

Contents lists available at ScienceDirect

# **Bioorganic & Medicinal Chemistry Letters**

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



# Heterobiaryl purine derivatives as potent antiproliferative agents: Inhibitors of cyclin dependent kinases. Part II

Michael P. Trova<sup>a</sup>, Keith D. Barnes<sup>a,\*</sup>, Luis Alicea<sup>a</sup>, Travis Benanti<sup>a</sup>, Mark Bielaska<sup>a</sup>, Joseph Bilotta<sup>a</sup>, Brian Bliss<sup>a</sup>, Thuy Nguyen Duong<sup>a</sup>, Simon Haydar<sup>a</sup>, R. Jason Herr<sup>a</sup>, Yu Hui<sup>a</sup>, Matthew Johnson<sup>a</sup>, John M. Lehman<sup>b</sup>, Denise Peace<sup>b</sup>, Matthew Rainka<sup>a</sup>, Patricia Snider<sup>a</sup>, Susan Salamone<sup>b</sup>, Steven Tregay<sup>a</sup>, Xiaozhang Zheng<sup>a</sup>, Thomas D. Friedrich<sup>b</sup>

<sup>a</sup> AMRI, Medicinal Chemistry Department, 26 Corporate Circle, PO Box 15098, Albany, NY 12212-5098, USA <sup>b</sup> Albany Medical College, Center for Immunology and Microbial Disease, 47 New Scotland Avenue, Albany, NY 12208-3479, USA

#### ARTICLE INFO

Article history: Received 21 August 2009 Revised 1 October 2009 Accepted 5 October 2009 Available online 12 October 2009

*Keywords:* Purines Cdk inhibitor Antiproliferative agent

#### ABSTRACT

C-6 Biarylmethylamino purine derivatives of roscovitine (1) inhibit cyclin dependent kinases and demonstrate potent antiproliferative activity. Replacement of the aryl rings of the C-6 biarylmethylamino group with heterobiaryl rings has provided compounds with significantly improved activity. In particular, derivatives **18g** and **9c** demonstrated 1000-fold and 1250-fold improvements, respectively, in the growth inhibition of HeLa cells compared to roscovitine (1).

© 2009 Elsevier Ltd. All rights reserved.

In the search for potential drug targets to combat cancer, attention has focused on the cyclin dependent kinases (Cdks) which drive and control the cell cycle progression.<sup>1–5</sup> Uncontrolled cell proliferation, the trademark of tumor cells is a result of cell cycle dysfunction. Since Cdk-related events are among the most common genetic changes found in tumors,<sup>6,7</sup> the targeting of the Cdks may be a means of preventing cell proliferation.

Numerous scaffolds have been identified as Cdk inhibitors<sup>8–17</sup> with the purine<sup>18–22</sup> ring system providing a number of potent inhibitors. Many of these, such as olomoucine<sup>23</sup> and roscovitine  $(\mathbf{1})^{24}$  have also maintained selectivity toward other kinases.

We communicated previously<sup>25–28</sup> and reported in detail in the accompanying paper<sup>29</sup> the discovery of the *trans*-4-aminocyclo-hexylamino group as a potent C-2 substituent in the C-6 biarylmethylamino series, affording compounds such as **2** with significantly improved potencies against Cdks and high antiproliferative activities, compared to roscovitine (**1**) (Fig. 1).

The improved in vitro activities realized by the introduction of a biarylmethylamino group at C-6 suggested the potential for further activity improvement by variation of this position. Using compound **2** as a starting scaffold, the replacement of the C-6 biaryl moieties with heterobiaryl moieties was investigated<sup>21,22</sup> and further details are presented in this Letter. Preliminary molecular

\* Corresponding author. *E-mail address:* keith.barnes@amriglobal.com (K.D. Barnes).

0960-894X/\$ - see front matter @ 2009 Elsevier Ltd. All rights reserved. doi:10.1016/j.bmcl.2009.10.011

modeling studies (unpublished results) have indicated that the aryl rings of the C-6 substituent extend into a solvent exposed region of the Cdk2 enzyme; hence we felt that this would be a suitable site for the introduction of heteroatoms in order to enhance solubility and bioavailability. The replacement of the proximal and distal aryl rings with heteroaryl rings was investigated, as well as the simultaneous replacement of both rings with heteroaryl rings.

