Bioorganic & Medicinal Chemistry Letters 18 (2008) 4242–4248

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a novel class of 2-ureido thiophene carboxamide checkpoint kinase inhibitors

James W. Janetka [a,*], Lynsie Almeida [a], Susan Ashwell [a], Patrick J. Brassil [a], Kevin Daly [a], Chun Deng [a], Thomas Gero [a], Roberta E. Glynn [a], Candice L. Horn [a,†], Stephanos Ioannidis [a], Paul Lyne [a], Nicholas J. Newcombe [b], Vibha B. Oza [a], Martin Pass [b], Stephanie K. Springer [a,‡], Mei Su [a], Dorin Toader [a], Melissa M. Vasbinder [a], Dingwei Yu [a], Yan Yu [a], Sonya D. Zabludoff [a]

[a] AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451, USA
[b] AstraZeneca R&D Alderley Park, Cheshire, Macclesfield SK10 4TG, UK

ARTICLE INFO

Article history:
Received 17 April 2008
Revised 1 May 2008
Accepted 2 May 2008
Available online 6 May 2008

ABSTRACT

Checkpoint kinase-1 (Chk1, CHEK1) is a Ser/Thr protein kinase that mediates the cellular response to DNA-damage. A novel class of 2-ureido thiophene carboxamide urea (TCU) Chk1 inhibitors is described. Inhibitors in this chemotype were optimized for cellular potency and selectivity over Cdk1.

Checkpoint kinase-1 (Chk1, CHEK1) is a Ser/Thr protein kinase that mediates the cellular response to DNA-damage. In response to DNA-damage, the ATM and ATR kinases activate Chk1 by phosphorylation on Ser-317 and/or Ser-345. Chk1 mediated signaling then leads to S phase or G2/M cell-cycle arrest primarily driven by Cdk inhibition. A Chk1 inhibitor would consequently permit a cell with damaged DNA to progress through the cell-cycle ultimately leading to mitotic catastrophe and/or apoptosis.[1] Therefore, Chk1 inhibitors have been highly sought after as chemo- or radiopotentiation agents.[2] We report here on the identification and SAR of a new class of thiophene carboxamide urea (TCU) Chk1 inhibitors.

Several diverse structural classes of Chk1 inhibitors shown in Figure 1 were identified via a high-throughput Chk1 kinase scintillation proximity assay. The three major series are exemplified by triazolones (**1**; IC_{50} 0.8 μM), pyrazoles (**2**; IC_{50} 0.6 μM), and thiophene carboxamides (**3**; IC_{50} 0.15 μM). All three chemotypes showed good potency inhibiting Chk1 activity and were pursued as lead series. The discovery and initial optimization of the TCU series exemplified by compound **3** will be discussed herein while the optimization and SAR of other Chk1 inhibitor series will be reported in the near future.

TCU **3**, while showing good potency toward Chk1, also showed activity against other kinases. This was not a surprise since the TCU's have been previously reported as kinase inhibitors.[3] We found early on that a variety of substitutions on the 4-position ether of the phenyl ring are tolerated (Table 1). Albeit not selective for Chk1, the most potent analogs generally contain a basic secondary or tertiary amine sidechain as in compounds **3** and **4a**–**c**. It is interesting that the non-basic compounds **4d**–**h** show only weak inhibition of Chk1. Matched pair analysis of the compounds in Table 1 shows that 3-position analogs are equipotent while those on the 2-position lead to reduced potency.

To demonstrate the mechanism of action, Chk1 inhibitors were profiled in a cellular assay where HT29 tumor cells were pretreated with the DNA-damaging agent Camptothecin for 2 h causing cell-cycle arrest at G2/M. The ability of a compound to abrogate this cell-cycle arrest is then quantified by levels of the marker of mitosis (M phase), phospho histone-H3. Although compound **3** displays potent enzyme inhibition, it does not abrogate the G2/M block (EC_{50} > 12.5 μM), a marker of Chk1 inhibition at the cellular level. Similarly, other closely related analogs fail to produce any substantial cellular activity.

