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Synthesis and biological evaluation of 3-ethylidene-1,3-dihydro-indol-2-ones as novel checkpoint 1 inhibitors

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Abstract—Chk1 inhibitors have emerged as a novel class of neoplastic agents for abrogating the G2 DNA damage checkpoint arrest. Analogs of the Chk1 inhibitor, 3-ethylidene-1,3-dihydro-indol-2-one, were synthesized and tested in vitro for their inhibitory activities. The most promising compound identified from this series is analog **28**, which possesses potent enzymatic and cellular activities.

In response to DNA damage, cells arrest at various cell cycle checkpoints (G1, S, or G2) to initiate repair programs. Upon completion of these programs, cells proceed through the cell cycle. If the DNA damage is not repairable, they undergo apoptosis.[1–3] Most tumor cells, through the loss of p53 or Rb function, are deficient in the G1 checkpoint and must arrest at either S or G2 to repair DNA damage.[4–8] If the S and G2 checkpoints are abrogated, the cells will undergo premature mitotic entry/premature chromosome condensation (PCC), leading to apoptosis, mitotic catastrophe, and cell death. In contrast, normal cells will arrest at G1 in response to DNA damage and will be less affected by S and G2 checkpoint abrogation.[9–15]

Checkpoint kinase 1 (Chk1), a serine/threonine kinase, was initially identified as a link between DNA damage and cell cycle arrest at G2.[16,17] In response to DNA damage, Chk1 phosphorylates and inactivates the Cdc25 family of phosphatases (Cdc25A, B, and C). It was discovered that upon phosphorylation by Chk1, Cdc25C is rapidly deactivated through its sequestration to the cytosol.[18,19] The consequences of this are hyperphosphorylation of the Cdc25C substrate Cdc2, and inactivation of cyclin B/Cdc2 complex, which results in cell cycle arrest at G2.

Recently, we and others have demonstrated that Chk1 also plays a key role in the S checkpoint induced by various DNA damaging agents.[20–24] Activation of Chk1 through phosphorylation by ATM or ATR kinase in response to various types of DNA damage induces degradation of Cdc25A. The resulting diminished cellular level of Cdc25A has been shown to be directly responsible for the S-phase arrest. Correspondingly, elimination of Chk1 with siRNA leads to abrogation of both Cdc25A proteolysis and the S- or G2 checkpoints.[20,24] Collectively, these results demonstrate that Chk1 plays an important role in both the S- and G2 checkpoints, largely mediated by Cdc25A.

Cancer cells often lack one or more genes for G1 checkpoint control, either through loss of p53 or Rb function, or by overexpression of proto-oncogenes (cyclins and CDKs).[25] Inhibition of the remaining checkpoints, at S and G2, by Chk1 inhibitors should make tumor cells selectively more sensitive to anticancer therapies, such as γ-radiation or DNA damaging drugs.[12,26–28] In addition, tumor cells deficient in p53 are usually more resistant to cancer therapeutics. Thus, Chk1 inhibitors can potentially enhance the cytotoxicity of DNA damaging agents and overcome this resistance. There is ample

