Roscovitine-Derived, Dual-Specificity Inhibitors of Cyclin-Dependent Kinases and Casein Kinases 1

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Cyclin-dependent kinases (CDKs) and casein kinases 1 (CK1) are involved in the two key molecular features of Alzheimer's disease, production of amyloid-β peptides (extracellular plaques) and hyper-phosphorylation of Tau (intracellular neurofibrillary tangles). A series of 2,6,9-trisubstituted purines, structurally related to the CDK inhibitor roscovitine, have been synthesized. They mainly differ by the substituent on the C-6 position. These compounds were screened for kinase inhibitory activities and antiproliferative effects. Several biaryl derivatives displayed potent inhibition of both CDKs and CK1. In particular, derivative **13a** was a potent inhibitor of CDK1/cyclin B (IC_{50}: 220 nM), CDK5/p25 (IC_{50}: 80 nM), and CK1 (IC_{50}: 14 nM). Modeling of these molecules into the ATP-binding pocket of CK1δ provided a rationale for the increased selectivity toward this kinase. **13a** was able to prevent the CK1-dependent production of amyloid-β in a cell model. CDK/CK1 dual-specificity inhibitors may have important applications in Alzheimer's disease and cancers.

Introduction

Protein phosphorylation is catalyzed by protein kinases. The human kinome comprises 518 kinase genes: 40 atypical and 478 classical protein kinases. These latter consist of 338 serine/threonine kinases, 90 tyrosine kinases, and 50 sequences that lack a functional catalytic site.[1–4] Abnormalities in protein phosphorylation have been observed in numerous major human diseases, strongly encouraging the search for pharmacological inhibitors of protein kinases.[5–7]

Our laboratory has focused its efforts on the search for, optimization, and characterization of inhibitors of several disease-relevant kinases, such as cyclin-dependent kinases (CDKs), glycogen synthase kinases-3 (GSK-3),[a] and casein kinases 1 (CK1). Inhibitors of CDKs have a great potential in the treatment of pathological conditions such as solid tumors, leukemia, neurodegenerative disorders (Alzheimer's and Parkinson's diseases, stroke, traumatic brain injury), renal diseases (polycystic kidney disease, glomerulonephritis), pain, inflammation, diabetes, and some viral diseases (reviewed in refs 8, 9). Inhibitors of GSK-3 are being studied for their potential use in Alzheimer's disease, manic depression, diabetes, etc., (reviewed in ref 10). CK1s are less studied as therapeutic targets, but their implication in multiple physiological events and some human diseases (reviewed in ref 11) provides a growing impetus for the discovery of selective CK1 inhibitors.[12]

Purines represent a large family of biologically active molecules and constitute the scaffold of a wide variety of promising drugs, including kinase inhibitors (reviewed in refs 13–15). Among the large diversity of purines that have been synthesized, 2,6,9-trisubstituted purines have generated considerable interest as protein kinase inhibitors (reviewed in ref 16). The most advanced molecule, roscovitine (or CYC202 or Seliciclib),[17–19] developed by Cyclacel Pharmaceuticals, is currently in phase 2b clinical trials against non-small cell lung cancer, in phase 2 against nasopharyngeal cancer, in phase 1 against various renal diseases (glomerulonephritis), and in preclinical evaluation against polycystic kidney disease,[20] Alzheimer's disease (in preparation), stroke,[21] and inflammation.[22] The metabolism of roscovitine, which is orally available, is well established. The main metabolic pathways are the oxidation of the alcohol of the C-2 side chain into a carboxylate group, the conjugation of a glucose or glucuronic acid residue on this alcohol, and the loss of the isopropyl group on N9.[23,24] Interestingly, the benzyl group has not been reported to be affected by metabolism. Although frequently considered as rather selective for some CDKs (CDK1, CDK2, CDK5, CDK7, CDK9), roscovitine interacts with several other targets, although with a lower affinity (DYRK1A, CK1, pyridoxal kinase).[25–28]

All of this information first suggests that the trisubstituted purine motive of roscovitine remains an attractive scaffold to develop new derivatives with reasonable chances of generating useful bioactivity associated with moderate toxicity. Inhibitors should be selected more on the basis of their inhibitory profile on several key relevant kinases implicated in a particular pathology rather than on potent inhibition of a single target. For instance, in Alzheimer's disease, hyperphosphorylation of Tau is carried out by a small set of kinases, CDK5, GSK3, and CK1 being the most important ones (reviewed in refs 29, 30). In addition, amyloid-β formation from its β-amyloid precursor

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[a] Abbreviations: Aβ, amyloid β peptide; β-APP, β-amyloid precursor protein; CDK, cyclin-dependent kinase; CK1, casein kinase 1; FCS, fetal calf serum; GSK-3, glycogen synthase kinase-3; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H* -tetrazolium.

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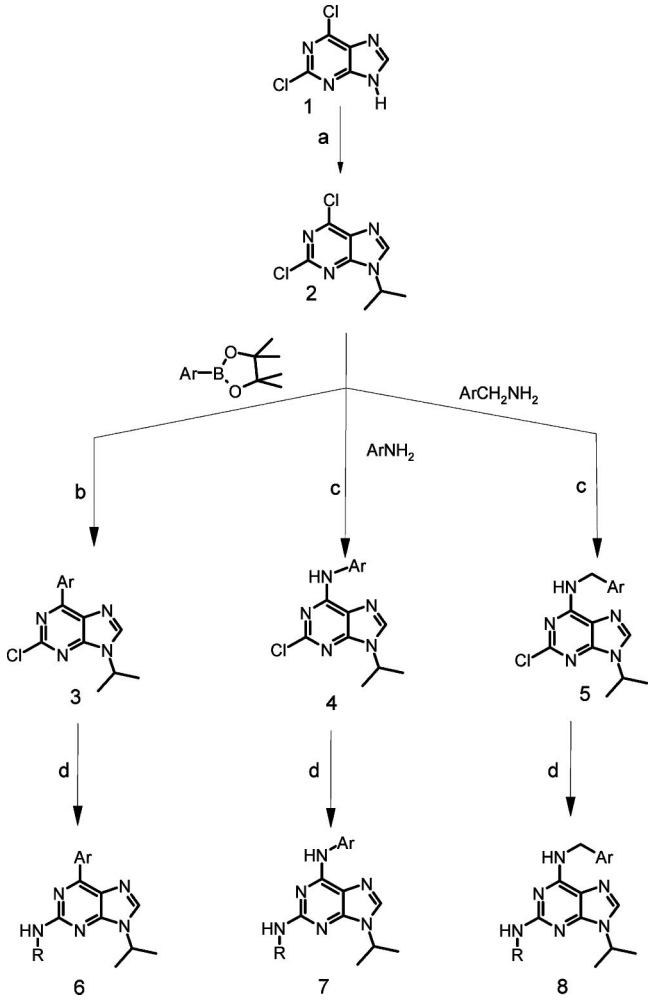
Scheme 1[a]



[a] Reagents and conditions: (a) 2-bromopropane, K_2CO_3, DMSO 15−18 °C; (b) boronate, Na_2CO_3, $Pd[P(C_6H_5)_3]_4$, H_2O, dioxane, 100 °C; (c) $ArNH_2$ or $ArCH_2NH_2$, NEt_3, BuOH 100 °C; (d) RNH_2, NBu_3, DMSO, 165 °C.

protein (β-APP) also involves CDK5,[31] GSK-3,[32,33] and CK1.[34] Thus CDK1,[35–37] CDK5,[38,39] GSK-3,[32,40–42] and CK1[34,43–45] all appear to be involved in the two diagnostic features of Alzheimer's disease, amyloid-β production (responsible for formation of amyloid plaques) and Tau hyperphosphorylation (responsible for the formation of neurofibrillary tangles of paired helical filaments). Multitarget inhibitors acting on this selection of kinases could therefore become of great therapeutic value.

The aim of the present study was to synthesize and evaluate a series of trisubstituted purines that mainly differed by the C-6 substituent. The prepared compounds were evaluated on the four main kinases involved in Alzheimer's disease, CDK1, CDK5, GSK-3, and CK1. This study uncovered CDK/CK1 dual specificity inhibitors, which should be evaluated for their potential therapeutic applications, especially in the Alzheimer's disease field.

Results and Discussion

Chemistry. Most of the trisubstituted purine derivatives described herein were obtained following a three steps synthetic route outlined in Scheme 1. Starting from commercially available 2,6-dichloropurine **1**, alkylation of N9 with 2-bromopropane provided 2,6-dichloro-9-isopropylpurine **2**. Displacement of the 6-chloro was accomplished either by Suzuki cross-coupling or by nucleophilic substitution with appropriate amines. In the last step, nucleophilic substitution of the 2-chlorine afforded the expected molecules. However, we failed to achieve the nucleophilic substitution of the 6-chlorine with anilines bearing a pyridyl group (Scheme 2). Reaction of intermediate **2** with 4-bromoaniline afforded the corresponding amine **9**. Suzuki coupling of boronic ester with bromoderivative **9** did not lead to the expected biaryl but to compound **12**. Reaction of boranylanilines with **2** afforded the corresponding amines **10**, which subsequently reacted with bromopyridines to afford the expected biaryls **11**. Finally, reaction with amino-alcohols afforded the trisubstituted purines **13**.

The synthesis of a large number of 2,6,9-trisubstituted purines, including combinatorial methods, has been previously described.[46–50] Generally, the synthesis of 2,6,9-trisubstituted purines start from commercially available 2,6-dichloropurine **1** and most authors start with the substitution on C6-position. We found easier to achieve first the preparation of the key intermediate **2**. Regiospecific N9 alkylation was achieved by precise control of the reaction temperature.

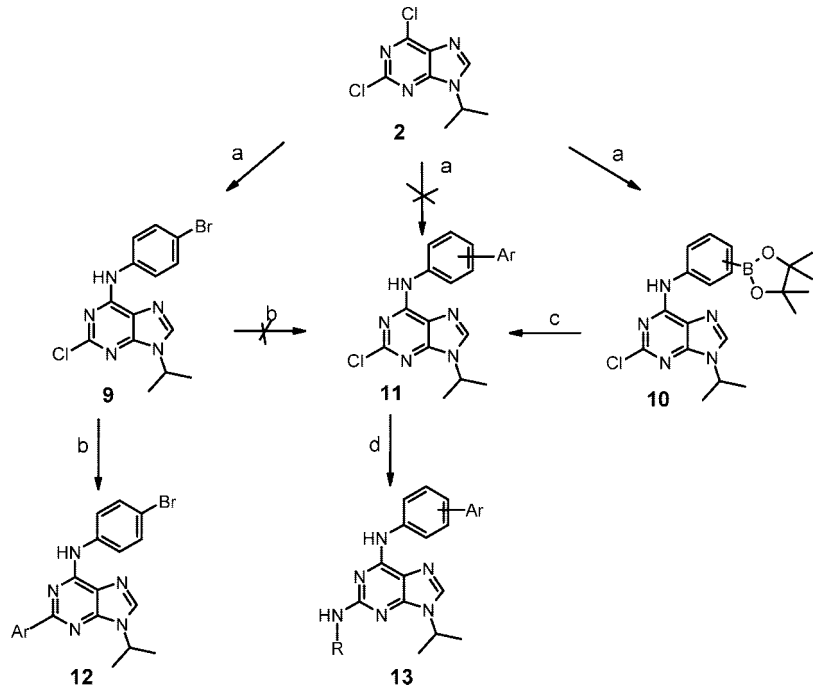
Biological Evaluation: Purified Protein Kinases. All synthesized compounds were evaluated for their potential inhibitory effects on purified kinases (CDK1/cyclin B, CDK5/p25, GSK-3α/β, CK1δ/ε) as described in the Experimental Section. IC_{50} values were determined from dose−response curves and compared to those of the reference molecules, (*R*)-roscovitine **8a** and (*S*)-roscovitine **8b** (Tables 1, 2, and 3).

All three 6-aryl-substituted purines (**6a**, **6b**, **6c**) were completely inactive, confirming the essential hydrogen bond donating N6 present in all active purines.[18,19,52] All other compounds showed submicromolar activities on CDK1 and CDK5. The substituent in C2-position had generally limited influence. The most potent inhibitors were the biarylanilines series **13** with the pyridine nucleus meta to the amine. In contrast, all roscovitine analogues remained either inactive or poorly active on GSK-3 (IC_{50} in the 2−50 μM range), despite the observation that many CDK inhibitors are also excellent GSK-3 inhibitors.[53] IC_{50} values for CDK1 and CDK5 were very similar, a likely consequence of the close similarity of both kinases, especially in their ATP-binding site.

Results showed the appearance of significant CK1 inhibitory activity among many derivatives or analogues of roscovitine (Tables 1, 2, and 3), especially compounds **13a**−**13f**. The most potent CK1 inhibitors shared a biaryl substituent on N6. (*R*)-DRF053 (**13a**) was the most potent inhibitor of CK1 (IC_{50}: 0.014 μM), compared to (*R*)-roscovitine (**8a**) (IC_{50}: 2.3 μM). In fact, **13a** appears to be one of the most potent known inhibitor of CK1.[12]

Modeling of the CK1/Purine Interactions. The conversion of (*R*)-roscovitine (**8a**) to C&R8 (**8m**) and then to DRF053 (**13a**) (Figure 1) showed a gradual improvement of biological activity toward CK1 (Table 1–3). Enhanced affinity of **13a** was also demonstrated for CDK1 and CDK5, however to a lesser degree. To provide a structural rationale for this biological data, we tried to locate and evaluate differences in the interaction mode of these analogues with the two groups of kinases. A sequence alignment of CDK1, CDK2, CDK5, and various isoforms of CK1 (Figure 2) showed that there are several important variations of residues located in the vicinity of the ATP binding pocket between the CDKs and the CK1 isoforms. These variations are located either at the ribose binding site or at the glycine loop. More specifically, at the ribose site $Asp86^{CDK2}$, an important residue that in many cases interacts directly either with the natural substrate (ATP-CDK2; 1B38) or with inhibitors (roscovitine-CDK5; 1UNL) is replaced by a serine ($Ser88^{CK1\text{-}\delta}$). Moreover, $Lys89^{CDK2}$ is replaced by an aspartate in the CK1

Scheme 2[a]



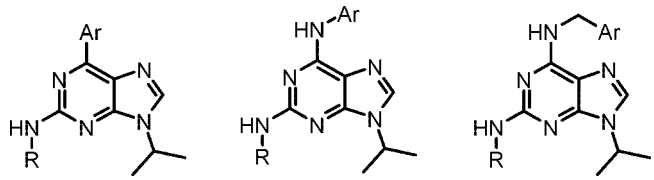
[a] Reagents and conditions: (a) $ArNH_2$, NEt_3, BuOH 100 °C; (b) boronate, Na_2CO_3, $Pd[P(C_6H_5)_3]_4$, H_2O, dioxane, 100 °C; (c) ArBr, Na_2CO_3, $Pd[P(C_6H_5)_3]_4$, H_2O, dioxane, 100 °C; (d) RNH_2, NBu_3, DMSO 165 °C.

isoforms ($Asp91^{CK1\text{-}\delta}$). Two additional residue variations appear at the glycine loop: $Glu8^{CDK2}$ is replaced by a basic residue, arginine in CK1-α, -δ ($Arg13^{CK1\text{-}\delta}$), and -$\varepsilon$ isoforms and lysine in CK1-γ1, -γ2, and -γ3 isoforms. $Lys20^{CDK2}$ is replaced by a leucine in CK1 ($Leu25^{CK1\text{-}\delta}$).

