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Discovery of pyrazolo[1,5-*a*]pyrimidine-based CHK1 inhibitors: A template-based approach—Part 1

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ABSTRACT

The synthesis and hit-to-lead SAR development of a pyrazolo[1,5-*a*]pyrimidine hit **4** is described leading to a series of potent, selective CHK1 inhibitors such as compound **17r**. In the Letter, the further utility of the pyrazolo[1,5-*a*]pyrimidine template for the development of potent, selective kinase inhibitors is detailed.

Checkpoint kinase 1 (CHK1) is a serine/threonine kinase that controls the cellular response to DNA damage. In response to a DNA-damaging agent, CHK1 is activated by phosphorylation via ATR and ATM to arrest cells at various cell-cycle checkpoints (G1, S and G2) in order to initiate the DNA repair process.[1] Inhibition of CHK1 abrogates cell-cycle arrest resulting in genomic instability and ultimately progression into mitosis and cell death.[2] The inhibition of CHK1 creates a 'synthetic lethal' response by which aberrant cells can not replicate which should impede the progression of cancer. In contrast, normal cells still arrest at the G1 checkpoint, via p53, to repair the DNA damage caused by these agents. Due to the fact that inhibition of CHK1 represents a targeted approach to enhance the cytotoxicity of DNA-damaging agents toward tumor cells while having a lesser effect on normal cells, it has been an attractive target in the oncology field.[3]

A number of small-molecule CHK1 inhibitors have been described recently and several comprehensive reviews have provided overviews of the emerging CHK1 small-molecule chemotypes.[4] In addition, several checkpoint kinase inhibitors, such as PF-00477736 (**1**)[5] and AZD7762 (**2**)[6] (Fig. 1), have recently entered the clinic in combination with various DNA-damaging agents. Due to the therapeutic value of a CHK1 inhibitor as a chemopotentiator,[7] efforts were directed towards the identification of additional, novel CHK1 inhibitors.

Screening of an internal compound library identified compounds **3** and **4**, as early CHK1 program hits (Fig. 2). While these initial hits possessed better in vitro potency versus CDK2 than CHK1, it was rationalized that proper substitution around the pyrazolo[1,5-*a*]pyrimidine core might improve the potency and selectivity of this series for CHK1. Previously, the pyrazolo-[1,5-*a*]pyrimidine core was shown to be a viable template for the preparation of CDK2 inhibitors such as compound **5** which was orally bioavailable and found to be efficacious in a mouse tumor xenograft model (Fig. 2).[8] With compound **4** as a starting point,

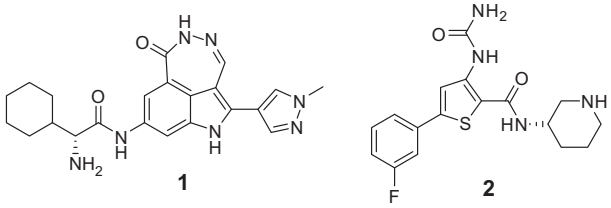

Figure 1. CHK1 inhibitors currently under clinical evaluation.

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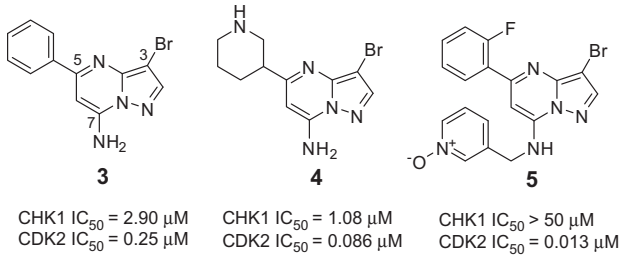
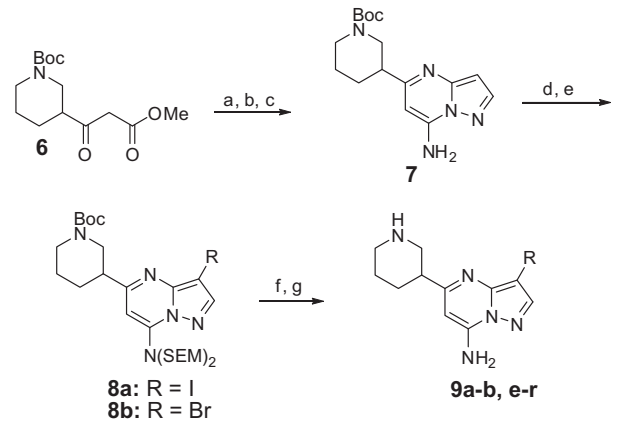


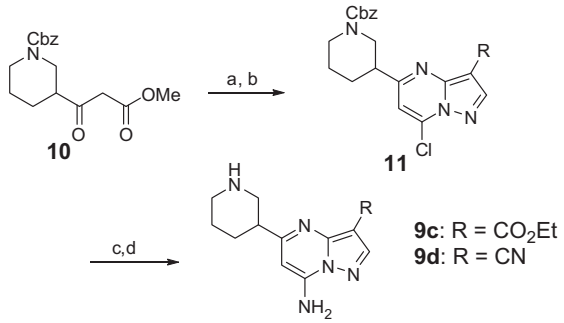
Figure 2. Initial pyrazolo[1,5-*a*]pyrimidine CHK1 Hits.



