Bioorganic & Medicinal Chemistry Letters 20 (2010) 7216–7221

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and SAR of thienopyridines as potent CHK1 inhibitors

Lianyun Zhao [a,*], Yingxin Zhang [a], Chaoyang Dai [a], Timothy Guzi [a], Derek Wiswell [b], Wolfgang Seghezzi [b], David Parry [b], Thierry Fischmann [c], M. Arshad Siddiqui [a]

[a] Department of Chemistry, Merck Research Laboratories, 320 Bent Street, Cambridge, MA 02141, USA
[b] Merck Research Laboratories, 901 California Avenue, Palo Alto, CA 94304, USA
[c] Drug Design Department, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

ARTICLE INFO

Article history:
Received 2 September 2010
Revised 20 October 2010
Accepted 21 October 2010
Available online 26 October 2010

Keywords:
CHK1 inhibitors
Thienopyridines
Antitutor agent
Kinase inhibitor

ABSTRACT

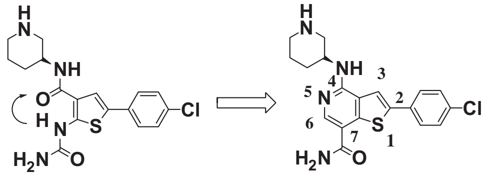
A novel series of CHK1 inhibitors based on thienopyridine template has been designed and synthesized. These inhibitors maintain critical hydrogen bonding with the hinge and conserved water in the ATP binding site. Several compounds show single digit nanomolar CHK1 activities. Compound **70** shows excellent enzymatic activity of 1 nM.

Development of DNA damage agents for cancer chemotherapy is an area of great interest.[1] However, mechanism based toxicity to normal cells has limited their usage.[2] Therefore, development of antitumor agents with lower toxicity has been attracting more attentions.[3]

Following DNA damage, normal cells arrest and attempt to repair at the cell cycle checkpoints G1 and S phases through tumor suppressor p53, and at G2 and S phases through checkpoint 1 kinase (CHK1).[4] However, the p53 gene is one of the most commonly mutated gene in human cancer cells and most of the tumor cells are short of p53 function and must rely on CHK1 to induce arrest for repair and survival.[5] Therefore, the inhibition of CHK1 should selectively sensitize tumor cells to DNA damage while normal cells still arrest at G1 and S phases.[6] Thus, the identification of CHK1 inhibitors has attracted attention of researchers in the past few years,[7–13] and some of the CHK1 inhibitors are being evaluated in clinical trials as an anticancer agent.[14]

Compound **1** (Fig. 1) has been reported as a CHK1 inhibitor.[15] The X-ray crystal structure of compound **1** (Fig. 2) indicated that the carbonyl and NH_2 of the urea group form hydrogen bonds (H-bonds) with the hinge kinase backbone of Glu85 and Cys87. Additionally, the carbonyl group of the amide forms H-bond interactions with a conserved water molecule in the selectivity pocket and the NH of the piperidine is involved in a network of H-bond interactions with Asp148 and Glu134. An intramolecular H-bond between the carbonyl of amide and the NH of urea freezes the inhibitor conformation in a putative six-membered ring. We reasoned that this conformation shown by X-ray crystal structure could be further rigidified into the thienopyridine class of inhibitors **2a** (Fig. 1). It was expected that the constrained core would likely maintain all key interactions with CHK1, including the H-bond between the conserved water molecule and the nitrogen of the thienopyridine ring as well as the hinge H-bonds with Glu85 and Cys87. The compound **2a** was synthesized and the X-ray crystal structure of **2a** bound to CHK1 was determined (Fig. 2)[18] confirming our design strategy. The chemistry and biological results of this series of compounds are presented in the following sections.

Thienopyridine compounds **2** were prepared according to Scheme 1. The Suzuki reaction of **3** with 4-chlorophenyl boronic acid produced **4** in high yield. Compound **4** was reacted with malonic acid in pyridine in the presence of catalytic amount of piperidine using Knoevenagel reaction to provide acrylic acid **5**.[16]



1　　**2a**

Figure 1. Structures of compound **1** and designed compound **2a**.