Docking of **13a** in CK1-δ resulted in two distinct binding modes. In both of them, the usually observed adenine type interaction between the ligand and the receptor was sustained with purine N6 donating and N7 receiving a hydrogen bond from the receptor backbone of the binding cavity (Figure 3A−B). However, two possible orientations of the 3-pyridinylphenylamino moiety were obtained. The first binding mode is characterized by a mixed hydrogen bond stacking interaction of pyridine with the side chain of Arg16 of the glycine loop. The pyridine nitrogen forms a hydrogen bond with the guanidine moiety, while the phenyl ring is oriented parallel to Arg guanidinium, stabilized through a $\pi-\pi$ interaction (Figure 3B). In the second binding mode, the phenylamino N6−C1′ bond demonstrates a ∼60° rotation, which orients the 3-pyridinylphenylamino group toward the C-terminal lobe where it forms hybrophobic interactions with $Pro87^{CK1\text{-}\delta}$ and stacking with $Phe95^{CK1\text{-}\delta}$. Docking of **8m** in CK1-δ (Figure 3C) resulted in two binding poses that were similar to the structures, which resulted in the case of **13a-**CK1-δ complex.

The favorable interaction of pyridine with $Arg13^{CK1\text{-}\delta}$ proposed by the first binding mode of both **13a** and **8m**, which does not exist in the case of roscovitine **8a** (Figure 3D), could provide an explanation of the improved affinity of 3- and 4-pyridinyl substituted analogues with respect to CK1. In the case of CDKs, the corresponding residue of $Arg13^{CK1\text{-}\delta}$ is a conserved acidic residue ($Glu8^{CDK1,\ 2,\ 5}$) with no capacity to act either as a hydrogen bond donor or as a stacking partner for pyridine. A 2-aromatic substituted pyridine (calculated pK_a 4.25) can accommodate mainly $\pi-\pi$ aromatic stacking and cation−π interactions or hydrogen bonds where pyridine nitrogen serves as hydrogen bond acceptor (Figure 3B−C). The absence of an interaction in the CDKs similar to that between pyridine and $Arg13^{CK1\text{-}\delta}$ in CK1 explains the fact that **8m** does not demonstrate an improved affinity for CDK1 and CDK5. However, the increased binding affinity of **13a** toward both CK1 and CDKs with respect to **8m** could not be interpreted by docking calculations but only by considering the alteration of N6 stereoelectronic properties. The conversion of the 4-pyridinylbenzyl substitution of **8m** N6 to a 3-pyridinylphenyl in **13a** increases the hydrogen bonding potential of N6. This is caused by the increased polarization of the N−H bond resulting from the enhancement of the sp2 character of the N6 of **13a**, which carries two aromatic substitutions compared to analogue **8m**. An ab initio single-point energy calculation of **13a** and **8m** at the B3LYP/6-31+G* level validated the aforementioned hypothesis. The gap between the negative partial charge of nitrogen and the positive charge of hydrogen is 1.14e for **13a** and 0.58e for **8m**. The stronger hydrogen bonding potential of **13a** N6H is consistent with experimental data showing that *N*-methylaniline exhibits half of the hydrogen bond acidity compared to diphenylamine.[51] To reproduce this experimental result theoretically in our system and further examine the influence of substitutions on the hydrogen bonding potential of secondary amines, we have performed ab initio interaction energy calculations. The second-order Møller−Plesset perturbation theory method was chosen in order to include electron correlation effects and obtain accurate results. Interaction energy calculations of the complexes of diphenylamine−CK1 and *N*-methylaniline−CK1 were performed. The results obtained at the MP2/6-31+G* level showed that the interaction energy was 1.07 kcal/mol more favorable in the case of the diphenylamine−receptor complex. The experimental $\Delta\Delta G$ between **13a** and **8m**, which is in the range of 1.45−1.97 kcal/mol, is in very good agreement with the above calculated values, considering the approximations for the ab initio calculations (see Experimental Section). These results suggest that the improved affinity of **13a**, where N6 carries two aromatic substituents, compared to that of **8a**, with only one aromatic substituent, could be mainly

Table 1. Effects of Purines **6a**−**8h** on Four Protein Kinases and on the Survival of SH-SY5Y and HEK293 Cells[a]



6 **7** **8**

compd	Ar	R	CDK1	CDK5	GSK-3α/β	CK1	SH-SY5Y	HEK293
6a	phenyl	(*R*)-1-hydroxy-but-2-yl	>100	>100	>100	>50	>100	>100
6b	3-pyridyl	(*R*)-1-hydroxy-but-2-yl	>100	>100	>100	>50	>100	>100
6c	4-pyridyl	(*R*)-1-hydroxy-but-2-yl	>100	>100	>100	>10	>100	>100
7a	phenyl-2 -phenyl	(*R*)-1-hydroxy-but-2-yl	29	17	>100	>50	29	6.6
7b	phenyl-3-phenyl	(*R*)-1-hydroxy-but-2-yl	1.1	0.4	>100	0.05	37.6	−
7c	phenyl-4-phenyl	(*R*)-1-hydroxy-3-methylbut-2-yl	0.23	0.1	90	0.08	26.8	−
7d	phenyl-3-phenyl	(*R*)-1-hydroxy-3-methylbut-2-yl	0.05	0.02	>100	0.6	11	15.6
7e	phenyl-4-phenyl	1-hydroxy-2-methylprop-2-yl	0.28	0.08	50	0.048	28.6	−
7f	phenyl-3-phenyl	(*R*)-1-hydroxy-but-2-yl	0.09	0.05	>10	0.6	11.6	16.8
8a	phenyl	(*R*)-1-hydroxy-but-2-yl	0.35	0.20	>10	2.3	17.5	46.7
8b	phenyl	(*S*)-1-hydroxy-but-2-yl	0.55	0.25	−	6.0	−	−
8c	2-pyridyl	(*R*)-1-hydroxy-but-2-yl	0.74	1.00	14.5	7	165.1	190
8d	2-pyridyl	(*S*)-1-hydroxy-but-2-yl	1.15	1.70	>33	13	−	−
8e	3-pyridyl	(*R*)-1-hydroxy-but-2-yl	0.13	0.16	>10	2.2	7.7	20.5
8f	3-pyridyl	(*S*)-1-hydroxy-but-2-yl	0.13	0.14	>10	4.1	−	−
8g	4-pyridyl	(*R*)-1-hydroxy-but-2-yl	0.33	0.23	>10	2.0	64.8	73.7
8h	4-pyridyl	(*S*)-1-hydroxy-but-2-yl	0.39	0.41	>10	2.2	−	−
8i	phenyl-3-phenyl	(*R*)-1-hydroxy-but-2-yl	3.2	2.60	>100	1.1	2.4	2.5
8j	phenyl-3-(2-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.25	0.2	3.3	0.9	3.3	2.8.
8k	phenyl-3-(3-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.12	0.08	>10	0.9	7.4	5.2
8l	phenyl-3-(4-pyridyl)	(*R*)-1-hydroxy-but-2-yl	1.6	1.6	58	1.2	6.5	20
8m	phenyl-4-(2-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.13	0.13	12	0.6	0.43	0.56
8n	phenyl-4-(2-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.08	0.08	10	0.6	0.76	0.90
8o	phenyl-4-(2-pyridyl)	1-hydroxy-2-methylprop-2-yl	0.21	0.21	16	0.68	0.94	−
8p	phenyl-4-(3-pyridyl)	(*R*)-1-hydroxy-but-2-yl	1.9	2.0	84	0.73	1.25	2.2
8q	phenyl-4-(3-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.9	0.9	6	0.7	0.74	0.76
8r	phenyl-4-(4-pyridyl)	(*R*)-1-hydroxy-but-2-yl	2.3	2.2	35.5	1.1	1.69	1.54
8s	phenyl-4-phenyl	(*R*)-1-hydroxy-but-2-yl	3.0	2.52	>100	0.8	0.75	1.33

[a] Purines were tested at various concentrations on CDK1/cyclin B, CDK5/p25, GSK-3α/β, and CK1δ/ε, as described in Experimental Section. IC_{50} values, calculated from the dose−response curves, are reported in μM. The compounds were tested at various concentrations for their effects on SH-SY5Y and HEK293 cell survival after 48 h incubation as estimated using the MTS reduction assay. IC_{50} values, calculated from the dose−response curves, are reported in μM. −: not tested. When IC_{50} values are reported as >10, 33, or 100 μM, this indicates that the compound did not display any inhibitory activity at the highest concentration tested (10, 33, or 100 μM). Some compounds were essentially inactive at 10 μM (indicated as >10 μM), they were not tested at higher concentrations. Some displayed a small but significant inhibition at this concentration and were thus tested up to 100 μM, when solubility was not an issue. **8a**, (*R*)-roscovitine; **8b**, (*S*)-roscovitine; **8m**, (*R*)-C&R8.

attributed to the enthalpic gain offered by the hydrogen bond formed between N6 and the protein backbone carbonyl.

The dual CDK−CK1 specificity character gained by analogues **13a** and **8m** with respect to roscovitine can be considered as a combined result of the favorable interactions provided by the 3- or 4-pyridinyl substitution toward CK1 and the enhanced hydrogen bonding potential of N6 carrying two aromatic substituents toward both CDK and CK1 receptors.

Biological Evaluation: Cell Proliferation Assays. All compounds were tested for their antiproliferative effects. Human neuroblastoma SH-SY5Y and human embryonic kidney cells were exposed for 48 h to various concentrations of each compound. Viability was then estimated by the MTS reduction assay as described in the Experimental Section. The three 6-aryl-substituted purines (**6a**, **6b**, **6c**) and compounds **8c** and **12a** were completely inactive. All other compounds displayed significant cell death inducing activity, the IC_{50} values ranging from 0.43 to 65 μM.

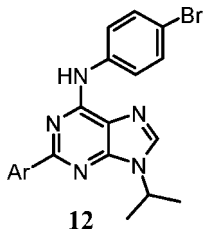
Structure−activity relationships based on CDKs and CK1 inhibition were difficult to correlate with the antiproliferative effects determined on both cell lines. For example, biarylmethyamines compounds (e.g., compounds **8m** and **8n**), which displayed the highest antiproliferative effects (on SH-SY5Y cell line, IC_{50} values were 0.43 and 0.76 μM, respectively), were far from being the most potent kinase inhibitors. Further, another interesting aspect of the SAR studies was the observation that the replacement of an heterobiaryl moiety in **8m** and **8n** by a biphenyl in **8s** reduced the inhibitory potency on CDK1 and CDK2 (IC_{50} >2 μM). Nevertheless, a potent antiproliferative effect was observed with this compound.

Altogether, the synthesized compounds can be divided in three groups in terms of biological activity (kinase and cell survival):

(1) Inactive on both kinases and cell proliferation: compounds **6a**−**6c**.

(2) Active on both kinases and cell proliferation: the biarylmethylamine series (compounds **8i**−**8s**) demonstrated high antiproliferative action, which contrasted with variable enzyme inhibition. No clear correlation could be established between kinase inhibition and antiproliferative effects. Different cellular properties (cell permeability, intracellular stability, intracellular distribution, or targets) may account for this lack of direct correlation. The **8i**−**8s** series of molecules could be developed as potential anticancer agents. As an illustration, we have investigated in detail the cellular effects of the most active compound, **8m** (C&R8).[54]

Table 2. Effects of Purines **12** on Four Protein Kinases and on the Survival of SH-SY5Y and HEK293 Cells[a]



12

compd	Ar	CDK1	CDK5	GSK-3α/β	CK1	SH-SY5Y	HEK293
12a	3-pyridyl	0.41	0.73	>100	0.091	>100	–
12b	4-pyridyl	0.59	0.6	>100	0.083	60	–

[a] Purines were tested at various concentrations on CDK1/cyclin B, CDK5/p25, GSK-3α/β, and CK1δ/ε, as described in Experimental Section. IC_{50} values, calculated from the dose−response curves, are reported in μM. The compounds were tested at various concentrations for their effects on SH-SY5Y and HEK293 cell survival after 48 h incubation as estimated using the MTS reduction assay. IC_{50} values, calculated from the dose−response curves, are reported in μM. −: not tested. When IC_{50} values are reported as >10, 33, or 100 μM, this indicates that the compound did not display any inhibitory activity at the highest concentration tested (10, 33, or 100 μM). Some compounds were essentially inactive at 10 μM (indicated as >10 μM), they were not tested at higher concentrations. Some displayed a small but significant inhibition at this concentration and were thus tested up to 100 μM, when solubility was not an issue.

(3) Active on kinases but poor antiproliferative activity: the biarylamine type compounds (series **13**) were highly potent CDK/CK1 inhibitors but displayed low antiproliferative effect. As a general rule, CK1 inhibition inversely correlated with antiproliferative activity. This series of molecules could be developed as protective agents, especially against neurodegenerative diseases, as illustrated below with compound **13a**.

Biological Evaluation: Inhibition of Amyloid-β Production. Recently, CK1 was demonstrated to regulate the production of amyloid-β (Aβ) formation and CK1 inhibitors to prevent Aβ production.[34] To test whether our molecules could act as CK1 inhibitors in a cellular system, we exposed N2A cells stably expressing amyloid-β precursor protein (APP) (N2A-APP_{695} cells) to various concentrations of the selection of three compounds, (*R*)-roscovitine (**8a**), (*R*)-C&R8 (**8m**), and (*R*)-DRF053 (**13a**), for a 3 h period. Cell supernatants were then collected and A-β40 levels measured by an ELISA assay. Results show a dose-dependent inhibitory effect on A-β40 production for the three compounds, (*R*)-DRF053 (**13a**) being the most efficient (Figure 4). Although these results might suggest that the three compounds are able to inhibit CK1 in a cellular context, leading to reduced production of amyloid-β from the amyloid-β precursor protein, this needs to be formally demonstrated and there are alternative explanations, including an effect through other targets (kinases and non-kinases). The use of chemically different CK1 inhibitors, specific siRNAs, and compounds that do not affect other kinases than CK1, will shed some light on the link between CK1 and amyloid-β production.

Conclusion

In this study, the introduction of a biarylamine or a biarylmethylamine on the 6 position of the purine system led to potent inhibitors of CDKs. Unexpectedly, some compounds displayed potent inhibition of CK1 as well. Docking calculations suggested that potency enhancement toward the CDKs and CK1 result from interactions between the protein binding pocket and two separate parts of the inhibitor molecule. Introducing a substituent at purine position 6 that can interact favorably with an arginine (or alternatively a lysine) located in the glycine loop of CK1α, γ, δ, and ε isoforms takes advantage of the conserved variation of this residue in the CDKs and inhibitors like **8m** gain 1 order of magnitude in potency with respect to CK1 compared to roscovitine. Further improvement of potency but in a common trend in both families of kinases is achieved by the transformation of the biarylmethylamine substituent of purine position 6 to a biarylamine. The amine group attached at position 6 acts as a hydrogen bond donor that stabilizes the protein−inhibitor complex. The bond formed in the case of the biarylamines is stronger than that formed in the case of the biarylmethylamine analogues. The stronger bonding potential of biarylamines like **13a** was confirmed with a high level ab initio interaction energy calculation.

