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Biaryl purine derivatives as potent antiproliferative agents: Inhibitors of cyclin dependent kinases. Part I

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ABSTRACT

The introduction of an aryl ring onto the 4-position of the C-6 benzyl amino group of the Cdk inhibitor roscovitine (**2**), maintained the potent Cdk inhibition demonstrated by roscovitine (**2**) as well as greatly improving the antiproliferative activity. A series of C-6 biarylmethylamino derivatives was prepared addressing modifications on the C-6 biaryl rings, N-9 and C-2 positions to provide compounds that displayed potent cytotoxic activity against tumor cell lines. In particular, derivative **21h** demonstrated a >750-fold improvement in the growth inhibition of HeLa cells compared to roscovitine (**2**).

The cyclin dependent kinases (Cdks) are a family of serine–threonine protein kinases which play key roles in eukaryotic cell division, a process regulated by a series of events defined as the cell cycle.[1–5] The activity of the kinases as their name implies is dependent on association with cyclin partners. The Cdks drive the cell cycle through the individual phases of the process with different Cdk/cyclin pairs active during each phase of the cell cycle. Cell cycle dysfunction is a characteristic feature of tumor cells, with Cdk-related events among the most common genetic changes found in human tumors.[6,7] The observations of the connection between Cdks and the molecular pathology of cancer suggest the Cdks as potential therapeutic targets for the treatment of proliferative diseases.

A variety of chemical classes have been identified as Cdk inhibitors. Examples of these include staurosporine derivatives,[8] flavonoids,[9,10] oxindoles,[11,12] indolinones,[13] pyrrolopyrazines,[14] pyridopyrimidine,[15] quinazolines,[16] indenopyrazole,[17] and purines.[18–22] The 2,6,9-substituted purines form a class of inhibitors which include olomoucine (**1**)[23] and roscovitine (**2**).[24] Olomoucine (**1**), which contains a benzylamino group in the 6-position, was one of the first purine Cdk inhibitors discovered which demonstrated modest Cdk inhibitory activity and selectivity with respect to a number of kinases. Optimization of this structure gave rise to a second generation purine, roscovitine (**2**), which had increased steric bulk at the 2- and 9-positions. Roscovitine (**2**) demonstrated an increase in potency for a number of Cdks while also maintaining selectivity toward other kinases.

Using 2,6,9-trisubstituted purines as a starting scaffold, we[25–28] found that the installation of a C-6 biarylmethylamino group onto the purine nucleus resulting in compound **3a**, gave Cdk inhibition similar to roscovitine, but afforded much greater antiproliferative activity as evidenced by a 50-fold improvement in the inhibition of the cell growth (GI_{50}) of HeLa cells (see Fig. 1). Based on these findings, a systematic structure–activity relationship study investigating purines containing biarylmethylamino groups at C-6 was undertaken. The focus of this study was directed toward examining the effects of substitution at C-2, N-9 as well as the C-6 aminomethylene linker and C-6 biphenyl substitution. A full accounting of these results is presented herein. Subsequently, others[29] have also noted that several C-6 biarylmethylamino roscovitine analogues demonstrated moderate antiproliferative properties.

The synthesis of a series of C-2 modified analogues is shown in Scheme 1. Commercially available 2,6-dichloropurine (**4**) was treated with 4-phenyl benzyl amine to afford **5** followed by alkylation of the N-9 with 2-iodopropane. Nucleophilic displacement of the C-2 chloro with (*R*)-(−)-2-amino-1-butanol afforded **3a**. Analogues 3b–**o**, maintaining the C-6 *p*-biphenylmethylamino and N-9 isopropyl functionalities of **3a** were readily prepared by displacement of the C-2 chloro of **6** with a variety of amines.