Early on it was determined that benzylic amine substitution in the 5-position of the thiophene led to increased potency against Chk1 (Table 2), which is consistent with the previous result indicating that ether-linked amines were preferred substituents. Once again, a variety of substituents are tolerated. Unfortunately, this set of compounds also showed only limited cellular activity (**5f**; EC_{50} 0.27 μM).

It was hypothesized that limited or lack of cellular activity could indicate poor cellular permeability, an efflux liability, or additional off-target activities (e.g., Cdk1) which would mask the cellular effects of Chk1 inhibition. At this juncture, it was concluded that the combination of Chk1 selectivity and cellular potency could not be achieved simply through modulation of substitution on the 5-position aryl ring.

* Corresponding author. Tel.: +1 781 839 4434; fax: +1 781 839 4230.
E-mail address: james.janetka@astrazeneca.com (J.W. Janetka).
† Present address: Lilly, Indianapolis, IN, USA.
‡ Present address: Amgen, Cambridge, MA, USA.

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.05.016

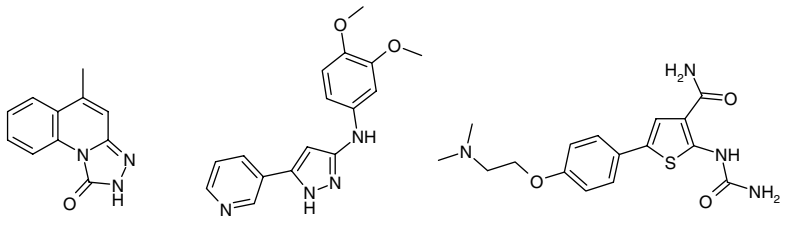
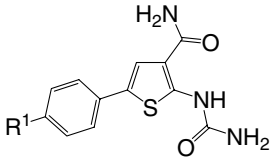


Figure 1. Chk1 inhibitors identified from high-throughput screening.

Table 1
Aryl ether substituted thiophene carboxamide ureas



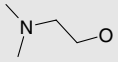
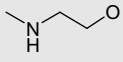
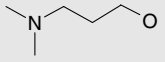
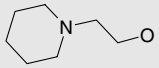
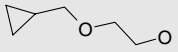
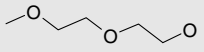
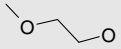
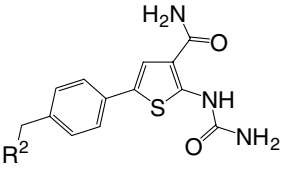
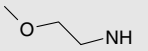
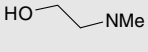
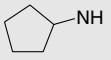
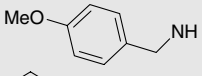
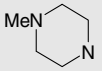
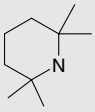
Compound	R^1	Chk1 IC_{50} (μM)
3		0.15
4a		0.36
4b		0.23
4c		0.23
4d		23
4e		4.1
4f		6.7
4g	OMe	5
4h	OCF_3	2.6

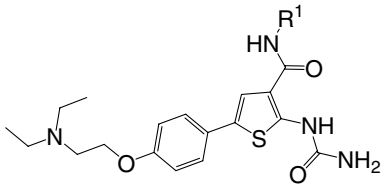
Table 2
Benzylamine thiophene carboxamide ureas

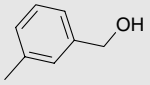
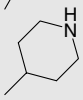
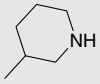


Compound	R^2	Chk1 IC_{50} (μM)
5a	NMe	>0.3
5b		0.072
5c		0.031
5d		0.039
5e		0.029
5f		0.037
5g		0.041
5h		0.017