As both CK1 and CDK families of kinases are involved in the development of Alzheimer's disease, these modifications on the 2,6,9-trisubstituted purines deserve to be further explored in terms of both pharmacological tools for the study of neurodegeneration pathways in cellular and animal models and possibly for potential therapeutic applications.

Experimental Section

Chemistry. General Methods. Melting points were determined on a Kofler hot-stage (Reichert) and are uncorrected. NMR spectra were recorded on Bruker Avance 400 MHz (100 MHz for ^{13}C NMR). Chemical shifts are given in ppm downfield of tetramethylsilane (TMS) used as an internal standard. Infrared spectra were recorded on a FTIR Schimadzu 8300. Reactions were monitored by TLC using Merk silica gel 60F-254 thin layer plates. Column chromatography was carried out on SDS Chromagel 60 ACC, 40−63 μm. The HPLC analyses were carried out on a system consisting of a Waters 600 system controller, a Jones Chromatography heater−chiller oven, and a Waters 994 photodiode array detector. Microanalysis (C, H, N) of compounds **6**, **7**, **8**, and **13** agreed (±0.4) with calculated values. Biarylamines were prepared by Suzuki−Myaura coupling.[24] Results of compounds microanalysis are presented in the Supporting Information.

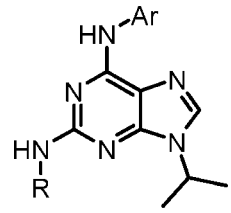
2,6-Dichloro-9-iso-propylpurine (2). To a solution of 2,6-dichloro-9*H*-purine **1** (9.00 g, 47.6 mmol) in DMSO (50 mL) at 15 °C was added K_2CO_3 (19.74 g, 142.8 mmol) and 2-bromopropane (21.85 mL, 238 mmol). After 8 h stirring at 15−18 °C, water was added and the solution was extracted with AcOEt. The organic layers were assembled, washed with H_2O (3 × 20 mL), dried (Na_2SO_4), concentrated, and chromatographed on a silica gel column (Cyclohexane/AcOEt 1:9) to yield a white solid (7.80 g, 71%); mp 149−152 °C. ^1H NMR ($CDCl_3$): δ 1.65 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$), 4.91 (hept, 1H, J = 6.8 Hz, $CH(CH_3)_2$), 8.17 (s, 1H, H-8).

General Procedure for the Synthesis of 6-Aryl-2-chloropurines (3). To solution of **2** (2.31 g, 10 mmol) in 40 mL dioxane under argon were added $Pd[P(C_6H_5)_3]_4$ (0.575 g, 0.5 mmol). After 5 min stirring under N_2, 1 M Na_2CO_3 aqueous solution (20 mL) and pinacolboronate derivatives (11 mmol) were added. The reaction mixture was refluxed under N_2 for 6 h and then cooled to room temperature. The mixture was concentrated in vacuo to half of its initial volume and extracted with AcOEt (3 × 15 mL). The combined organic extracts were washed with H_2O and brine and then dried (Na_2SO_4). The solvent was removed in vacuo. Compounds **3** crystallized upon trituration with cyclohexane.

2-Chloro-6-phenyl-9-iso-propylpurine (3a). Yield 87%, mp 112−113 °C. ^1H NMR ($CDCl_3$): δ 1.66 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$), 4.97 (hept, 1H, $CH(CH_3)_2$), 7.54−7.67 (m, 3H, H_{phenyl}), 7. 75−7.90 (m, 2H, H_{phenyl}), 8.17 (s, 1H, H-8).

2-Chloro-6-(3-pyridyl)-9-iso-propylpurine (3b). Yield 53%, mp 163−164 °C. ^1H NMR ($CDCl_3$): δ 1.66 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$), 4.97 (hept, 1H, $CH(CH_3)_2$), 7.30 (m, 1H, $H_{pyridyl}$), 7.90 (m, 2H, $H_{pyridyl}$), 8.22 (s, 1H, H-8), 8.65 (d, 1H, $H_{pyridyl}$).

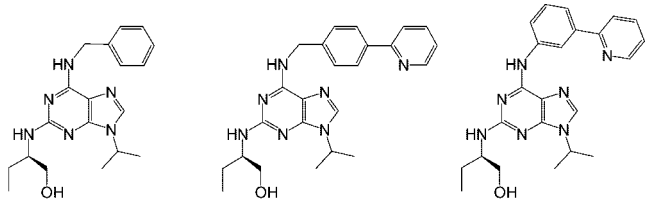
Table 3. Effects of Purines **13** on Four Protein Kinases and on the Survival of SH-SY5Y and HEK293 Cells[a]



13

compd	Ar	R	CDK1	CDK5	GSK-3α/β	CK1	SH-SY5Y	HEK293
13a	phenyl-3-(2-pyridyl)	(*R*)- 1-hydroxy-but-2-yl	0.22	0.08	4.1	0.014	17.2	–
13b	phenyl-3-(2-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.18	0.08	5.1	0.031	16.8	–
13c	phenyl-3-(2-pyridyl)	1-hydroxy-2-methylprop-2-yl	0.48	0.16	4.9	0.020	16.0	–
13d	phenyl-3-(3-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.18	0.05	60	0.05	15.4	–
13e	phenyl-3-(3-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.05	0.01	13	0.08	7.5	9.2
13f	phenyl-3-(4-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.18	0.05	20	0.11	15.8	11.6
13g	phenyl-4-(2-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.05	0.03	40	0.13	10	10.4
13h	phenyl-4-(2-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.06	0.05	90	0.4	17.5	14.6
13i	phenyl-4-(3-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.18	0.02	7.5	0.22	6.5	7.4
13j	phenyl-4-(3-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.18	0.02	8.0	0.63	8.9	8.3
13k	phenyl-4-(4-pyridyl)	(*R*)-1-hydroxy-but-2-yl	0.02	0.02	1.9	0.23	7	8
13l	phenyl-4-(4-pyridyl)	(*R*)-1-hydroxy-3-methylbut-2-yl	0.03	0.02	12	1.2	6.6	6.3

[a] Purines were tested at various concentrations on CDK1/cyclin B, CDK5/p25, GSK-3α/β, and CK1δ/ε, as described in Experimental Section. IC_{50} values, calculated from the dose−response curves, are reported in μM. The compounds were tested at various concentrations for their effects on SH-SY5Y and HEK293 cell survival after 48 h incubation as estimated using the MTS reduction assay. IC_{50} values, calculated from the dose−response curves, are reported in μM. −: not tested. When IC_{50} values are reported as >10, 33, or 100 μM, this indicates that the compound did not display any inhibitory activity at the highest concentration tested (10, 33, or 100 μM). Some compounds were essentially inactive at 10 μM (indicated as >10 μM), they were not tested at higher concentrations. Some displayed a small but significant inhibition at this concentration and were thus tested up to 100 μM, when solubility was not an issue.



(R)-roscovitine (8a) **CDK5**: IC_{50}= 0.200 μM **CK1**: IC_{50}= 2.300 μM

(R)-C&R8 (8m) **CDK5**: IC_{50}= 0.130 μM **CK1**: IC_{50}= 0.600 μM

(R)-DRF053 (13 a) **CDK5**: IC_{50}= 0.080 μM **CK1**: IC_{50}= 0.014 μM

Figure 1. Structure of (*R*)-roscovitine (**8a**), (*R*)-C&R8 (**8m**), and DRF053 (**13a**). IC_{50} values for CDK5/p25 and CK1 are indicated under each structure in μM.

2-Chloro-6-(4-pyridyl)-9-iso-propylpurine (3c). Yield 76%, mp 145−148 °C. ^{1}H NMR ($CDCl_3$): δ 1.66 (d, 6H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.97 (hept, 1H, $CH(CH_3)_2$), 8.22 (s, 1H, H-8), 8.63 (bd, 2H, *J* = 5.5 Hz, $H_{pyridyl}$), 8.82 (bd, 2H, $H_{pyridyl}$).

General Procedure for the Synthesis of 6-Amino-2-chloropurines (Preparation of Compounds 4, 5, 9, 10). To a solution of **2** (2.31 g, 10 mM) in 15 mL *n*-BuOH was added the primary amine (12 mM) and NEt_3 (2.20 mL, 16 mM). After 2 h heating at 100 °C, *n*-BuOH was evaporated in vacuo. To the residue, 10 mL water were added and this mixture was extracted with AcOEt (3 × 20 mL). The combined organic extracts were dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was chromatographied on a silica gel column using (CH_2Cl_2/AcOEt, various ratio) as eluent.

2-Chloro-6-(2-phenylphenylamino)-9-iso-propylpurine (4a). Yield 56%, mp 68 °C. ^{1}H NMR ($CDCl_3$): δ 1.66 (d, 6H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.97 (hept, 1H, $CH(CH_3)_2$), 6.90−7.47(m, 10H, H_{phenyl}), 8.22(s, 1H, H-8).

2-Chloro-6-(3-phenylphenylamino)-9-iso-propylpurine (4b). Yield 75%, mp 117−119 °C. ^{1}H NMR ($CDCl_3$): δ 1.66 (d, 6H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.97 (hept, 1H, $CH(CH_3)_2$), 6.90−7.35 (m, 10H, H_{phenyl}), 8.22 (s, 1H, H-8).

2-Chloro-6-(4-phenylphenylamino)-9-iso-propylpurine (4c). Yield 65%, mp 123−126 °C. ^{1}H NMR ($CDCl_3$): δ 1.54 (d, 6H, *J* = 6 Hz, $CH(CH_3)_2$), 4.82 (hept, 1H, $CH(CH_3)_2$), 7.25−7.82(m, 10H, H_{phenyl}), 8.10(s, 1H, H-8).

2-Chloro-6-[(2-pyridyl)methylamino]-9-iso-propylpurine (5a). Yield 45%, mp 67−69 °C. ^{1}H NMR ($CDCl_3$): δ 1.49 (d, 6H, *J* = 6.6 Hz, $CH(CH_3)_2$), 4.55 (hept, 1H, $CH(CH_3)_2$), 4.88 (bs, 2H, $NHCH_2$), 6.53(bs, 1H, NH), 7.10 (dd, 1H, *J* = 7.6 Hz and *J′* = 5.0 Hz, $H_{pyridyl}$), 7.29 (d, 1H, $H_{pyridyl}$), 7.47 (s, 1H, H-8), 7.54 (td, 1H, *J* = 7.6 Hz and *J′* = 1.8 Hz, $H_{pyridyl}$), 8.48 (dd, 1H, $H_{pyridyl}$).

2-Chloro-6-[(3-pyridyl)methylamino]-9-iso-propylpurine (5b). Yield 56%, mp 184−186 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (d, 6H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.83 (hept, 1H, $CH(CH_3)_2$), 4.85−4.90 (m, 2H, $NHCH_2$), 6.22 (bs, 1H, NH), 7.24−7.29 (m, 1H, $H_{pyridyl}$) 7.73 (m, 1H, $H_{pyridyl}$), 7.78 (s, 1H, H-8), 8.54 (m, 1H, $H_{pyridyl}$), 8.66 (bs, 1H, $H_{pyridyl}$).

2-Chloro-6-[(4-pyridyl)methylamino]-9-iso-propylpurine (5c). Yield 77%, mp 120−122 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (d, 6H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.84 (hept, 1H, $CH(CH_3)_2$), 4.85−4.91 (m, 2H, $NHCH_2$), 6.25 (bs, 1H, $NHCH_2$), 7.29 (d, 2H, $H_{pyridyl}$), 7.80 (s, 1H, H-8), 8.56 (d, 2H, *J* = 5.6 Hz, $H_{pyridyl}$).

2-Chloro-6-[3-(phenyl)phenylmethylamino]-9-iso-propylpurine (5d). Yield 45%, mp 105−107 °C. ^{1}H NMR ($CDCl_3$): δ 1.35 (d, 6H, *J* = 6.7 Hz, $CH(CH_3)_2$), 4.60 (hept, 1H, $CH(CH_3)_2$), 4.80 (bs, 2H, $NHCH_2$), 7.26−7.49 (m, 10H, H_{phenyl}), 7.82 (s, 1H, H-8).

2-Chloro-6-[4-(phenyl)phenylmethylamino]-9-iso-propylpurine (5e). Yield 32%, mp 98−101 °C. ^{1}H NMR ($CDCl_3$): δ 1.57 (d, 6H, *J* = 6.7 Hz, $CH(CH_3)_2$) 4.64 (hept, 1H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.82 (bs, 2H, $NHCH_2$), 6.65 (brs, 1H, NH); 7.30−7.34 (m, 1H, H_{phenyl}), 7.44 (t, 2H, *J* = 8 Hz, H_{phenyl}), 7.52−7.63 (m, 5H, H_{phenyl} + H-8), 7.81−7.84 (d, 2H, *J* = 8.8 Hz, H_{phenyl}).

