz, C*H*(CH$_3$)$_2$), 5.03 (1H, bd, J = 7.4 Hz, N*H*), 7.51 (d, 2H, J = 8.1 Hz, H$_{phenyl}$), 7.54 (s, 1H, 8-H), 7.81 (bs, 1H, N*H*), 7.88 (d, 2H, J = 8.1 Hz, H$_{phenyl}$), 8.89 (s, 2H, H$_{pyrimidinyl}$), 9.11 (s, 1H,

$H_{pyrimidinyl}$). ^{13}C NMR: δ 19.0, 19.4, 22.4, 22.5, 30.0, 46.6, 59.6, 65.3, 115.1, 120.3, 127.2, 127.9, 133.8, 135.3, 140.3, 150.8, 151.9, 154.4, 156.9, 159.8. MS (ES^+) *m/z* 433 $(M+H)^+$, 455 $(M+Na)^+$.

(S)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6h)*** Yield 41 %. ^1H NMR($CDCl_3$): δ 0.99 (d, 3H, $J = 6.8$ Hz, CH_3CH_2),1.51 (d, 6H, $J = 6.8$ Hz, $NCH(CH_3)_2$), 1.90–2.03 (m, 1H, $(CH_3)_2CH$), 3.60–3.71 (m, 1H, CH_2OH), 3.82–3.90 (m, 2H, CH_2OH + CHNH), 4.59 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 5.06 (1H, bd, $J = 7.6$ Hz, NH), 7.52 (d, 2H, $J = 8.6$ Hz, H_{phenyl}), 7.57 (s, 1H, 8-H), 7.93 (d, 2H, $J = 8.6$ Hz, H_{phenyl}), 8.40 (bs, 1H, NH) 8.90 (s, 2H, $H_{pyrimidinyl}$), 9.10 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 19.0, 19.4, 22.4, 22.5, 30.0, 46.6, 59.6, 65.4, 115.1, 120.3, 127.3, 128.0, 133.8, 135.3, 140.3, 152.0, 154.4, 156.9, 159.8. MS (ES^+) *m/z* 433 $(M+H)^+$, 455 $(M+Na)^+$.

(S)-2-(1-Hydroxy-4-methylpent-2-ylamino)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6i)*** Yield 49 %. ^1H NMR($CDCl_3$): δ 0.83–1.02 (m, 6H, $(CH_3)_2CHCH_2$), 1.36–1.48 (m, 2H, $CHCH_2CH$), 1.57 (d, 6H, $J = 6.5$ Hz, $(CH_3)_2CH$), 1.71–1.84 (m, 1H, $(CH_3)_2CHCH_2$), 3.84 (d, 1H, $J = 11.2$ Hz, CH_2OH), 4.07–4.25 (m, 2H, CH_2OH + CHNH), 4.64 (hept, 1H, $J = 6.5$ Hz, $CH(CH_3)_2$), 5.01 (1H, bd, $J = 7.1$ Hz, NH), 7.56 (d, 2H, $J = 8.5$ Hz, H_{phenyl}), 7.61 (s, 1H, 8-H), 7.85 (bs, 1H, NH), 7.93 (d, 2H, $J = 8.5$ Hz, H_{phenyl}), 8.95 (s, 2H, $H_{pyrimidinyl}$), 9.17 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 22.3, 22.4, 23.1, 24.8, 41.0, 46.5, 52.0, 67.2, 114.9, 120.3, 127.1, 127.8, 133.8, 135.2, 140.3, 150.9, 151.9, 154.3, 156.7, 159.4, MS (ES^+) *m/z* 447 $(M+H)^+$, 469 $(M+Na)^+$.

