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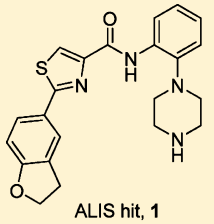
Discovery of a Novel Series of CHK1 Kinase Inhibitors with a Distinctive Hinge Binding Mode

Xiaohua Huang,*[,†] Cliff C. Cheng, Thierry O. Fischmann,[‡] José S. Duca,[§] Xianshu Yang,[†] Matthew Richards,[†] and Gerald W. Shipps, Jr.[†]

Merck Research Laboratories, 320 Bent Street, Cambridge, Massachusetts 02141, United States

Supporting Information

ABSTRACT: A novel series of CHK1 inhibitors with a distinctive hinge binding mode, exemplified by 2-aryl-*N*-(2-(piperazin-1-yl)phenyl)thiazole-4-carboxamide, was discovered through high-throughput screening using the affinity selection−mass spectrometry (AS-MS)-based Automated Ligand Identification System (ALIS) platform. Structure-based ligand design and optimization led to significant improvements in potency to the single digit nanomolar range and hundred-fold selectivity against CDK2.



KEYWORDS: *affinity selection−mass spectrometry (AS-MS), Automated Ligand Identification System (ALIS), CHK1 protein kinase, structure-based drug design, thiazole-4-carboxamide*

Cancer is the leading cause of death globally.[1] Among the treatments used in cancer clinics, DNA-damaging chemotherapies have received widespread use despite their severe side effects on highly proliferative tissues and vulnerability to drug resistance.[2] There is an urgent need in cancer therapy for improved tolerability and longer lasting efficacy. When cells are exposed to agents that induce DNA damage, DNA repair pathways are activated by arresting at various cell cycle check points, G1, S, and G2/M.[3] While normal cells could arrest in the G1 phase through the tumor suppressor protein p53, most cancer cells lack this option because of p53 mutations and will have to rely on the S or G2/M checkpoint for DNA repair and survival. Such reliance in p53 mutant cancer cells would offer a significant opportunity for targeted cancer therapy.[4] CHK1 (checkpoint kinase 1) is a serine/threonine kinase and the key mediator in S and G2/M checkpoints.[5] The abrogation of CHK1 and the remaining checkpoints consequentially will cause cancer cells with DNA damage premature entry into mitosis and result in cell death.[6] Therefore, an enlarged therapeutic window would be expected in anticancer therapies utilizing a combination of a DNA-damaging agent with a CHK1 inhibitor.

Over the past years, extensive research efforts in CHK1 inhibition have been undertaken in oncology. Several small molecule CHK1 inhibitors have been discovered and entered into clinical trials.[7−11] In our efforts to discover novel small molecule CHK1 inhibitors, the AS-MS ALIS (affinity selection−mass spectrometry-based Automated Ligand Identification System) platform was used to identify hits from mixture-based combinatorial libraries.[12] ALIS has demonstrated its unique capability in screening label-free mixture-based libraries and finding hits that were subsequently developed into lead compounds in various classes of protein targets, including the anti-infective target *Escherichia coli* dihydrofolate reductase,[13] antibacterial AccC (acetyl coenzyme-A carboxylase),[14] HCV NS5B (hepatitis C virus nonstructural protein 5B) polymerase,[15−17] protein kinase CDK2 (cyclin-dependent kinase 2),[18] KSP (kinesin spindle protein) in oncology,[19] FABP4 (fatty acid binding protein-4)[20] and the lipid phosphatase SHIP2 (SH2 domain-containing inositol 5-phosphatase 2)[21] in diabetes, MK2 (mitogen-activated protein kinase-activated protein kinase 2)[22] and TACE (TNF-α converting enzyme)[23] in inflammation, and the GPCR (G-protein-coupled receptor) muscarinic M_2 acetylcholine receptor.[24] Herein, we describe another example using ALIS to screen for novel inhibitors of the CHK1 protein and subsequent medicinal chemistry optimization.