2-Chloro-6-[3-(2-pyridyl)phenylmethylamino]-9-iso-propylpurine (5f). Yield 44%, mp 120−122 °C. ^{1}H NMR ($CDCl_3$): δ 1.34 (d, 6H, *J* = 6.8 Hz, $CH(CH_3)_2$), 4.78 (hept, 1H, $CH(CH_3)_2$); 4.86 (bs, 2H, $NHCH_2$), 5.80 (brs, 1H, NH); 7.23 (m, 2H, $H_{pyridyl}$), 7.80

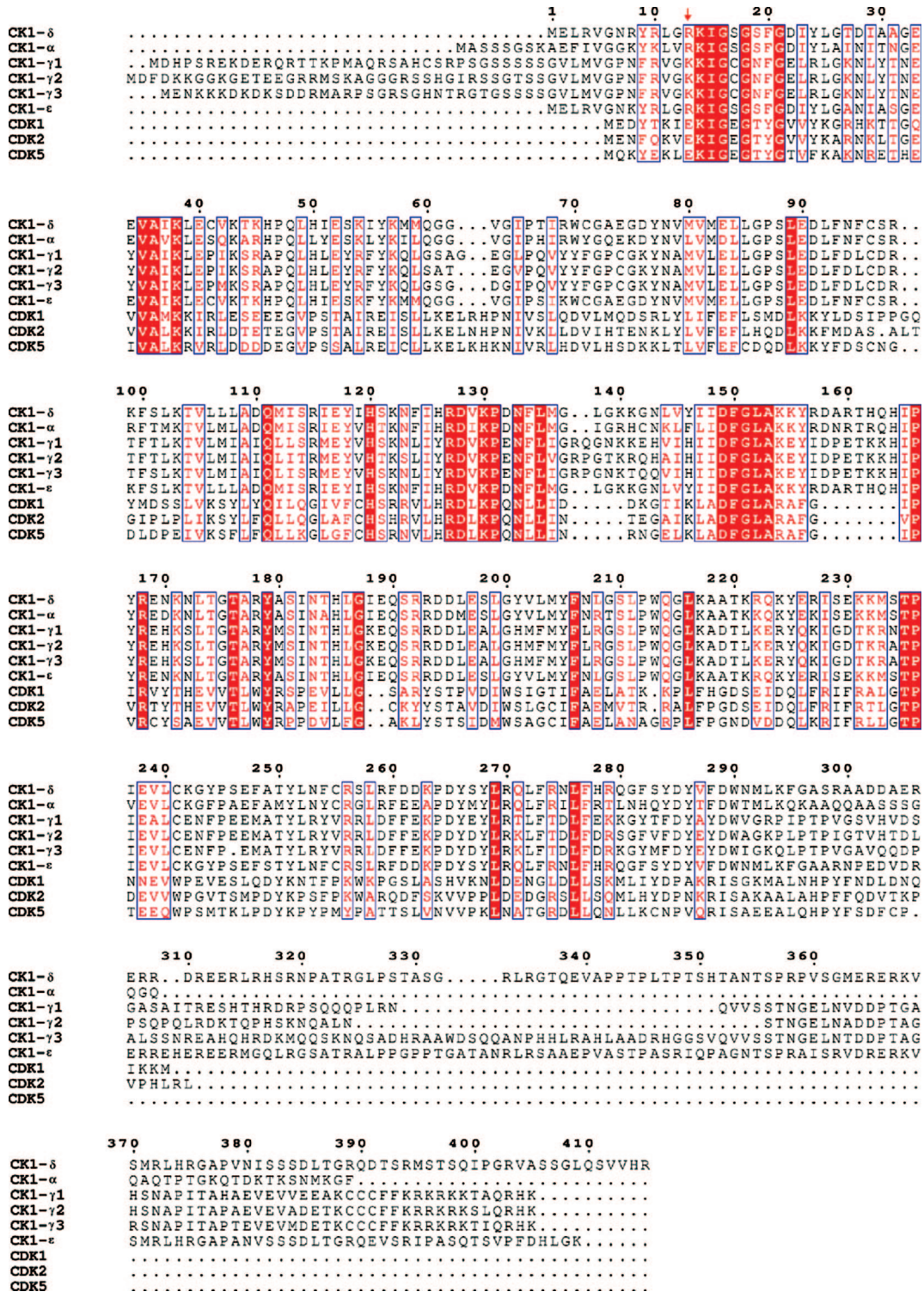


Figure 2. An alignment of the sequences of CDK1, 2, and 5 and five isoforms of CK1. Identical residues are enclosed in red-filled boxes and similar residues in blue-bordered boxes. The red arrow indicates $Arg13^{CK1\text{-}\delta}$. This position is occupied by a basic residue, arginine or lysine, in all CK1 isoforms, while in the CDKs, it is a glutamate. A favorable interaction between this residue and the pyridinyl ring of C&R8 (**8m**) and **13a** in CK1, and its absence in the CDKs could account for the specificity gain of the latter two analogues with respect to roscovitine for CK1.

(dd, 1H, $H_{pyridyl}$), 7.38−7.65 (m, 5H, H_{phenyl} + H-8), 8.63 (d, 1H, J = 2.5 Hz, $H_{pyridyl}$).

2-Chloro-6-[3-(3-pyridyl)phenylmethylamino]-9-iso-propylpurine (5g). Yield 65%, mp 115−117 °C. ^{1}H NMR ($CDCl_3$): δ 1.55 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$), 4.81 (hept, 1H, $CH(CH_3)_2$), 4.90 (d, 2H, $NHCH_2$), 6.66 (bs, 1H, N*H*), 7.30 (dd, 1H, J = 2.8, 8 Hz, $H_{pyridyl}$), 7.41−7.52 (m, 3H, H_{phenyl}), 7.61 (s, 1H, H-8), 7.69 (s, 1H, H_{phenyl}), 7.86 (dd, 1H, J = 1.6 and J' = 8 Hz, $H_{pyridyl}$), 8.59 (dd, 1H, J = 1.6 Hz and J' = 4.8 Hz, $H_{pyridyl}$), 8.85 (d, 1H, J = 2.4 Hz, $H_{pyridyl}$).

2-Chloro-6-[3-(4-pyridyl)phenylmethylamino]-9-iso-propylpurine (5h). Yield 48%, mp 145−148 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (d, 6H, J = 6.8 Hz, $CH(CH_3)_2$), 4.79 (hept, 1H, $CH(CH_3)_2$), 4.85 (bs, 2H, $NHCH_2$), 6.59 (bs, 1H, N*H*), 7.20−7.49 (d, 2H, J

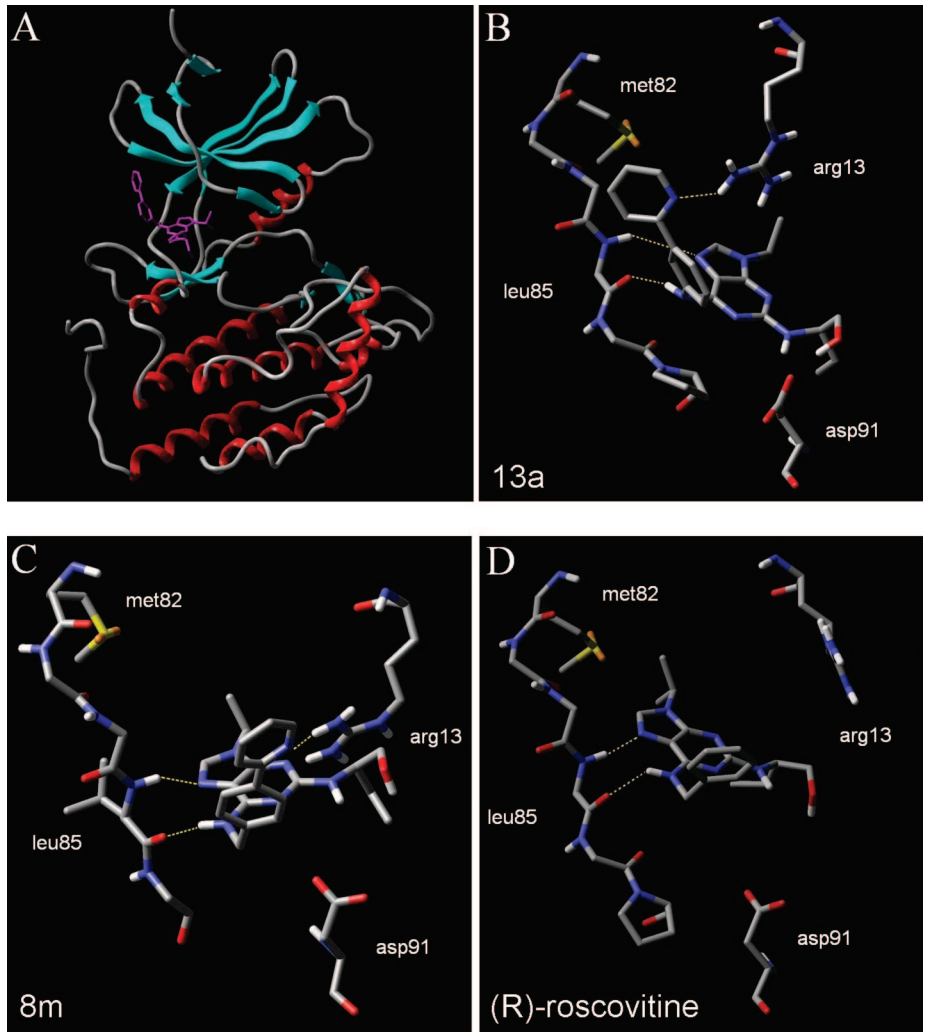


Figure 3. (A) Overall structure of CK1-δ complexed with analogue **13a** (magenta). The kinase is depicted as a ribbon and colored according to its secondary structure (red: α helix; cyan: beta-sheet; gray: random coil). (B) Analogue **13a** docked in the ATP-binding pocket of CK1-δ. The hydrogen bonds formed between the ligand and the kinase backbone as well as with the side chain of Arg13 are depicted as yellow dashed lines. The gatekeeper residue Met82 near the hydrophobic cavity of the pocket is also visible. (C) Analogue **8m** docked in the ATP-binding pocket of CK1-δ. Same representation as in (B). (D) (*R*)-Roscovitine **8a** docked in the ATP-binding pocket of CK1-δ. While the two hydrogen bonds formed with the receptor backbone are present, Arg13 does not interact with the ligand and adopts a different orientation.

= 8.4 Hz, H_{phenyl}) 7.65(dd, 2H, pyridyl), 7.76 (s, 1H, 8-H), 7.98 (d, 2H, J = 8.4 Hz, H_{phenyl}), 8.79(dd, 2H, $H_{pyridyl}$).

2-Chloro-6-[4-(2-pyridyl)phenylmethylamino]-9-iso-propylpurine (5i). Yield 56%, mp 176−179 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 4.79 (hept, 1H, CH(CH_3)$_2$), 4.85 (bs, 2H, NHCH_2), 6.59 (bs, 1H, NHCH$_2$), 7.20−7.23 (m, 1H, $H_{pyridyl}$), 7.49 (d, 2H, J = 8 Hz, H_{phenyl}), 7.73−7.71 (m, 2H, $H_{pyridyl}$), 7.79 (s, 1H, H-8), 7.98 (d, 2H, H_{phenyl}), 8.71 (d, 1H, J = 4.8 Hz, $H_{pyridyl}$).

2-Chloro-6-[4-(3-pyridyl)phenylmethylamino]-9-iso-propylpurine (5j). Yield 68%, mp 158−160 °C. ^{1}H NMR ($CDCl_3$): δ 1.56 (d, 6H, J = 7.2 Hz, CH(CH_3)$_2$), 4.84 (hept, 1H, CH(CH_3)$_2$), 4.88 (bs, 2H, NHCH_2), 7.37 (dd, 1H, J = 2.8 Hz and J' = 7.6 Hz, $H_{pyridyl}$), 7.52 (d, 2H, J = 8.4 Hz, H_{phenyl}), 7.57 (d, 2H, J = 8.4 Hz, H_{phenyl}), 7.80 (s, 1H, H-8), 7.82 (dt, 1H, J = 1.6 Hz and J' = 8.4 Hz, $H_{pyridyl}$), 8.60 (d, 1H, J = 3.6 Hz, $H_{pyridyl}$), 8.84 (d, 1H, J = 1.6, $H_{pyridyl}$).

2-Chloro-6-[4-(4-pyridyl)phenylmethylamino]-9-iso-propylpurine (5k). Yield 56%, mp 127−132 °C. ^{1}H NMR ($CDCl_3$): δ 1.53 (d, 6H, J = 7 Hz, CH(CH_3)$_2$), 4.84 (hept, 1H, CH(CH_3)$_2$), 4.88 (bs, 2H, NHCH_2), 7.52 (d, 2H, J = 8.4 Hz, H_{phenyl}), 7.57 (d, 2H, H_{phenyl}), 7.80 (s, 1H, H-8), 7.62 (dd, 2H, $H_{pyridyl}$), 8.82 (dd, 2H, $H_{pyridyl}$).

Amination by Nucleophilic Substitution of the 2 Chlorine. Preparation of 6, 7, 8, and 13. A mixture of compound **3**, **4**, **5**, or **11**, (2 mmol), NBu_3 (10 mmol), amino-alcohol (10 mmol), and DMSO 0.5 mL was heated at 165 °C for 3 h. After cooling to rt, 5 mL of H_2O was added and the mixture was extracted with AcOEt (3 × 10 mL). The residue was chromatographied using AcOEt-EtOH-NEt_3 as eluent.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-phenyl-9-iso-propylpurine (6a). Yield 56%, mp 100−104 °C. ^{1}H NMR ($CDCl_3$): δ 1.01 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.53 (d, 6H, J = 6.6 Hz, CH(CH_3)$_2$), 1.71 (m, 2H, CH$_3$CH_2), 3.62 (dd, 1H, J = 10,3 Hz and J' = 7.5 Hz, CH_2OH), 3.75 (dd, 1H, J = 10.3 Hz and J' = 2.4 Hz, CH_2OH), 4.60 (hept, 1H, CH(CH_3)$_2$), 7.48−7.66(m, 3H, H_{phenyl}), 7. 75−7.92 (m, 2H, H_{phenyl}), 8.22 (s, 1H, H-8).

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-(3-pyridyl)-9-iso-propylpurine (6b). Yield 42%, mp 98−100 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, J = 7.4 Hz, CH_3CH_2), 1.54 (d, 6H, J = 6.9 Hz, CH(CH_3)$_2$), 1.72 (m, 2H, CH_3CH_2), 3.66 (dd, 1H, J = 6.6 Hz and J′ = 10.8 Hz, CH_2OH), 3.83 (dd, 1H, J = 3.2 Hz and J′ = 10.8 Hz, CH_2OH), 4.02 (m, 1H, CHNH), 4.66 (hept, 1H, CH(CH_3)$_2$), 5.20 (d, 1H, J = 6.5 Hz, CHNH), 7.35 (dd, 1H, J = 4.8 Hz and J′ = 8.1 Hz, $H_{pyridyl}$), 7.78 (s, 1H, H-8), 8.64 (d, 1H, J = 4.5 Hz, $H_{pyridyl}$), 8.88 (d, 1H, J = 7.8 Hz, $H_{pyridyl}$), 9.73 (s, 1H, $H_{pyridyl}$). ^{13}C NMR: δ 9.8,

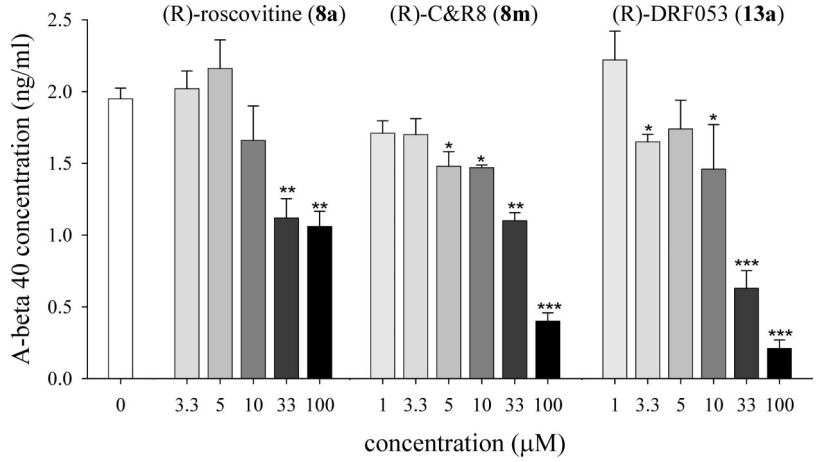


Figure 4. Dose-dependent inhibition of amyloid-β production in N2A-APP$_{695}$ cells by (*R*)-roscovitine (**8a**), (*R*)-C&R8 (**8m**), and (*R*)-DRF053 (**13a**). N2A cells stably expressing APP-695 were incubated in the absence or presence of various concentrations of (*R*)-roscovitine (**8a**), (*R*)-C&R8 (**8m**), and (*R*)-DRF053 (**13a**). Cell supernatants were collected after 3 h of incubation and subjected to Aβ40 ELISA assays. Data from at least three experiments (mean ± SEM) were compared (GraphPad Prism v4.0) with the control conditions (DMSO). (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; one-tailed Student's *t* test, 95% significance level).