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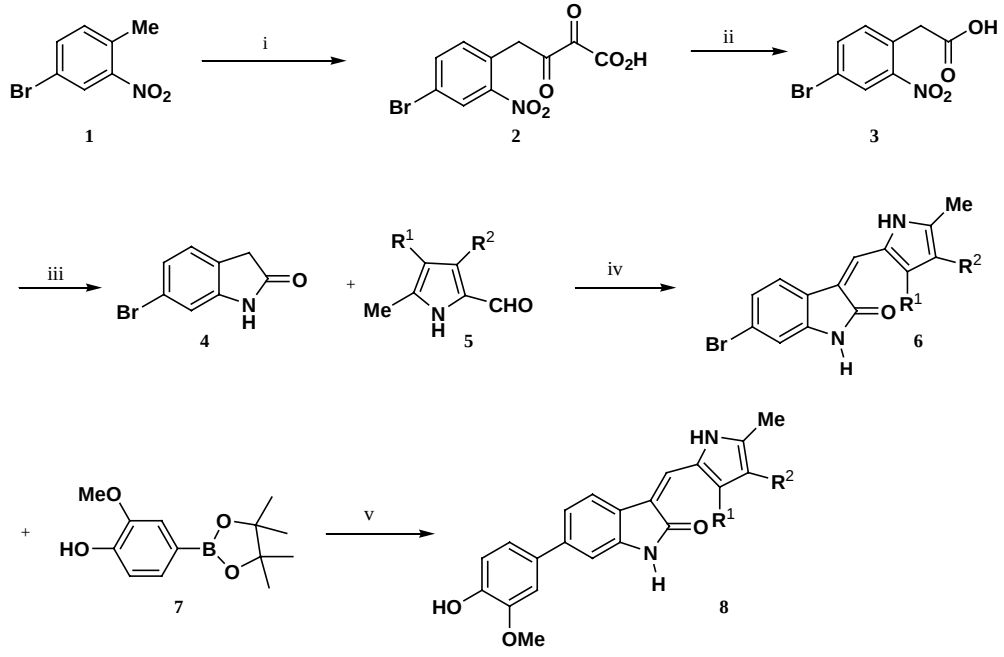
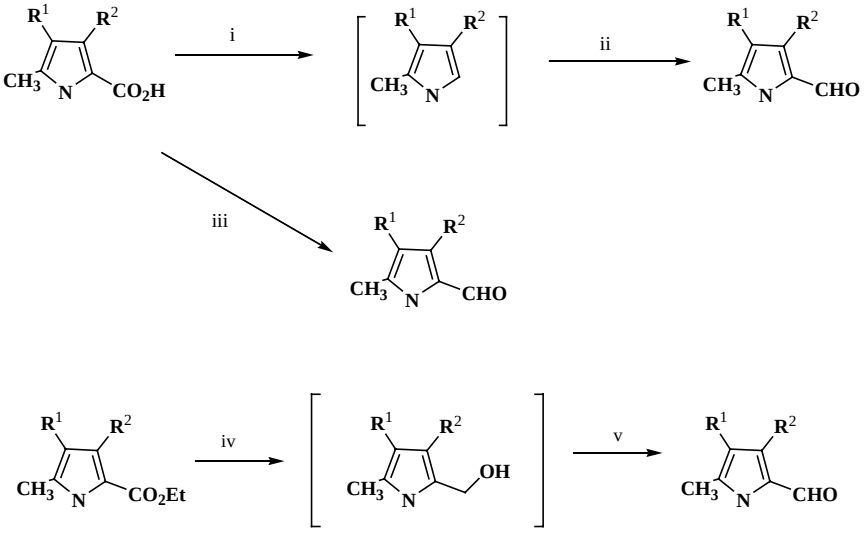
experimental evidence to demonstrate that the abrogation of the S- and G2 checkpoints, through inactivation of Chk1, can selectively sensitize cells deficient in the G1 checkpoint to DNA damage.[12–16,26,29,30]

We and others have disclosed ureas as a new class of small molecule Chk1 inhibitors.[31–34] To continue our search for a structurally different Chk1 inhibitor that possesses potent activity against Chk1 enzyme, we initiated an extensive medicinal chemistry effort based on high throughput screen hits. In this paper, we report the synthesis and preliminary structure–activity relationship of a series of these compounds, 3-ethylidene-1,3-dihydro-indol-2-one.

Indolinone analogs were prepared using the reaction sequences shown in Schemes 1 and 2. The non-commercially available starting keto acid **2** was prepared from the corresponding toluene by reaction with dimethylmalonate in the presence of sodium (Scheme 1) Oxidation of the keto acid with hydrogen peroxide followed by reduction of the nitro group to an amine gave the 6-bromo-indolinone **4**. The indolinone was deprotonated with piperidine, and addition of the resultant anion to the pyrrole aldehyde **5** provided the desired ethylidene indolinone **6**. Coupling of the substituted indolinone bromides **6** with various substituted phenylboronic acids such as **7** gave the indolinone analogs **8** that are described in Tables 1–3.