1,3-(Dihydroxyprop-2-yl)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6j)*** Yield 25 %. ^1H NMR($CDCl_3$): δ 1.58 (d, 6 H, $J = 6.6$ Hz $(CH_3)_2CH$), 3.70–3.90 (m, 7H, $(CH_2OH)_2$ + $(CH_2OH)_2$ + $CH(CH_2OH)_2$), 4.66 (hept, 1H, $J = 6.6$ Hz, $CH(CH_3)_2$), 5.80 (bd, 1H, $J = 6.0$ Hz, NH), 7.58 (d, 2H, $J = 8.1$ Hz, H_{phenyl}), 7.64 (s, 1H, 8-H), 8.03 (d, 2H, $J = 8.1$ Hz, H_{phenyl}), 8.19 (s, 2H, $H_{pyrimidinyl}$), 8.33 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR(DMSO): 22.3, 46.5, 55.1, 60.5, 114.4, 120.6, 127.0, 127.4, 133.4, 136.9, 141.5, 151.6, 152.1, 154.4, 156.8, 158.8. MS (ES^+) *m/z* 443 $(M+Na)^+$.

2-(1-Hydroxy-2-methylprop-2-yl)-6-[4-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(6k)*** Yield 29 %, m.p. 235–238 °C. ^1H NMR($CDCl_3$): δ 1.44 (s, 6H, $C(CH_3)_2$), 1.60 (d, 6H, $J = 6.5$ Hz, $(CH_3)_2CH$), 3.75 (bs, 2H, CH_2OH), 4.62 (hept, 1H, $J = 6.5$ Hz, $(CH_3)_2CH$), 5.16 (bs, 1H, NH), 7.58 (d, 2H, $J = 8.3$ Hz, H_{phenyl}), 7.62 (s, 1H, 8-H), 7.81 (bs, 1H, NH), 7.90 (d, 2H, $J = 8.3$ Hz, H_{phenyl}), 8.96 (s, 2H, $H_{pyrimidinyl}$), 9.18 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 22.3, 24.9, 47.0, 55.3, 71.5, 114.9, 120.6, 127.2, 128.2, 133.7, 135.3, 139.9, 149.9, 152.2, 154.3, 156.8, 158.2.

(R)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7a)*** Yield 31 %, m.p. 117–124 °C. ^1H NMR($CDCl_3$): 1.00 (t, 3H, $J = 8$ Hz, CH_3CH_2), 1.51 (m, 6H, $CH(CH_3)_2$), 1.58–1.67 (m, 2H, CH_3CH_2), 3.62–3.80(m, 2H, CH_2OH), 4.00–4.08 (m, 1H, CHNH), 4.59 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 7.14–7.20 (m, 1H, $H_{pyrmidinyl}$), 7.43 (t, 1H, $J = 7$ Hz, H_{phenyl}), 7.55 (s, 1H, 8-H), 7.58 (m, 1H, H_{phenyl}), 7.67–7.74 (m, 1H, H_{phenyl}) 8.09 (d, 1H, $J = 7$ Hz, H_{phenyl}), 8.76 (d, 2H, $J = 8$ Hz, $H_{pyrimidinyl}$). ^{13}C NMR: δ 10.9, 22.5, 22.6, 25.0, 46.4, 56.0, 68.1, 119.2, 119.4, 122.1, 122.8, 128.7, 129.1, 130.9, 132.4, 135.1, 138.1, 139.6, 152.2, 157.2, 159.6, 164.6. 167.7. MS (ES^+) *m/z* 419 $(M+H)^+$, 441 $(M+Na)^+$.

(S)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7b)*** Yield 35 %, m.p. 118–125 °C. ^1H NMR($CDCl_3$): δ 1.01 (t, 3H, $J = 7,2$ Hz, CH_3CH_2), 1.57 (d, 6H, $J = 6,8$ Hz, $CH(CH_3)_2$), 1.63-1.67 (m, 2H, CH_3CH_2), 3.70–3.85(m, 2H, CH_2OH), 3.96–4.02 (m, 1H, CHNH), 4.63 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 7.23 (t, 1H, $J = 4.8$ Hz, $H_{pyrmidinyl}$), 7.48 (t, 1H, $J = 7.6$ Hz, H_{phenyl}), 7.61 (s, 1H, 8-H), 7.68 (m, 1H, H_{phenyl}), 7.72–7.82 (m, 1H, H_{phenyl}) 8.15 (d, 1H, $J = 7.6$ Hz, H_{phenyl}), 8.83 (d, 2H, $J = 4.8$ Hz, $H_{pyrimidinyl}$). ^{13}C NMR: δ 10.8, 22.5, 22.6, 25.0, 46.4, 55.9, 66.9, 115.0, 119.2, 119.4, 122.1, 122.7, 129.1, 135.1, 138.1, 139.7, 150.8, 152.2, 157.2, 159.6, 164.5. MS (ES^+) *m/z* 441 $(M+Na)^+$.