Scheme 1. Reagents and conditions: (a) 3-aminopyrazole, $PhCH_3$, 73%; (b) $POCl_3$, *N*,*N*-dimethylaniline, 71%; (c) NH_3, 2-propanol, H_2O, 98%; (d) SEMCl, DIPEA, $(CH_2Cl)_2$, 76%; (e) NBS, CH_3CN or NIS, CH_3CN, 92%; (f) $RB(OH)_2$, $PdCl_2dppf$, K_3PO_4, DME/H_2O or Bu_3SnR, $Pd[PPh_3]_4$, dioxane, 29–89%; (g) 3 N HCl/EtOH, 23–78%.

we focused upon making systematic modifications around the pyrazolo[1,5-*a*]pyrimidine core in order to enhance the in vitro potency for CHK1 while monitoring the selectivity versus CDK2. It was rationalized that selectivity for CHK1 over CDK2 was required since the inhibition of the CDK function may antagonize CHK1 ablation/inhibition phenotypes.[9]

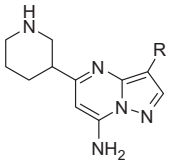
Since a limited set of substituents had been tolerated at the C3 position in the pyrazolo[1,5-*a*]pyrimidine core in our previous CDK2 program,[8] initial synthetic efforts focused upon modifications of the 3-position of compound **4** to further explore potential differences in this region for CHK1. The preparation of C3 analogs of compound **4** is illustrated in Schemes 1 and 2.[10] Cyclization of β-keto ester **6** with 3-aminopyrazole followed by chlorination and amination afforded **7**. Diprotection of the resultant C7 amino group[11] followed by introduction of either the C3 iodide via NIS

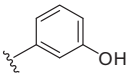
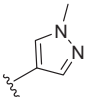


Scheme 2. Reagents and conditions: (a) 3-aminopyrazole-4-carbonitrile or 3-amino-4-carboethoxypyrazole, $PhCH_3$, 41–61%; (b) $POCl_3$, *N*,*N*-dimethylaniline, 91–98%; (c) NH_3, 2-propanol, H_2O, 82–90%; (d) TMSI, MeOH, 31–45%.

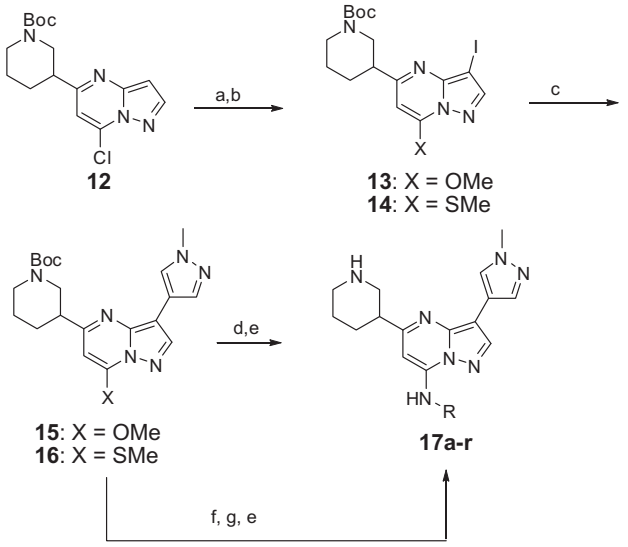
treatment or C3 bromide via NBS treatment afforded intermediates **8a** or **8b**. Treatment of **8a, b** under either Suzuki or Stille coupling conditions followed by global deprotection with 3 N HCl in EtOH afforded the C3 analogs **9a–b, e–r** which are summarized in Table 1. Alternatively in Scheme 2, cyclization of the Cbz-protected β-keto ester with either the cyano or ethyl ester substituted

Table 1
CHK1 and CDK2 inhibitory activity of C3 substituted pyrazolo[1,5-*a*]pyrimidines **9a–r**



Compd	R	CHK1 IC_{50}[a] (μM)	CDK2/cyclin A IC_{50}[b] (μM)
9a	H	36	7.8
9b		18	0.08
9c		>50	nt
9d		2.1	0.81
9e		9.4	0.27
9f		5.1	0.71
9g		4.5	4.6
9h		1.7	0.64
9i		0.28	5.4
9j		0.29	1.2
9k		0.54	nt
9l		1.1	0.59
9m		1.3	0.69
9n		0.82	nt
9o		2.0	2.2
9p		>50	10
9q		0.060	6.1
9r		0.070	0.79

nt = not tested; values are means of two experiments.
[a] Assay conditions can be found in Ref. 12.
[b] Assay conditions can be found in Ref. 8a.