Starting from 2,6-dichloropurine (**3**), all analogues were prepared by two general routes. The heterobiarylmethylamine groups at C-6 were in some instances introduced in the first step of the synthesis by displacement of the C-6 chloro with the appropriate



 $\begin{array}{ll} {\rm IC}_{50} = 4.6 \ \mu {\rm M} \ ({\rm Cdk2/cyclin} \ {\rm A}) & {\rm IC}_{50} = 0.40 \ \mu {\rm M} \ ({\rm Cdk2/cyclin} \ {\rm A}) \\ {\rm IC}_{50} = 0.52 \ \mu {\rm M} \ ({\rm Cdk2/cyclin} \ {\rm E}) & {\rm IC}_{50} = 0.10 \ \mu {\rm M} \ ({\rm Cdk2/cyclin} \ {\rm E}) \\ {\rm GI}_{50} = 20 \ \mu {\rm M} \ ({\rm HeLa} \ {\rm cells}) & {\rm GI}_{50} = 0.077 \ \mu {\rm M} \ ({\rm HeLa} \ {\rm cells}) \end{array}$ 

Figure 1.

heterobiarylmethylamine, followed by alkylation of N-9 with iodopropane, nucleophilic displacement of the C-2 chloro with *trans*-1,4-diaminocyclohexane, and then subsequent treatment with HCl gave the corresponding salts (Schemes 1, 3 and 6). Alternatively, analogues were prepared by construction of the C-6 heterobiarylmethylamine groups in a stepwise fashion (Schemes 2, 4 and 5). The C-6 chloro of **3** was initially displaced with 4-bromo- or 4iodobenzylamine or with a bromo substituted heteroarylmethylamine, followed by introduction of the N-9 isopropyl and the C-2 *trans*-4-aminocyclohexylamine groups. Subsequent Suzuki couplings of aryl/heteroaryl boronic acids with the halogen atoms on the purine intermediates gave the targeted derivatives.

Analogues wherein the proximal ring is phenyl were prepared as shown in Schemes 1 and 2. In Scheme 1 the C-6 chloro of **3** was displaced with 4-heteroarylbenzylamines then further elaborated to **4a–d**. Treatment of **4c** and **4d** with HCl gave the corresponding salts **5c** and **5d**. Following the alternate route (Scheme 2), the C-6 chloro of **3** was displaced with either 4-iodobenzylamine or 4-bromobenzylamine. Late stage Suzuki coupling with thienylboronic acids gave **4e–i** and coupling with 2-furanylboronic acid gave **4j**. Compounds **4f** and **4g** were converted to their respective HCl salts **5f** and **5g**.

Schemes 3 and 4 show the preparations of the analogues in which the proximal C-6 ring is a heteroaryl ring, while the distal C-6 ring is phenyl. Analogues with the proximal ring as pyridine or thiazole were prepared as illustrated in Scheme 3 by initial displacement of the C-6 chloro of **3** with the appropriate phenylpyridylmethylamine or phenylthiazoylmethylamine. Analogues wherein the proximal ring is thienyl or furanyl were prepared as shown in Scheme 4. The 5-phenylthiophen-3-yl, **13**, and 5-phenylfuran-3-yl, **14**, analogues were prepared by displacement of the C-6 chloro of **3** with (5-bromothiophen-3-yl)methylamine and (5-bromofuran-3-yl)methylamine, respectively, then subsequent reaction with phenylboronic acid. A series of 5-arylthiophen-2-yl derivatives, **15a-e**, were prepared by displacement of the C-6 chloro of **3** with (5-bromothiophen-2-yl)methylamine, then subsequent reaction with a series of substituted phenyl boronic acids.

The preparations of analogues wherein both the proximal and distal C-6 rings are heteroaryl were prepared as illustrated in Schemes 5 and 6. As shown in Scheme 5, a series of C-6 5-heteroarylthiophen-2-yl analogues, **16a–d**, was prepared by reaction of **12** with a series of heteroaryl boronic acids. Alternatively as shown



**Scheme 1.** Reagents and conditions: (a) the appropriate amine (2.15 equiv), 4:1 EtOH/water, reflux, 24 h; (b) *i*-Prl (3.0 equiv),  $K_2CO_3$  (5.0 equiv), DMSO, rt, 24 h; (c) *trans*-1,4-diaminocyclohexane (15–30 equiv), EtOH, 170–190 °C (sealed tube) 18–24 h; (d) HCl, MeOH, rt.