From the CHK1 ALIS screening of mixture-based libraries, compound **1** emerged as a high affinity ligand (ALIS K_d <100 nM), and the series was selected for additional optimization. Biochemical assays confirmed its inhibitory activity against CHK1 with an IC_{50} value of 75 nM with a clearly detectable in-cell activity (~10 μM) that was hydroxyurea (HU)-dependent. However, an initial selectivity assessment revealed only a modest selectivity (~3-fold) against CDK2, the inhibition of which was anticipated to mitigate the inhibition of CHK1.[25,26] Structure-based ligand design and empirical structure−activity relationship (SAR)-driven optimization were initiated with the minimal goals of achieving a 10-fold improvement in potency and demonstrating a 100-fold in selectivity against CDK2.

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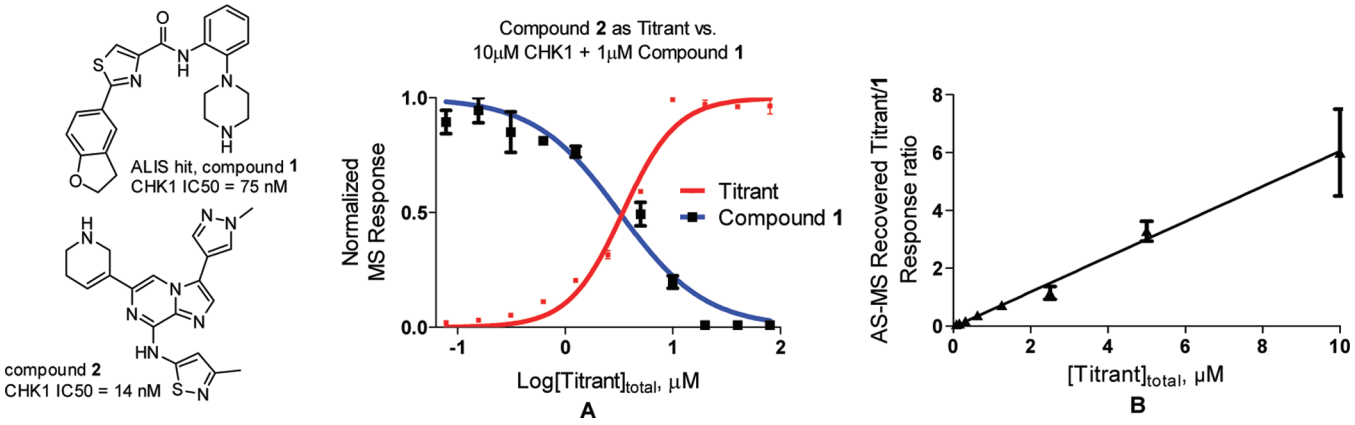

Figure 1. Affinity competition experiment. For all figures, data points and error bars reflect the average and standard error of duplicate samples.