---

* Corresponding author. Tel.: +1 617 499 3569; fax: +1 617 499 3535.
E-mail address: lianyun.zhao@merck.com (L. Zhao).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.10.105

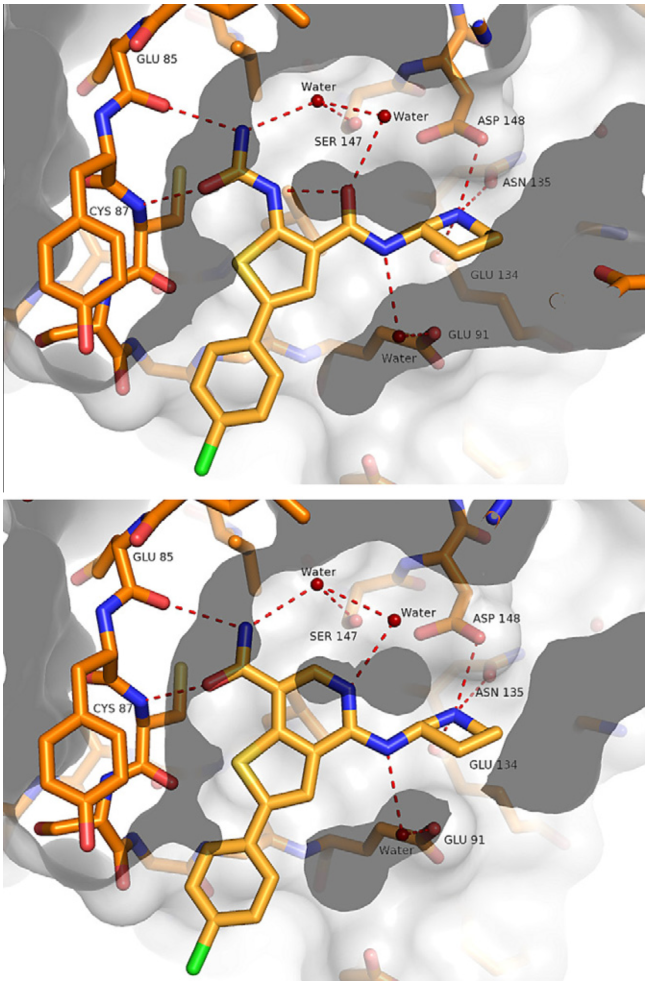


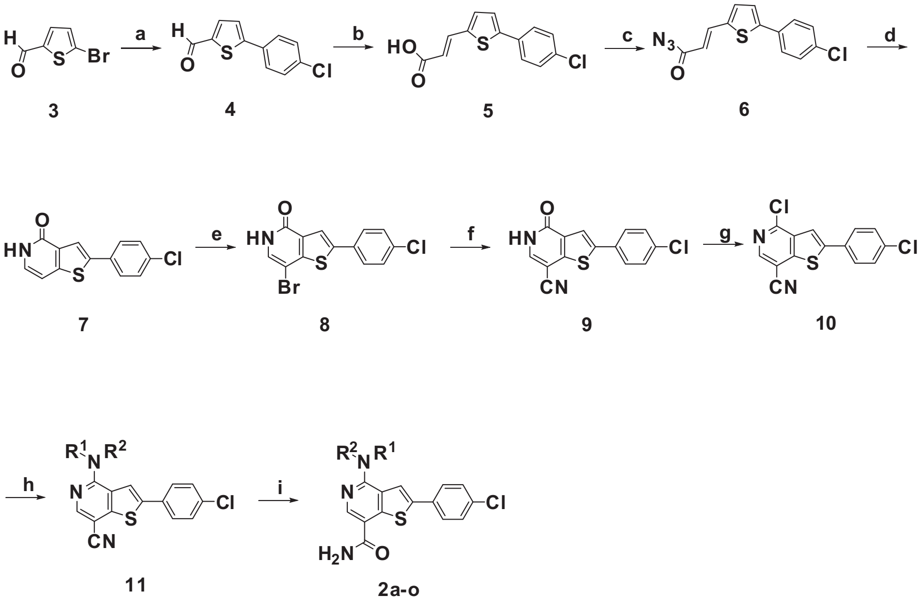
Figure 2. X-ray structures of compounds **1** and **2a** in CHK1 enzyme. Pictures generated with PyMol.[17,18]

Acyl azide formation of **5** yielded precursors **6**. Curtius rearrangement followed by intramolecular cyclization in phenyl ether with the presence of NBu_3 at 230 °C gave the thienopyridone **7** in 86% yield. Regioselective bromination with NBS provided compound **8** in 78% yield. Compound **8** was treated with CuCN followed by $FeCl_3$ to yield thienopyridone nitrile **9**. Chlorination of **9** with $P(O)Cl_3$ followed by displacement with desired amines and hydrolysis of nitrile with concentrated H_2SO_4 gave the desired compounds **2a–l**, listed in Table 1.