(R)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7c)*** Yield 23 %, m.p. 115–122 °C. ^1H NMR($CDCl_3$): δ 1.03 (d, 3H, $J = 6.7$ Hz, CH_3CH), 1.05 (d, 3H, $J = 6.7$ Hz, CH_3CH), 1.55 (d, 3H, $J = 6.8$ Hz, CH_3CHN), 1.56 (d, 3H, $J = 6.8$ Hz, CH_3CHN), 2.03–2.14 (m, 1H, $(CH_3)_2CH$ CH), 3.76–3.82 (m, 2H, CH_2OH), 4.06–4.16 (m, 1H, $CHCH_2OH$), 4.65 (hept, 1H, $J = 6.7$ Hz, $(CH_3)_3CH$), 5.01–5.10 (m, 1H, NH), 7.22 (t, 1H, $J = 4.8$ Hz, $H_{pyrimidinyl}$), 7.47 (t, 1H, $J = 7.9$ Hz, H_{phenyl}), 7.60 (s, 1H, 8-H), 7.70–7.82 (m, 1H, H_{phenyl}), 7.82–7.87 (m, 1H, H_{phenyl}), 8.15 (d, 1H, $J = 7.9$ Hz, H_{phenyl})), 8.82 (d, 2H, $J = 4.8$ Hz, $H_{pyrimidinyl}$), 9.00–9.20 (m, 1H, NH). ^{13}C NMR: δ 19.6, 22.5, 22.6, 29.6, 46.3, 59.3, 114.9, 119.2, 119.4, 122.1, 122.6, 129.0, 135.0, 138.1, 139.8, 152.2, 157.2, 159.8, 164.5. MS (ES^+) *m/z* 433 $(M+H)^+$, 455 $(M+Na)^+$.

(S)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7d)*** Yield 22 %, m.p. 126–135 °C. ^{1}H NMR($CDCl_3$): δ 1.01–1.07 (m, 6H, $(CH_3)_2CH$), 1.52–1.58 (m, 6H, 1.55 (d, 3H, $J = 6.8$ Hz, $CH(CH_3)_2$), 2.03–2.13 (m, 1H, $(CH_3)_2CH$ CH), 3.77–3.83 (m, 2H, CH_2OH), 4.02–4.16 (m, 1H, $CHCH_2OH$), 4.64 (hept, 1H, $J = 6.7$ Hz, $(CH_3)_3CH$), 5.02–5.12 (m, 1H, NH), 7.22 (t, 1H, $J = 4.7$ Hz, $H_{pyrimidinyl}$), 7.47 (t, 1H, $J = 7.8$ Hz, H_{phenyl}), 7.60 (s, 1H, 8-H), 7.71–7.80 (m, 1H, H_{phenyl}), 7.82–7.88 (m, 1H, H_{phenyl}), 8.15 (d, 1H, $J = 7.6$ Hz, H_{phenyl})), 8.82 (d, 2H, $J = 4.8$ Hz, $H_{pyrimidinyl}$), 8.98–9.12 (m, 1H, NH). ^{13}C NMR: δ 19.6, 22.5, 22.6, 29.6, 46.3, 59.3, 114.9, 119.2, 119.4, 122.0, 122.7, 129.0, 135.1, 138.0, 139.7, 149.0, 152.1, 157.2, 159.8, 164.5. MS (ES^+) *m/z* 433 $(M+H)^+$, 455 $(M+Na)^+$.