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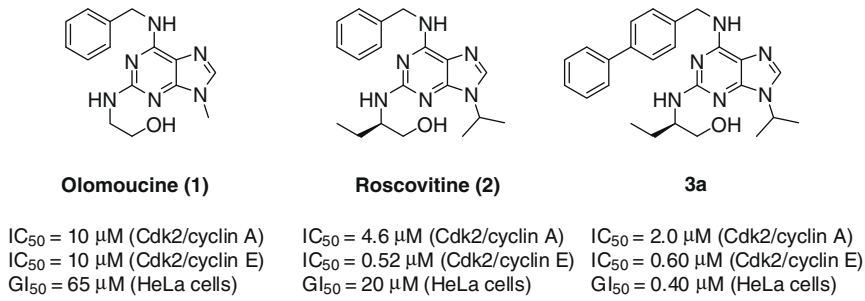
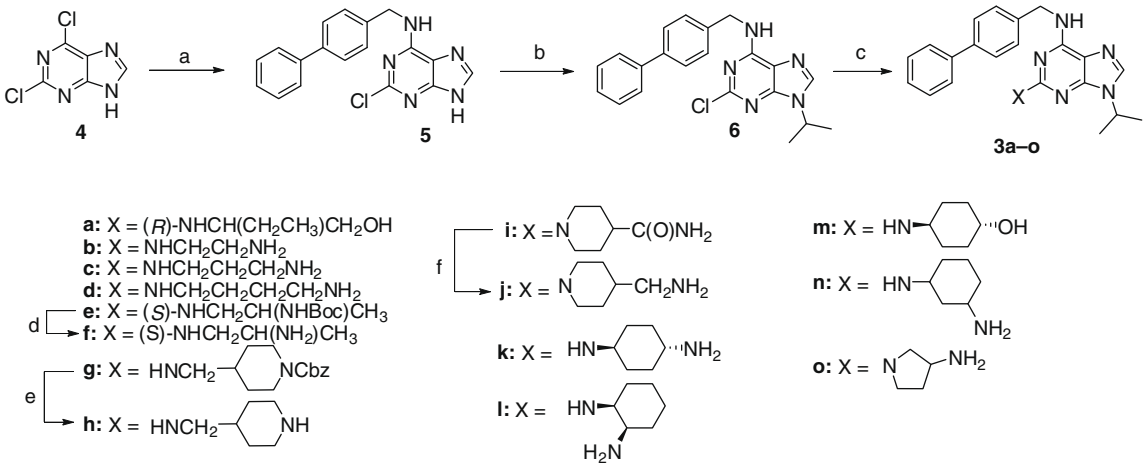


Figure 1.

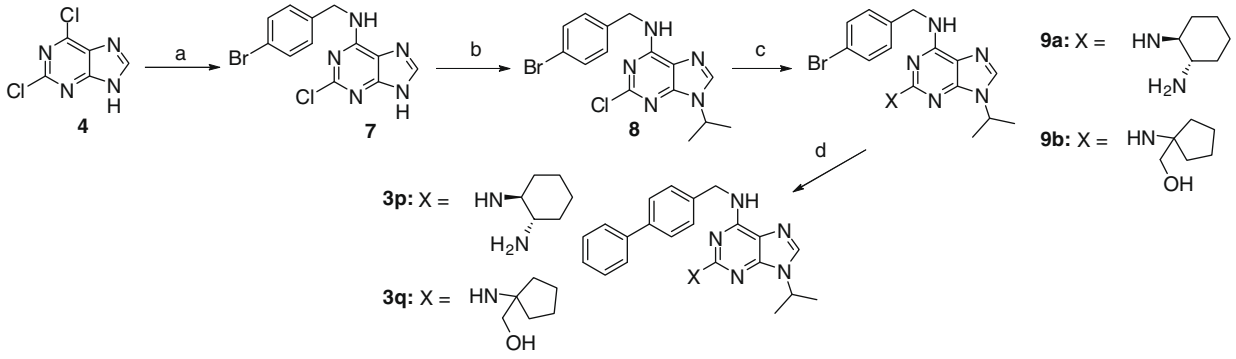


Scheme 1. Reagents and conditions: (a) 4-phenyl benzyl amine (1.01 equiv), EtN(*i*-Pr)$_2$ (1.95 equiv), water, reflux, 5 h; (b) *i*-PrI (4.0 equiv), K_2CO_3 (8.0 equiv), DMSO, rt, overnight; (c) amine (5–20 equiv), EtOH, 150–190 °C (sealed tube), 2–60 h; (d) HCl, Et_2O, CH_2Cl_2, MeOH, 16 h, rt; (e) Pd/C, NH_4HCO_2, MeOH, reflux, 4 h; (f) BH_3, THF, reflux, 2 h.