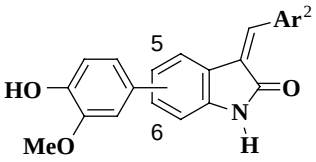


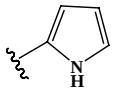
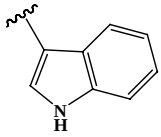
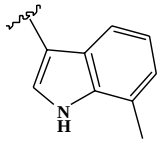
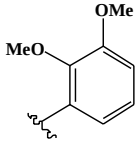
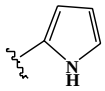
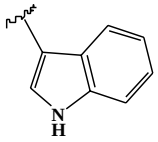
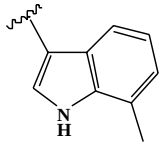
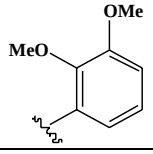
Scheme 1. Reagents: (i) Na, dimethylmalonate; (ii) H_2O_2, NaOH; (iii) Zn, H_2SO_4; (iv) piperidine, MeOH; (v) $Pd(PPh_3)_4$, CsF.



Scheme 2. Reagents: (i) Heat, neat or DMF; (ii) $POCl_3$, DMF; (iii) CF_3CO_2H, $CH(OEt)_3$; (iv) $LiAlH_4$; (v) MnO_2.

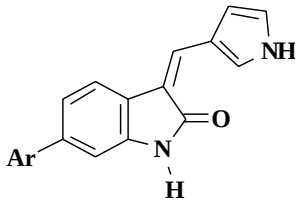
Table 1. Inhibition data for indolinone analogs[a]

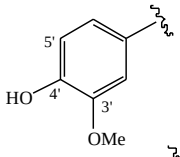
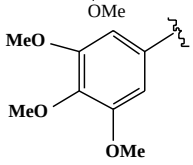
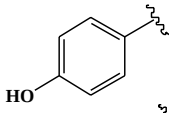
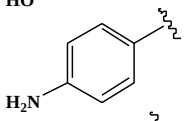
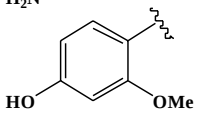
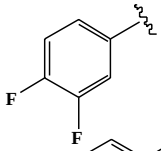
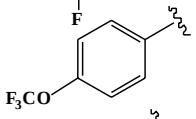
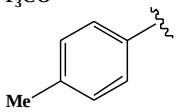


Compound	Cx	Ar^2	IC_{50} (nM)
9	C5		2839
10	C5		>50,000
11	C5		3465
12	C5		>50,000
13	C6		7
14	C6		92
15	C6		1306
16	C6		>50,000

[a] See Ref. 34 for assay method.

Table 2. Inhibition data for pyrrole analogs[a]



Compound	Ar	IC_{50} (nM)
13		7
17		>10,000
18		29
19		1033
20		10
21		>10,000
22		>10,000
23		>10,000

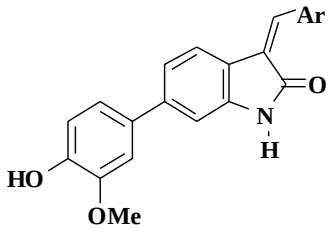
[a] See Ref. 34 for assay method.

Scheme 2 illustrates three routes that were used to prepare the substituted pyrrole aldehydes. Thus, commercially available substituted pyrrole acids were thermally decarboxylated to provide pyrrole intermediates, which could be formylated with phosphorous oxychloride in dimethylformamide to obtain the corresponding aldehydes. Alternatively, the pyrrole aldehydes were formed directly from the corresponding acids by treatment with trifluoroacetic acid and triethyl orthoformate. Finally, aldehydes could also be obtained by reduction of the corresponding ester followed by oxidation of the resultant alcohol with manganese oxide.

In this study, several phenyl and pyrrole analogs of 3-ethylidene-1,3-dihydro-indol-2-one were tested for their inhibitory activities against Chk1 enzyme. The effects of varying the substituents at the C4-position of the pyrrole moiety have been studied in detail. We have also investigated the impact of positional isomers and substituents on phenyl ring on the inhibitory potency.