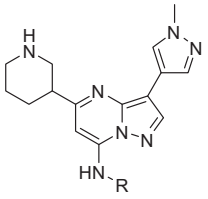
Scheme 3. Reagents and conditions: (a) NIS, CH_3CN, 97%; (b) NaOMe or NaSMe, THF, 85–97%; (c) 1-methylpyrazole-4-boronic acid pinacol ester, $PdCl_2dppf$, K_3PO_4, DME/H_2O, 52–75%; (d) H_2NR, NaH, DMF, 38–98%; (e) TFA, CH_2Cl_2, 13–98%; (f) MCPBA, CH_2Cl_2, 85%; (g) H_2NR, *n*-BuOH, 100 °C, 21–85%.

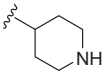
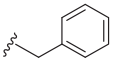
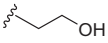
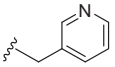
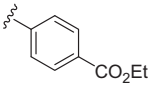
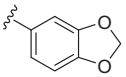
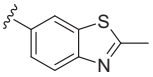
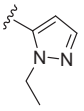
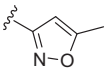
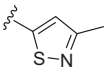
pyrazole followed by subsequent chlorination under standard conditions afforded **11**. Treatment with NH_3 in isopropanol followed by Cbz deprotection with TMSI afforded the final adducts **9c** and **9d**.

As shown in Table 1, small linear substituents (**9b–f**) at the C3 position improved the CHK1 potency versus the 3-H adduct (**9a**) with the exception of ester **9c**. Despite the potency improvements for CHK1, these analogs also retained good potency for CDK2. Incorporation of aryl (**9g,h**) and heteroaryl substituents into the C3 position substituents (**9i–n**) resulted in marked improvement in CHK1 potency and selectivity versus CDK2 in the biochemical assay. Additionally, several heteroaryl derivatives in Table 1 (**9o,p)** showed weaker CHK1 activity which suggested that the proper placement of heteroatoms in the heteroaryl ring at C3 is critical to retain CHK1 potency. From this SAR survey of the C3 position, compound **9q** emerged as a key compound which demonstrated good CHK1 potency (IC_{50} = 60 nM) and nearly 100-fold selectivity for CHK1 over CDK2. Compound **9q** was selected as a lead structure for further SAR optimization efforts.

With compound **9q** in hand, attention turned toward the exploration of additional substitution of the primary amine located at the C7 position of this compound. The preparation of these analogs is outlined in Scheme 3.[10] Iodination of **12** followed by treatment with either sodium methoxide or sodium thiomethoxide in THF afforded compounds **13** or **14**, respectively. Treatment of **13** or **14** with 1-methylpyrazole-4-boronic acid pinacol ester under Suzuki coupling conditions afforded the corresponding coupled products **15** and **16**, respectively. For the preparation of C7 amino derivatives bearing either aryl or heteroaryl functionality, compound **15** was treated with the anion of the anilinic/heteroaryl coupling partners using NaH followed by TFA treatment to afford the title compounds **17i–r** (Table 2). Surprisingly, treatment of **15** with simple alkyl and benzyl amines with heating yielded none of the desired addition product but only the demethylated (7-OH) adduct. This issue was circumvented by utilization of the corresponding thiomethyl adduct **16**. Oxidation of the thiomethyl group with mCPBA afforded a mixture of sulfoxide/sulfone which was treated with either alkyl or branched alkyl amine derivatives in hot *n*-BuOH in the presence of Et_3N to afford the desired addition products. Deprotection of the intermediate Boc adducts with TFA afforded the C7 substituted amino derivatives **17a–h** listed in Table 2.

Table 2
CHK1 and CDK2 inhibitory activity of C7 substituted amino pyrazolo[1,5-*a*]pyrimidines **9q**, **17a–r**



Compd	R	CHK1 IC_{50}[a] (μM)	CDK2/cyclin A IC_{50}[b] (μM)
9q	H	0.06	6.06
17a	Me	0.14	na
17b	Et	0.48	na
17c		1.5	na
17d		5.0	na
17e		6.8	na
17f		1.0	31
17g		0.86	na
17h		0.41	na
17i		0.087	na
17j		0.051	na
17k		0.39	na
17l		0.61	na
17m		34	nt
17n		2.2	na
17o		0.028	na
17p		0.028	na
17q		0.021	na
17r		0.009	40

na = not active up to >50 μM; nt = not tested.
[a] Assay conditions can be found in Ref. 12.
[b] Assay conditions can be found in Ref. 8a.