21.3, 21.4, 23.9, 45.7, 54.8, 65.6, 122.3, 124.8, 130.8, 135.8, 138.5, 149.6, 150, 152, 152.8, 158.4.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-(4-pyridyl)-9-iso-propylpurine (6c). Yield 58%, mp 136−138 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, $J = 7.4$ Hz, CH_3CH$_2$), 1.54 (d, 6H, $J = 6.8$ Hz, CH(CH_3)$_2$), 1.77−1.31 (m, 2H, CH$_3$CH_2), 3.70 (m, 1H, CH_2OH), 3.83 (dd, 1H, $J = 3.4$ Hz and $J' = 10.9$ Hz, CH_2OH), 3.99−4.09 (m, 1H, C*H*NH), 4.68 (hept, 1H, C*H*(CH$_3$)$_2$), 5.20 (d, 1H, $J = 3.4$ Hz, CHN*H*), 7.81 (s, 1H, H-8), 8.43 (d, 2H, $J = 2.8$ Hz, H$_{pyridyl}$), 8.70 (d, 2H, $J = 2.8$ Hz, H$_{pyridyl}$). ^{13}C NMR: δ 9.8, 21.3, 21.4, 23.8, 45.8, 54.8, 65.5, 122.2 (2×), 125.3, 138.9, 142.2, 149.2 (2×), 151.6, 153.3, 158.4.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[2-(phenyl)-phenylamino]-9-iso-propylpurine (7a). Yield 65%, mp 134−138 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, $J = 7.2$ Hz, CH_3CH$_2$), 1.43 (d, 6H, $J = 6.8$ Hz, CH(CH_3)$_2$), 1.48−1.60 (m, 2H, CH$_3$CH_2), 3.54 (dd, 1H, $J = 3.2$ Hz and $J' = 10.4$ Hz, CH_2OH), 3.71 (dd, 1H, $J = 2.4$ and $J' = 10$ Hz, CH_2OH), 3.81−3.85 (m, 1H, C*H*NH), 4.51 (hept, 1H, C*H*(CH$_3$)$_2$), 5.1 (d, 1H, $J = 6$ Hz, CHN*H*), 7.10 (t, 1H, $J = 7.6$ Hz, H$_{phenyl}$), 7.23−7.40 (m, 8H, H-8 + H$_{phenyl}$), 7.54 (s, 1H, N*H*$_{phenyl}$), 8.25 (d, 1H, $J = 8.4$ Hz, H$_{phenyl}$). ^{13}C NMR: δ 10.9, 22.4, 22.5, 24.8, 46.5, 56.0, 67.4, 115.1, 123.0, 123.9, 127.7, 127.8, 128.8 (×2), 129.3, 130.4, 133.6, 135.1 (×2), 135.4, 138.4, 150.5, 152.6, 159.6.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(phenyl)phenylamino)-9-iso-propylpurine (7b). Yield 78%, mp 100−105 °C. ^{1}H NMR ($CDCl_3$): δ 1.02 (t, 3H, $J = 7.6$ Hz, CH_3-CH$_2$), 1.56 (d, 6H, $J = 6.8$ Hz, CH(CH_3)$_2$), 1.59−1.69 (m, 2H, CH$_3$CH_2), 3.65 (dd, 1H, $J = 7.2$ Hz and $J' = 10$ Hz, CH_2OH), 3.85 (d, 1H, $J = 10$ Hz, CH_2OH), 3.95−4.03 (m, 1H, C*H*-NH), 4.63 (hept, 1H, C*H*(CH$_3$)$_2$), 4.98 (d, 1H, $J = 6.4$ Hz, CHN*H*), 7.14−7.75 (m, 10H, H$_{phenyl}$ + H-8). ^{13}C NMR: δ 10.9, 22.5, 24.9, 46.7, 56.1, 67.5, 115.1, 118.8, 121.9, 127.2, 127.4, 128.7, 129.2, 135.2, 139.4, 141.1, 142.0, 152.4 (×2), 159.7.

(*R*)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[3-(phenyl)phenylamino]-9-iso-propylpurine (7c). Yield 56%, mp 90−105 °C. ^{1}H NMR ($CDCl_3$): δ 1.01 (d, 6H, $J = 6.4$ Hz, CHCH(CH_3)$_2$), 1.56 (d, 6H, $J = 6.8$ Hz, NCH(CH_3)$_2$), 2.00 (hept, 1H, $J = 6.4$ Hz, CHC*H*(CH$_3$)$_2$), 3.71 (dd, 1H, $J = 7.2$ Hz and $J' = 11$ Hz, CH_2OH), 3.87 (d, 1H, $J = 11$ Hz, CH_2OH), 4.09−4.24 (m, 1H, C*H*NH), 4.64 (hept, 1H, NC*H*(CH$_3$)$_2$), 5.01 (d, 1H, $J = 6.4$ Hz, CHN*H*), 7.14−7.75 (m, 10H, H$_{phenyl}$). ^{13}C NMR: δ 18.7, 19.5, 22.5 (×2), 30.0, 46.5, 59.8, 65.8, 115.2, 118.8, 121.8, 127.2 (×2), 127.4 (×3), 128.7, 129.2, 135.1, 139.5, 141.1, 141.9, 152.3 (×2), 160.0.

(*R*)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(phenyl)phenylamino]-9-iso-propylpurine (7d). Yield 45%, mp 115−118 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (d, 6H, $J = 6.8$ Hz, CHCH(CH_3)$_2$), 1.57 (d, 6H, $J = 6.8$ Hz, NCH(CH_3)$_2$), 2.00−2.10 (m, 1H, CHC*H*(CH$_3$)$_2$), 3.72−3.77 (m, 1H, CH_2OH), 3.87−3.93 (m, 2H, CH_2OH and C*H*NH), 4.64 (hept, 1H, NC*H*(CH$_3$)$_2$), 5.01 (d, 1H, $J = 5.1$ Hz, CHN*H*), 7.30−7.34 (m, 1H, H$_{phenyl}$), 7.44 (t, 2H, $J = 8$ Hz, H$_{phenyl}$), 7.59−7.61 (m, 5H, 8-H + H$_{phenyl}$), 7.67 (s, 1H, N*H*$_{phenyl}$), 7.81−7.84 (d, 2H, $J = 8.8$ Hz, H$_{phenyl}$).

2-(1-Hydroxy-2-methylprop-2-ylamino)-6-[3-(phenyl)-phenylamino]-9-iso-propylpurine (7e). Yield 27%, mp 80−85 °C. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 6H, (HOCH$_2$CC*H*$_3$)$_2$), 1.59 (d, 6H, $J = 6.8$ Hz, NHC*H*(CH$_3$)$_2$), 3.72 (d, 2H, $J = 6$ Hz, CH_2OH), 4.61 (hept, 1H, NC*H*(CH$_3$)$_2$), 7.14−7.75 (m, 10H, H$_{phenyl}$). ^{13}C NMR: δ 22.4 (×2), 25.1 (×2), 46.9, 55.4, 72.3, 115.0, 119.2, 119.5, 122.3, 127.2 (×2), 127.3 (×2), 128.6, 129.2, 135.2, 139.0, 141.0, 142.0, 149.6, 152.6, 158.4.

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[4-(phenyl)-phenylamino]-9-iso-propylpurine (7f). Yield 42%, mp 76−79 °C. ^{1}H NMR ($CDCl_3$): δ 1.06 (t, 3H, $J = 7.4$ Hz, CH_3CH$_2$), 1.57 (d, 6H, $J = 6.8$ Hz, CH(CH_3)$_2$), 1.62−1.72 (m, 2H, CH$_3$CH_2), 3.69−3.72 (m, 1H, CH_2OH), 3.83−3.89 (m, 1H, CH_2OH), 3.93−4.00 (m, 1H, C*H*NH), 4.64 (hept, 1H, C*H*(CH$_3$)$_2$), 5.00 (bs, 1H, CHN*H*), 7.30−7.34 (m, 1H, H$_{phenyl}$), 7.42−7.46 (m, 2H, H$_{phenyl}$), 7.59−7.75 (m, 5H, 8-H + H$_{phenyl}$), 7.81−7.84 (m, 2H, H$_{phenyl}$). ^{13}C NMR: δ 10.9, 22.5, 22.6, 24.9, 46.7, 56.2, 67.7, 115.3, 120.2 (×2), 126.8 (×2), 126.9, 127.5 (×2), 128.7 (×2), 135.2, 135.8, 138.3, 140.7, 152.3, 153.3, 159.7.

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[(2-pyridyl)-methylamino]-9-iso-propylpurine (8c). Yield 44%, mp 109−113 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 3H, $J = 7.3$ Hz, CH_3CH$_2$), 1.47 (d, 6H, $J = 6.6$ Hz, CH(CH_3)$_2$), 1.55 (m, 2H, CH$_3$CH_2), 3.60 (dd, 1H, $J = 10.8$ Hz and $J' = 7.1$ Hz, CH_2OH), 3.75 (dd, 1H, $J = 10.8$ Hz and $J' = 3.0$ Hz, CH_2OH), 3.85 (m, 1H, C*H*NH), 4.55 (hept, 1H, C*H*(CH$_3$)$_2$), 4.83 (bs, 2H, NHCH_2), 5.08 (d, 1H, $J = 6.3$ Hz, CHN*H*), 7.10 (dd, 1H, $J = 7.6$ Hz and $J' = 5.0$ Hz, H$_{pyridyl}$), 7.25 (bs, 1H, N*H*CH$_2$), 7.29 (d, 1H, $J = 7.6$ Hz, H$_{pyridyl}$), 7.47 (s, 1H, 8-H), 7.54 (td, 1H, $J = 7.6$ Hz and $J' = 1.8$ Hz, H$_{pyridyl}$), 8.46 (dd, 1H, $J = 5.0$ Hz and $J' = 1.8$ Hz, H$_{pyridyl}$). ^{13}C NMR: δ 10.9, 22.4, 22.5, 24.8, 45.5, 46.3, 55.9, 67.2, 114.5, 121.6, 122.0, 134.5, 136.6, 148.9, 154.7, 158.2, 159.9.

(*S*)-2(1-Hydroxybut-2-ylamino)-6-[(2-pyridyl)methylamino]-9-iso-propylpurine (8d). Yield 54%, mp 117−120 °C. ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, $J = 7.3$ Hz, CH_3CH$_2$), 1.47 (d, 6H, $J = 6.6$ Hz, CH(CH_3)$_2$), 1.57 (m, 2H, CH$_3$CH_2), 3.58 (dd, 1H, $J = 10.8$ Hz and $J' = 7.1$ Hz, CH_2OH), 3.75 (dd, 1H, $J = 10.8$ Hz and $J' = 3.0$ Hz, CH_2OH), 3.85 (m, 1H, C*H*NH), 4.55 (hept, 1H, C*H*(CH$_3$)$_2$), 4.83 (bs, 2H, NHCH_2), 5.08 (d, 1H, $J = 6.3$ Hz, CHN*H*), 7.10 (dd, 1H, $J = 7.6$ Hz and $J' = 5.0$ Hz, H$_{pyridyl}$), 7.25 (bs, 1H, N*H*CH$_2$), 7.29 (d, 1H, $J = 7.6$ Hz, H$_{pyridyl}$), 7.47 (s, 1H, 8-H), 7.54 (td, 1H,

$J = 7.6$ Hz and $J' = 1.8$ Hz, $H_{pyridyl}$), 8.48 (dd, 1H, $J = 5.0$ Hz and $J' = 1.8$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[(3-pyridyl)methylamino]-9-iso-propylpurine (8e). Yield 55%, mp 117−120 °C. ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H, $J = 7.5$ Hz, CH_3CH_2), 1.47 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.61 (m, 2H, CH_3CH_2), 3.60 (dd, 1H, $J = 10.6$ Hz and $J' = 7.3$ Hz, CH_2OH), 3.77 (dd, 1H, $J = 10.7$ Hz and $J' = 2.9$ Hz, CH_2OH), 3.88 (m, 1H, CHNH), 4.59 (hept, 1H, $CH(CH_3)_2$), 4.73 (m, 2H, $NHCH_2$), 4.96 (d, 1H, $J = 6.6$ Hz, CHNH), 6.65 (bs, 1H, $NHCH_2$), 7,21 (dd, 1H, $J = 7.8$ Hz and $J' = 4.8$ Hz, $H_{pyridyl}$), 7.46 (s, 1H, 8-H), 7.66 (m, 1H, $H_{pyridyl}$), 8.47 (dd, 1H, $J = 4.8$ Hz and $J' = 1.5$ Hz, $H_{pyridyl}$) 8.60 (d, 1H, $J = 1.8$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 11.1, 22.6, 22.7, 25.4, 42.4, 47.9, 55.8, 65.3, 114.6, 125.2, 136.6, 137.5, 137.9, 148.5, 149.4, 152.0, 155.9, 161.0.

(*S*)-2-(1-Hydroxybut-2-ylamino)-6-[(3-pyridyl)methylamino]-9-iso-propylpurine (8f). Yield 70%, mp 115−116 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, $J = 7.5$ Hz, CH_3CH_2), 1.50 (d, 6H, $J = 6.5$ Hz, $CH(CH_3)_2$), 1.64 (m, 2H, CH_3CH_2), 3.62 (dd, 1H, $J = 10.0$ Hz and $J' = 7.3$ Hz, CH_2OH), 3.77 (dd, 1H, $J = 10.0$ Hz and $J' = 2.9$ Hz, CH_2OH), 3.92 (m, 1H, CHNH), 4.59 (hept, 1H, $CH(CH_3)_2$), 4.75 (m, 2H, $NHCH_2$), 5.21 (d, 1H, $J = 6.6$ Hz, CHNH), 6.63 (bs, 1H, $NHCH_2$), 7,22 (dd, 1H, $J = 7.8$ Hz and $J' = 4.8$ Hz, $H_{pyridyl}$), 7.41 (s, 1H, 8-H), 7.63 (m, 1H, $H_{pyridyl}$), 8.45 (dd, 1H, $J = 4.8$ Hz and $J' = 1.5$ Hz, $H_{pyridyl}$) 8.61 (d, 1H, $J = 1.8$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[(4-pyridyl)methylamino]-9-iso-propylpurine (8g). Yield 67%, mp 130−132 °C. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 1.53 (d, 6H, $J = 6.7$ Hz, -$CH(CH_3)_2$), 1.61 (m, 2H, CH_3CH_2), 3.60 (dd, 1H, $J = 10.8$ Hz and $J' = 7.5$ Hz, -CH_2OH), 3.78 (dd, 1H, $J = 10.8$ Hz and $J' = 2.4$ Hz, CH_2OH), 3.85 (m, 1H, CHNH), 4.60 (hept, 1H, $CH(CH_3)_2$), 4.77 (m, 2H, $NHCH_2$), 4.92 (d, 1H, $J = 6.1$ Hz, CHNH), 6.55 (bs, 1H, $NHCH_2$), 7.26 (d, 2H, $J = 5.8$ Hz, $H_{pyridyl}$), 7.51 (s, 1H, 8-H), 8.51 (d, 2H, $J = 2.8$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 10.5, 22.0, 22.1, 24.5, 42.7, 46.1, 55.7, 67.5, 114.1, 121.8 (×2), 134.5, 148.1, 149.5 (×2), 154.3 (×2), 159.4.