In order to explore SAR at 2-position of the thienopyridine ring, analogs listed in Table 2 were prepared following Scheme 2. Selective bromination of compound **12** with NBS followed by the treatment with CuCN gave thienopyridone nitrile **14**. Regioselective bromination of compound **14** with NBS at 60 °C afforded compound **15**. Chlorination of **15** followed by amination with (*S*)-1-Boc-3-aminopiperidine provided the key intermediate **17**. Suzuki coupling of **17** with different boronic acids followed by hydrolysis of nitrile group yielded compounds **19–57** shown in Table 2. Starting from **14**, compound **59** was obtained with H at 2-position. Whereas compound **58** was derived from **15** through $P(O)Cl_3$ mediated chlorination, amination with 1-Boc-3-aminopiperidine and hydrolysis.

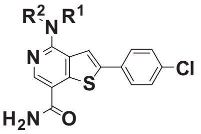
To explore the effect of the sulfur atom positions in thienopyridine ring **60,** an efficient synthetic route was devised. Preparation of compound **60** in Scheme 3 began with 3-(3-thienyl)acrylic acid **61** with the similar method described in Scheme 2. Acyl azide formation followed by Curtius rearrangement and intramolecular cyclization at high temperature gave compound **62**. Bromination of **62** with NBS followed by treatment with CuCN and subsequent bromination gave compound **65**. Chlorination of **65** with $P(O)Cl_3$ followed by amination with (*S*)-1-Boc-3-aminopiperidine and Suzuki coupling with 4-chlorophenyl boronic acid provided intermediate **68**. Compound **60** was obtained through the treatment of **68** with concentrated H_2SO_4.

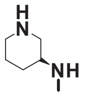
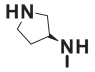
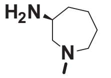
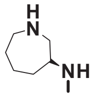
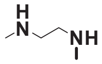
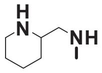
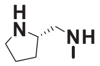
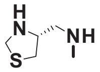
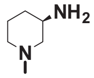
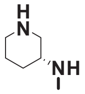
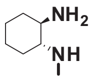
To further understand the role of nuclear nitrogen in thiophene ring, the thiazolopyridine analog **69** was prepared according to Scheme 4 by following methods and conditions used for compound **2** in Scheme 1 starting from 2-bromo-5-formylthiazole.



Scheme 1. Reagents and conditions: (a) 4-chlorophenyl boronic acid, $Pd(dppf)Cl_2$, K_3PO_4, dioxane, 80 °C, overnight, 83%; (b) malonic acid, piperidine, Py, reflux, overnight, 83%; (c) Et_3N, ethyl chloroformate, acetone, 0 °C to rt, 20 min then NaN_3, 0 °C to rt, 30 min; (d) NBu_3, phenyl ether, 230 °C, 30 min, 65% in two steps; (e) NBS, DMF, rt, overnight, 90%; (f) CuCN, DMF, reflux, overnight, then $FeCl_3$, HCl, 70 °C, 15 min, 75%; (g) $P(O)Cl_3$, reflux, overnight; 83% (h) amines, K_2CO_3, NMP, 80 °C, 1.5 h, 88%; (i) concd H_2SO_4, rt, 30 min, 66%.

Table 1
SAR at 4-position of thienopyridine

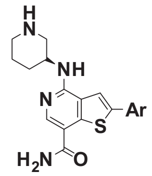


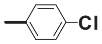
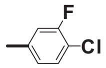
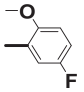
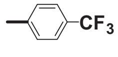
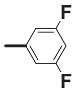
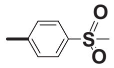
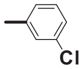
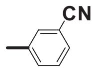
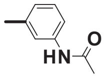
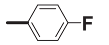
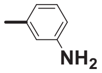
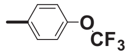
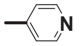
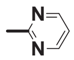
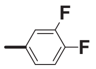
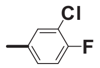
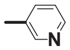
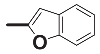
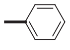
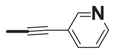
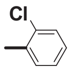
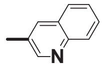
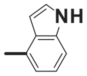
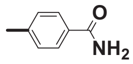
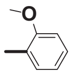
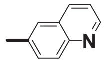
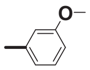
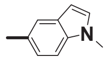
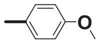
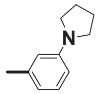
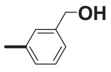
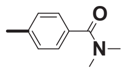
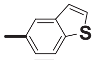
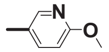
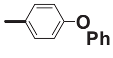
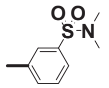
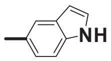
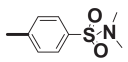
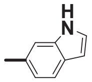
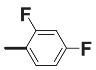
Compound	R^1	R^2	CHK1 IC$_{50}$ (nM)
1	–	–	2
2a	H		3
2b	H		14
2c	H		707
2d			746
2e	H		3
2f	H		7
2g	H		292
2h	H		197
2i	H		937
2j			148
2k	H		211
2l	H		27,143