(S)-2-(1-Hydroxy-4-methylpent-2-ylamino)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7g)*** Yield 27 %, m.p. 114–120 °C. ^{1}H NMR($CDCl_3$): δ 0.84 (d, 3H, $J = 6.5$ Hz, $(CH_3)_2CHCH_2$), 0.89 (d, 3H, $J = 6.4$ Hz, $(CH_3)_2CHCH_2$), 1.34–1.44 (m, 2H, $CHCH_2CH$), 1.49 (d, 6H, $J = 6.8$ Hz, $(CH_3)_2CH$), 1.68–1.81 (m, 1H, $(CH_3)_2CHCH_2$), 3.56–3.63 (m, 1H, CH_2OH), 3.66–3.76 (m, 1H, CH_2OH), 4.19–4.30 (m, 1H, CHNH), 4.59 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 7.16 (t, 1H, $J = 4.9$ Hz, $H_{pyrimidinyl}$), 7.40 (t, 1H, $J = 8.1$ Hz, H_{phenyl}), 7.54 (s, 1H, 8-H), 7.60–7.71 (m, 1H, H_{phenyl}), 7.73–7.80 (m, 1H, H_{phenyl}), 8.09 (d, 1H, $J = 7.7$ Hz, H_{phenyl})), 8.76 (d, 2H, $J = 4.8$ Hz, $H_{pyrimidinyl}$), 8.94–9.06 (m, 1H, NH). ^{13}C NMR: δ 22.2, 22.4, 22.5, 23.3, 24.8, 41.2, 46.3, 52.3, 67.4, 114.8, 119.1, 119.4, 122.1, 122.7, 129.0, 135.0, 138.0, 139.7, 152.2, 157.2, 159.4, 164.6. MS (ES^+) *m/z* 447 $(M+H)^+$, 469 $(M+Na)^+$.

1,3-(Dihydroxyprop-2-yl)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7f)*** Yield 30 %, m.p. 136–144 °C. ^{1}H NMR(DMSO): δ 1.46 (d, 6 H, $J = 6.3$ Hz, $(CH_3)_2CH$), 3.55–3.68 (m, 5H, $(CH_2OH)_2 + CH(CH_2OH)_2$), 4.56 (hept, 1H, $J = 6.3$ Hz, $CH(CH_3)_2$), 5.74–5.84 (m, 1H, NH), 7.22–7.27 (m, 1H, $H_{pyrimidinyl}$), 7.33 (t, 1H, $J = 7.6$ Hz, H_{phenyl}), 7.71 (s, 1H, 8-H), 7.87–8.00 (m, 3H, H_{phenyl}), 8.73–8.80 (d, 2H, $J = 4.5$ Hz, $H_{pyrimidinyl}$). ^{13}C NMR(DMSO): δ 22.1, 40.3, 54.5, 60.6, 113.7, 119.7, 121.6, 121.7, 128.7, 136.0, 137.6, 140.3, 150.9, 151.8, 157.4, 158.3, 163.6. MS (ES^+) *m/z* 421 $(M+H)^+$, 443 $(M+Na)^+$.

2-(1-Hydroxy-2-methylprop-2-yl)-6-[3-(2-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7e)*** Yield 20 %, m.p. 159–162 °C. ^{1}H NMR($CDCl_3$): δ 1.35 (s, 6H, $C(CH_3)_2$), 1.51 (d, 6H, $J = 6.5$ Hz, $(CH_3)_2CH$), 3.65–3.71 (m, 2H, CH_2OH), 4.35 (hept, 1H, $J = 6.5$ Hz, $(CH_3)_2CH$), 5.11 (bs, 1H, NH), 7.14 (t, 1H, $J = 4.5$ Hz, $H_{pyrimidinyl}$), 7.26 (t, 1H, $J = 7.8$ Hz, H_{phenyl}), 7.53 (s, 1H, 8-H), 7.71–7.74 (m, 1H, H_{phenyl}), 7.99 (d, 1H, $J = 8.3$ Hz, H_{phenyl}), 8.09 (d, 1H, $J = 8.2$ Hz, H_{phenyl})), 8.55 (bs, 1H, NH), 8.75 (d, 2H, $J = 4.5$ Hz, $H_{pyrimidinyl}$). ^{13}C NMR: δ 22.4, 25.1, 46.8, 55.3, 72.0, 115.0, 119.1, 119.9, 122.8, 123.0, 129.1, 135.1, 138.2, 139.2, 149.6, 152.6, 157.1, 158.3, 164.3. MS (ES^+) *m/z* 419 $(M+H)^+$, 441 $(M+Na)^+$.