**Scheme 2.** Reagents and conditions: (a) 4-bromobenzylamine or 4-iodobenzylamine (2.0 equiv),  $EtN(i-Pr)_2$  (2.0 equiv), water, reflux, 5–24 h; (b) *i*-Prl (3.0 equiv),  $K_2CO_3$  (5.0 equiv), DMSO, rt, overnight; (c) *trans*-1,4-diaminocyclohexane (10–15 equiv), EtOH, 120–190° (sealed tube), 24–60 h; (d) The appropriate heteroaryl boronic acid (3.0 equiv),  $Pd_2(dba)_3$  (0.03 equiv),  $PPh_3$  (0.5 equiv)  $Na_2CO_3$ , water, DME, reflux, 24–48 h; (e) HCl, MeOH, rt.



**Scheme 3.** Reagents and conditions: (a) the appropriate amine  $RCH_2NH_2$  (1.1–2.15 equiv),  $EtN(i-Pr)_2$ , water, EtOH, reflux, 24 h; (b) *i*-PrI (3.0 equiv),  $K_2CO_3$  (5.0 equiv), DMSO, rt, overnight; (c) *trans*-1,4-diaminocyclohexane (5–30 equiv), EtOH, 120–150 °C (sealed tube), 24–96 h; (d) HCl, MeOH, rt.

in Scheme 6, a series 5-heteroarylthiophen-2-yl analogues **17a–c**, as well as a series of 6-heteroarylpyridyl-3-yl analogues, **17d–g**, was prepared by the initial displacement of the C-6-chloro of **3** with the appropriate heterobiaryl amines.



Scheme 4. Reagents and conditions: (a) the appropriate amine RCH<sub>2</sub>NH<sub>2</sub> (1.1–2.15 equiv), EtN(*i*-Pr)<sub>2</sub>, water, EtOH, reflux, 24 h; (b) *i*-Prl (3.0 equiv), K<sub>2</sub>CO<sub>3</sub> (5.0 equiv), DMSO, rt, overnight; (c) *trans*-1,4-diaminocyclohexane (5–30 equiv), EtOH, 120–170 °C (sealed tube), 24–48 h; (d) phenyl boronic acid or the appropriately substituted phenyl boronic acid (3.6 equiv), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.3 equiv), Na<sub>2</sub>CO<sub>3</sub>, DME, water; (e) HCl, MeOH, rt.

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.

Maintaining the proximal phenyl ring of the C-6 biphenylmethylamino moiety, a series of compounds (**4a**, **4b**, **4f**, **4h–j**, and **5c–e**, **5g**) was prepared wherein a variety of heterocycles were introduced in place of the distal phenyl ring of **2**. A number of these had very potent HeLa cell growth inhibitory activity with the 2pyridyl **4a**, 2-thienyl **4f**, 3-thienyl **4i**, 2-furanyl **4j**, and 1-pyrrolyl **5d** derivatives demonstrating improved antiproliferative activities compared to **2**. Several substituted 2-thienyl analogues (**4h**, **5e**, and **5g**) were prepared and had weaker HeLa cell growth inhibitory activities compared to the parent 2-thienyl compound **4f**. Although the 1-pyrrolyl derivative **5d**, demonstrated particularly strong antiproliferative activity, replacement of the 1-pyrrolyl moiety with a 1-pyrazolyl moiety as in **5c**, resulted in an 8.5-fold decrease in HeLa cell growth inhibitory activity relative to **5d**, while simultaneously maintaining Cdk2/cyclin inhibitory activity.

Compounds **8a**, **8b**, **9d**, **9e**, **13**, **14**, and **15a–e** examined the effects on activity when the distal phenyl ring of **2** is retained and the proximal phenyl ring is replaced with a heteroaryl ring. Only the



**Scheme 5.** Reagents and conditions: (a) the appropriate heteroaryl boronic acid (3.6 equiv),  $Pd_2(dba)_3$  (0.03 equiv),  $PPh_3$  (0.5 equiv)  $Na_2CO_3$ , water, DME, reflux, 24–48 h, (b) HCl, MeOH, rt.