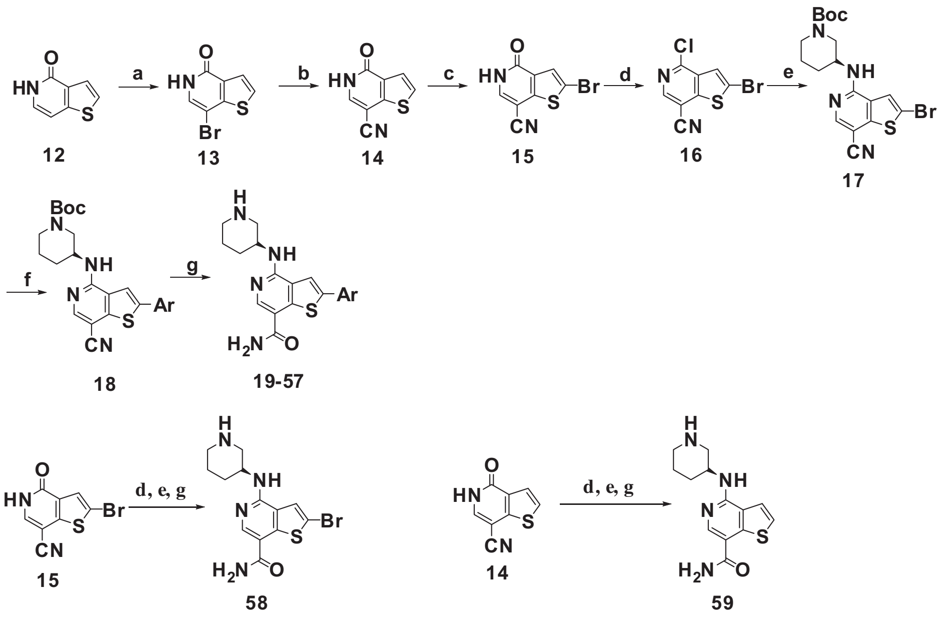
In order to explore the effect of additional nitrogen in the pyridine part of the thienopyridine scaffold, the thienopyridazine **70** was prepared via a synthetic route devised as shown in Scheme 5. Compound **71** was treated with LDA at −78 °C followed by diethyl oxalate, then cyclization with hydrazine in ethanol at 60 °C gave product **72**. Chlorination of **72** with P(O)Cl$_3$ followed by amination with (*S*)-1-Boc-3-aminopiperidine provided key intermediate **74**. The desired compound **70** was obtained by treating **74** with concentrated ammonia in sealed tube at 80 °C followed by deprotection with 4 N HCl in dioxane.

Table 1, containing compounds **2a–l**, summarizes the SAR at 4-position of the thienopyridine. Compound **2a**, with (*S*)-3-aminopiperidine-3-yl group at 4-position of thienopyridine ring, showed potent enzymatic activity (IC$_{50}$ = 3 nM).[19] However, its enantiomer **2k** with (*R*)-3-aminopiperidine-3-yl group showed approximately 100-fold loss in enzyme potency suggesting that the NH of the piperidine ring needs to be correctly aligned to form key H-bond interactions with Asp148 and Glu134, as shown by the X-ray crystal structure (Fig. 2). Also, compounds **2b** and **2e**, with five- and seven-membered ring amines with S stereochemistry maintained most of

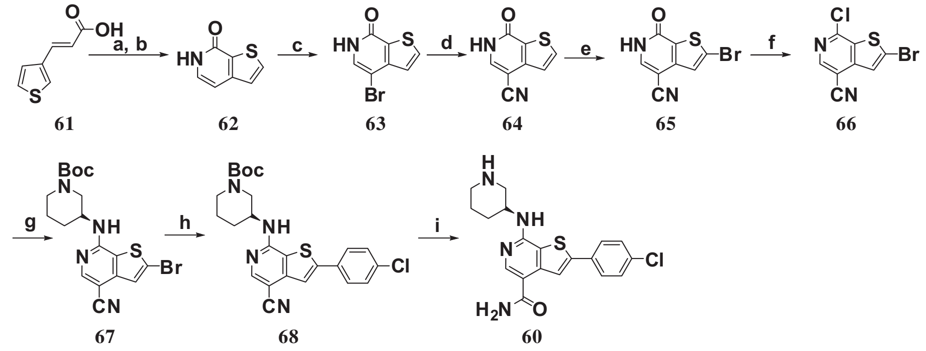
Table 2
SAR at 2-position of thienopyridine