Prior work on this series[3] had revealed that other kinase activity could be diminished by amide substitution and it was postulated that these inhibitors could possess a different binding mode to Chk1[4] that would accommodate substitution at this position. Therefore, a small set of exploratory amides was prepared holding the para-substituted basic aryl sidechain or 'solvent tail' constant as seen in Table 3. Indeed, a variety of amide substituents were not only allowed but also afforded improved potency and selectivity against Cdk1. The lack of activity against Cdk1 is important for an effective Chk1 inhibitor since Cdk1 inhibition leads to cell-cycle arrest at G2/M phase of the cell-cycle and is counterproductive to the desired G2/M checkpoint abrogation. The piperidinyl amides **6d** and **6e** both displayed excellent potency but only **6e** showed modest cellular activity. The large drop-off from enzyme to cell potency was partly attributed to the dibasic nature of these inhibitors likely leading to poor cellular permeability. Having shown that that Chk1 selectivity and potency was possible through amide modification and that basic moieties were preferred, the basic solvent tail was eliminated with the goal of improving cellular potency by eliminating the dibasic attribute of these inhibitors. Therefore, a diverse library slightly biased toward amides with basic amines and holding the 5-position phenyl constant was synthesized as outlined in Scheme 1. The original route utilized a Gewald[5] synthesis consisting of a Knovenagel condensation of benzaldehyde **8** with methylcyanoacetate followed by cyclization with elemental sulfur under basic conditions to construct the 2-amino-3-carboxy-5-phenyl thiophene **9**. Hydrolysis of ester **9** required forcing conditions by refluxing in 3 N methanolic sodium hydroxide. EDC coupling of the resultant carboxylic acid with various amines produced the desired amides **10a**. It is important to note, however, that in many cases the use of HOBt in these couplings resulted in the generation of an unreactive benzotriazole intermediate[6] and only a small amount of amide product. Finally, installation of the urea was accomplished via a two-step reaction of aminothiophene **10a** with trichloroacetyl isocyanate to give **11** followed by deprotection with ammonia yielding the desired TCU's **12**. An improved, more divergent synthesis using a Weinreb amidation reaction[7] as the key step was also utilized to synthesize the target compounds **12**. Reaction of the trichloroacetyl protected urea **13**

Table 3
Ethylamino phenyl ether substituted amides



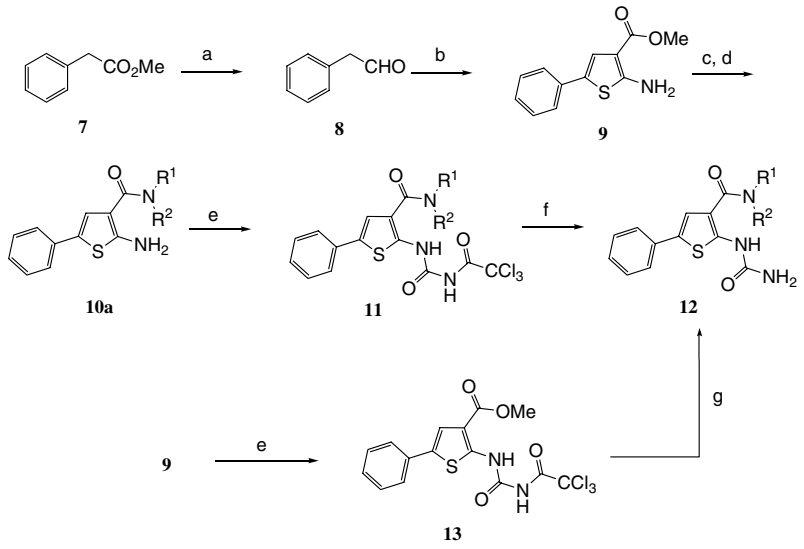
Compound	R^1	Chk1 IC_{50} (μM)	Cdk1 IC_{50} (μM)	Abrogation EC_{50} (μM)
6a		0.05	>100	>12.5
6b		0.1	44	>12.5
6c		0.1	>100	>12.5
6d		0.035	>69	>12.5
6e		0.01	4.8	0.71

with trimethyl-aluminate complex of primary or secondary amine results in dual amide formation and deprotection to afford compounds **12** in good yield.

As seen in Table 4, a large array of substitution is allowed including alkyl, aryl, benzyl, and various heterocycles. However, as determined previously alkyl linked and cyclic amines were preferred. In this set of compounds, a clear preference for the 3*S*-piperidinyl amide was seen with compound **12n**. Incredibly, amide **12n** has an IC_{50} of 7 nM for Chk1 while its enantiomer **12o** shows a 24-fold drop in activity with an IC_{50} of only 0.17 μM. Furthermore, piperidine **12n** was shown to potently abrogate the G2/M checkpoint in the abrogation assay with an EC_{50} of 20 nM versus only weak abrogation with **12o** with an EC_{50} of 1.9 μM. Substitution with tertiary amines like methylpiperidine **12p** and quinuclidine **12w** diminishes both enzyme and cellular Chk1 activity. In addition, tertiary amides exemplified by morpholine **12g**, piperazine **12h**, and **12l** all show reduced or weak Chk1 activity.