(6-phenylpyridin-3-yl)methylamine derivative **8a** demonstrated antiproliferative activity comparable to **2**, and improved Cdk2/cyclin inhibition. As we reported in the accompanying Letter,<sup>19</sup> introduction of a 3-substituent onto the distal phenyl ring of the C-6 biphenylmethylamino group afforded compounds with improved antiproliferative activities compared to the unsubstituted analogue. Similarly we found that introduction of a 3-F substituent onto the distal phenyl ring of **8a** to afford **9c**, resulted in a 4.4-fold improvement in antiproliferative activity over **8a**, but no improvement in Cdk2/cyclin inhibition.



**Scheme 6.** Reagents and conditions: (a) the appropriate amine  $RCH_2NH_2$  (1.1–2.2 equiv),  $EtN(i-Pr)_2$ , water, EtOH, reflux, 16–24 h; (b) *i*-PrI (3.0 equiv),  $K_2CO_3$  (5.0 equiv), DMSO, rt, overnight; (c) *trans*-1,4-diaminocyclohexane (10–36 equiv), EtOH, 120–150 °C (sealed tube), 20–48 h; (d) HCl, MeOH, rt.

Table 1

In vitro Inhibition of Cdks and effect on cell proliferation for compounds **4a**, **4b**, **4f**, **4h–j**, **5c–e**, **5g**, **8a**, **8b**, **9c–e**, **13**, **14**, **15a–e**, **16a–d**, **17e**, **17f**, and **18a–d**, **18g** 

<b>2</b> 0.40 0.10 0.077	
<b>4a</b> 0.2 0.1 0.044	
<b>4b</b> 0.3 0.07 0.14	
<b>4f</b> 0.1 0.09 0.048	
<b>4h</b> 0.4 0.3 0.38	
<b>4i</b> 0.4 0.2 0.04	
<b>4j</b> 0.15 0.09 0.03	
<b>5c</b> 0.14 0.95 0.17	
5d 0.2 0.2 0.02	
5e 0.5 0.2 0.27	
5g 0.6 0.2 0.4	
<b>8a</b> 0.1 0.04 0.071	
<b>8b</b> 0.5 0.4 0.5	
<b>9c</b> 0.08 0.04 0.016	
<b>9d</b> <0.05 <0.05 0.2	
<b>9e</b> 0.7 0.4 4	
<b>13</b> 0.25 0.085 0.37	
<b>14</b> 0.3 0.15 0.33	
<b>15a</b> 0.4 0.08 0.3	
<b>15b</b> 0.25 0.09 0.19	
<b>15c</b> 0.5 0.3 0.39	
<b>15d</b> 0.7 0.2 0.2	
<b>15e</b> 0.3 0.1 0.16	
<b>16a</b> 0.3 0.2 0.15	
<b>16b</b> 0.2 0.1 0.3	
<b>16c</b> 0.6 0.2 2	
<b>16d</b> 0.2 0.1 0.2	
<b>17e</b> 0.1 <0.03 0.027	
<b>17f</b> 0.09 0.03 0.31	
<b>18a</b> 0.14 0.055 0.3	
<b>18b</b> 0.1 <0.04 0.04	
<b>18C</b> 1.5 0.044 0.27	
<b>180</b> 0.9 <0.04 0.098	
18g 0.07 0.05 0.02	

A series of derivatives was prepared wherein heteroaryl rings were incorporated for both the proximal and distal phenyl rings of **2**. For compounds **16a–d** and **18a–c** the proximal ring was a 2-thienyl ring substituted at the 5-position with a variety of heterocycles. Only **18b** demonstrated improved antiproliferative activity and Cdk2/cyclin inhibition relative to **2**. Compounds **8a** and **8c** having the 3-pyridyl proximal ring had very potent antiproliferative activities; therefore, compounds **17e**, **17f**, **18d** and **18g** with a proximal 3-pyridyl ring substituted with heterocycles at the 6-position were prepared. The compounds with the 3-thienyl **17e** and 1-pyrazole **18g** rings appended to the 6-position demonstrated excellent antiproliferative activities. Compounds **17e**, **17f**, **18a–d**, and **18g** all demonstrated outstanding Cdk2/cyclinE inhibition properties as well.