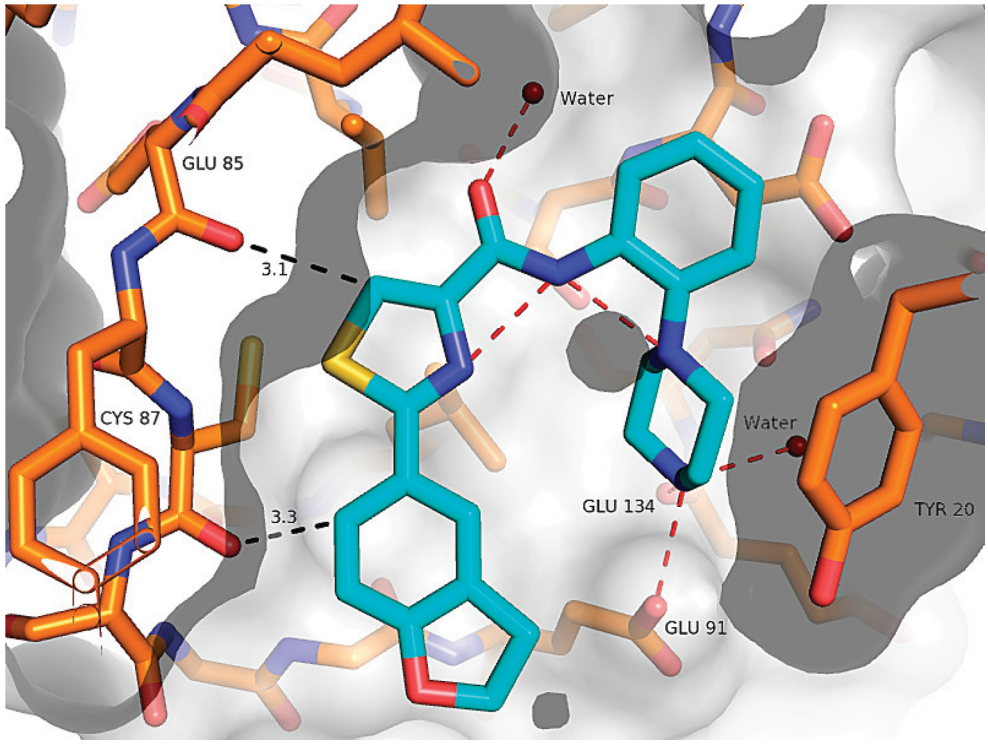

Figure 2. X-ray of crystal structure of compound **1** bound to CHK1 protein.

The characterization of the binding mode of CHK1 to compound **1** was analyzed using an affinity competition experiment (Figure 1).[27] In these experiments, a constant concentration of compound **1** and serially increasing concentrations of the titrant (compound **2**, a previously reported ATP-competitive CHK1 inhibitor with IC_{50} value of 14 nM[11,28]) were incubated together with the CHK1 protein. The protein−ligand complexes were separated from the unbound compounds by size-exclusion chromatography. The protein-bound compound **1** and the titrant were dissociated from the protein using reverse-phase chromatography and quantified by a mass spectrometer. The AS-MS recovery of compound **1** in the presence of the titrant suggested the binding site. As shown in Figure 1A, compound **1** was fully displaced from binding to the CHK1 protein by the titrant at a higher concentration. The linear plot of titrant/**1** response ratio versus total titrant concentration in Figure 1B provided compelling evidence of direct competition between compound **1** and the titrant for binding with CHK1.

To further establish the binding site and gain insight into the specific interactions between compound **1** and CHK1, the X-ray crystal structure of the complex was successfully pursued.[29] The X-ray crystal structure of compound **1** bound to CHK1 at 1.85 Å (Figure 2) illuminated key interactions that were noteworthy for kinase−ligand complexes. Compound **1** overlaps with the ATP binding pocket. As most kinase inhibitors, it interacts with several main-chain polar atoms from the hinge region exposed to the binding site. A first interaction is between the 2,3-dihydrobenzofuran 6-C-H and the Cys87 carbonyl. A second interaction is between the Glu85 main-chain carbonyl and the 5-C-H of the thiazole. In both cases, the distances between atoms, 3.3 and 3.1 Å, respectively, are consistent with an interaction with partial H-bond character. This is the first reported example in a kinase[30] where the C−H next to a S in a thiazole ring forms a