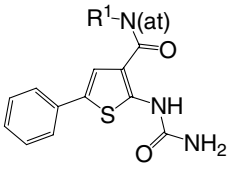
This exciting result with compound **12n** prompted a comprehensive evaluation of SAR around the 5-position substitution of the 2-ureido thiophene while keeping the amide constant as 3*S*-piperidinyl. In order to improve the synthetic efficiency, a more divergent synthesis employing a Suzuki coupling as the key pivotal step was designed (shown in Scheme 2). Reaction of 2-amino thiophene ester **14** with trichloroacetyl isocyanate followed by bromination afforded bromide **15** in 85% yield over two steps. Subsequent urea deprotection gave urea **16** which was then subjected to Weinreb amidation using 2–3 equiv of chiral *N*-1-Boc-3*S*-aminopiperidine to give the amide and key intermediate **17**. An excess of amine was required to obtain good yields. Suzuki coupling of bromide **17** under standard conditions was accomplished at 80 °C. In some cases especially for more difficult couplings a microwave reaction was employed. Finally, deprotection of the *tert*-butoxycarbonyl carbamate produced the target compound **18** in excellent yields.

A summary of the compounds with results is compiled in Table 5. A number of diverse functional groups on the 5-position phenyl are tolerated and in many cases substitution increases potency in the Chk1 enzyme assay. For example, a variety of other simple aryl substituents such as F (**18d**–**f**), Cl, (**18g**–**h**) Me (**18i**), CN (**18j**), and CF_3 (**18k**) are all essentially equipotent. Anilines and ethers are also tolerated as seen with **18a**–**c** and **18l**–**o**. With the exception of dimethylaniline **18m**, all these analogs show a drop-off in cellular potency. It is interesting that **18c** and **18l**–**o** all contain a weakly acidic residue. It was later elucidated that these analogs are efflux substrates which could rationalize this result. A similar result was found with the amides **18v**–**z′**. Compounds **18a** and **18b** both have a basic amine linker and as a result are both dibasic compounds. It is presumable that the lack of cellular potency seen in this



Scheme 1. Synthesis of 5-phenyl thiophene substituted amides. Reagents and conditions: (a) DIBAL, toluene, −78 °C; (b) $NCCH_2CO_2Me$, S, DIEA, DMF; (c) 3 N NaOH, MeOH, reflux; (d) R_1R_2NH, EDCI, DMF; (e) trichloroacetyl isocyanate, THF; (f) NH_3, MeOH; (g) $AlMe_3$, R_1R_2NH, THF.

Table 4
Library of 5-phenyl thiophene substituted amides



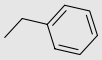
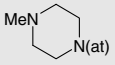
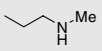
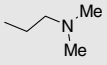
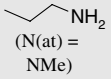
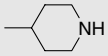
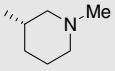
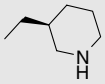
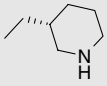
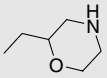
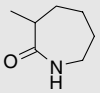
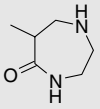
Compound	R^1	Chk1 IC_{50} (μM)	Abrogation EC_{50} (μM)
12a	Me	0.89	ND[a]
12b		1	ND[a]
12c		>10	ND[a]
12d		2.1	ND[a]
12e		3.9	ND[a]
12f		5.1	ND[a]
12g		>30	ND[a]
12h		7.1	ND[a]
12i		0.03	0.96
12j		0.10	0.58
12k		0.11	4.1
12l	 (N(at) = NMe)	5.5	ND[a]
12m		0.11	ND[a]
12n		0.007	0.02
12o		0.17	1.9
12p		0.16	2.3
12q		0.1	2.1
12r		0.1	6.2

Table 4 (*continued*)