In summary, a series of purines was prepared using 2 as the starting scaffold in which the biphenylmethylamino group at C-6 was replaced with heterobiarylmethylamino groups. Although the antiproliferative activity did not correlate well with the Cdk inhibitory activity, many of the compounds had excellent antiproliferative activity. This lack of correlation between Cdk inhibition and antiproliferative activity may be due to additional mechanisms of action. Select compounds were screened against a panel of kinases at PanLabs<sup>32</sup> and a panel of kinases by the NCI,<sup>33</sup> 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. Typically 10 to 100-fold improvements in the Cdk inhibitory activities compared to roscovitine (1) were noted for many of the compounds, and some had significantly improved antiproliferative activities. In particular, derivatives **18g** and **9c** demonstrated 1000-fold and 1250-fold improvements respectively, in the growth inhibition of HeLa cells compared to roscovitine (**1**).

### Acknowledgments

The authors thank the NCI and PanLabs for conducting the kinase panel screens.

## **References and notes**

- 1. Norbury, C.; Nurse, P. A. Annu. Rev. Biochem. 1992, 61, 441.
- 2. Sanchez, I.; Dynlacht, B. D. Semin. Cell Dev. Biol. 2005, 16, 311.
- 3. Hochegger, H.; Takeda, S.; Hunt, H. Nat. Rev. Mol. Cell. Bio. 2008, 9, 910.
- 4. Morgan, D. O. Nature 1995, 374, 131.
- 5. Pines, J. Nat. Cell Biol. **1999**, 1, E73.
- 6. Hall, M.; Peters, G. Adv. Cancer Res. 1996, 68, 67.
- 7. Malumbres, M.; Barbacid, M. Nat. Rev. Cancer 2009, 9, 153.
- 8. Akinaga, S.; Sugiyama, K.; Akiyama, T. *Anti-Cancer Drug Des.* **2000**, *15*, 43. 9. Carlson, B. A.; Dubay, M. M.; Sausville, E. A.; Brizuella, L.; Worland, P. J. *Cancer*
- Res. **1996**, *56*, 2973. 10. Losiewicz, M. D.; Carlson, B. A.; Kaur, G.; Suasville, E. A.; Worland, P. J. Biochem.
- Distewicz, M. D., Carison, B. A., Kau, G., Sussvine, E. K., Worland, F. J. Biothem. Biophys. Res. Commun. **1994**, 201, 589.
  Bramson, H. N.; Corona, J.; Davis, S. T.; Dickerson, S. H.; Edelstein, M.; Frye, S.
- Bramson, H. N.; Corona, J.; Davis, S. I.; Dickerson, S. H.; Edelstein, M.; Frye, S. V.; Gampe, R. T.; Harris, P. A.; Hassell, A.; Holmes, W. D.; Hunter, R. N.; Lackey, K. E.; Lovejoy, B.; Luzzio, M. J.; Montana, V.; Rocque, W. J.; Rusnak, D.; Shewchuk, L.; Veal, J. M.; Walker, D. H.; Kuyper, L. E. J. Med. Chem. 2001, 44, 4339.
- 12. Dermatakis, A.; Kuk, K.-C.; DePinto, W. Bioorg. Med. Chem. 2003, 11, 1873.
- Andreani, A.; Cavalli, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Garnier, M.; Meijer, L. *Anti-Cancer Drug Des.* **2000**, *15*, 447.
  Mettey, Y.; Gompel, M.; Thomas, V.; Garnier, M.; Leost, M.; Ceballos-Picot, I.;
- Mettey, Y.; Gompel, M.; Thomas, V.; Garnier, M.; Leost, M.; Ceballos-Picot, I. Noble, M.; Endicott, J.; Vierfond, J.-M.; Meijer, L. J. Med. Chem. 2003, 46, 222.
- Barvian, M.; Boschelli, D. H.; Cossrow, J.; Dobrusin, E.; Fattaey, A.; Fritsch, A.; Fry, D.; Harvey, P.; Keller, P.; Garrett, M.; La, F.; Leopold, W.; McNamara, D.; Quin, M.; Trumpp-Kallmeyer, S.; Toogood, P.; Wu, Z. P.; Zhang, E. J. Med. Chem. 2000, 43, 4606.