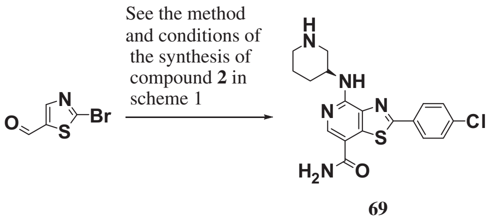
Compound	Ar	IC$_{50}$ (nM)	Compound	Ar	IC$_{50}$ (nM)
2a		3	**39**		9
19		24	**40**		5
20		5	**41**		2
21		4	**42**		7
22		2	**43**		5
23		2	**44**		9
24		3	**45**		60
25		2	**46**		4
26		5	**47**		2
27		4	**48**		67
28		21	**49**		34
29		5	**50**		1
30		19	**51**		5
31		3	**52**		8
32		3	**53**		3
33		3	**54**		1
34		4	**55**		3
35		4	**56**		20
36		60	**57**		16
37		16	**58**	Br	56
38		9	**59**	H	717



Scheme 2. Reagents and conditions: (a) NBS, DMF, rt, overnight, 95%; (b) CuCN, DMF, reflux, overnight, then $FeCl_3$, HCl, 70 °C, 15 min, 73%; (c) NBS, DMF, 60 °C, 30 min, 89%; (d) $P(O)Cl_3$, reflux, overnight, 87%; (e) (*S*)-1-Boc-3-aminopiperidine, K_2CO_3, NMP, 80 °C, 1.5 h, 85%; (f) boronic acids, Pd(dppf)Cl_2, K_3PO_4, dioxane, 80 °C, overnight, yield 65–97%; (g) concd H_2SO_4, rt, 30 min, yield 33–83%.



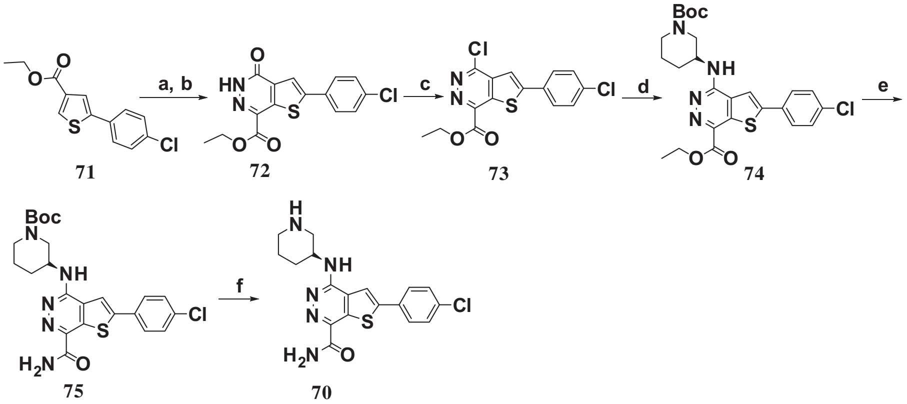
Scheme 3. Reagents and conditions: (a) Et_3N, ethyl chloroformate, acetone, 0 °C to rt, 20 min then NaN_3, 0 °C to rt, 30 min; (b) NBu_3, phenyl ether, 230 °C, 30 min, 66% in two steps; (c) NBS, DMF, rt, overnight, 88%; (d) CuCN, DMF, reflux, overnight, then $FeCl_3$, HCl, 70 °C, 15 min, 77%; (e) NBS, DMF, 60 °C, 30 min, 89%; (f) $P(O)Cl_3$, reflux, overnight, 83%; (g) (*S*)-1-Boc-3-aminopiperidine, K_2CO_3, NMP, 80 °C, 1.5 h, 88%; (h) 4-chlorophenyl boronic acid, Pd(dppf)Cl_2, K_3PO_4, dioxane, 80 °C, overnight, 79%; (i) concd H_2SO_4, rt, 30 min, 63%.