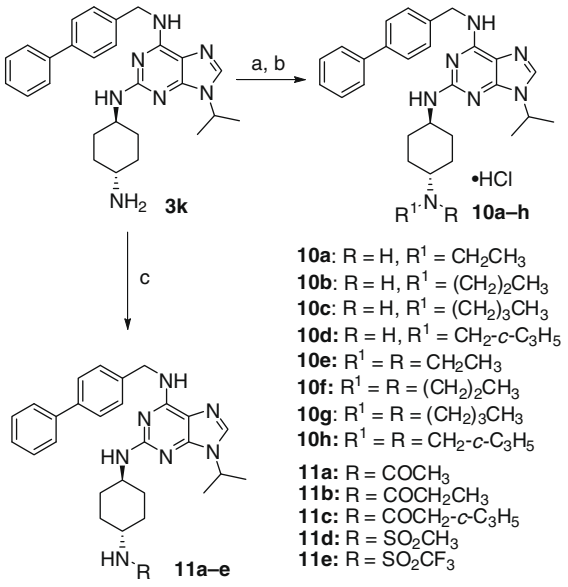
Alternatively the C-2 amine derivatives **3p** and **3q**, maintaining the C-6 *p*-biphenylmethylamino and N-9 isopropyl functionalities can be prepared has shown in Scheme 2. The C-6 chloro was displaced with 4-bromo benzyl amine to afford **7**, which was converted to the N-9 isopropyl intermediate **8**, followed by reaction with the appropriate amines to yield **9a** and **9b**. Suzuki reaction of **9a** or **9b** with phenyl boronic acid gave the corresponding biphenyl derivatives **3p** and **3q**, respectively.

Compound **3k** demonstrated outstanding antiproliferative activity with a GI_{50} = 0.077 μM, therefore a series of analogues was prepared as shown in Scheme 3, wherein the distal amino group of **3k** was derivatized. These modifications were directed toward modulation of the solubility and permeability of the molecules, adjustment of the basicity of this group and the introduction of steric bulk. Reductive amination of a series of aldehydes, provided mixtures of the mono-alkyl and bis-alkyl derivatives which were separated by chromatography and converted to their respective HCl salts **10a–h**. Acylations of **3k** afforded **11a–c** and sulfonylations gave **11d** and **11e**.

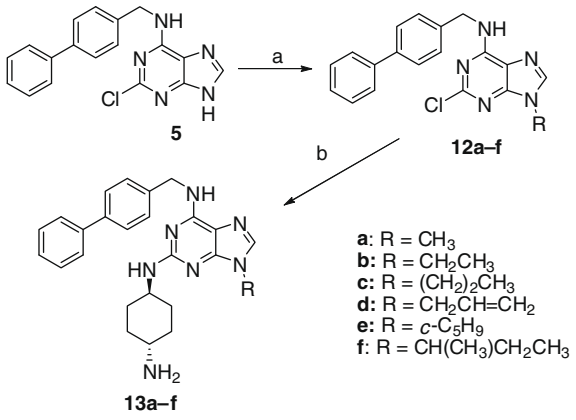
Compounds in which the C-6 *p*-biphenylmethylamino and C-2 *trans*-4-aminocyclohexylamine were maintained and the N-9 alkyl group varied were prepared as shown in Scheme 4. Alkylation of **5**



Scheme 2. Reagents and conditions: (a) 4-bromo benzyl amine (2.15 equiv), EtN(*i*-Pr)$_2$ (4.0 equiv), 1:1 water/EtOH, reflux, overnight; (b) *i*-PrI (3.0 equiv), K_2CO_3 (5.0 equiv), DMSO, rt, overnight; (c) amine (7–15 equiv), 150–190 °C, 25–60 h; (d) PhB(OH)$_2$ (3.6 equiv), Pd(PPh$_3$)$_4$ (0.27 equiv), Na_2CO_3, DME, water, reflux, 18 h.



