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Discovery of pyrazolo[1,5-*a*]pyrimidine-based CHK1 inhibitors: A template-based approach—Part 2

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ABSTRACT

Previous efforts by our group have established pyrazolo[1,5-*a*]pyrimidine as a viable core for the development of potent and selective CDK inhibitors. As part of an effort to utilize the pyrazolo[1,5-*a*]pyrimidine core as a template for the design and synthesis of potent and selective kinase inhibitors, we focused on a key regulator in the cell cycle progression, CHK1. Continued SAR development of the pyrazolo[1,5-*a*]pyrimidine core at the C5 and C6 positions, in conjunction with previously disclosed SAR at the C3 and C7 positions, led to the discovery of potent and selective CHK1 inhibitors.

As one of the key regulators of the cell cycle progression, CHK1 is a serine/threonine kinase which has been an attractive target in oncology.[1] A number of small-molecule ATP-competitive CHK1 inhibitors have been described[2] and several compounds have recently entered the clinic including PF-00477736 (**1**)[3] and AZD7762 (**2**)[4] (Fig. 1). Herein, we describe our continued SAR development

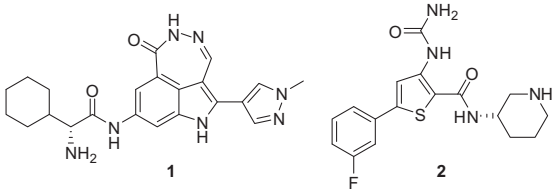

Figure 1. CHK1 inhibitors currently under clinical evaluation.

of the pyrazolo[1,5-*a*]pyrimidine core to develop potent and selective CHK1 inhibitors.

Initial screening of our internal compound library identified compound **3** as a CHK1 program hit which was derived from an earlier CDK program (Fig. 2). Initial SAR optimization work on the pyrazolo[1,5-*a*]pyrimidine scaffold at both the C3 and C7–NH_2 positions led to several promising targets, including **4** and **5**, which displayed improved in vitro potency for CHK1 and increased selectivity against CDK2 versus the early hit **3** (Fig. 2).[5]

As discussed in the preceding Letter,[5] the SAR study of the C3 position demonstrated that the 4-*N*-methylpyrazole moiety conferred optimal CHK1 potency while maintaining selectivity against CDK2. Additionally, structural modifications of the C7–NH_2 position were extremely challenging as a majority of substituents

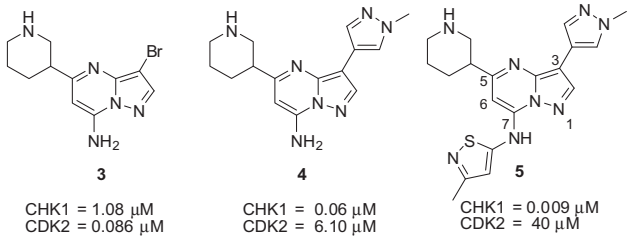

Figure 2. Pyrazolo[1,5-*a*]pyrimidine hits for CHK1 program.

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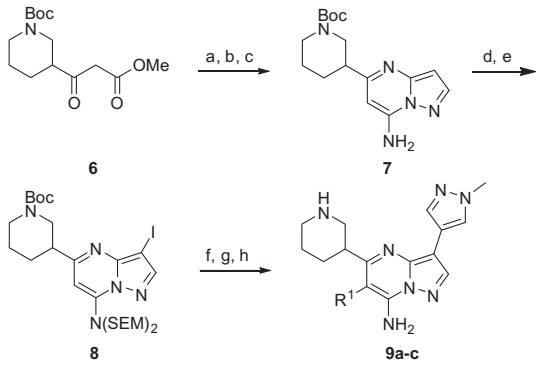
incorporated in this region displayed a loss in CHK1 potency versus the C7–NH_2 analog with the exception of compound **5**. These observations could, in part, be attributed to the deleterious effect of the C7–NH_2 substituents on the crucial H-bonding motif in the hinge region.[5] In light of these observations, we felt there might be additional opportunities to explore the solvent exposed region using C6 substitution. Initial synthetic efforts focused upon the preparation of the 6-halo compounds **9a–c** which maintained key functionality at C3 (*N*-methyl pyrazole) and C5 (3-piperdine) that was previously noted for CHK1 potency.[5] It was envisioned that the C6 halo functionality could serve as handles for further elaboration at the C6 position. The preparation of these analogs was discussed previously and shown in Scheme 1.[5,6] Interestingly, the 6-halo compounds **9a–c** demonstrated a 20-fold improvement in CHK1 activity relative to the parent compound **4** (Table 1).