Compound	R^1	Chk1 IC_{50} (μM)	Abrogation EC_{50} (μM)
12s		0.3	ND[a]
12t		0.003	0.11
12u		23	>13
12v		0.11	ND[a]
12w		0.13	ND[a]
12x		0.98	ND[a]

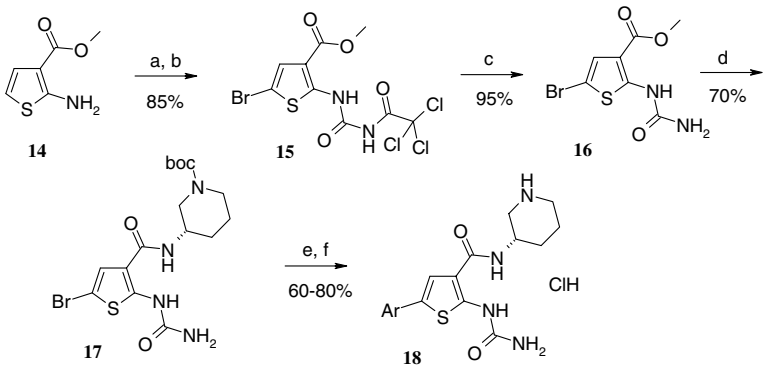
[a] ND, not determined.

case could be rationalized by a lack of cellular permeability. Some of the most potent analogs identified were heterocyclic replacements for the phenyl ring including thiophenes **18r–s** and pyridyls **18t–u**. As seen before with a number of other analogs, compounds with pyridyl substitution such as **18t** and **18u** are also prone to cellular efflux. This is in contrast with the high cellular potency observed with the thiophenes **18r** and **18s**. Furthermore, all the Chk1 inhibitors in Table 5 exhibit excellent selectivity over the cell-cycle kinase Cdk1. In summary, a large number of very potent Chk inhibitors were identified from this 5-position library. In addition, this class of 3*S*-piperidinyl amide TCU Chk inhibitors, as exemplified by lead **12n**, displays excellent physical and drug-like properties, including high solubility, low protein binding, and good in vivo PK (Fig. 2).

In conclusion, a novel class of potent thiophene carboxamide urea or TCU Chk1 inhibitors was discovered and optimized for Chk1 selectivity and cellular potency through SAR exploration of the 5-position aryl ring and 3-position amide substitution. A preference for the chiral 3*S*-aminopiperidinyl amide was discovered resulting in large improvements in both Chk1 enzyme and cellular potency. This series of TCU Chk1 inhibitors possess drug-like properties and further optimization of lead compound **12n** resulted in the identification of the candidate drug checkpoint kinase inhibitor AZD7762[8] that is currently undergoing phase I clinical evaluation in combination with various DNA-damaging agents. Further details on the optimization of the TCU series of Chk1 inhibitors and discovery of AZD7762 are forthcoming.

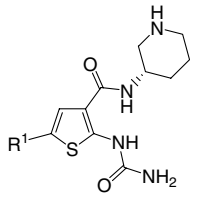
Acknowledgments

We thank Anne White and Graham Walker for their respective works on the development and implementation of the high-throughput Chk1 kinase screening assay. We also thank Elizabeth Mouchet for the development of the cellular abrogation assay.



Scheme 2. Synthesis of 5-arylthiophene (3*S*)-piperidinyl amides. Reagents and conditions: (a) trichloroacetyl isocyanate, THF; (b) Br_2, AcOH; (c) NH_3, MeOH; (d) $AlMe_3$, *N*-1 Boc-3*S*-aminopiperidine, THF; (e) $ArB(OH)_2$, $Pd(Ph_3P)_4$, Cs_2CO_3, dioxane, H_20, 80 °C; (f) 4 N HCl, dioxane.