(R)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7h)*** Yield 30 %, m.p. 116–122 °C. ^{1}H NMR($CDCl_3$): δ 1.03 (t, 3H, $J = 8$ Hz, CH_3CH_2), 1.58 (d, 6H, $J = 8$ Hz, $CH(CH_3)_2$), 1.62–1.74 (m, 2H, CH_3CH_2), 3.64–3.77 (m, 1H, CH_2OH), 3.86 (dd, 1H, $J = 12$ Hz and $J' = 4$ Hz, CH_2OH), 3.95–4.04 (m, 1H, CHNH), 4.65 (hept, 1H, $J = 8$ Hz, $CH(CH_3)_2$), 4.99 (d, 1H, $J = 5$ Hz, NH), 7.28–7.30 (m, 1H, H_{phenyl}), 7.49 (t, 1H, $J = 9$ Hz, H_{phenyl}), 7.62 (s, 1H, 8-H), 7.70–7.78 (m, 2H, H_{phenyl}), 8.15 (bs, 1H, NH), 9.00 (s, 2H, $H_{pyrimidinyl}$), 9.22 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 10.9, 22.4, 22.5, 24.8, 46.6, 56.0, 67.1, 115.0, 118.2, 120.2, 121.3, 129.9, 134.3, 134.9, 135.4, 140.2, 152.1, 154.9, 157.4, 159.6. MS (ES^+) *m/z* 441 $(M+Na)^+$.

(S)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7i)*** Yield 31 %, m.p. 120–127 °C. ^{1}H NMR($CDCl_3$): δ 0.95 (t, 3H, $J = 8$ Hz, CH_3CH_2), 1.50 (d, 6H, $J = 8$ Hz, $CH(CH_3)_2$), 1.57–1.67 (m, 2H, CH_3CH_2), 3.60 (dd, 1H, $J = 12$ Hz and $J' = 8$ Hz, CH_2OH), 3.79 (dd, 1H, $J = 12$ Hz and $J' = 4$ Hz, CH_2OH), 3.88–3.97 (m, 1H, CHNH), 4.58 (hept, 1H, $J = 8$ Hz, $CH(CH_3)_2$), 4.93 (d, 1H, $J = 8$ Hz, NH), 7.20–7.22 (m, 1H, H_{phenyl}), 7.42 (t, 1H, $J = 9$ Hz, H_{phenyl}), 7.55 (s, 1H, 8-H), 7.78–7.82 (bs, 1H, H_{phenyl}), 8.09 (bs, 1H, NH), 8.93 (s, 2H, $H_{pyrimidinyl}$), 9.15 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 10.9, 22.4, 22.5, 24.7, 46.7, 56.0, 67.2, 115.1, 118.2, 120.2, 121.3, 129.9, 134.3, 134.9, 135.4, 140.2, 152.1, 154.9, 157.4, 159.6. MS (ES^+) *m/z* 419 $(M+H)^+$, 441 $(M+Na)^+$, 457 $(M+K)^+$.

(R)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[3-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine ***(7j)*** Yield 23 %. ^{1}H NMR($CDCl_3$): δ 0.98–1.03 (m, 6H, CH_3CH), 1.57 (d, 6H, $J = 6.6$ Hz, $NCH(CH_{3)2}$), 1.95–2.03 (m, 1H, $(CH_3)_2CH$), 3.68–3.76 (m, 1H, CH_2OH), 3.83–3.90 (m, 2H, $CH_2OH + CHNH$), 4.67 (hept, 1H, $J = 6.6$ Hz, $CH(CH_3)_2$), 5.13 (1H, bd, $J = 7.8$ Hz, NH), 7.27–7.30 (m, 1H, H_{phenyl}), 7.50 (t, 1H, $J = 8.8$ Hz, H_{phenyl}), 7.64 (s, 1H, 8-H), 7.84–7.91 (m, 1H, H_{phenyl}), 8.17–8.30 (m, 2H, H_{phenyl} + NH), 9.02 (s, 2H, $H_{pyrimidinyl}$), 9.22 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 18.9, 19.4, 22.4, 22.5, 29.9, 46.6, 59.7, 65.4, 118.2, 120.2, 121.2, 129.9, 134.3, 134.8, 135.3, 139.5, 140.2, 152.1, 154.9, 157.4, 159.8. MS (ES^+) *m/z* 433 $(M+H)^+$, 455 $(M+Na)^+$.