(*S*)-2-(1-Hydroxybut-2-ylamino)-6-[(4-pyridyl)methylamino]-9-iso-propylpurine (8h). Yield 55%, mp 128−130 °C. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 1.53 (d, 6H, $J = 6.7$ Hz, $CH(CH_3)_2$), 1.61 (m, 2H, CH_3CH_2), 3.60 (dd, 1H, $J = 10.8$ Hz and $J' = 7.5$ Hz, CH_2OH), 3.78 (dd, 1H, $J = 10.8$ Hz and $J' = 2.4$ Hz, CH_2OH), 3.85 (m, 1H, CHNH), 4.60 (hept, 1H, $CH(CH_3)_2$), 4.77 (m, 2H, $NHCH_2$), 4.92 (d, 1H, $J = 6.1$ Hz, CHNH), 6.55 (bs, 1H, $NHCH_2$), 7.26 (d, 2H, $J = 5.8$ Hz, $H_{pyridyl}$), 7.51 (s, 1H, 8-H), 8.51 (d, 2H, $J = 2.8$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[3-(phenyl)phenylmethylamino]-9-iso-propylpurine (8i). Yield 42%, mp 100−102 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, $J = 7.3$ Hz, CH_3CH_2), 1.40 (d, 6H, $J = 6.7$ Hz, $CH(CH_3)_2$), 1.45−1.56 (m, 2H, CH_3CH_2), 3.54 (m, 1H, CH_2OH), 3.78 (d, 1H, $J = 10.6$ Hz, CH_2OH), 3.79−3.82 (m, 1H, CHNH), 4.50 (hept, 1H, $CH(CH_3)_2$), 4.74 (bs, 2H, $NHCH_2$), 4.87 (d, 1H, $J = 5,7$ Hz, CHNH), 6.48 (bs, 1H, $NHCH_2$), 7.23−7.52 (m, 10H, H-8). ^{13}C NMR ($CDCl_3$): δ 10.2, 21.4, 21.5, 23.9, 43,4, 45.4, 55.2, 67.2, 113.6, 118.5, 125.1, 125.6, 126.1 (2×), 126.3, 127.7 (2×), 128, 133.5 (2×), 138.5, 139.9, 140.5, 153.9, 159.

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[3-(2-pyridyl)phenylmethylamino]-9-iso-propylpurine (8j). Yield 32%, mp 163−165 °C. ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H, $J = 7.3$ Hz, CH_3CH_2), 1.40 (d, 6H, $J = 6.7$ Hz, $CH(CH_3)_2$), 1.45−1.56 (m, 2H, CH_3CH_2), 3.54 (m, 1H, CH_2OH), 3.78 (d, 1H, $J = 10.6$ Hz, CH_2OH), 3.79−3.82 (m, 1H, CHNH), 4.50 (hept, 1H, $CH(CH_3)_2$), 4.74 (bs, 2H, $NHCH_2$), 4.87 (brs, 1H, NH) 6.48 (bs, 1H, NH), 7.32−7.62 (m, 6H, H_{phenyl} + $H_{pyridyl}$); 7.76 (s, 1H, 8-H), 8.66 (d, 1H, $J = 4.5$ Hz, $H_{pyridyl}$), 8.85 (d, 1H $J = 2.8$ Hz $H_{pyridyl}$).

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[3-(3-pyridyl)phenylmethylamino]-9-iso-propylpurine (8k). Yield 67%, mp 123−126 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (t, 3H, $J = 6.8$ Hz, CH_3CH_2), 1.60 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.90−2.00 (m, 2H, $CH_2CH_3)_2$, 3.67 (dd, 1H, $J = 2.4$ Hz and $J' = 10.4$ Hz, CH_2OH), 3.78−3.87 (m, 2H, CHNH and CH_2OH), 4.60 (hept, 1H, $CH(CH_3)_2$), 4.83 (bs, 2H, $NHCH_2$), 4.99 (d, 1H, NHCH), 6.25 (bs, 1H, NH), 7.36 (dd, 1H, $J = 3.2$ Hz and $J' = 7.4$ Hz, $H_{pyridyl}$), 7.48 (d, 2H, $J = 8$ Hz, H_{phenyl}), 7.50 (s, 1H, 8-H), 7.53 (d, 2H, $J = 8$ Hz, H_{phenyl}), 7.86 (dt, 1H, $J = 2$ Hz and $J' = 7.6$ Hz, $H_{pyridyl}$), 8.58 (dd, 1H, $J = 1.6$ Hz and $J' = 8.6$ Hz, $H_{pyridyl}$), 8.82 (d, 1H, $J = 2$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[3-(4-pyridyl)phenylmethylamino]-9-iso-propylpurine (8l). Yield 78%, mp 144−146 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H, $J = 6.8$ Hz, CH_3CH_2), 1.60 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.90−2.00 (m, 2H, CH_2CH_3), 3.70 (dd, 1H, $J = 2.4$ Hz and $J' = 10.0$ Hz, CH_2OH), 3.75−3.85 (m, 2H, CHNH + CH_2OH), 4.62 (hept, 1H, $CH(CH_3)_2$), 4.80 (bs, 2H, $NHCH_2$), 5.15 (d, 1H, NHCH), 6.22 (bs, 1H, NH),7.45−7.63(m, 5H, H_{phenyl} + 8-H), 8.45 (d, 2H, $J = 2.8$ Hz, $H_{pyridyl}$), 8.68 (d, 2H, $J = 2.8$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[4-(2-pyridyl)phenylmethylamino]-9-iso-propylpurine (8m). Yield 66%, mp 89−93 °C. ^{1}H NMR ($CDCl_3$): δ 0.95 (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 1.47 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.50−1.60 (m, 2H, CH_3CH_2), 3.56 (dd, 1H, $J = 2.8$ Hz and $J' = 10.8$ Hz, CH_2OH), 3.75 (dd, 1H, $J = 2.4$ Hz and $J' = 10.8$ Hz, CH_2OH), 3.79−3.85 (m, 1H, CHNH), 4.54 (hept, 1H, $CH(CH_3)_2$), 4.76 (bs, 2H, $NHCH_2$), 4.90 (bs, 1H, CHNH), 6.10 (bs, 1H, $NHCH_2$), 7.16 (td, 1H, $J = 1.6$ and $J' = 5.4$ Hz, $H_{pyridyl}$), 7.41 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 7.45 (s, 1H, 8-H), 7.63−7.70 (m, 2H, $H_{pyridyl}$), 7.89 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 8.62 (d, 1H, $J = 4.8$ Hz, $H_{pyridyl}$). ^{13}C NMR ($CDCl_3$): δ 10.5, 22.3, 22.4, 24.1, 38.0, 45.9, 55.0, 66.8, 113.6, 120.0, 120.9, 125.5, 126.8 (×2), 127.7, 130.0, 134.1, 135.9, 137.7, 140.0, 149.1, 154.1, 156.8, 159.1.

(*R*)-2-(1-Hydroxy-3-methylbut-2-yl)-6-[4-(2-pyridyl)phenylmethylamino]-9-iso-propylpurine (8n). Yield 76%, mp 128−135 °C. ^{1}H NMR ($CDCl_3$): δ 0.94 (d, 6H, $J = 6.8$ Hz, $CHCH(CH_3)_2$), 1.47 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.67−1.80 (m, 1H, $CHCH(CH_3)_2$, 3.60−3.65 (m, 1H, CH_2OH), 3.76−3.79 (m, 2H, CHNH and CH_2OH), 4.55 (hept, 1H, $CH(CH_3)_2$), 4.78 (bs, 2H, $NHCH_2$), 6.10 (bs, 1H, $NHCH_2$), 7.14−7.18 (m, 1H, $H_{pyridyl}$), 7.41 (d, 2H, $J = 8.2$ Hz, H_{phenyl}), 7.48 (s, 1H, H-8), 7.63−7.70 (m, 2H, $H_{pyridyl}$), 7.89 (d, 2H, $J = 8.2$ Hz, H_{phenyl}), 8.61 (d, 1H, $J = 5.2$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 15.2, 18.9, 19.4, 22.5, 22.6, 30.1, 46.4, 59.9, 65.8, 114.6, 120.4, 122.0, 127,1 (×2), 128.0 (×2), 134.5 (×2), 134.7, 138.4, 139.7, 149.6, 154.8, 157.1, 160.2.

2-(1-Hydroxy-2-methylprop-2-yl)-6-[4-(2-pyridyl)phenylmethylamino]-9-iso-propylpurine (8o). Yield 65%, mp 110−112 °C. ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 6H, $C(CH_3)_2$), 1.56 (d, 6H, $J = 6.8$ Hz, $NCH(CH_3)_2$), 2.5 (bs, 1H, CH_2OH), 3.74 (s, 2H, CH_2OH), 4.59 (hept, 1H, $NCH(CH_3)_2$), 4.81 (bs, 2H, $NHCH_2$), 6.15 (bs, 1H, $NHCH_2$), 7.14−7.18 (m, 1H, $H_{pyridyl}$), 7.40 (d, 2H, $J = 8.0$ Hz, H_{phenyl}), 7.52 (s, 1H, 8-H), 7.65−7.72 (m, 2H, $H_{pyridyl}$), 7.93 (d, 2H, $J = 8.0$ Hz, H_{phenyl}), 8.65 (d, 1H, $J = 5.0$ Hz, $H_{pyridyl}$).

(*R*)- 2-(1-Hydroxybut-2-ylamino)-6-[4-(3-pyridyl)phenylmethylamino]-9-iso-propylpurine (8p). Yield 62%, mp 104−106 °C. ^{1}H NMR ($CDCl_3$): δ 0.97(t, 3H, $J = 7.5$ Hz, CH_3CH_2), 1.48 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.61 (m, 2H, CH_3CH_2), 3.62 (dd, 1H, $J = 10.6$ Hz and $J' = 7.3$ Hz, CH_2OH), 3.77 (dd, 1H, $J = 10.7$ Hz and $J' = 2.9$ Hz, CH_2OH) 4.60 (hept, 1H, $CH(CH_3)_2$), 4.80 (bs, 2H, $NHCH_2$), 5.03 (brt, 1H, $J = 6.8$ Hz, $NHCH_2$), 7.36 (dd, 1H, $J = 3.2$ and $J = 7.4$ Hz, $H_{pyridyl}$), 7.48 (d, 2H, $J = 8$ Hz, H_{phenyl}), 7.50 (s, 1H, 8-H), 7.53 (d, 2H, $J = 8$ Hz, H_{phenyl}), 7.86 (dt, 1H, $J = 2$ Hz and $J' = 7.6$ Hz, $H_{pyridyl}$), 8.58 (dd, 1H, $J = 1.6$ Hz and $J' = 4.6$ Hz, $H_{pyridyl}$), 8.85 (d, 1H, $J = 2$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(3-pyridyl)phenylmethylamino]-9-iso-propylpurine (8q). Yield 89%, mp 83−85 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (d, 6H, $J = 6.8$ Hz, $CHCH(CH_3)_2$), 1.60 (d, 6H, $J = 6.8$ Hz, $NCH(CH_3)_2$), 1.90−2.00 (m, 1H, $CHCH(CH_3)_2$, 3.67 (dd, 1H, $J = 2.4$ Hz and $J' = 10.4$ Hz, -CH_2OH), 3.78−3.87 (m, 2H, CHNH + CH_2OH), 4.60 (hept, 1H, $CH(CH_3)_2$), 4.83 (bs, 2H, $NHCH_2$), 4.99 (d, 1H, $J = 6.8$ Hz, NHCH), 6.25 (bs, 1H, $NHCH_2$), 7.36 (dd, 1H, $J = 3.2$ Hz and $J' = 7.4$ Hz, $H_{pyridyl}$), 7.48 (d, 2H, $J = 8$ Hz, H_{phenyl}), 7.50 (s, 1H, 8-H), 7.53 (d, 2H, $J = 8$ Hz, H_{phenyl}), 7.86 (dt, 1H, $J = 2$ Hz and $J' = 7.6$ Hz, $H_{pyridyl}$), 8.58 (dd, 1H, $J = 1.6$ Hz and $J' = 8.6$ Hz, $H_{pyridyl}$), 8.82 (d, 1H, $J = 2$ Hz, $H_{pyridyl}$).

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(4-pyridyl)phenylmethylamino]-9-iso-propylpurine (8r). Yield 56%, mp 70−72 °C. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, 3H, $J = 7.5$ Hz, CH_3CH_2), 1.49 (d, 6H,

J = 6.8 Hz, CH(CH_3)$_2$), 1.61 (m, 2H, CH_3CH_2), 1.55−1.71 (m, 2H, CH_3CH_2), 3.57 (dd, 1H, J = 7.8 Hz and J' = 10.7 Hz, CH_2OH), 3.75 (dd, 1H, J = 2.4 Hz, J' = 10.7 Hz, CH_2OH), 3.79−3.82 (m, 1H, C*H*NH), 4.56 (hept, 1H, C*H*(CH$_3$)$_2$), 4.71 (bs, 2H, NHCH_2), 7.41 (d, 2H, J = 6 Hz, H$_{pyridyl}$), 7.48−7.75 (m 5H, H$_{phenyl}$ + H-8), 8.60 (d, 2H, H$_{pyridyl}$).

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(phenyl)phenylmethylamino]-9-iso-propylpurine (8s). Yield 67%, mp 117−120 °C. ^{1}H NMR (CDCl$_3$): δ 0.97 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 1.47 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 1.55−1.71 (m, 2H, CH_3CH_2), 3.58 (dd, 1H, J = 7.8 Hz and J' = 10.8 Hz, CH_2OH), 3.75 (dd, 1H, J = 2.4 Hz, J' = 10.8 Hz, CH_2OH), 3.79−3.82 (m, 1H, C*H*NH), 4.55 (hept, 1H, C*H*(CH$_3$)$_2$), 4.74 (bs, 2H, NHCH_2), 4.82−4.84 (m, 1H, CHN*H*), 6.04 (bs, 1H, N*H*CH$_2$), 7.24−7.56 (m, 10H, 8-H). ^{13}C NMR: δ 10.9, 22.5, 22.6, 25.0, 38.7, 46.4, 56.4, 68.6, 113.6, 118.5, 125.1, 125.6 (×2), 127, 127.2, 127.3, 128.1, 128.7, 134.6 (2×), 137.8, 140.3, 140.8, 154.8, 159.9.

2-Chloro-6-(4-bromophenylamino)-9-iso-propylpurine (9). Yield 71%, mp 172 °C. ^{1}H NMR (CDCl$_3$): δ 1.54 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 4.79 (hept, 1H, C*H*(CH$_3$)$_2$), 7.42 (d, 2H, J = 8.8 Hz, H$_{phenyl}$), 7.62 (d, 2H, J = 8.8 Hz, H$_{phenyl}$), 7.82 (s, 1H, 8-H).