Table 5
Library of 5-aryl thiophene 3*S*-piperidinyl amides



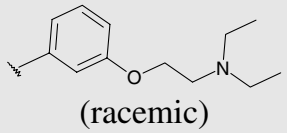
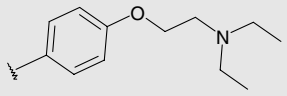
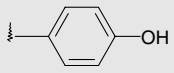
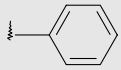
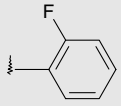
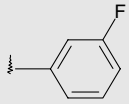
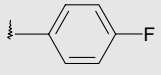
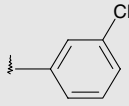
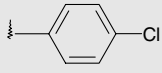
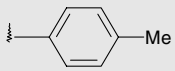
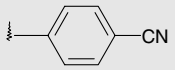
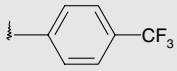
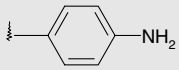
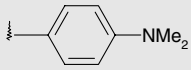
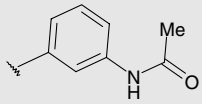
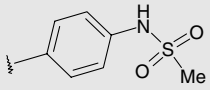
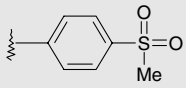
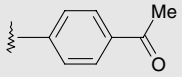
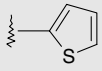
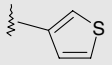
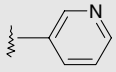
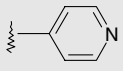
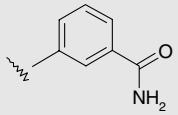
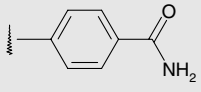
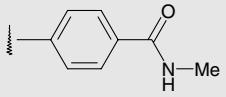
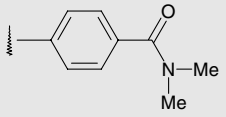
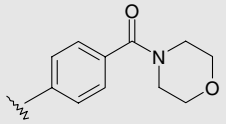
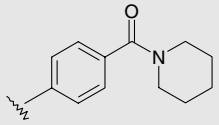
Compound	R^1	Chk1 IC_{50} (μM)	Cdk1 IC_{50} (μM)	Cdk1/Chk1 IC_{50} ratio	Abrogation EC_{50} (μM)
18a	 (racemic)	0.01	27	2700	2.60
18b		<0.01	3.6	>360	1.10
18c		0.007	10.2	1457	1.20
12n		0.007	5.1	729	0.02
18d		0.009	ND[a]	—	0.08
18e		0.011	5.6	509	0.02
18f		0.009	3.3	367	0.03
18g		0.014	8.8	629	0.04
18h		0.010	3.7	370	0.04

Table 5 (*continued*)

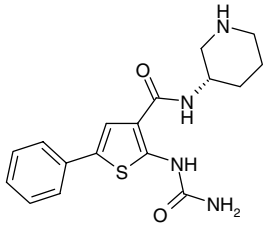
Compound	R^1	Chk1 IC_{50} (μM)	Cdk1 IC_{50} (μM)	Cdk1/Chk1 IC_{50} ratio	Abrogation EC_{50} (μM)
18i		0.010	5.5	550	0.07
18j		0.006	3.6	600	0.08
18k		0.019	5.4	284	0.17
18l		0.013	ND[a]	–	0.92
18m		0.041	8.4	205	0.09
18n		0.008	ND[a]	–	1.80
18o		0.041	ND[a]	–	2.20
18p		0.006	2.2	367	0.20
18q		0.007	ND[a]	–	0.06
18r		0.003	5.6	1867	<0.008
18s		0.006	5.3	883	<0.009
18t		0.006	3.3	550	0.30
18u		0.004	1.8	450	0.34
18v		0.005	ND[a]	–	8.10
18w		0.005	ND[a]	–	3.20

(*continued on next page*)

Table 5 (*continued*)

Compound	R^1	Chk1 IC_{50} (μM)	Cdk1 IC_{50} (μM)	Cdk1/Chk1 IC_{50} ratio	Abrogation EC_{50} (μM)
18x		0.009	ND[a]	—	4.30
18y		0.009	3.7	411	0.32
18z		0.010	ND[a]	—	0.61
18z′		0.011	10.8	982	0.13

[a] ND= not determined.