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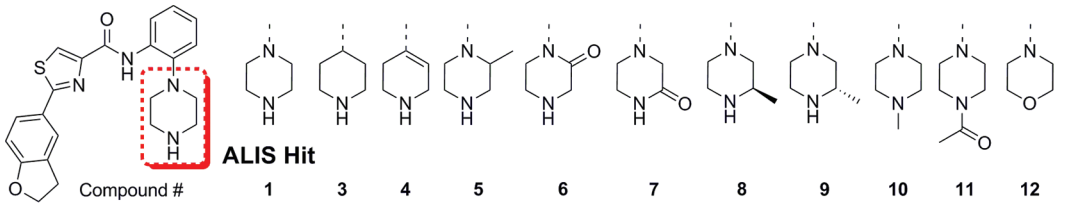
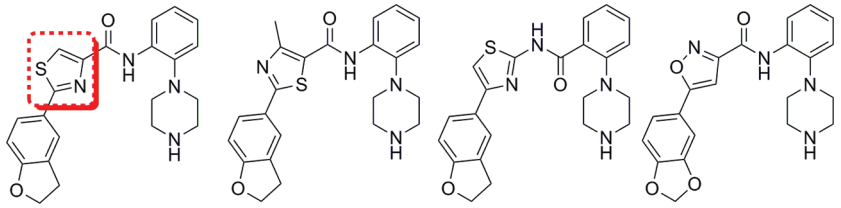
Table 1. Optimization To Probe Intramolecular Hydrogen Bonding and Salt Bridge Interactions



Compound #	1	3	4	5	6	7	8	9	10	11	12
CHK1 IC50 (nM)[a]	75	4500	760	280	> 50000	> 50000	120	86	2000	4100	> 50000
CDK2/CHK1[a,b]	2.7	1.8	1.7	nd	nd	nd	2	3	0.16	0.34	nd

[a]Biochemical data represent average values of duplicates or triplicates with a standard deviation of ±10%. [b]nd, not determined.

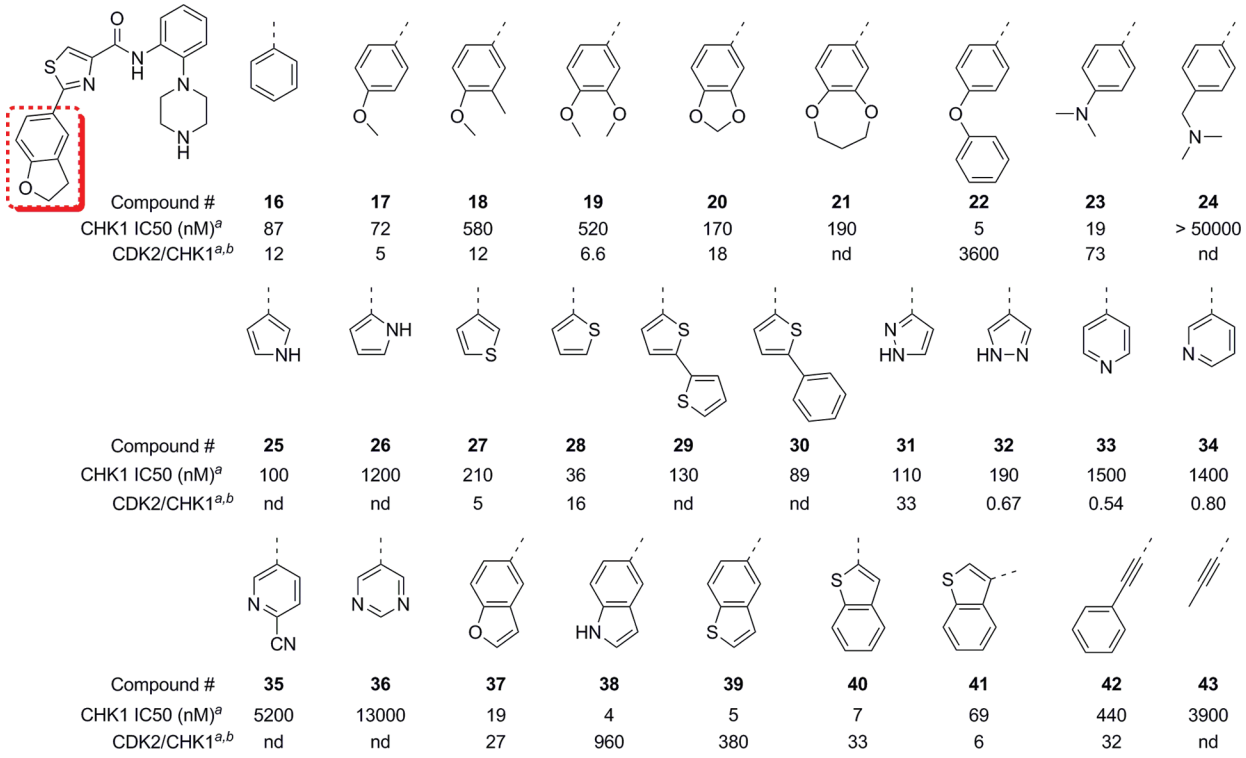
Table 2. Optimization To Probe Interaction with Hinge Binding