The effects of moving the 3-methoxy-4-hydroxyphenyl moiety from the C5 position to the C6 position are shown in Table 1. With the exception of dimethoxy analogs (**12** and **16**), it reveals that C5-substituted 3-methoxy-4-hydroxylphenyl analogs are consistently less potent than the corresponding C6-substituted

Table 3. Inhibition data for pyrrole analogs[a]



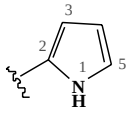
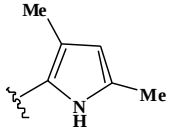
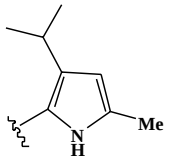
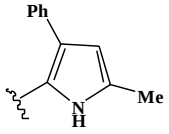
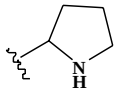
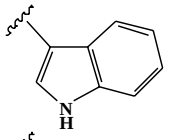
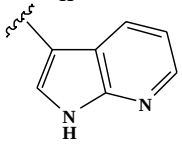
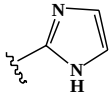
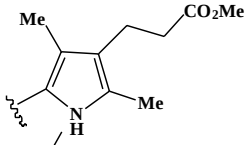
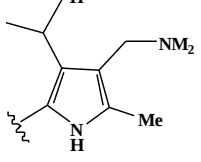
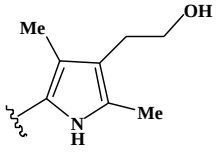
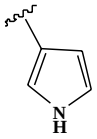
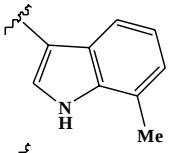
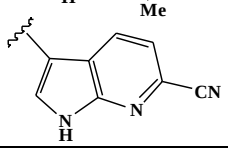
Compound	Ar	IC_{50} (nM)
13		7
24		120
25		3465
26		>10,000
27		3972
14		92
28		4
29		18
30		75
31		1280
32		47

Table 3 (*continued*)

Compound	Ar	IC_{50} (nM)
33		25
15		1306
34		445

[a] See Ref. 34 for assay method.

compounds against the Chk1 enzyme in the indolinone series. For instance, C5-substituted compound **9** shows a 405-fold decrease in potency compared with the corresponding C6-substituted analog **13**. A similar SAR trend is also observed when the pyrrole moiety is replaced with an indole group (**10** vs. **14** and **11** vs. **15**). The data also indicate that compound **13**, with a pyrrole group at C3 ethylidene, is more potent than the corresponding indole analogs **14** and **15**. Replacement of indole with dimethoxyphenyl results in a huge reduction in inhibitory potency. Since compound **13** provides the most potent inhibitor, we then turned our attention to the modification of 3-methoxy-4-hydroxyphenyl ring with various substituents. It was hoped that this change would increase enzymatic potency.

As indicated in Table 2, replacement of the hydroxyl group at the C4′- position of the phenyl ring (**18**) with an electron-donating amino group (**19**) causes a 36-fold decrease in potency. Further reduction is observed when the hydroxyl group is replaced with a methyl group. The introduction of electron-withdrawing groups such as fluoro or trifluoromethoxy groups at the C4 position resulted in complete loss of inhibition (cf. **21** and **22**). Apparently, electronic effects do not play an important role in determining the enzymatic potency. In contrast, shifting the methoxy group from the C3′ position to the C2′ position of phenyl ring has only minimal impact on the enzymatic potency (**13** vs. **20**). However, introduction of methoxy groups to the C3′, C4′, and C5′ positions (**17**) caused a huge reduction in potency. These SAR studies clearly indicate that the 3-methoxy-4-hydroxyphenyl moiety is still the best group for the C6 position of indolinone ring.

Since compound **13** provides the most potent enzymatic activity, we then focused our attention on SAR studies of the pyrrole ring in the indolinone series (Table 3). We have introduced various functionalities to the C3′-, C4′-, and C5′ positions of pyrrole ring. The inhibitory activity at the C3′-position of the pyrrole appears to be governed by steric effects. As Table 3 indicates, the phenyl analog (**26**) that has a large group at the

C3′-position is less potent than either the methyl (**24**) or isopropyl (**25**) analogs. In addition, the dimethyl compound **24** is 17-fold less potent than the parent pyrrole analog **13**.