Scheme 1. Reagents: (a) 3-aminopyrazole, $PhCH_3$, 75%; (b) $POCl_3$, *N*,*N*-dimethylaniline, 71%; (c) NH_3, 2-propanol, H_2O, 98%; (d) SEMCl, DIPEA $(CH_2Cl)_2$, 76%; (e) NIS, CH_3CN, 92%; (f) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $PdCl_2dppf$, K_3PO_4, DME, 81%; (g) HCl, EtOH, 90%; (h) NCS, CH_3CN, 72% or NBS or Br_2, *t*-$BuNH_2$, CH_2Cl_2, 73% or NIS, CH_3CN, 83%.

Unfortunately, all attempts to install additional functionality at the C6 position via the 6-halo precursors using either Suzuki or Stille coupling protocols were unsuccessful (not shown). While the limitations of using these coupling protocols in sterically congested systems has been documented,[7] the initial synthetic route had to be modified to allow for more facile incorporation of substituents at the C6 position which is shown in Scheme 2. Treatment of **7** with bromine in *t*-butylamine[8] yielded the corresponding 6-bromo derivative **10** for further elaboration (Scheme 2). Bisprotection of **10** with SEMCl[9] followed by Pd-mediated couplings introduced desired substitution at the C6 position to yield compound **11**. Functionalization of the C3 position was achieved by bromination, Suzuki or Stille coupling with the appropriate heteroaromatic group, and global deprotection to afford targets **9d–q**. The biochemical assay results for both CHK1 and CDK2 are summarized in Table 1 for compounds **4 and 9a–q**.

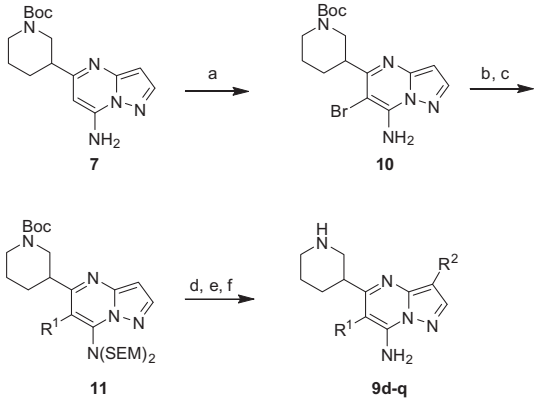
As evident from Table 1, small alkyl or cycloalkyl substituents at the C6 position retained reasonable CHK1 potency with varying levels of CDK2 selectivity (**9d–i**) similar to the 6-halo compounds **9a–c**. Incorporation of either aryl or heteroaryl derivatives at the C6 position generally led to a loss of CHK1 potency versus the smaller substituents while maintaining some selectivity over CDK2 (**9j–p**). Although it appears that the solvent exposed region would be more accommodating to a variety of substituents, the SAR observed at the C6 position may be a combination of both size and polarity requirements of the substituents. Additionally, the electron withdrawing substituents such as the 6-halo derivatives **9a–c** seem to be preferred at this position and may in fact play a role in modulating the acidity of the adjacent C7–NH_2 group.

Based upon the SAR observed in Table 1, we decided to briefly investigate a series of C3 heteroaromatic groups bearing the C6–Br to determine if we were maintaining optimal potency and selectivity among the C3, C6, and C7 substituents. Representative examples are depicted in Table 2. Although compound **9b** remained the most potent analog, other C3 heteroaromatic groups, for example, **12c**, demonstrated comparable CHK1 potency with a comparable selectivity profile against CDK2.

With the initial SAR investigations at the C3, C6, and C7–NH_2 positions of the pyrazolo[1,5-*a*]pyrimidine core complete, attention was turned toward exploration of the C5 position. From the X-ray structure of compound **5** bound to CHK1,[5] the NH of the C5 3-piperidinyl group was observed to form several key hydrogen bond interactions with several acidic residues as well as a conserved water molecule in this region. Initial SAR efforts at the C5 position focused upon further optimization of this key H-bonding interaction. In order to more rapidly examine the SAR in this region, an alternative synthesis was developed taking into account the optimal substituents at the C3, C6, and C7 positions of the pyrazolo[1,5-*a*]pyrimidine core. Retrosynthetically, we envisioned a more convergent assembly of the pyrazolo[1,5-*a*]pyrimidine core (e.g., **4)** via the cyclocondensation of β-keto nitrile **13** and 3-amino-1-methyl-1*H*-1′*H*-4,4′-bispyrazole **14** (Fig. 3).