Compound #	13	14	15
CHK1 IC50 (nM)[a]	21000	> 50000	3400

[a]Biochemical data represent the average values of duplicate or triplicate data sets with a standard deviation of ±10%.

Table 3. Optimization To Probe Solvent Front Interactions



Compound #	16	17	18	19	20	21	22	23	24
CHK1 IC50 (nM)[a]	87	72	580	520	170	190	5	19	> 50000
CDK2/CHK1[a,b]	12	5	12	6.6	18	nd	3600	73	nd

Compound #	25	26	27	28	29	30	31	32	33	34
CHK1 IC50 (nM)[a]	100	1200	210	36	130	89	110	190	1500	1400
CDK2/CHK1[a,b]	nd	nd	5	16	nd	nd	33	0.67	0.54	0.80

Compound #	35	36	37	38	39	40	41	42	43
CHK1 IC50 (nM)[a]	5200	13000	19	4	5	7	69	440	3900
CDK2/CHK1[a,b]	nd	nd	27	960	380	33	6	32	nd

[a]Biochemical data represent average values of duplicates or triplicates with a standard deviation of ±10%. [b]nd, not determined.

nonstandard hydrogen bond, whereas most of reported examples have the C−H adjacent to N in various heterocycles.[31] Quantum mechanical calculations suggest that the polarizability of the S in the thiazole ring contributes to this "unusual" hinge interaction. It is also evident from the X-ray crystal structure that the secondary amide NH serves as an intramolecular H-bond bridge between two proximal N's to confer a "U-shaped" topology to compound **1**.[32] Such intramolecular hydrogen bonding may significantly preorganize compound **1** to fit into the binding pocket with a reduced entropic penalty. The preferred orthogonal orientation of the piperazine with respect to the aryl group enables this conformation. Additional key interactions are evident: a salt bridge between Glu91 and the piperazine NH, which also shares H-bonds with Glu134 main-chain carbonyl and a water molecule, an H-bond between the amide oxygen and a