Table 3 also reveals that replacement of the pyrrole ring with an imidazole moiety is reasonably well tolerated (**13** vs. **29**), but reduction of the pyrrole ring to a pyrrolidine moiety results in a drastic reduction of potency in the enzymatic assay (**13** vs. **27**). It is interesting to note that introduction of polar long chained aliphatic functionalities to the C4′-position provides more potent Chk1 inhibitors compared with the C4′-unsubstituted analog (**30** vs. **24** and **32** vs. **24**). The same SAR trend was observed with 2′-isopropyl-5′-methyl pyrrole analog (**31** vs. **25**).

As described previously, replacement of the pyrrole ring with an indole moiety results in a 13-fold decrease in potency in the inhibition of Chk1 enzyme (**13** vs. **14**). In contrast to this result, replacement of the pyrrole ring with an azaindole moiety has only minimal impact on the enzymatic potency (**13** vs. **28**). Interestingly, the inhibitory activities of indole analogs appear to be governed by steric effects. Hence, as previously observed, the C7′-methyl analog **15** is less potent than compound **14**. A similar SAR trend was also observed with azaindole analogs. Thus, introducing a cyano group to azaindole moiety results in a decrease in potency of greater than 100-fold (**18** vs. **34**). The most potent compound identified in our SAR studies of this series is the azaindole analog **28** with an IC_{50} value of 4 nM.

To understand how these Chk1 inhibitors interact with the enzyme, the potent Chk1 inhibitor **13** was submitted for X-ray crystallographic study.[35] The X-ray co-crystal structure of compound **13** indicates that the 3′-OMe group forms a hydrogen bond with Lys38 of the backbone protein of the Chk1 enzyme, and the 4′-OH functionality forms another H-bond with Glu55. In addition, the hinge region of compound **13** forms two additional hydrogen bonds with both Glu85 and Cys 87 as shown in Figure 1.

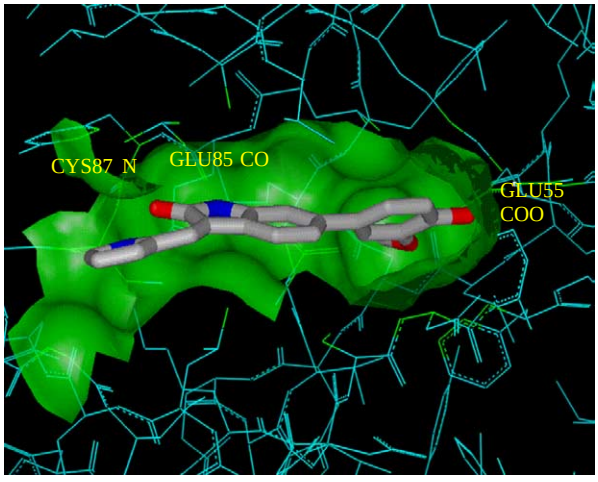


Figure 1. X-ray co-crystal structure of **13** with Chk1 enzyme.

The most potent compounds identified were submitted for enzymatic assays against other serine/threonine kinases. As shown in Table 4, analog **13** is the most selective Chk1 inhibitor identified in this study. It does not have any inhibitory activity against the other enzymes tested. Compound **28** has 91-fold selectivity against CDC2 enzyme, whereas compound **29** shows only 14-fold selectivity. This demonstrates that the group connected to the olefinic moiety in the indolinone series plays an important role in determining the enzymatic selectivity against other kinases.

The cellular activity of the most potent compound **28** was evaluated in the MTS assay. For routine screening, the antiproliferative EC_{50}'s of the compounds are determined in HeLa cells (a p53-deficient human cervical cancer cell line), either alone or in the presence of 150 nM of doxorubicin, a topoisomerase II inhibitor in clinical use known to confer G2/M checkpoint arrest at this concentration in HeLa cells. In the latter assay, the EC_{50}s are calculated from the percentages of inhibition by the test compounds at varying concentrations above the background inhibition by 150 nM doxorubicin. Thus, the ratio of the EC_{50}s of the compound alone and the compound with 150 nM doxorubicin represents the ability of the compound to sensitize HeLa cells. Compound **28** exhibits significant antiproliferative activity as a single agent (EC_{50} = 0.01 μM), probably indicating that this compound may inhibit other targets, consistent with its inferior selectivity. Compound **28** also shows potent antiproliferative activity (EC_{50} = 0.18 μM) in the presence of doxorubicin (150 nM). To confirm the target, additional characterization of the compound using other cellular assays is required.

