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Structure-based design of 2,6,7-trisubstituted-7*H*-pyrrolo[2,3-*d*]pyrimidines as Aurora kinases inhibitors

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ABSTRACT

This Letter reports the optimization of a pyrrolopyrimidine series as dual inhibitors of Aurora A/B kinases. This series derived from a pyrazolopyrimidine series previously reported as inhibitors of aurora kinases and CDKs. In an effort to improve the selectivity of this chemotype, we switched to the pyrrolopyrimidine core which allowed functionalization on C-2. In addition, the modeling rationale was based on superimposing the structures of Aurora-A kinase and CDK2 which revealed enough differences leading to a path for selectivity improvement. The synthesis of the new series of pyrrolopyrimidine analogs relied on the development of a different route for the two key intermediates 7 and 19 which led to analogs with both tunable activity against CDK1 and maintained cell potency.

The Aurora proteins form a small family of serine/threonine kinases which have become prominent targets for the modulation of cell cycles. The three types of Aurora kinases expressed in humans (Aurora-A, -B and -C) play a critical role during mitosis especially in chromosome segregation and cytokinesis.[1] Aurora-A and -B kinases are overexpressed in human tumors including breast, lung, colon, ovarian, and pancreatic cancers. Overexpressed early in mitosis and localized to the centrosome and proximal spindles, Aurora-A kinase phosphorylates a variety of proteins such as TPX2[2] TACC3,[3] and others. Inhibition of Aurora-A kinase in cancer cells delays the mitotic entry and results in accumulation of cells in the G2/M cell cycle phase. On the other hand, localized to the kinetochores during mitosis and to the midbody during cytokinesis, Aurora-B kinase phosphorylates several proteins such as INCEMP[4] (inner centromere protein), histone H3 and RacGAP. Inhibition of Aurora-B kinase prevents the proper alignment of chromosomes to the spindle plate, halts cytokinesis, and results in the formation of multinucleated cells.

As previously reported,[5] the pyrazolopyrimidine analogs of type I were non-selective inhibitors of CDKs, AKA and AKB. In order to increase their therapeutic window, we envisaged to improve their kinase selectivity profile with a focus on dialing out the inhibition of CDKs.[6] Comparison of crystal structures of Aurora A and CDK2 reveals differences between Aurora and CDK around the binding pocket (Fig. 1). First, Aurora A has an amino acid insertion (G216) compared to CDK2 in the hinge region, creating a bulge and giving a more open conformation to the inter-domain loop. Second, the gatekeeper, which is part of the back pocket, is Leu for Aurora A and Phe for CDK2. In this study, the design efforts were based on

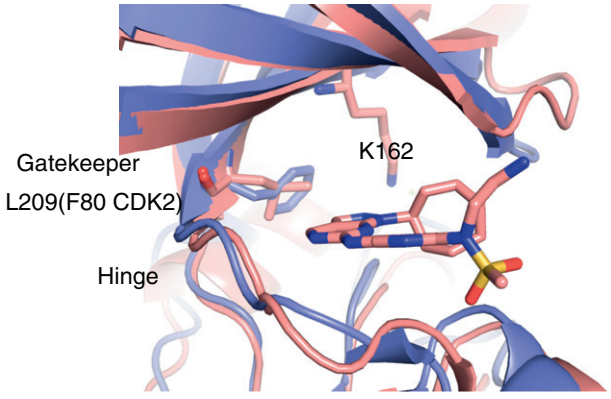

Figure. 1. Superimposed structure of Aurora A (pink, pdb code: 3R21) and CDK2 (purple, pdb code: 1OIT).

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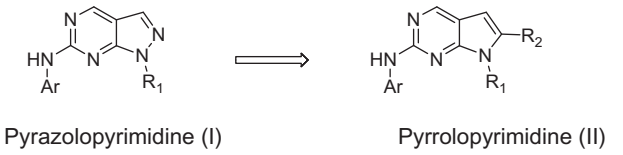
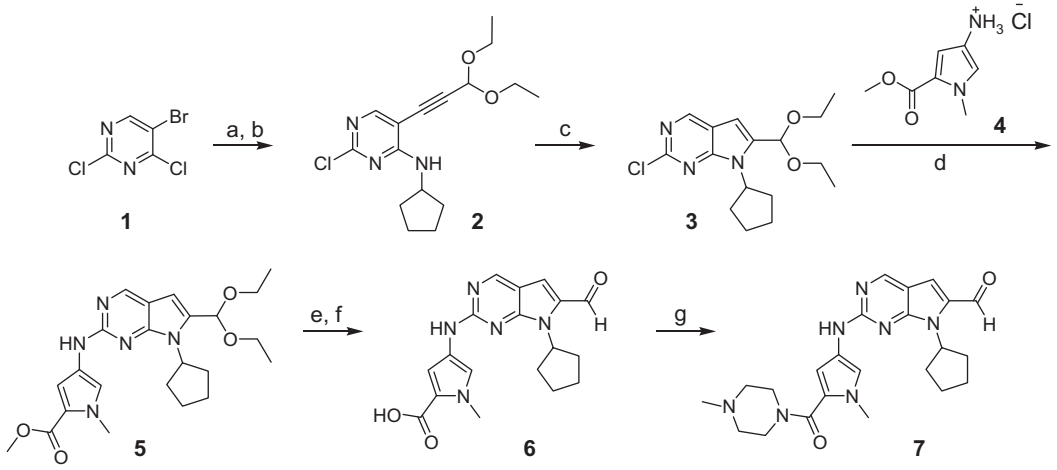


Figure. 2. Conversion of pyrazolopyrimidine to pyrrolopyrimidine core.