2-(1-Hydroxy-2-methylprop-2-yl)-6-[3-(5-pyrimidinyl)phenylamino]-9-iso-propylpurine (**7k**) Yield 20 %. ^{1}H NMR($CDCl_3$): δ 1.41 (s, 6H, $C(CH_3)_2$), 1.59 (d, 6H, $J = 7.1$ Hz, $(CH_3)_2CH$), 3.73 (bs, 2H, CH_2OH), 4.62 (hept, 1H, $J = 7.1$ Hz, $(CH_3)_2CH$), 5.09–5.13 (bs, 1H, N*H*), 7.25–7.30 (m, 1H, H_{phenyl}), 7.50 (t, 1H, $J = 7.5$ Hz, H_{phenyl}), 7.63 (s, 1H, 8-H), 7.82 (d, 2H, $J = 8.5$ Hz, H_{phenyl}), 7.95–7.98 (bs, 1H, H_{phenyl}), 7.99–8.02 (bs, 1H, N*H*), 8.98 (s, 2H, $H_{pyrimidinyl}$), 9.22 (s, 1H, $H_{pyrimidinyl}$). ^{13}C NMR: δ 22.4, 24.8, 25.1, 47.0, 55.4, 72.0, 114.9, 118.6, 119.0, 120.7, 121.7, 130.0, 134.2, 134.9, 135.3, 139.8, 149.8, 152.4, 154.9, 157.4, 158.3. MS (ES^+) *m/z* 419 $(M+H)^+$, 441 $(M+Na)^+$.

Biology–protein kinase assays

Biochemical reagents

Sodium orthovanadate, EGTA, EDTA, Mops, β-glycerophosphate, phenylphosphate, sodium fluoride, dithiothreitol (DTT), glutathione–agarose, glutathione, bovine serum albumin (BSA), nitrophenylphosphate, leupeptine, aprotinine, pepstatin, soybean trypsin inhibitor, benzamidine, and histone H1 (type III-S) were obtained from sigma Chemicals. [γ-^{33}P]-ATP was obtained from Amersham. The CK-S peptide (RRKHAAIGpSAYSITA) (pS stands for phosphorylated serine) was purchased from Millegen, and the GS-1 peptide (YRRAAVPPSPSLSRHSSPHQpSEDEEE) was obtained from GenScript Corporation.

Buffers

Buffer A: 10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris–HCl pH 7.5, 50 µg heparin/mL. *Buffer C*: 60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM 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 C, at 30 °C, at a final 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 DMSO.

CDK1/cyclin B (M phase starfish oocytes, native), *CDK2/cyclin A* (human, recombinant), and *CDK5/p25* (human, recombinant) were prepared as previously described (Bettayeb *et al.*, 2008). Their kinase activity was assayed in buffer C, with 1 mg histone H1/ml, in the presence of 15 µM [γ-^{33}P] ATP (3,000 Ci/mmol; 10 mCi/ml) in a final volume of 30 µl. After 30 min of incubation at 30 °C, 25µL 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/liter of water. The wet filter was counted in the presence of 1 mL of ACS (Amersham) scintillation fluid.

GSK-3α/β (porcine brain, native) was assayed, as described for CDK1, but in Buffer A and using a GSK-3-specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQ-SpEDEEE) (pS stands for phosphorylated serine) (Bach *et al.*, 2005).

CK1δ/ε (porcine brain, native) was assayed in threefold diluted buffer C as described for CDK1, but using 25 µM CKS peptide (RRKHAAIGpSAYSITA), a CK1-specific substrate (Reinhardt *et al.*, 2007).

DYRK1A (human, recombinant, expressed in *E. coli* as a GST fusion protein) was purified by affinity chromatography on glutathione-agarose and assayed in buffer A (+ 0.5 mg BSA/ml) with using Woodtide (KKISGRLSPIMTEQ) (1.5 µg/assay) as a substrate.

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