2-Chloro-6-[3-(tetramethyldioxaborolanyl)phenylamino]-9-iso-propylpurine (10a). Yield 65%, mp 213 °C. ^{1}H NMR (CDCl$_3$): δ 1.37 (s, 12 H, (CH_3)$_2$COB), 1.61 (d, 6H, J = 7.0 Hz, CH(CH_3)$_2$), 4.85 (hept, 1H, C*H*(CH$_3$)$_2$), 7.44 (t, 1H, J = 7.3 Hz, H$_{phenyl}$), 7.57 (d, 1H, = 7.3 Hz, H$_{phenyl}$), 7.72 (s, 1H, NH), 7.82 (s, 1H, H$_{phenyl}$), 7.87 (s, 1H, 8-H), 8.27 (d, 1H, J = 7.3 Hz, H$_{phenyl}$).

2-Chloro-6-[4-(tetramethyldioxaborolanyl)phenylamino]-9-iso-propylpurine (10g). Yield = 59%, mp 168 °C. ^{1}H NMR (CDCl$_3$): δ 1.34 (s, 12 H, (CH_3)$_2$COB), 1.61 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 4.87 (hept, 1H, C*H*(CH$_3$)$_2$), 7.78−7.91 (m, 4H, H$_{phenyl}$), 8.07 (s, 1H, H-8).

2-Chloro-6-[3-(2-pyridyl)phenylamino]-9-iso-propylpurine (11a). Yield 76%, mp 142−148 °C. ^{1}H NMR (CDCl$_3$): δ 1.61 (d, 6H, J = 6.7 Hz, CH(CH_3)$_2$), 4.85 (hept, 1H, C*H*(CH$_3$)$_2$), 7.53 (t, 1H, J = 7.5 Hz, H$_{phenyl}$), 7.67−7.75 (m, 3H, H$_{phenyl}$ + H$_{pyridyl}$), 7.77−7.85 (m, 2H, H$_{phenyl}$ + H$_{pyridyl}$), 7.91−7.95 (m, 1H, anilin or pyridin), 8.35 (bs, 1H, H$_{pyridyl}$), 8.69 (d, 1H, J = 4.1 Hz, H$_{pyridyl}$).

2-Chloro-6-[3-(3-pyridyl)phenylamino]-9-iso-propylpurine (11d). Yield 66%, mp 85−88 °C. ^{1}H NMR (CDCl$_3$): δ 1.62 (d, 6H, J = 6.6 Hz, CH(CH_3)$_2$), 4.86 (hept, 1H, C*H*(CH$_3$)$_2$), 7.36 (d, 1H, J = 7.6 Hz, H$_{pyridyl}$), 7.40 (dd, 1H, J = 4.2 Hz and J' = 7.6 Hz, H$_{pyridyl}$), 7.50 (t, 1H, J = 8.0 Hz, H$_{phenyl}$), 7.79 (d, 1H, J = 8.0 Hz, H$_{phenyl}$), 7.90 (s, 1H, 8-H), 7.95 (d, 1H, J = 8.0 Hz, H$_{phenyl}$), 8.07 (s, 1H, H-8), 8.62 (d, 1H, J = 4.2 Hz, H$_{pyridyl}$), 8.91 (s, 1H, H$_{pyridyl}$).

2-Chloro-6-[3-(4-pyridyl)phenylamino]-9-iso-propylpurine (11f). Yield 45%, mp 160−165 °C. ^{1}H NMR (CDCl$_3$): δ 1.55 (d, 6H, CH(CH_3)$_2$), 4.81 (sept, 1H, J = 6.8 Hz, C*H*(CH$_3$)$_2$), 7.35 (d, 1H, J = 7.6 Hz, H$_{phenyl}$), 7.44 (t, 1H, J = 7.6 Hz, H$_{phenyl}$), 7.52 (d, 2H, J = 4 Hz, H$_{pyridyl}$), 7.74 (d, 1H, J = 7.6 Hz, H$_{phenyl}$), 7.85 (s, 1H, H$_{phenyl}$), 7.99 (s, 1H, 8-H), 8.63 (d, 2H, J = 4 Hz, H$_{pyridyl}$).

2-Chloro-6-[4-(2-pyridyl)phenylamino]-9-iso-propylpurine (11g). Yield 57%, mp 170 °C. ^{1}H NMR (CDCl$_3$): δ 1.62 (d, 6H, CH(CH$_3$)$_2$), 4.86 (hept, 1H, C*H*(CH$_3$)$_2$), 7.70−7.91 (m, 5H, H$_{phenyl}$, H$_{pyridyl}$ and 8-H), 7.92 (d, 1H, J = 8.4 Hz, H$_{pyridyl}$), 8.07 (d, 2H, J = 8.8 Hz, H$_{phenyl}$), 8.70 (d, 1H, J = 4 Hz, H$_{pyridyl}$).

2-Chloro-6-[4-(3-pyridyl)phenylamino]-9-iso-propylpurine (11h). Yield 52%, mp 204−207 °C. ^{1}H NMR (CDCl$_3$) δ 1.56 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 4.82 (hept, 1H, C*H*(CH$_3$)$_2$), 7.30 (dd, 1H, J = 4.8 Hz and J' = 8 Hz, H$_{pyridyl}$), 7.57 (d, 2H, J = 8.8 Hz, H$_{phenyl}$), 7.82 (dt, 1H, J = 1.6 Hz and J' = 8 Hz, H$_{pyridyl}$), 7.86 (d, 2H, J = 8.8 Hz, H$_{phenyl}$), 7.94 (s, 1H, 8-H), 8.51 (dd, 1H, J = 1.6 Hz and J'= 4.8 Hz, H$_{phenyl}$), 8.80 (d, 1H, J = 1.6 Hz, H$_{pyridyl}$).

2-Chloro-6-[4-(4-pyridyl)phenylamino]-9-iso-propylpurine (11k). Yield 70%, yellow oil. ^{1}H NMR (CDCl$_3$): δ 1.56 (d, 6H, J = 6.5 Hz, CH(CH_3)$_2$), 4.85 (hept, 1H, C*H*(CH$_3$)$_2$), 7.10−7.20 (m, 2H, H$_{phenyl}$), 7.40 (d, 2H, J = 7.7 Hz, H$_{phenyl}$), 7.54 (d, 2H, J = 5.0 Hz, H$_{pyridyl}$), 7.75 (s, 1H, 8-H), 8.00 (d, 2H, J = 5.0 Hz, H$_{pyridyl}$).

2-(3-Pyridyl)-6-(4-bromophenylamino)-9-iso-propylpurine (12a). Yield 26%, mp 122−125 °C. ^{1}H NMR (CDCl$_3$): δ 1.68 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 4.96 (hept, 1H, C*H*(CH$_3$)$_2$), 7.37−7.45 (m, 1H, H$_{pyridyl}$), 7.54 (d, 2H, J = 8.4 Hz, H$_{phenyl}$), 7.81 (d, 2H, J = 8.4 Hz, H$_{phenyl}$), 7.94 (s, 1H, 8-H), 8.62−8.75 (m, 2H, H$_{pyridyl}$), 9.70 (s, 1H, H$_{pyridyl}$). ^{13}C NMR: δ 22.5, 47.9, 115.5, 120.1, 127.7, 123.2, 123.4, 132.0, 139.1, 149.9, 144.8, 151.4, 156.2.

2-(4-Pyridyl)-6-(4-bromophenylamino)-9-iso-propylpurine (12b). Yield 20%, mp 200−215 °C. ^{1}H NMR (CDCl$_3$): δ 1.63 (d, 6H, J = 6.4 Hz, CH(CH_3)$_2$), 4.90 (hept, 1H, C*H*(CH$_3$)$_2$), 7.48 (d, 2H, J = 8.6 Hz, H$_{phenyl}$), 7.75 (d, 2H, J = 8.6 Hz, H$_{phenyl}$), 7.90 (s, 1H, H-8), 8.25 (d, 2H, J = 4.8 Hz, H$_{pyridyl}$), 8.69 (d, 2H, J = 4.8 Hz, H H$_{pyridyl}$). ^{13}C NMR: δ 22.6, 47.4, 115.3, 119.6, 120.1, 121.2, 121.8, 128.0, 130.3, 131.7, 137.7, 139.3, 145.4, 149.9, 151.1, 155.9.

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[3-(2-pyridyl)phenylamino]-9-isopropylpurine (13a). Yield 79%, mp 77−85 °C. ^{1}H NMR (CDCl$_3$): δ 0.98 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.49 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 1.67−1.79 (m, 2H, CH_3CH_2), 3.64 (m, 1H, CH_2OH), 3.74 (dd, 1H, J = 2.4 Hz and J' = 10,6 Hz, CH_2OH), 3.96−4.07 (m, 1H, C*H*NH), 4.58 (hept, 1H, C*H*(CH$_3$)$_2$), 4.94 (m, 1H, CHN*H*), 7.38 (t, 1H, J = 7.2 Hz, H$_{phenyl}$), 7.50−7.54 (m, 2H, H$_{phenyl}$ + H$_{pyridyl}$) 7.68−7.71 (m, 3H, H$_{phenyl}$ + H$_{pyridyl}$) 7.86 (s, 1H, H-8), 8.62 (d, 1H, J = 4.3 Hz, H$_{pyridyl}$). ^{13}C NMR: δ 10.8, 22.5, 22.6, 24.9, 46.4, 55.9, 66.7, 114.9, 118.6, 120.3, 121.0, 121.4, 122.2, 129.1, 135.1, 136.9, 139.8, 140.1, 149.4, 152.2, 157.3, 159.6.

(*R*)-2(1-Hydroxy-3-methyl-but-2-ylamino)-6-[3-(2-pyridyl)phenylamino]-9-isopropylpurine (13b). Yield 50%, mp 95−110 °C. ^{1}H NMR (CDCl$_3$): δ 0.94 (d, 6H, J = 6.8 Hz, CHCH(CH_3)$_2$), 1.49 (d, 6H, J = 6.8 Hz, NCH(CH_3)$_2$), 1.95−2.06 (m, 1H, CHC*H*(CH$_3$)$_2$, 3.62−3.77 (m, 2H, CH_2OH), 3.91−3.99 (m, 1H, C*H*NH), 4.58 (hept, 1H, NC*H*(CH$_3$)$_2$), 4.91 (m, 1H, CHN*H*), 7.36 (t, 1H, J = 7.2 Hz, H$_{phenyl}$), 7.50−7.73 (m, 6H, H$_{phenyl}$, 8-H), 8.62 (d, 1H J = 4.2 Hz, H$_{pyridyl}$). ^{13}C NMR: δ 18.4, 19.7, 22.5, 22.6, 29.5, 46.4, 59.6, 65.2, 115.1, 118.6, 120.3, 121.0, 121.3, 122.3, 129.2, 135.0, 136.9, 139.7, 140.1, 149.5, 152.3, 157.4, 159.9.

2-(1-Hydroxy-2-methylprop-2-ylamino)-6-[3-(2-pyridyl)phenylamino]-9-iso-propylpurine (13c). Yield 48%, mp 103−109 °C. ^{1}H NMR (CDCl$_3$): δ 1.40 (s, 6H, C(CH_3)$_2$), 1.56 (d, 6H, J = 6.8 Hz, NCH(CH_3)$_2$), 2.54(bs, 1H, CH_2OH), 3.74 (s, 2H, CH_2OH), 4.59 (hept, 1H, NC*H*(CH$_3$)$_2$), 6.81 (bs, 1H, N*H*), 7.24 (td, 1H, J = 1.6 and J' = 6.8 Hz, H$_{pyridyl}$), 7.45 (t, 1H, J = 8 Hz, H$_{phenyl}$), 7.59 (s, 1H, N*H*Phenyl), 7.65 (d, 1H, J = 3.2 Hz, H$_{phenyl}$), 7.70−7.77 (m, 2H, H$_{pyridyl}$) 7.90 (d, 1H, J = 8.4 Hz, H$_{phenyl}$), 7.99 (s, 1H, H-8), 8.23 (s, 1H, H$_{pyridyl}$) 8.69 (d, 1H, J = 4.8 Hz, H$_{pyridyl}$). ^{13}C NMR: δ 22.4 (×2), 25.1, 46.9, 55.4 (×2), 72.1, 115.0, 118.9, 120.7, 120.9, 121.8, 122.2, 129.2, 135.2, 136.7, 139.3, 140.1, 149.5, 149.6, 152.6, 157.1, 158.4.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(3-pyridyl)phenylamino]-9-iso-propylpurine (13d). Yield 60%, mp 82−89 °C. ^{1}H NMR (CDCl$_3$): δ 0.94 (t, 3H, J = 6.8 Hz, CH_3CH$_2$), 1.49 (d, 6H, J = 6.8 Hz, CH(CH_3)$_2$), 1.53−1.66 (m, 2H, CH_3CH_2), 3.61 (dd, 1H, J = 7.2 Hz and J' = 10.4 Hz, CH_2OH), 3.78 (dd, 1H, J = 2.4 Hz and J' = 10.4 Hz, CH_2OH), 3.88−3.98 (m, 1H, C*H*NH), 4.56 (hept, 1H, J = 6.8 Hz, C*H*(CH$_3$)$_2$), 4.94 (d, 1H, J = 6 Hz, CHN*H*), 7.29 (dd, 1H, J = 4 Hz and J = 7.6 Hz, H$_{pyridyl}$), 7.37 (t, 1H, J = 7.6 Hz, H$_{pyridyl}$), 7.53 (s, 1H, H-8), 7.62−7.70 (m, 2H, H$_{phenyl}$ and H$_{pyridyl}$), 7.82 (d, 1H, J = 7.6 Hz, H$_{phenyl}$), 7.99 (s, 1H, H$_{phenyl}$), 8.52 (d, 1H, J = 4 Hz, H$_{pyridyl}$), 8.81 (s, 1H, H$_{pyridyl}$). ^{13}C NMR: δ 10.8, 22.6, 24.8, 46.7, 56.0, 67.2, 115.1, 118.5, 119.5, 121.6, 123.5, 129.5, 134.5, 135.3, 136.5, 138.5, 139.8, 148.3, 148.5, 152.3, 159.6.