In summary, we have shown that variation of the substituent pattern at the phenyl or pyrrole rings of a 6-substituted indolinone compound of structure **8** alters the inhibitory properties of these compounds. The assay data indicate that substituents are not well tolerated at the C5-position of the phenyl ring (Table 1). In general, compounds having a hydroxyl group at the C4′-position as shown in Table 2 possess potent inhibitory activities against the Chk1 enzyme. Unsubstituted pyrrole or azaindole analogs possesses the most potent activity against the Chk1 enzyme. Introduction of substituents to either the pyrrole or the azaindole

Table 4. Inhibition data of Chk1 inhibitors against various kinases

Compound	Chk1	Chk2	CDC2	CHK2	CK2	EGFR	MAPKAP2	PKCγ	PKCδ	SGK	SRC	Tie2
13	7	21730	>50000	>50000	>50000	>50000	>50000	>50000	>50000	>50000	>50000	>50000
28	4	22679	454	368	41690	NT	>50000	NT	NT	>50000	2377	NT
29	18	14649	251	NT	NT	>50000	NT	>50000	>50000	1500	>50000	23273

results in a decrease in potency as shown in Table 3. This study has also shown that reduction of the pyrrole moiety decreases the potency. However, only a small effect is observed when the pyrrole is replaced with an imidazole ring. The most potent analog identified from this study is compound **28**, possessing an IC_{50} value of 4 nM.