Scheme 4. Reagents and conditions are the same as described in Scheme 1.

the potency at 3 and 14 nM, respectively, presumably preserving H-bond interactions with Asp148 and Glu134. Compound **2c** with four-membered ring showed significant loss of activity with IC_{50} of 707 nM, perhaps due to the inaccessibility of the azetidine NH to form key H-bond interactions with Asp148 and Glu134. Introduction of a flexible 2-methylaminoethylamine group (compound **2f**) maintained most of the activity at 7 nM. Inhibitors containing basic amines (**2g–i**) where aminomethyl group were connected to the 4-position of thienopyridine core showed modest activity against CHK1 enzyme, again presumably due to the misalignment of the basic NH group of inhibitors with Asp148 and Glu134. In summary, specific orientation of basic groups is required for more favorable interactions with CHK1 protein and the most optimal group at the 4-position of the thienopyrididne was found to be (*S*)-3-aminopiperidine-3-yl group (compound **2a**).

Table 2 lists compounds prepared to explore SAR at the 2-position of the thienopyridine while keeping (*S*)-3-aminopiperidine-3yl group at the 4-position of the core. In general, diverse aromatic hydrophobic groups are preferred at this position providing compounds with activity against CHK1 enzyme in low nM range. The predominant interaction is the hydrophobic packing between these aromatic groups and Leu15, located at the 'roof' of the binding pocket (not shown in Fig. 2). The aromatic groups project into the solvent-exposed opening of the active site of CHK1. The absence of the hydrophobic groups at 2-position of thienopyridine core resulted in significant loss of potency (compound **59**). It is interesting

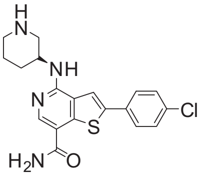
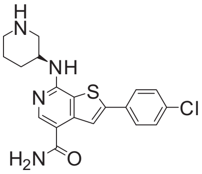
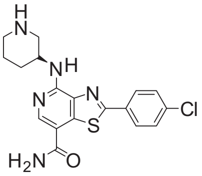
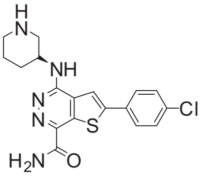


Scheme 5. Reagents and conditions: (a) LDA, diethyl oxalate, THF, −78 °C, 30 min; (b) hydrazine, ethanol, 60 °C, 10 min, followed by rt, 30 min, 57% in two steps; (c) $P(O)Cl_3$, 90 °C, 3 h, 87%; (d) (*S*)-1-Boc-3-aminopiperidine, K_2CO_3, NMP, 80 °C, 1.0 h, 91%; (e) amonnia, 80 °C, overnight, 83%; (f) 4 N HCl in dioxane, rt, 10 min, 97%.

to note that CHK1 activity does not alter significantly by 2-position hydrophobic groups with electron-donating groups, like compound **32** with methoxy group at 4-postion of the phenyl ring, or electron-withdrawing groups, like compound **43** with fluoro substituted group. Compounds **45** and **48** with 2-pyrimidine and 3-pyrido-alkyne groups, respectively, slightly deviate from above mentioned SAR trends where there was slight loss of CHK1 activity. In summary, SAR at the 2-position of thienopyridine core reflects its adjacency to the solvent-exposed opening of CHK1 and it is relatively insensitive to changes in aromatic hydrophobic groups.

Table 3 summarized the SAR of the core modification. Switching position of sulfur atom in thienopyridine core from the 1-position to the 3-position (compound **60**) resulted in dramatic loss of potency, presumably due to misalignment of key interactions of inhibitor with the CHK1 protein and the different direction angle of aromatic ring at 2-position led to the hydrophobic packing between these aromatic groups and Leu15 was not favorable to the potency. Introduction of an additional nitrogen atom at 3-position in thienopyridine ring (compound **69**) had very little impact on the potency. Whereas insertion of an additional nitrogen at the 6-position of the thienopyridine ring (compound **70**) showed improvement in potency, due to interaction of the additional nitrogen with the conserved water molecule as determined by X-ray crystallography (Fig. 3).[18] Since the conserved water molecule which has interaction with the additional water molecule also has the interaction with the primary amide, it may further locked the confirmation of the amide to benefit the potency. Compare to compound **1**, the compound **70** showed improved enzymatic activity (1 nM) while compound **1** showed 2 nM activity at biochemical assay.