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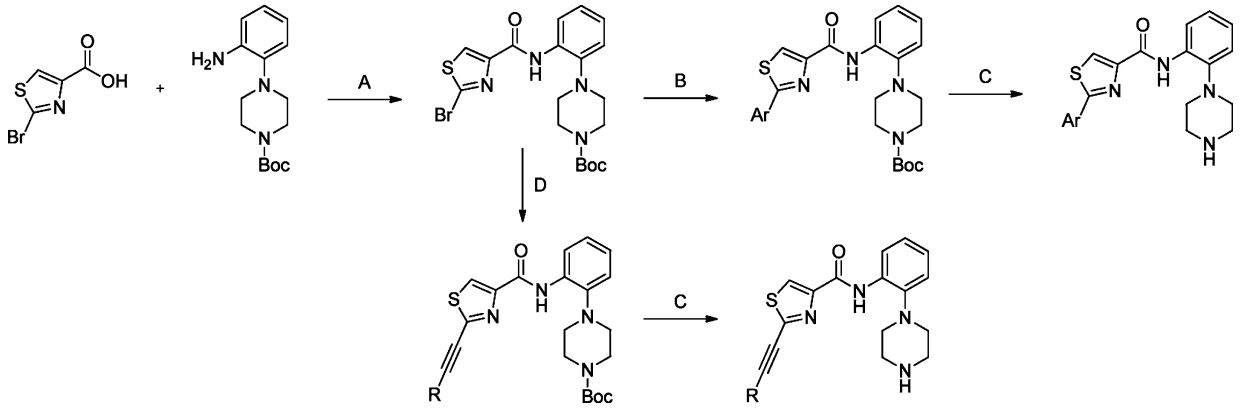
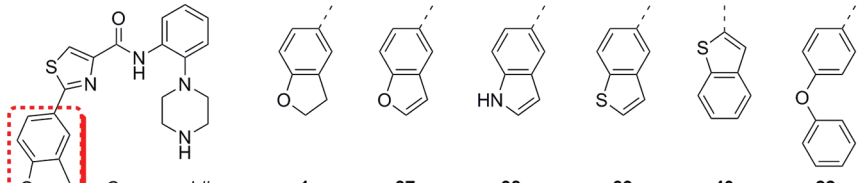
Scheme 1. Synthesis of 2-Aryl-*N*-(2-(piperazin-1-yl)phenyl)thiazole-4-carboxamide[a]



[a]Reagents and conditions: (A) HATU (*N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate), DIEA (*N*,*N*-diisopropylethylamine), DMF, room temperature. (B) $Pd(OAc)_2$, S-Phos (2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl), $ArB(OH)_2$, K_3PO_4, dioxane, 100 °C. (C) TFA (trifluoroacetic acid). (D) $Pd(OAc)_2$, X-Phos (2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl), alkyne, Cs_2CO_3, MeCN, 85 °C.

Table 4. Cell-Based Activity



Compound #	**1**	**37**	**38**	**39**	**40**	**22**
CHK1 IC50 (nM)[a]	75	19	4	5	7	5
CDK2/CHK1[a]	2.7	27	960	380	33	3600
H2AX EC50 (nM)[a]	10000	2000	3500	2500	1500	> 50000

[a]Biochemical and cell-based data represent average values of duplicates or triplicates with a standard deviation of ±10%.

conserved water,[33,34] and contacts between the 2,3-dihydrobenzofuran moiety and the solvent front. Inhibitor binding induces a conformational rearrangement of the glycine-rich loop. The phenol of Tyr20 at the tip of the glycine-rich loop moves to stack itself against a hydrophobic edge of the piperazinyl.

Identifying modifications of the piperazine portion of compound **1** which improved potency proved to be challenging (Table 1). A significant loss of potency was observed in changes to the tertiary aniline, such as the replacement of a nitrogen with either an sp^3 (**3**) or an sp^2 (**4**) carbon or adding a methyl (**5**) or carbonyl (**6**) group adjacent to the tertiary nitrogen. Such sensitivity to structural change strongly suggested the necessity of intramolecular hydrogen bonding between the tertiary N and N−H of the secondary amide. Attempts to probe the salt bridge interaction between the distal secondary amino group and the Glu91 were made, and loss of potency was observed. While adding a methyl group adjacent to the distal nitrogen in either stereo configuration (**8** and **9**) gave a slight decrease in potency, complete potency loss was observed in the change from a basic amino group to a lactam (**7**). Capping with a methyl (**10**) or acetyl (**11**) group or replacement with O (**12**) yielded a much less potent compound.

Modifications to probe the interaction between the C−H of the thiazole and the carbonyl of Glu85 in the hinge region were attempted (Table 2), such as substitution with a methyl group (**13**), different connectivity at the thiazole (**14**), and replacement with isoxazole (**15**). All of these changes resulted in a significant loss of potency and reinforced the value of the C−H/Glu85 to the overall binding affinity.