Scheme 3. Reagents and conditions: (a) the appropriate aldehyde (0.9–1.1 equiv), $Na(OAc)_3BH$ (1.25–1.55 equiv), 1,2-dichloroethane, rt, 1.5–3 h; (b) 2 N HCl, EtOAc, rt; (c) the appropriate acyl chloride or sulfonyl chloride (1.1–3.0 equiv), pyridine or $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt, 1–18 h.



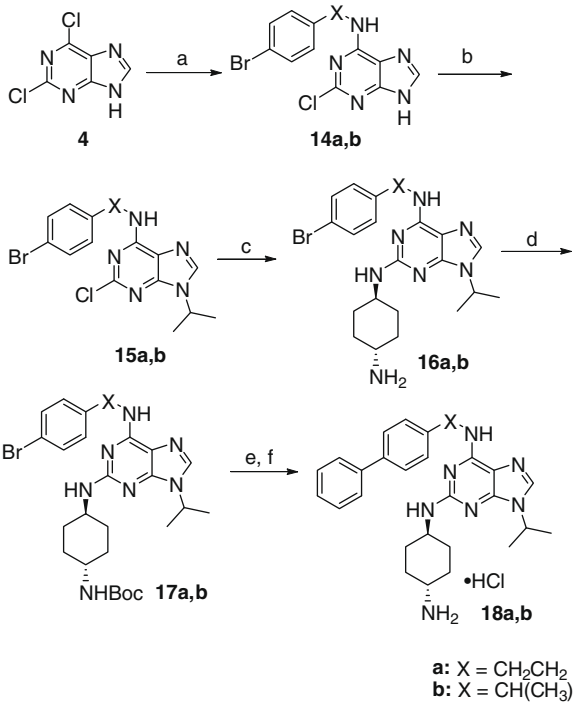
Scheme 4. Reagents and conditions: (a) the appropriate alkyl halide (4.0 equiv), K_2CO_3 (8.0 equiv), DMSO, rt, 18–48 h; (b) *trans*-1,4-diaminocyclohexane (10 equiv), EtOH, 140–160 °C (sealed tube), 24–72 h.

with the corresponding alkyl halides, gave compounds **12a–f** which upon reaction with *trans*-1,4-diaminocyclohexane gave **13a–f**, respectively.

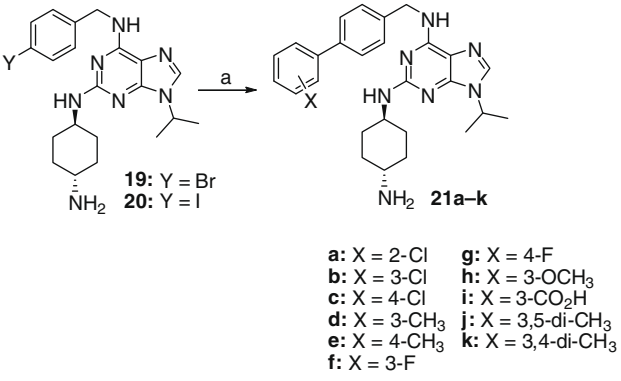
C-6 Aminomethylene modified analogues were prepared as shown in Scheme 5. Reaction of **4** with 2-(4-bromophenyl)ethanamine and 1-(4-bromophenyl)ethanamine afforded **14a** and **14b**, respectively. N-Alkylation with isopropyl iodide and displacement of the C-2 chloro with *trans*-1,4-diaminocyclohexane gave **16a,b** which were converted to the respective biphenyl derivatives **17a,b** by Suzuki coupling with phenyl boronic acid. Treatment with HCl gave the salts **18a,b**.

Analogues investigating substitution on the distal phenyl ring of **3k** were prepared as shown in Scheme 6, introducing the various substitutions by Suzuki reactions of **19** or **20** with substituted phenyl boronic acids.