As summarized in Table 2, incorporation of simple alkyl, branched alkyl, and cycloalkyl groups at the C7 amino group (**17a–e**) resulted in poorer in vitro potency for CHK1 versus the parent NH_2 derivative (**9q**). Simple alkyl modifications with pen-

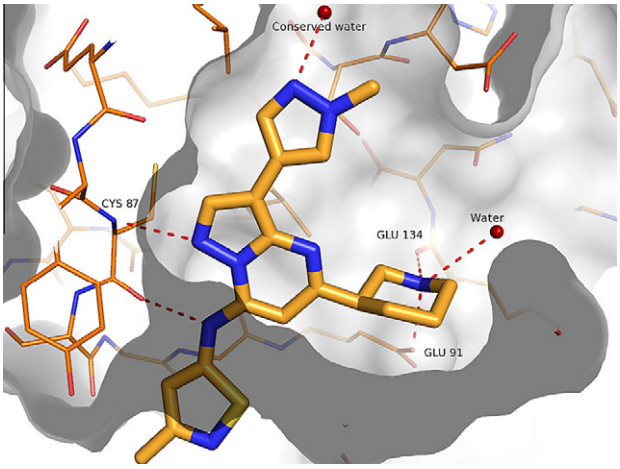


Figure 3. X-ray of crystal structure of **17r** in CHK1.[13]

dant functionality (**17f–h**) resulted in derivatives with modest CHK1 activity. All of the C7 substituted amino derivatives in Table 2 demonstrated improved selectivity versus CDK2 (IC_{50} >30 μM). While simple aryl derivatives or heteroaryl derivatives demonstrated good CHK1 activity (**17i–j, o–p**), the proper placement of heteroatom functionality in these motifs was important for retaining CHK1 potency as illustrated by **17k–n**. Aminoisothiazole **17r** emerged from the SAR work at the C7 position with excellent potency for CHK1 (<10 nM) and very good selectivity over CDK2.

In order to better understand the SAR trends observed in Tables 1 and 2, a single crystal X-ray structure of **17r** bound to CHK1 (shown in Fig. 3) was obtained.[13] In the X-ray structure, three key interactions were observed between **17r** and the CHK1 protein. First, the N1 moiety and C7 NH of the pyrazolo[1,5-*a*]pyrimidine core bind to the peptide backbone in the hinge area. Secondly, the 1-methylpyrazole moiety at the C3 position interacts with an array of ordered water molecules in the kinase specificity domain of CHK1. The SAR observed for the C3 heterocyclic derivatives shown in Table 1 may be explained in part by the propensity of these motifs to interact favorably with these water molecules which may mediate interactions with other amino acids. Lastly, the C5 piperidine nitrogen interacts with the carboxylate of Glu 91 as well as the amide carbonyl of Glu 134. Interestingly, the SAR observed at the C7 amino group (Table 2) is difficult to rationalize since this substitution projects into the solvent-exposed region based upon the X-ray structure depicted in Figure 3. Additional SAR efforts directed toward trying to elucidate the structural/electronic requirements in this region of this class of CHK1 inhibitors will be discussed in the accompanying manuscript.[14]

In summary, systematic optimization of both the C3 and C7 positions of pyrazolo[1,5-*a*]pyrimidine CHK1 hit **4** led to the discovery of potent, selective CHK1 inhibitors represented by **17r**. Single X-ray crystallography of **17r** in CHK1 elucidated several key interactions with the protein that appear to be critical to the improvement of CHK1 potency and selectivity versus CDK2 for this class of compounds. Additional SAR development and further analysis of this novel class of CHK1 inhibitors is found in the accompanying paper.[14]

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10. Full experimental details have appeared elsewhere: Guzi, T. J.; Paruch, K.; Dwyer, M. P.; Parry, D. A. US 2007/0082900.
11. Protection of the C7 amino group as the di-SEM analog proved to be optimal for efficient coupling reactions using either the Suzuki or Stille coupling protocols.
12. *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.
13. The coordinates of compound **17r** bound to CHK1 have been deposited in the Protein Databank: pdb ID 3OT8.
14. Labroli, M.; Paruch, K.; Dwyer, M. P.; Alvarez, C.; Keertikar, K.; Poker, C.; Rossman, R.; Fischmann, T. O.; Duca, J. A.; Madison, V.; Parry, D.; Davis, N.; Seghezzi, W.; Wiswell, D.; Guzi, T. J. *Bioorg. Med. Chem. Lett.* **2010**, *21*, 471.