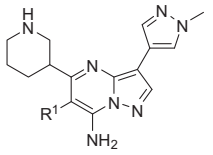
The preparation of aminopyrazole **14**[11] began with Vilsmeier-Haack formylation of *N*-methyl-1*H*-pyrazole **15** to afford aldehyde **16** (Scheme 3). Treatment of **16** with tosyl methyl isocyanide[12] (TosMIC) in the presence of potassium *t*-butoxide in DME resulted in the one-step homologation and cyanation process to yield the acetonitrile **17**. α-Formylation, followed by cyclization with hydrazine monohydrochloride in ethanol yielded the final bispyrazole **14**. It should be noted that this preparation of bispyrazole **14** was amenable to large-scale synthesis and the use of TosMIC avoids the need for toxic cyanide reagents commonly employed in other cyanation protocols.

With the bispyrazole **14** in hand, the syntheses of the C5 targets **22a–k** were accomplished utilizing the route displayed in Scheme 4. Treatment of acids **19** with 1,1-carbonyldiimidazole followed by addition of the anion of acetonitrile provided the substituted β-keto nitriles **20** in good yield. Cyclocondensation of **20** with bispyrazole **14** from Scheme 4 in EtOH or toluene provided the core **21**. Regioselective bromination with NBS followed by acid depro-



Scheme 2. Reagents: (a) Br_2, *t*-$BuNH_2$, CH_2Cl_2, 79%; (b) SEMCl, DIPEA $(CH_2Cl)_2$, 39%; (c) $R^1B(OH)_2$, $PdCl_2dppf$, K_3PO_4, DME, H_2O or Bu_3SnR^1, $Pd(PPh_3)_4$, dioxane, 27–83%; (d) NBS, CH_3CN, 66–92%; (e) $R^2B(OH)_2$, $PdCl_2dppf$, K_3PO_4, DME, H_2O or Bu_3SnR^2, $Pd[PPh_3]_4$, dioxane, 41–90%; (f) HCl, EtOH, 17–80%.

Table 1
CHK1 and CDK2 inhibitory activity of pyrazolo[1,5-*a*]pyrimidines **4** and **9a–p**



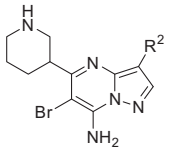
Compds	R^1	CHK1 IC_{50}[a] (μM)	CDK2/cyclin A IC_{50}[b] (μM)
4	H	0.060	6.1
9a	Cl	0.003	0.31
9b	Br	0.003	0.16
9c	I	0.003	0.33
9d	Et	0.031	0.33
9e	Propynyl	0.045	0.52
9f	Cypr	0.017	0.17
9g	$(CH_2)_2OH$	0.026	0.048
9h	CH=CH–CH_2OMe	0.052	2.2
9i	CN	0.10	1.7
9j	Ph	0.56	8.3
9k	2-Thienyl	0.086	0.99
9l	3-Thienyl	0.16	2.2
9m	3-Furyl	0.34	2.3
9n	3-Pyridyl	0.51	1.5
9o	4-Pyridyl	0.23	11
9p	4-*N*-Methylpyrazolyl	0.10	22

Values reported are means of two experiments.
[a] Assay conditions can be found in Ref. 5.
[b] Assay conditions can be found in Ref. 10.

tection of Boc intermediates, when necessary, provided the desired targets **22a–k** listed in Table 2.

As summarized in Table 3, the SAR at C5 clearly demonstrates the necessity of both the proper positioning and basicity of the piperdine NH for optimal CHK1 potency and selectivity against other kinases, specifically CDK2. Loss of the piperdine NH (e.g., **22a**) as

Table 2
CHK1 and CDK2 inhibitory activity of C3-substituted pyrazolo[1,5-*a*]pyrimidines **9b** and **12a–c**



Compds	R^2	CHK1 IC_{50}[a] (μM)	CDK2/cyclin A IC_{50}[b] (μM)
9b		0.003	0.16
12a		0.017	0.17
12b		0.025	0.10
12c		0.008	0.20

Values reported are means of two experiments.
[a] Assay conditions can be found in Ref. 5.
[b] Assay conditions can be found in Ref. 10.

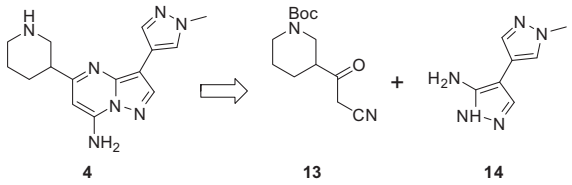
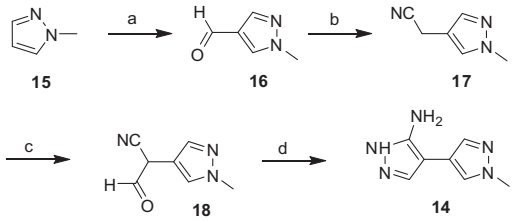


Figure 3. Retrosynthetic analysis of **4**.