- Sielecki, T. M.; Johnson, T. L.; Liu, J.; Muckelbauer, J. K.; Grafstrom, R. H.; Cox, S.; Boylan, J.; Burton, C. R.; Chen, H.; Smallwood, A.; Chang, C.-H.; Boisclair, M.; Benfield, P. A.; Trainor, G. L.; Seitz, S. P. *Bioorg. Med. Chem. Lett.* 2001, *11*, 1157.
- Yue, E. W.; Higley, C. A.; DiMeo, S. V.; Carini, D. J.; Nugiel, D. A.; Benware, C.; Benfield, P. A.; Burton, C. R.; Cox, S.; Grafstrom, R. H.; Sharp, D. M.; Sisk, L. M.; Boylan, J. F.; Muckelbauer, J. K.; Smallwood, A. M.; Chen, H.; Chang, C.-H.; Seitz, S. P.; Trainor, G. L. J. Med. Chem. 2002, 45, 5233.
- 18. Meijer, L.; Raymond, E. Acc. Chem. Res. 2003, 36, 417.
- Schow, S. R.; Mackman, R. L.; Blum, C. L.; Brooks, E.; Horsma, A. G.; Joly, A.; Kerwar, S. S.; Lee, G.; Shiffman, D.; Nelson, M. G.; Wang, X.; Wick, M. M.; Zhang, X.; Lum, R. T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2697.
- 20. Gray, N. S.; Kwon, S.; Schultz, P. G. Tetrahedon Lett. 1997, 38, 1161.
- 21. Lum, R. T.; Blum, C. L.; Mackman, R.; Wick, M. M.; Schow, S. R.; Zablocki, J.; Ibrahim, P. W02000044750, 2000.
- 22. 22 Dumont, J. A.; Bitonti, A. J.; Borcherding, D. R.; Peet, N. P.; Munson, H. R.; Shum, P. W. US 6,479,487, 2002.
- Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J. Y.; Detivaud, L.; Leclerc, S.; Meijer, L. *Eur. J. Biochem.* 1994, 224, 771.
- Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P. J.; Blow, J. J.; Inagaki, M.; Delcros, J. G.; Moulinoux, J. P. *Eur. J. Biochem.* **1997**, 243, 527.
- 25. Trova, M. WO2000055161, 2000.
- 26. Trova, M. WO2003022805, 2003.
- 27. Trova, M. WO2003022219, 2003.
- 28. Trova, Michael P.; Friedrich, Thomas D.; Alicea, L. R.; Barford, C. A.; Barnes, K. D.; Benanti, T.; Bergeron, M. E.; Bielaska, M.; Bilotta, J. A.; Burry, L. C.; Davisdon, M. R.; Duong, T. N.; Haydar, S. N.; Hui, Y.; Johnson, M. R.; Johnson, R. E.; Lu, J.; Murphy, C. M.; O'Grady, H. R.; Peace, D.; Rainka, M. P.; Russell, M.; Salamone, S.; Smith, J. L.; Snider, P. A.; Toporowski, J. W.; Tregay, S. W.; Wilson, A. C.; Wyle, M. J.; Yao, X.; Zheng, X. Presented at the 31st Northeast Regional Meeting of the American Chemical Society, Saratoga Springs, NY, June 2003; Paper 58.
- Trova, M. P.; Barnes, K. D.; Barford, C.; Benanti, T.; Bielaska, M.; Burry, L.; Lehman, J. M.; Murphy, C.; O'Grady, H.; Peace, D.; Salamone, S.; Smith, J.; Snider, P.; Toporowski, J.; Tregay, S.; Wilson, A.; Wyle, M.; Zheng, X.; Friedrich, T. D. Bioorg. Med. Chem. Lett. 2009, 19, 6608.
- 30. 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<sub>2</sub>, 1 mM DTT, 0.1 mg/mL histone H1, 0.016 mM ATP, 1 μCi [γ <sup>32</sup>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 phosphorinaging. IC<sub>50</sub> values were calculated using GraphPad Prism data analysis software.

 Growth inhibition (GI<sub>50</sub>) 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.

- 32. The Panlabs kinase panel consisted of PKC alpha, Ca/Calmodulin dependent PKII, EGF Receptor, ERK1, LCK and PKA.
- 33. The NCI kinase panel consisted of c-RAF, MEK-1, MAPK2, MKK6, SAPK2b, SAPK4, MAPKAP-K2, MKK4, JNKα1, JNKα2, SGK, GSK3β, ROCKII, CK2, LCK, p70S6K, 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.