A breakthrough in potency and selectivity against CDK2 was achieved through the modification of the aryl group connected to thiazole (Table 3). These analogues were prepared by Suzuki cross-coupling of bromothiazole following Scheme 1.[35] The compound with a plain phenyl group (**16**) is close to compound **1** in potency but with slightly better selectivity against CDK2. *para*-MeO substitution of the phenyl group (**17**) afforded a compound comparable to **1** in potency and selectivity. Additional substitution of a methyl (**18**) or methoxy (**19**) group at the *meta* position resulted in a potency decrease of 7-fold. Bridging the two oxygen atoms at the 3- and 4-position of the phenyl group through either one carbon (**20**) or three carbons (**21**) gave compounds with potency 2-fold less than compound **1**. Interestingly, pronounced improvements in potency and selectivity against CDK2 were observed when the phenyl group was substituted with a *para*-phenoxy group (**22**). While *para*-NMe_2 substitution of phenyl group (**23**) resulted in a slight increase in potency, insertion of a methylene bridge between the dimethylamino and phenyl group (**24**) led to the complete loss of activity.

The replacement of phenyl moieties with selected heterocycles did not result in appreciable improvements in IC_{50}. While the 2-pyrrole (**26**) is 10-fold weaker in potency than the 3-pyrrole (**25**), 2-thiophene (**28**) is a few fold better in potency and selectivity than the 3-thiophene (**27**) and compound **1** as well. Substitution of the 2-thiophene group with thienyl (**29**) or the phenyl (**30**) afforded a slight potency improvement. Both

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the 3- (**31**) and the 4-pyrazole (**32**) were slightly less potent than compound **1**; however, encouraging selectivity toward CDK2 was demonstrated by the 3-pyrazole (**31**). Other *N*-containing heterocycles such as pyridines (**33**, **34**, and **35**) and pyrimidine (**36**) showed reduced CHK1 inhibition. Replacement with fused bicyclic aromatics was also explored. The replacement of the 2,3-dihydrobenzofuran group in compound **1** with benzofuran (**37**) gave slight improvements in potency and selectivity. The change of oxygen to other heteroatoms such as NH (**38**) or S (**39**) led to further improvement in IC_{50} to single-digit nanomolar as well as selectivity against CDK2 to several hundred-fold. Other connections at benzothiophene were also explored, and 3-benzothiophene (**41**) was found to be less potent and selective than the 2- (**40**) and the 5-benzothiophene (**39**). Analogues with an alkyne group attached to the thiazole were also prepared by employing Sonogashira cross-coupling of bromothiazole.[36] While less potency was observed, a 10-fold improvement in selectivity against CDK2 was obtained in **42** in comparison to compound **1**.

Top compounds in biochemical activity were tested in cell-based assays using γ-H2AX as the cellular biomarker for the DNA double-strand breaks (Table 4).[6] Compounds **37**−**40** showed improved cell-based activity by 3−6-fold. Compound **22** showed no cell-based activity despite its marked biochemical potency and selectivity against CDK2, possibly due to lack of membrane permeability, compound efflux from the cell, or a confounding off-target effect.

In summary, a new series of small molecules with distinctive hinge binding modes, 2-aryl-*N*-(2-(piperazin-1-yl)phenyl)-thiazole-4-carboxamide, were identified via ALIS screening of mixture-based libraries and developed into potent inhibitors to CHK1. Hit-to-lead chemistry efforts resulted in significant improvements in biochemical potency and selectivity against CDK2. Additional SAR development and improvement in cell-based activity will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, spectroscopic characterization of key compounds, and assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: xiaohua.huang@merck.com.

Present Addresses

[†]Merck Research Laboratories, 33 Avenue Louis Pasteur, Boston, Massachusetts 02115.

[‡]Merck Research Laboratories, 2000 Galloping Hill Road, Kenilworth, New Jersey 07033.

[§]Novartis Institutes for BioMedical Research, 100 Technology Square, Cambridge, Massachusetts 02139.

Notes

The authors declare no competing financial interest.

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