References and notes

1. Martin, N. M. *J. Photochem. Photobiol. B* **2001**, *63*(1–3), 162.
2. Heinen, C. D.; Schmutte, C.; Fishel, R. *Cancer Biol. Ther.* **2002**, *1*, 477.
3. Sampath, D.; Rao, V. A.; Plunkett, W. *Oncogene* **2003**, *22*, 9063.
4. Ozbun, M. A.; Butel, J. S. *Adv. Cancer Res.* **1995**, *66*, 71.
5. Bartek, J.; Lukas, J. *Curr. Opin. Cell Biol.* **2001**, *13*, 738.
6. Bartek, J.; Lukas, J. *FEBS Lett.* **2001**, *490*, 117.
7. Hahn, W. C.; Weinberg, R. A. *Nat. Rev. Cancer* **2002**, *2*, 331.
8. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
9. Russell, K. J.; Wiens, L. W.; Demers, G. W.; Galloway, D. A.; Plon, S. E.; Groudine, M. *Cancer Res.* **1995**, *55*, 1639.
10. Powell, S. N.; DeFrank, J. S.; Connell, P.; Eogan, M.; Preffer, F.; Dombkowski, D.; Tang, W.; Friend, S. *Cancer Res.* **1995**, *55*, 1643.
11. Bunch, R. T.; Eastman, A. *Cell Growth Differ.* **1997**, *8*, 779.
12. Koniaras, K.; Cuddihy, A. R.; Christopoulos, H.; Hogg, A.; O'Connell, M. J. *Oncogene* **2001**, *20*, 7453.
13. Kohn, E. A.; Ruth, N. D.; Brown, M. K.; Livingstone, M.; Eastman, A. J. *Biol. Chem.* **2002**, *277*, 26553.
14. Kohn, E. A.; Yoo, C. J.; Eastman, A. *Cancer Res.* **2003**, *63*, 31.
15. Eastman, A.; Kohn, E. A.; Brown, M. K.; Rathman, J.; Livingstone, M.; Blank, D. H.; Gribble, G. W. *Mol. Cancer Ther.* **2002**, *1*, 1067.
16. Liu, Q.; Guntuku, S.; Cui, X.-S.; Matsuoka, S.; Cortez, D.; Tamai, K.; Luo, G.; Carattini-Rivera, S.; DeMayo, F.; Bradley, A.; Donehower, L. A.; Elledge, S. J. *Gene Dev.* **2000**, *14*, 1448.
17. Sanchez, Y.; Wong, C.; Thoma, R. S.; Richman, R.; Wu, Z.; Piwnica-Worms, H.; Elledge, S. J. *Science* **1997**, *277*, 1497.
18. Peng, C. Y.; Graves, P. R.; Thoma, R. S.; Wu, Z.; Shaw, A. S.; Piwnica-Worms, H. *Science* **1997**, *277*, 1501.
19. Graves, P. R.; Lovly, C. M.; Uy, G. L.; Piwnica-Worms, H. *Oncogene* **2001**, *20*, 1839.
20. Xiao, Z.; Chen, Z.; Gunasekera, A. H.; Sowin, T. J.; Rosenberg, S. H.; Fesik, S.; Zhang, H. *J. Biol. Chem.* **2003**, *278*, 21767.
21. Mailand, N.; Falck, J.; Lukas, C.; Syljuåsen, R. G.; Welcker, M.; Bartek, J.; Lukas, J. *Science* **2000**, *288*, 1425.
22. Zhou, B. B.; Elledge, S. J. *Nature* **2000**, *408*, 433.
23. Cliby, W. A.; Lewis, K. A.; Lilly, K. K.; Kaufmann, S. H. *J. Biol. Chem.* **2002**, *277*, 1599.
24. Zhao, H.; Watkins, J. L.; Piwnica-Worms, H. *Proc. Nat. Acad. Sci. U.S.A.* **2002**, *99*, 14795.
25. Lukas, J.; Herzinger, T.; Hansen, K.; Moroni, M. C.; Resnitzky, D.; Helin, K.; Reed, S. I.; Bartek, J. *Gene Dev.* **1997**, *11*, 1479.
26. Suganuma, M. et al. *Cancer Res.* **1999**, *59*(23), 5887.
27. Luo, Y. *Neoplasia (New York)* **2001**, *3*, 411.
28. Takai, H.; Tominaga, K.; Motoyama, N.; Minamishima, Y. A.; Nagahama, H.; Tsukiyama, T.; Ikeda, K.; Nakayama, K.; Nakanishi, M.; Nakayama, K. *Gene Dev.* **2000**, *14*, 1439.
29. Jackson, J. R.; Gilmartin, A.; Imburgia, C.; Winkler, J. D.; Marshall, L. A.; Roshak, A. *Cancer Res.* **2000**, *60*, 566.
30. Chen, Z.; Xiao, Z.; Chen, J.; Ng, S.-C.; Sowin, T.; Sham, H.; Rosenberg, S.; Fesik, S.; Zhang, H. *Mol. Cancer Ther.* **2003**, *2*, 543.
31. Li G.; Li Q.; Li T.; Lin N-H.; Mantei R. A.; Sham H. L.; Wang G. T. US Patent Application 2004034038, **2004**.
32. Keegan K.; Kesicki E. A.; Gaudino J. J.; Cook A. W.; Cowen S. D.; Gurgess, L. E. WO 02/070494, **2002**.
33. Boyle R. G.; Imogai H. J.; Cherry M. WO 03/101444, **2003**.
34. The Chk1 enzymatic assay was carried out using a recombinant Chk1 kinase domain. A human Cdc25C peptide was used as substrate in the assay. The reaction mixture contained 25 mM of Hepes at pH 7.4, 10 mM $MgCl_2$, 0.08 mM Triton X-100, 0.5 mM DTT, 5 μM ATP, 4 nM ^{33}P ATP, 5 μM Cdc25C peptide substrate, and 5 nM of the recombinant Chk1 protein. For potent compound with K_i below 1 nM, 0.5 nM of the recombinant Chk1 protein and 8 nM ^{33}P were used in the assays. Compound vehicle DMSO was maintained at 2% in the final reaction. After 30 min at room temperature, the reaction was stopped by addition of an equal volume of 4 M NaCl and 0.1 M EDTA, pH 8. A 40 μL aliquot of the reaction was added to a well in a FlashPlate (NEN Life Science Products, Boston, MA) containing 160 μL of phosphate-buffered saline (PBS) without calcium chloride or magnesium chloride and incubated at room temperature for 10 min. The plate was then washed three times in PBS with 0.05% of Tween 20 and counted in a Packard TopCount counter (Packard BioScience Company, Meriden, CT).
35. The coordinates of the CHK1 complex have been deposited with the RCSB Protein Data Bank. The entry 'Crystal Structure of CHK1 with an Indole Inhibitor' has been assigned the RCSB ID code 'rcsb034461' and PDB ID code '2AYP'.