Table 3
SAR of core modification of thienopyridine

Compound	Structure	CHK1 IC_{50} (nM)
2a		3
60		4582
69		9
70		1

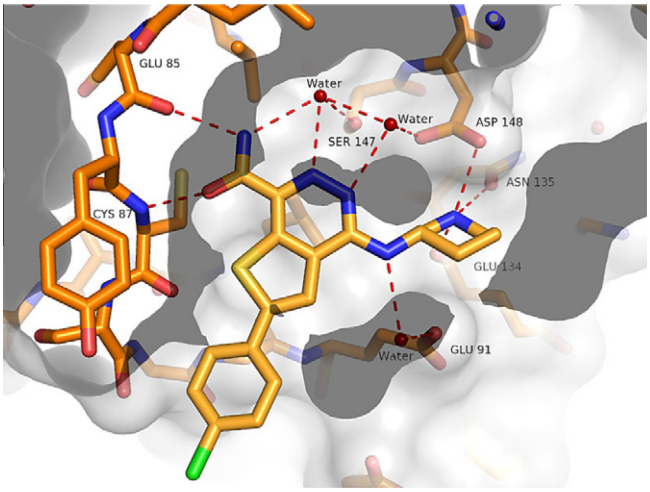


Figure 3. X-ray structures of compound **70** in CHK1 enzyme.[17,18]

In summary, we have rationally designed and synthesized potent CHK1 inhibitors based on the thienopyridine scaffold. SAR studies at the 4-position of the thienopyridine scaffold identified (*S*)-3-aminopiperidine-3-yl group as one of the optimal groups whereas at the 2-position most aromatic groups were tolerated including 4-chlorophenyl. Scaffold changes from thienopyridine to thiazolopyridine (compound **69**) and to thienopyridazine (compound **70**) were tolerated. Thienopyridazine compound **70** showed the highest CHK1 potency.