(*R*)-2(1-Hydroxy-3-methyl-but-2-ylamino)-6-[3-(3-pyridyl)phenylamino]-9-iso-propylpurine (13e). Yield 51%, mp 90−97 °C. ^{1}H NMR (CDCl$_3$): δ 0.93 (d, 6H, J = 6.8 Hz, CH-CH(CH_3)$_2$), 1.48 (d, 6H, J = 6.8 Hz, NCH(CH_3)$_2$), 1.95 (hept, 1H, J = 6.8 Hz, CHC*H*(CH$_3$)$_2$, 3.68 (dd, 1H, J = 7.6 Hz, J' = 10,8 Hz, CH_2OH), 3.84 (dd, 1H, J = 2.6 Hz and J' = 10.8 Hz, CH_2OH), 3.84−3.90 (m, 1H, C*H*NH), 4.55 (hept, 1H, J = 6.8 Hz, NC*H*(CH$_3$)$_2$), 4.91 (d, 1H, J = 6.6 Hz, CHN*H*), 7.30 (dd, 1H, J = 4.8 Hz and J = 7,6 Hz, H$_{pyridyl}$), 7.38 (t, 1H, J = 7.6 Hz, H$_{phenyl}$), 7.53 (s, 1H, 8-H), 7.61−7.70 (m, 2H, H$_{phenyl}$), 7.84 (d, 1H, J = 7.6 Hz, H$_{pyridyl}$), 8.00

(s, 1H, H_{phenyl}), 8.53 (d, 1H, $J = 4$ Hz, $H_{pyridyl}$), 8.82 (s, 1H, $H_{pyridyl}$). ^{13}C NMR: δ 18.8, 19.4, 22.5, 29.9, 46.6, 59.7, 65.6, 115.1, 118.5, 119.5, 121.6, 123.6, 129.6, 134.5, 135.4, 136.6, 138.6, 140.0, 148.3, 148.7, 152.2, 159.9.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[3-(4-pyridyl)phenylamino]-9-iso-propylpurine (13f). Yield 88%, mp 85−97 °C. ^{1}H NMR ($CDCl_3$): δ 0.96 (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 1.50 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.52−1.66 (m, 2H, CH_3CH_2), 3.60 (dd, 1H, $J = 7.2$ Hz, $J' = 10.6$ Hz, CH_2OH), 3.79 (dd, 1H, $J = 2.4$ Hz and $J' = 10.6$ Hz, CH_2OH), 3.89−3.96 (m, 1H, CHNH), 4.58 (hept, 1H, $CH(CH_3)_2$), 4.92 (d, 1H, $J = 6.4$ Hz, CHNH), 7.26 (d, 1H, $J = 7.6$ Hz, H_{phenyl}), 7.39 (t, 1H, $J = 7.6$ Hz, H_{phenyl}), 7.46 (d, 2H, $J = 6$ Hz, $H_{pyridyl}$), 7.55 (s, 1H, H), 7.69 (s, 1H, H_{phenyl}), 7.73 (d, 1H, $J = 7.6$ Hz, H_{phenyl}), 8.60 (d, 2H, $J = 6$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 10.8, 22.3, 25.1, 46.7, 56.3, 67.2, 115.3, 118.5, 120.6, 121.5, 121.6, 129.4, 135.4, 138.8, 140.1, 148.5, 150.0, 152.1, 159.4.

(*R*)-2-(1-Hydroxy-but-2-ylamino)-6-[4-(2-pyridyl)phenylamino]-9-iso-propylpurine (13g). Yield 76%, yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.98 (t, 3H, $J = 7.2$ Hz, CH_3CH_2), 1.46 (d, 6H, $J = 6.8$ Hz, $CH(CH_3)_2$), 1.52−1.68 (m, 2H, CH_3CH_2), 3.63 (dd, 1H, $J = 6.8$ Hz and $J' = 10,8$ Hz, CH_2OH), 3.81 (dd, 1H, $J = 2.8$ Hz, $J' = 10,8$ Hz, CH_2OH), 3.90−3.98 (m, 1H, CHNH), 4.53 (hept, 1H, $J = 6.8$ Hz, $CH(CH_3)_2$), 5.05 (d, 1H, $J = 6.8$ Hz, CHNH), 7.09−7.14 (m, 1H, $H_{pyridyl}$), 7.51 (s, 1H, H-8), 7.60−7.68 (m, 2H, $H_{pyridyl}$), 7.80 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 7.90 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 8.59 (d, 1H, $J = 4.8$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 10.4, 21.9, 24.5, 45.8, 55.4, 65.8, 114.3, 119.5, 119.6, 121.1, 127.0, 132.7, 134.7, 136.4, 140.1, 148.7, 150.5, 151.4, 159.7, 158.8.

(*R*)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(2-pyridyl)phenylamino]-9-iso-propylpurine (13h). Yield 87%, mp 78−82 °C. ^{1}H NMR ($CDCl_3$): δ 0.98 (d, 6H, $J = 7$ Hz, $CHCH(CH_3)_2$), 1.48 (d, 6H, $J = 6.8$ Hz, $NCH(CH_3)_2$), 1.97 (m, 1H, $CHCH(CH_3)_2$), 3.68 (dd, 1H, $J = 7,6$ Hz, $J' = 11$ Hz, CH_2OH), 3.84 (dd, 1H, $J = 2.6$ Hz and $J' = 11$ Hz, CH_2OH), 3.86−3.88 (m, 1H, CHNH), 4.54 (hept, 1H, $J = 6.8$ Hz, $NCH(CH_3)_2$), 5.03 (d, 1H, $J = 6.8$ Hz, CHNH), 7.10−7.15 (m, 1H, $H_{pyridyl}$), 7.53 (s, 1H, 8-H), 7.64−7.68 (m, 2H, $H_{pyridyl}$), 7.80 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 7.93 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 8.60 (d, 1H, $J = 4.6$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 18.7, 19.8, 21.7, 29.5, 46.4, 51.8, 59.4, 114.2, 119.2, 119.6, 121.1, 126.9, 133.0, 134.5, 136.2, 139.5, 148.9, 151.4, 156.2, 159.2.

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[4-(3-pyridyl)phenylamino]-9-iso-propylpurine (13i). Yield 79%, mp 85−105 °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, $CH(CH_3)_2$), 1.60−1.70 (m, 2H, CH_3CH_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, CHNH), 4.65 (hept, 1H, $CH(CH_3)_2$), 5.04 (d, 1H, $J = 8$ Hz, CHNH), 7.35 (dd, 1H, $J = 4.8$ Hz, $J' = 7.6$ Hz, $H_{pyridyl}$), 7.56−7.62 (m, 3H, $H_{phenyl} + H_{pyridyl}$), 7.68 (s, 1H, 8-H), 7.88 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 8.57 (d, 1H, $J = 4.8$ Hz, $H_{pyridyl}$), 8.86 (s, 1H, $H_{pyridyl}$). ^{13}C NMR: δ 10.9, 22.5, 24.9, 46.7, 56.0, 67.3, 115.2, 120.3, 123.6, 127.4, 132.0, 133.8, 135.3, 136.2, 139.3, 148.0, 152.1, 159.7.

(*R*)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(3-pyridyl)phenylamino]-9-iso-propylpurine (13j). Yield 84%, mp 116−120 °C. ^{1}H NMR ($CDCl_3$) δ 1.05 (d, 6H, $J = 6.8$ Hz, $CHCH(CH_3)_2$), 1.57 (d, 6H, $J = 6.8$ Hz, $NCH(CH_3)_2$), 2.01 (hept, 1H, $J = 7$ Hz, $CHCH(CH_3)_2$), 3.75 (dd, 1H, $J = 8.0$ Hz and $J' = 10.4$ Hz, CH_2OH), 3.90 (d, 1H, $J = 10.4$ Hz, CH_2OH), 4.09−4.17 (m, 1H, CHNH), 4.64 (hept, 1H, $NCH(CH_3)_2$), 5.08 (d, 1H, $J = 6$ Hz, CHNH), 7.35 (dd, 1H, $J = 4.8$ Hz, $J' = 7.6$ Hz, $H_{pyridyl}$), 7.56−7.62 (m, 3H, $H_{phenyl} + H_{pyridyl}$), 7.68 (s, 1H, 8-H), 7.88 (d, 2H, $J = 8.4$ Hz, H_{phenyl}), 8.57 (d, 1H, $J = 4.8$ Hz, $H_{pyridyl}$), 8.86 (s, 1H, $H_{pyridyl}$). ^{13}C NMR: δ 19.0, 19.2, 22.3, 30.1, 46.7, 59.7, 65.2, 115.2, 120.1, 123.7, 127.5, 132.2, 133.7, 135.0, 136.3, 139.2, 147.9, 152.0, 159.9.

(*R*)-2-(1-Hydroxybut-2-ylamino)-6-[4-(4-pyridyl)phenylamino]-9-iso-propylpurine (13k). Yield 37%, mp 126−130 °C. ^{1}H NMR ($CDCl_3$): δ 0.94−0.99 (m, 3H, CH_3CH_2), 1.44−1.52 (m, 6H, $CH(CH_3)_2$), 1.65−1.80 (m, 2H, CH_3CH_2), 3.66−3.75 (m, 1H, CH_2OH), 3.82−3.90 (m, 1H, CH_2OH), 3.97−4.05 (m, 1H, CHNH), 4.58−4.65 (m, 1H, $CH(CH_3)_2$), 5.00−5.05 (m, 1H, CHNH), 7.43−7.47 (m, 2H, H_{phenyl}), 7.53−7.65 (m, 3H, H_{phenyl} + 8-H), 7.89−7.95 (m, 2H, $H_{pyridyl}$), 8.60−8.65 (m, 2H, $H_{pyridyl}$). ^{13}C NMR: δ 11.0, 22.8, 25.4, 47.1, 53.5, 56.3, 120.4, 121.5, 127.6, 128.6, 129.4, 132.4, 135.7, 140.6, 148.2, 150.6, 152.2, 159.7.

(*R*)-2-(1-Hydroxy-3-methylbut-2-ylamino)-6-[4-(4-pyridyl)phenylamino]-9-iso-propylpurine (13l). Yield 41%, mp 121−123 °C. ^{1}H NMR ($CDCl_3$): δ 0.99 (d, 6H, $J = 7.9$ Hz, $CHCH(CH_3)_2$), 1.51 (d, 6H, $J = 7.9$ Hz, $NCH(CH_3)_2$), 1.97−1.99 (m, 1H, $CHCH(CH_3)_2$, 3.61−3.71 (m, 2H, CH_2OH), 3.82−3.85 (m, 1H, CHNH), 4.55−4.62 (m, 1H, $NCH(CH_3)_2$), 4.99−5.01 (m, 1H, CHNH), 7.44 (d, 2H, $J = 4.1$ Hz, H_{phenyl}), 7.55 (s, 1H, 8-H), 7.59 (d, 2H, $J = 4.1$ Hz, H_{phenyl}), 7.84 (d, 2H, $J = 6.2$ Hz, $H_{pyridyl}$), 8.57 (d, 2H, $J = 6.2$ Hz, $H_{pyridyl}$). ^{13}C NMR: δ 19.0, 19.5, 22.4, 30.0, 46.6, 59.7, 64.0, 115.1, 119.9, 121.0, 127.3, 131.9, 135.0, 140.3, 147.7, 150.0, 152.0, 160.0.

Docking on CK1. The multiple sequence alignment of the proteins was created using ClustalW 1.82 (http://www.ch.embnet.org/software/ClustalW.html). The crystal structure of CK1-δ (1CKJ) with sequence numbering based on SwissProt entry P48730 was used for all CK1 calculations. The ligands were placed in the receptor binding cavity and were minimized with the two hydrogen bonds formed between the receptor backbone and the purine ring constrained at their crystallographic distances and angles.[55] Then a Monte Carlo local search of 1000 steps as implemented in Macromodel v.9[56] was performed with all residues in a sphere of 6 Å around the ligand free to move and all remaining residues frozen. In all calculations the TNCG minimizer, the AMBER* force field and the GB/SA implicit solvent model were used. Prior to docking, the three ligand molecules were subjected to a 1000-step conformational search. An ab initio single point calculation of the global minimum conformation was performed at the DFT level using B3LYP method and the 6-31+G* basis set of JAGUAR v.4 for partial electrostatic atomic charge calculation. The pK_a calculations were performed with Pallas v.3 software (Pallas 3.1.1.2, CompuDrug Chemistry Ltd.). Alignment depictions were created with ESPript.[57] For the interaction energy calculations, the local MP2 method as implemented in JAGUAR v.4[58] and the 6-31+G* basis set were used. The LMP2 method is designed to avoid basis set superposition error, so the BSSE was calculated only for the HF energies using the Boys−Bernardi counterpoise method.[59] Interaction energy calculations were performed by transforming the ligand of the minimized 13a−CK1 complex in diphenylamine or *N*-methylaniline, subtracting all protein atoms except the backbone carbonyl acting as hydrogen bond acceptor and its amide nitrogen, adding hydrogens to fill valences and performing single point calculations. Experimental $\Delta\Delta G$ was calculated from IC_{50} values using the equation $\Delta\Delta G = \Delta G^{13a} - \Delta G^{C\&R8} = RT \ln(IC_{50}{}^{13a}/IC_{50}{}^{C\&R8})$, approximating $IC_{50} \sim K_d$.

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, leupeptin, aprotinin, 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) and CDK5/p25 (human, recombinant) were prepared as previously described.[54] Their kinase activity was assayed in buffer C, with 1 mg 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 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/liter of water. The wet filters were 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 GS-1, a GSK-3 specific substrate.[27] CK1 (porcine brain, native) was assayed as described for CDK1 but using CK-S, a CK1-specific peptide substrate [12].

Cell Biology. Chemicals. Cell Titer 96 containing the MTS reagent and CytoTox 96 kits were purchased from Promega (Madison, WI). The protease inhibitor cocktail was from Roche (Penzberg, Germany). Unless otherwise stated, the nonlisted reagents were from Sigma.

Cell Lines and Culture Conditions. SH-SY5Y human neuroblastoma cells were grown in DMEM medium from Invitrogen (Cergy Pontoise, France). The HEK 293 human embryonic kidney cell line was grown in MEM medium from Invitrogen. Both media were supplemented with antibiotics (penicillin-streptomycin) from Lonza and 10% volume of FCS from Invitrogen. Cells were cultured at 37 °C with 5% CO_2. Drug treatments were performed on exponentially growing cultures at the indicated time and concentrations. Control experiments were carried using appropriate dilutions of DMSO.

Cell Viability Assessment. Cell viability was determined by measuring the reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS, Promega (Madison, WI) as previously described.[60]

Measurement of Amyloid β Production in N2A Cells. N2A cells expressing APP (N2A-APP_{695} cells) were plated in 12-well dishes at a density of 3×10^5 cells per well and grown to 60% confluence. Drugs were added to cell cultures in fresh medium (0.5% fetal bovine serum). Following incubation, cells were recovered with 100 μL of RIPA buffer, incubated for 30 min on ice, and centrifuged at 13000 rpm for 20 min at 4 °C. The supernatants and/or cells were collected and subjected to BCA (Pierce) quantification. Equal amounts of protein samples were subjected to Western blot analysis using appropriate antibodies as described above. For immunoprecipitation assays, media were incubated with antibody 4G8 (Signet) to detect Aβ and full length βAPP.

After immobilization of total Aβ (coated monoclonal antibody specific for the N-terminus of Aβ) from media, Aβ40/42 peptide determinations were made by sandwich ELISA (BioSource International) using rabbit polyclonal antibodies specific for the C-terminus of Aβ40 or Aβ42. Aβ levels were normalized to total protein levels. Aβ40 and Aβ42 standard curves were plotted as a sigmoidal dose response curve (variable slope) using GraphPad Prism v4.0 (GraphPad Software Inc., San Diego, CA). Data presented are the results of at least three independent experiments done in triplicate. Results were analyzed with GraphPad Prism v4.0.

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Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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