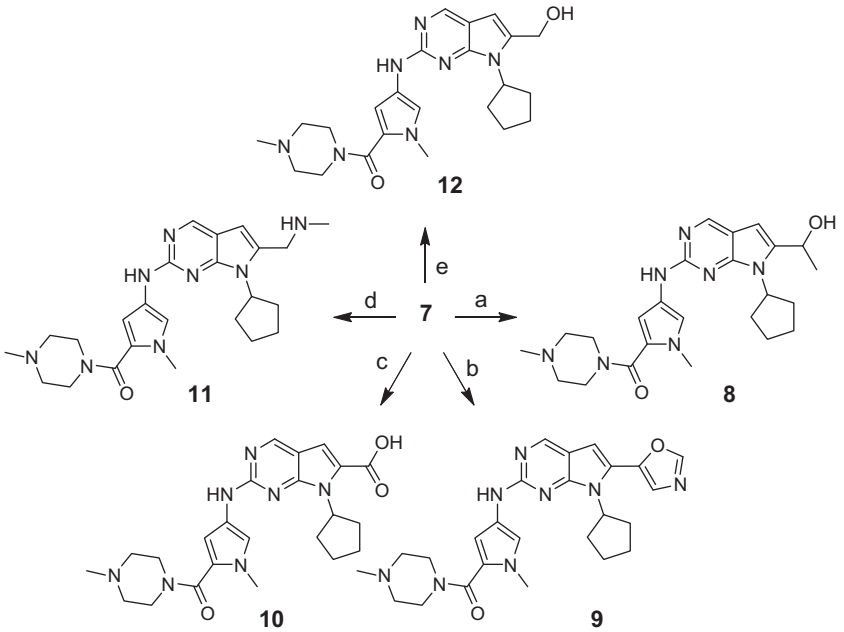
the previously reported crystal structure of pyrazolopyrimidine. Switching from the pyrazolopyrimidine to the pyrrolopyrimidine core (Fig. 2) allowed us to add a substituent at the C2 position. The substituents can potentially interact with the catalytic lysine[7] of aurora kinases for better potency, while reducing the potency for the CDKs due to the differences mentioned above.

Our initial efforts were focused on the small substituents off C2 position. The synthesis of the first set of pyrrolopyrimidine analogs started from the 5-bromo-2,4-dichloro-pyrimidine **1** which was treated with cyclopentylamine followed by a Sonogashira coupling with 3,3-diethoxy-propyne to give the compound **2** with moderated overall yield. After cyclization of **2** with TBAF,[8] **3** reacted with the aminopyrrole **4** to give **5** which, after hydrolysis of the ester, unmasking of the aldehyde and coupling with the *N*-methylpiperazine, produced the key amidoaldehyde intermediate **7** (Scheme 1). This latter was derivatized to the final compounds **8–15** using standard chemistry procedures (Schemes 2 and 3).

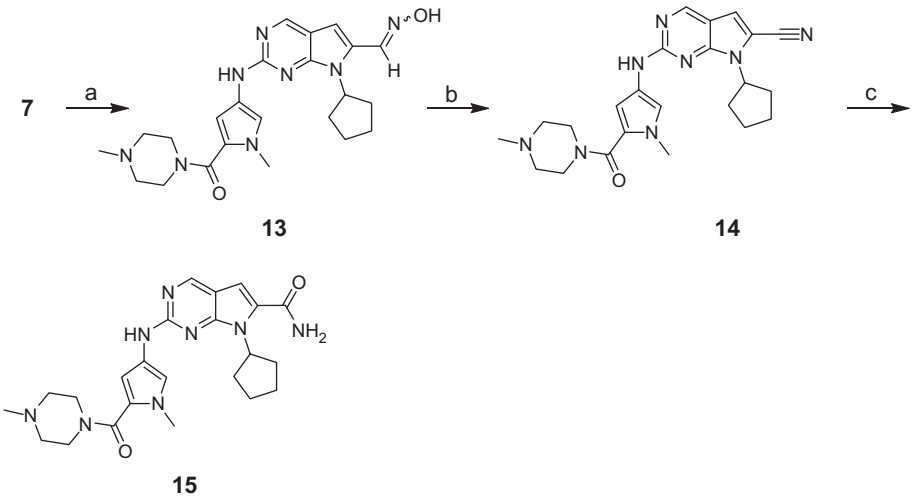
As mentioned in the introduction, the purpose of this study was to optimize the potency against AKA and AKB while dialing out activity against CDKs with a special focus on CDK1 by exploring R groups (Table 1). Without any substituent on C2 (R = H), the compound **16** exhibited moderate activity against CDK1 and suboptimum potency against AKA and AKB. Adding a CN or an oxime group (**13** or **14**) increased the potency against the three kinases by strengthening the interaction with the catalytic lysine supposedly without adding steric repulsion. A primary amide and a primary or secondary alcohol in this position (**15** and **8** or **12**)