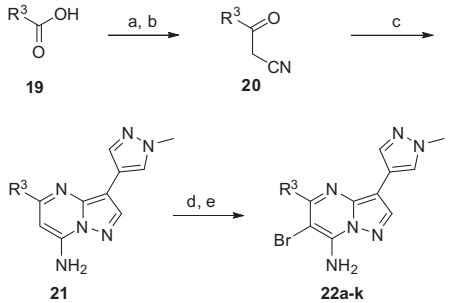
well as different positional isomers (**22b,c)** led to a loss of CHK1 potency. Additional heteroatoms are tolerated with varying degrees of CHK1 potency (e.g., **22d,e**) while ring-contracted (**22f**) or the acyclic amine derivative (**22g**) generally showed reduced CHK1 potency. Several homologated analogs (**22i,j**) as well as exocyclic amine analog **22k** demonstrated comparable CHK1 potency to **9b** with a comparable or improved selectivity profile against CDK2 (Table 3).

A single X-ray crystal structure of **22k** bound to CHK1 (Fig. 4) was obtained which confirmed a similar binding mode to that observed for compound **5**.[5] From Figure 4, the key H-bond network from the C3 pyrazole in **22k** with ordered waters is maintained while the C7–NH_2 maintains a key hinge contact with the C6–Br group projecting into the solvent exposed region as expected. Interestingly, the exocyclic NH_2 of **22k** occupies a similar position as the piperidine NH of **5** to maintain the key interactions with an acidic group and a conserved water molecule which is imperative for CHK1 potency as demonstrated in Table 3.

Starting with a CDK2 selective pyrazolo[1,5-*a*]pyrimidine CHK1 lead **3**, systematic SAR studies of the C3, C5, C6, and C7–NH_2 positions of the core led to the identification of potent and selective CHK1 inhibitors, for example, **5**, **9a–c**, **12c**, and **22i–k**.[5] Interestingly, potent CHK1 inhibition can be retained through the appropriate combination of C6 substitution with a primary amine at

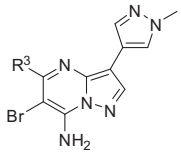


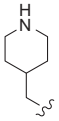
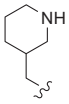
Scheme 3. Reagents: (a) $POCl_3$, DMF, 50%; (b) KO-*t*-Bu, TosMIC, DME, 65%; (c) ethyl formate, KO-*t*-Bu, DME, 82%; (d) N_2H_4–HCl, EtOH, 90%.



Scheme 4. Reagents and conditions: (a) CDI, THF; (b) LiHMDS, CH_3CN, THF, −78 °C, 22–78% overall for two steps; (c) **14**, EtOH, 45 °C, or toluene, 115 °C, 37–87%; (d) NBS, DCM, 71–89%; (e) TFA, DCM, 39–93%.

Table 3
CHK1 and CDK2 inhibitory activity of C5-substituted pyrazolo[1,5-*a*]pyrimidines **9b** and **22a–k**



Compds	R^3	CHK1 IC_{50}[a] (μM)	CDK2/cyclin A IC_{50}[b] (μM)
9b		0.003	0.16
22a		4.0	1.3
22b		2.9	28.7
22c		0.035	0.49
22d		0.13	1.2
22e		0.006	0.15
22f		0.031	0.51
22g		0.034	0.92
22h		0.087	2.8
22i		0.007	0.84
22j		0.007	2.4
22k[c]		0.005	0.44

Values reported are means of two experiments.
[a] Assay conditions can be found in Ref. 5.
[b] Assay conditions can be found in Ref. 10.
[c] 1′,3′-Syn.

C7. Approaches to differentiate between this novel class of pyrazolo[1,5-*a*]pyrimidines and those identified in the preceding Letter[5]

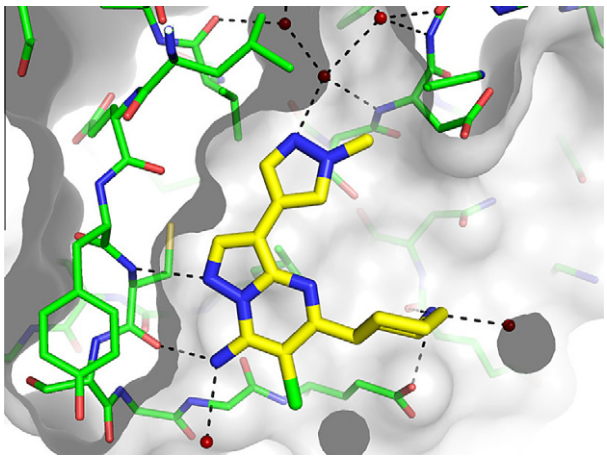


Figure 4. X-ray of crystal structure of **22k** in CHK1.[13]

as well as to calibrate the extent of CDK2 selectivity required in a CHK1 inhibitor utilizing a mechanistically based in-cell evaluation assay will be the subject of a future Letter.

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