The data in Table 1 summarizes the in vitro Cdk2/cyclinA and Cdk2/cyclinE inhibitory concentrations (IC_{50}) and the growth inhibition (GI_{50}) of HeLa cells for the compounds prepared. The series of compounds, **3a–q**, in which a variety of acyclic and cyclic amines were introduced at the C-2 position while maintaining the C-6 biphenylmethylamino and N-9 isopropyl groups, showed that



Scheme 5. Reagents and conditions: (a) the appropriate amine (1.1 equiv), $EtN(i\text{-}Pr)_2$ (2 equiv), water, EtOH, reflux; (b) *i*-PrI (4.0 equiv), K_2CO_3 (8.0 equiv), DMSO, rt, overnight; (c) *trans*-1,4-diaminocyclohexane, EtOH, 140–160 °C, 48–60 h; (d) Boc_2O, $NaHCO_3$, THF, water; (e) $PhB(OH)_2$ (3.6 equiv), $Pd(PPh_3)_4$ (0.27 equiv), Na_2CO_3, DME, water; (f) HCl, MeOH or 1:1 TFA/water.



Scheme 6. Reagents and conditions: (a) the appropriate substituted phenyl boronic acid (3.6 equiv), $Pd_2(dba)_3$ (0.04 equiv), PPh_3 (0.6 equiv), Na_2CO_3, DME, water, reflux, 12–20 h.

compound **3k**, in which the *trans*-4-aminocyclohexylamine group was introduced at C-2 was the best compound in this series with respect to both the Cdk inhibition and HeLa cell growth inhibitory activity. Antiproliferative activity of **3k** was fivefold greater than **3a**.

Derivatization of the distal amino group of **3k** afforded compounds **10a–h** and **11a–e**. Mono-alkyl derivatives **10a–d** demonstrated good antiproliferative activities with bis-alkylation **10e–h** resulting in a decrease in activity. The introduction of an acetyl group, compound **11a**, maintained potent HeLa cell inhibitory activity, which decreased with increasing size of the acyl group **11b** and **11c**. The methylsulfonyl analogue **11d** showed moderate activity, but the trifluoromethylsulfonyl compound **11e** was much less potent against the Cdks and HeLa cells.

Of the series of compounds **13a–f**, in which the N-9 substituent was varied, the *N*-ethyl derivative, **13b**, demonstrated antiproliferative activity similar to the *N*-isopropyl compound **3k**. Compounds

Table 1
In vitro inhibition of Cdk2 and effect of cell proliferation for compounds **3a–q**, **10a–h**, **11a–e**, **13a–f**, **18a**, **18b** and **21a–k**

Compds	Cdk2/cyclinA[32] IC_{50}, μM	Cdk2/cyclinE[32] IC_{50}, μM	HeLa[33] GI_{50}, μM	Compds	Cdk2/cyclinA IC_{50}, μM	Cdk2/cyclinE IC_{50}, μM	HeLa GI_{50} μM
3a	2	0.6	0.40	**11a**	0.5	0.3	0.097
3b	0.6	0.5	0.20	**11b**	0.5	0.2	0.30
3c	3	0.4	0.43	**11c**	0.6	0.3	0.30
3d	3	3	2.50	**11d**	0.5	0.2	0.20
3e	1	0.8	0.20	**11e**	>5	>5	3.0
3f	2	0.9	0.35	**13a**	1	0.8	0.12
3h	4	2.5	2.5	**13b**	NT	NT	0.048
3i	>5	>5	4.0	**13c**	NT	NT	0.47
3j	4	1	0.47	**13d**	NT	NT	0.43
3k	0.4	0.1	0.077	**13e**	0.1	0.09	0.40
3l	0.9	0.4	0.20	**13f**	NT	NT	1.0
3m	0.4	0.4	0.35	**18a**	1	0.8	5.0
3n	1	0.9	1.5	**18b**	5	4	3.5
3o	3	3	0.5	**21a**	1	0.6	0.40
3p	2	0.9	1.07	**21b**	0.4	0.4	0.084
3q	2	0.6	0.43	**21c**	0.8	1	10.5
10a	3	1	1.1	**21d**	0.3	0.2	0.054
10b	0.5	0.4	0.097	**21e**	0.4	0.2	2.0
10c	1	0.3	0.40	**21f**	1	0.6	0.15
10d	0.9	0.3	0.30	**21g**	0.5	0.4	1.0
10e	6	3	3.50	**21h**	0.35	0.15	0.026
10f	NT[a]	NT	1.26	**21i**	6	1	10
10g	NT	NT	3.0	**21j**	0.4	0.2	0.19
10h	NT	NT	3.0	**21k**	0.5	0.25	2.5

[a] NT = not tested.