12n

LogD 1.28
Solubility >2000 μM
PPB (% free) 7.7%

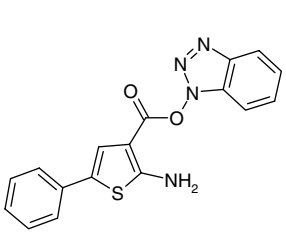
Mouse/Dog Cl 35.7/12.8 mL/min/kg
Mouse/Dog $t_{1/2}$ 1.5/5.2h
Dog F 78.8%

hERG IC_{50} >31.6 μM
CYP IC_{50} all isoforms >10 μM

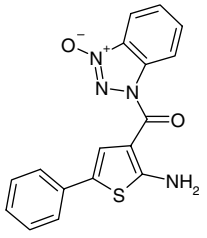
Figure 2. Lead TCU Chk1 inhibitor properties.

References and notes

1. (a) Bucher, N.; Britten, C. D. *Br. J. Cancer* **2008**, *98*, 523; (b) Luo, Y.; Leverson, J. D. *Expert Rev. Anticancer Ther.* **2005**, *5*, 333. (and references cited within).
2. (a) Janetka, J. W.; Ashwell, S.; Zabludoff, S.; Lyne, P. *Curr. Opin. Drug Discov. Dev.* **2007**, *10*, 473; (b) Arrington, K. L.; Dudkin, V. Y. *ChemMedChem* **2007**, *2*, 1571; (c) Tao, Z.-F.; Lin, N.-H. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 377; (d) Prudhomme, M. *Recent Patents Anti-Cancer Drug Discov.* **2006**, *11*, 55.
3. Baxter, A.; Brough, S.; Cooper, A.; Floettmann, E.; Foster, S.; Harding, C.; Kettle, J.; McInally, T.; Martin, C.; Mobbs, M.; Needham, M.; Newham, P.; Paine, S.; St.-Gallay, S.; Salter, S.; Unitt, J.; Xue, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2817.
4. Lyne, P. D.; Kenny, P. W.; Cosgrove, D. A.; Deng, C.; Zabludoff, S.; Wendoloski, J. J.; Ashwell, S. *J. Med. Chem.* **2004**, *47*, 1962.
5. (a) Buchstaller, H-P. H.-P.; Siebert, C. D.; Lyssy, R. H.; Frank, I.; Duran, A.; Gottschlich, R.; Noe, C. R. *Monatshefte fuer Chemie* **2001**, *132*, 279; (b) Sabnis, R. W.; Rangnekar, D. W.; Sonawane, N. D. *J. Heterocyclic Chem.* **1999**, *36*, 333.
6. No reaction seen even after heating with excess amine. Proposed benzotriazole ester intermediate **10b** or rearranged *N*-oxide amide **10c**:



10b



10c

Mahmoud, K. A.; Long, Y-T. Y.-T.; Schatte, G.; Kraatz, H-B. H.-B. *Eur. J. Inorg. Chem.* **2005**, *1*, 173.
7. (a) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989; (b) Lipton, M. F.; Basha, Anwer; Weinreb, S. M. *Org. Synth.* **1980**, *59*, 49.
8. (a) Ashwell, S. *Abstracts of Papers.* 2007 AACR Annual Meeting, Los Angeles, CA, April 14–18, 2007.; (b) Almeida, L.; Ashwell, S.; Ayres, D. W.; Brassil, P. J.; Daly, K.; Deng, C.; Gero, T.; Glynn, R. E.; Horn, C. L.; Ioannidis, S.; Janetka, J. W.; Lyne, P.; Oza, V. B.; Springer, S. K.; Su, M.; Toader, D.; Vasbinder, M. M.; Yu, D.; Yu, Y.; Zabludoff, S. D. *Abstracts of Papers.* 2007 EORTC-NCI-AACR International Meeting on Molecular Targets and Therapeutics, San Francisco, CA, October 22–26, 2007, p A233.; (c) Ashwell, S.; Caleb, B. L.; Green, S.; Grondine, M. R.; Haye, H. R.; Horn, C. L.; Janetka, J. W.; Liu, D.; Mouchet, E.; Ready, S.; Rosenthal, J. L.; Queva, C.; Taylor, K. J.; Sheehy, A. M.; Walker, G. E.; White, A. M.; Zabludoff, S. D. *Abstracts of Papers.* 2007 EORTC-NCI-AACR International Meeting on Molecular Targets and Therapeutics, San Francisco, CA, October 22–26, 2007, p A232.