References and notes

1. Tse, W. C.; Boger, D. L. *Chem. Biol.* **2004**, *11*, 1607.
2. Hurley, L. H. *Nat. Rev. Cancer* **2002**, *2*, 188.
3. Ding, J.; Miao, Z.-H.; Meng, L.-H.; Geng, M.-Y. *Trends Pharmacol. Sci.* **2006**, *27*, 338.
4. Kawabe, T. *Mol. Cancer Ther.* **2004**, *3*, 513.
5. (a) Zhou, B. B.; Bartek, J. *Nat. Rev. Cancer* **2004**, *4*, 1; (b) Chen, Y.; Sanchez, Y. *DNA Repair* **2004**, *3*, 1025.
6. (a) Bunch, R. T.; Eastman, A. *Clin. Cancer Res.* **1996**, *2*, 791; (b) Li, Q.; Zhu, G.-D. *Curr. Top. Med. Chem.* **2002**, *2*, 939.
7. Prudhomme, M. *Rec. Pat. Anti-Cancer Drug Discov.* **2006**, *1*, 55.
8. Li, G.; Tao, Z.-F.; Tong, Y.; Przytulinska, M. K.; Kovar, P.; Merta, P.; Chen, Z.; Zhang, H.; Sowin, T.; Rosenberg, S. H.; Lin, N.-H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6499.
9. Garbaccio, R. M.; Huang, S.; Tasber, E. S.; Fraley, M. E.; Yan, Y.; Munshi, S.; Ikuta, M.; Kuo, L.; Kreatsoulas, C.; Stirdivant, S.; Drakas, B.; Richert, K.; Walsh, E. S.; Hamilton, K. A.; Buser, C. A.; Hardwick, J.; Mao, X.; Beck, S. C.; Abrams, M. T.; Tao, W.; Lobell, R.; Sepp-Lorenzino, L.; Hartman, G. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6280.
10. Teng, M.; Zhu, J.; Johnson, M. D.; Chen, P.; Kornman, J.; Chen, E.; Blasina, A.; Register, J.; Anderes, K.; Rogers, C.; Deng, Y.; Ninkovic, S.; Grant, S.; Hu, Q.; Lundgren, K.; Peng, Z.; Kania, R. S. *J. Med. Chem.* **2007**, *50*, 5253.
11. Lin, N.-H.; Xia, P.; Kovar, P.; Park, C.; Chen, Z.; Zhang, H.; Rosenberg, S. H.; Sham, H. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 421.
12. Smaill, J. B.; Lee, H. H.; Palmer, B. D.; Thompson, A. M.; Squire, C. J.; Baker, E. N.; Booth, R. J.; Kraker, A.; Hook, K.; Denny, W. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 929.
13. (a) Tao, Z.-F.; Lin, N.-H. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 377; (b) Tao, Z.-F.; Wang, L.; Stewart, K.; Chen, Z.; Gu, W.; Bui, M.-H.; Merta, P.; Zhang, H.; Kovar, P.; Johnson, E.; Park, C.; Judge, R.; Rosenberg, S.; Sowin, T.; Lin, N.-H. *J. Med. Chem.* **2007**, *50*, 1514; (c) Tao, Z.-F.; Li, G.; Tong, Y.; Stewart, K. D.; Chen, Z.; Bui, M.-H.; Merta, P.; Park, C.; Kovar, P.; Zhang, H.; Sham, H. L.; Rosenberg, S.; Sowin, T. J.; Lin, N.-H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5944; (d) Tao, Z.-F.; Chen, Z.; Bui, M.-H.; Kovar, P.; Johnson, E.; Bouska, J.; Zhang, H.; Rosenberg, S.; Sowin, T.; Lin, N.-H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6593.
14. (a) Sauville, E. A.; Arbuck, S. G.; Messmann, R.; Headlee, D.; Bauer, K. S.; Lush, R. D.; Murgo, A.; Figg, W. D.; Lahusen, T.; Jaken, S.; Jing, X.-X.; Roberge, M.; Fuse, E.; Kuwabara, T.; Senderowicz, A. M. *J. Clin. Oncol.* **2001**, *19*, 2319; (b) Senderowicz, A. M. *Oncologist* **2002**, *7*, 12.
15. Ashwell, S.; Thomas, G.; Ioannidis, S.; Janetka, J.; Lyne, P.; Su, M.; Toader, D.; Yu, Y. WO2005066163, 2005.
16. Johnson, A. L. *J. Org. Chem.* **1976**, *41*, 1320.
17. DeLano, W. L. 'The PyMOL Molecular Graphics System'; DeLano Scientific LLC, San Carlos, CA, USA. http://www.pymol.org.
18. Protein expressed and purified following Chen, P.; Luo, C.; Deng, Y.; Ryan, K.; Register, J.; Margosiak, S.; Tempczyk-Russell, A.; Nguyen, B.; Myers, P.; Lundgren, K.; Kan, C.C.; M. O'Connor, P. *Cell* **2000**, *100*, 681. Protein crystallized by hanging drop using 2.0 µl protein mixed with 2.0 µl precipitant. Optimized precipitant consists of 0.1 M Tris–Cl, pH 7.5; 1% 1-butanol; 2.5% DMSO; 25% glycerol; 2.5 mM TCEP-Cl; 10 mM di-sodium dithionite and 6–12% PEG 3.25 K. Co-structures with inhibitor obtained by soaking for 5–7 days a crystal in a solution containing the ligand of interest at a concentration of 1 mM. The structures have been determined at a resolution of 1.4 Å ($R_{\text{work}} = 18.6\%$ $R_{\text{free}} = 20.8\%$), 1.9 Å ($R_{\text{work}} = 15.0\%$ $R_{\text{free}} = 25.5\%$) and 1.55 Å ($R_{\text{work}} = 19.3\%$ $R_{\text{free}} = 21.3\%$) for compounds **1**, **2a** and **70**, respectively. The respective PDB access codes are 3PA3, 3PA4 and 3PA5.
19. *CHK1 SPA assay.* An in vitro assay utilizing recombinant His-CHK1 expressed in the baculovirus expression system as an enzyme source and biotinylated peptide based upon CDC25C as substrate. His-CHK1 was diluted to 32 nM in kinase buffer containing 50 mM Tris pH 8.0, 10 mM $MgCl_2$, and 1 mM DTT. CDC25C (CDC25 Ser216 C-term biotinylated peptide, Research Genetics) peptide was diluted to 1.93 µM in kinase buffer. For each kinase reaction, 20 µL of 32 nM CHK1 enzyme solution and 20 µL of 1.926 µM substrate solution were mixed and combined with 10 µL of compound diluted in 10% DMSO, making final reaction concentrations of 6.2 nM CHK1, 385 nM CDC25C and 1% DMSO after addition of start solution. The reaction was started by addition of 50 µL of start solution consisting of 2 µM ATP and 0.2 µCi of 33P-ATP (Amersham, UK), making a final reaction concentration of 1 µM ATP, with 0.2 µCi of 33P-ATP per reaction. Kinase reactions ran for 2 h at room temperature and were stopped by the addition of 100 µL of stop solution consisting of 2 M NaCl, 1% H_3PO_4, and 5 mg/mL Streptavidin-coated SPA beads (Amersham, UK). SPA beads were captured using a 96-well GF/B filter plate (Packard/Perkin Elmer Life Sciences) and a Filtermate universal harvester (Packard/Perkin Elmer Life Sciences). Beads were washed twice with 2 M NaCl and twice with 2 M NaCl with 1% phosphoric acid. Signal was then assayed using a TopCount 96 well liquid scintillation counter (Packard/Perkin Elmer Life Sciences). Dose–response curves were generated from duplicate 8 point serial dilutions of inhibitory compounds. IC_{50} values were derived by nonlinear regression analysis.