18a–b wherein the C-6 aminomethylene linkage of the purine to the diphenyl group is modified, resulted in a significant loss of HeLa cell growth inhibitory activity.

Compounds **21a–k** investigating substitution on the distal phenyl ring demonstrated the best antiproliferative activities occurred with the 3-substituted derivatives. Within the series of 2-Cl, 3-Cl, and 4-Cl analogues, compounds **21a–c**, respectively, the 3-Cl was fivefold more potent than the 2-Cl and >100-fold more potent than the 4-Cl analogue. The 3-CH_3 **21d** and 3-OCH_3 **21h** analogues demonstrated excellent antiproliferative activities.

In conclusion, the introduction of a C-6 biphenylmethylamino group into the roscovitine scaffold initially afforded compound **3a** which showed a 50-fold improvement in the cell growth inhibition of HeLa cells relative to **2**. Systematic investigation of the C-6 biphenylmethylamino purine scaffold demonstrated the *trans*-4-aminocyclohexylamine group to be a potent C-2 substituent and substitution of the distal phenyl ring, especially *meta* substitution, further enhanced the antiproliferative activity. Compound **21h** demonstrated a >750-fold improvement in the growth inhibition of HeLa cells compared to roscovitine (**2**). A lack of correlation between Cdk inhibition and antiproliferative activity was noted, which may be due to additional mechanisms of action. Select compounds were screened against a panel of kinases at PanLabs[30] and a panel of kinases by the NCI,[31] with no significant inhibition of any of the kinases observed. A detailed reporting of this work and other biochemical mechanism of action studies that were conducted on select compounds will be the subject of future publications. Additionally, cytotoxicity profiling was conducted for select compounds in the NCI panel of 60-transformed cell lines as well as in vivo testing. These studies will also be reported in due course. Additional studies investigating the introduction of heterobiarylmethylamino substituents at C-6 were also conducted and are reported in detail in the accompanying paper.

Acknowledgments

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30. The Panlabs kinase panel consisted of PKC alpha, Ca/Calmodulin dependent PKII, EGF Receptor, ERK1, lck and PKA.
31. The NCI kinase panel consisted of c-RAF, MEK-1, MAPK2, MKK6, SAPK2b, SAPK4, MAPKAP-K2, MKK4, JNKα1, JNKα2, SGK, GSK3β, ROCKII, CK2, LCK, p70S6 K, CHK1, PKBα, cSRC, ZAP-70, JNK3, CK1, PKC-δ, MAPK1, CHK-2, PRK2 and AMPK. We thank the NCI for the results of these experiments.
32. The following are the conditions used for the Cdk2/cyclinA and Cdk2/cyclinE assays. Recombinant Cdk2/CyclinA (15 ng) and Cdk2/CyclinE (5 ng) (Upstate Biotechnology) assays were carried out in 50 mM Tris–HCl pH 7.4, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml histone H1, 0.016 mM ATP, 1 μCi [γ ^{32}P]ATP. A concentration range of each inhibitor dissolved in DMSO was added to the Cdk/Cyclin complexes in assay buffer in the absence of ATP. DMSO was kept constant at 0.04% in all reactions. The reaction was initiated by the addition of ATP and incubated at 30 °C for 30 min. The reactions were terminated by the addition of 2× SDS sample buffer and resolved by SDS–PAGE. H1 phosphorylation was quantified by phosphoimaging. IC_{50} values were calculated using GraphPad Prism data analysis software.
33. Growth inhibition (GI_{50}) values were measured with HeLa S-3 cells selected for growth on plastic. The procedure was based on the sulforhodamine B staining protocol of Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.