Scheme 1. Reagents and conditions: (a) Cyclopentylamine, DIEA, MeOH, rt, 80%; (b) 3,3-diethoxypropyne, CuI, $dppfPdCl_2$, DIEA, DMF, rt, 41%; (c) TBAF, THF, Δ, 66%; (d) **4**, $Pd(OAc)_2$, Xantphos, Cs_2CO_3, dioxane, 80 °C, 64%; (e) HCl concn, dioxane, rt, 71%; (f) KOH 5N, MeOH, DMSO, rt, 82%; (g) *N*-methylpiperidine, HATU, DIEA, DMF, 50 °C, 58%.

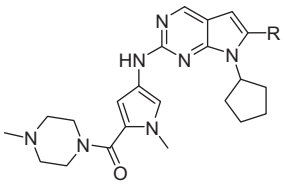


Scheme 2. Reagents and conditions: (a) MeMgBr, THF, −30 to 5 °C, 30%; (b) TosMIC, K_2CO_3, MeOH, Δ, 52%; (c) $NaClO_2$, NH_3SO_3, THF, water, rt, 13%; (d) $MeNH_2$, $NaCNBH_4$, rt, 20%; (e) $NaBH_4$, MeOH, 60%.



Scheme 3. Reagents and conditions: (a) NH_2OH–HCl, NaOAc, EtOH, Δ, 68%; (b) CDI, DCM, 5 °C, 71%; (c) KOH 5N, MeOH, dioxane, rt, 50%.

Table 1
In vitro SAR of 2-substituted pyrrolopyrimidines[a]



Compound	R	AKA IC_{50} (μM)	AKB IC_{50} (μM)	CDK1 IC_{50} (μM)	G2/M arrest (μM)	HCT116 IC_{50} (μM)
13	CHNOH	0.011	0.03	0.003	0.002	0.004
14	CN	0.0075	0.004	0.006	0.065	0.04
16	H	0.038	0.018	0.24	0.3	0.29
15	$CONH_2$	0.028	0.011	0.5	0.1	0.07
11	CH_2NHCH_3	0.04	0.06	0.7	0.3	0.21
8	CH(OH)Me	0.011	0.03	1.1	0.1	0.08
12	CH_2OH	0.02	0.04	1.3	0.1	0.13
10	COOH	0.35	1	10	10	12.3

[a] The IC_{50}s are determined based on a curve with 6 data points in duplicate. The G2/M arrest corresponds to the minimum concentration for a G2/M versus G1 peak ratio over 2.

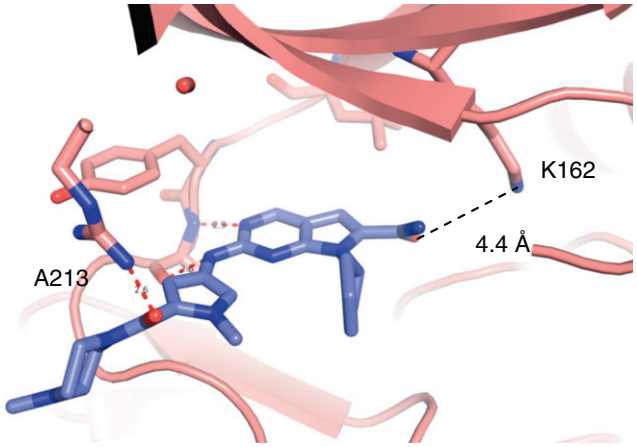


Figure. 3. Crystal structure of compound **15** (pdb code: 4DHF).

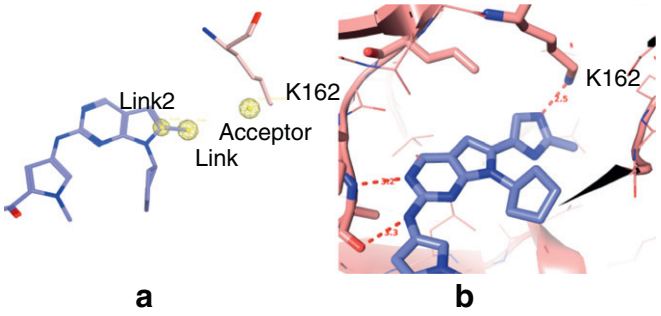
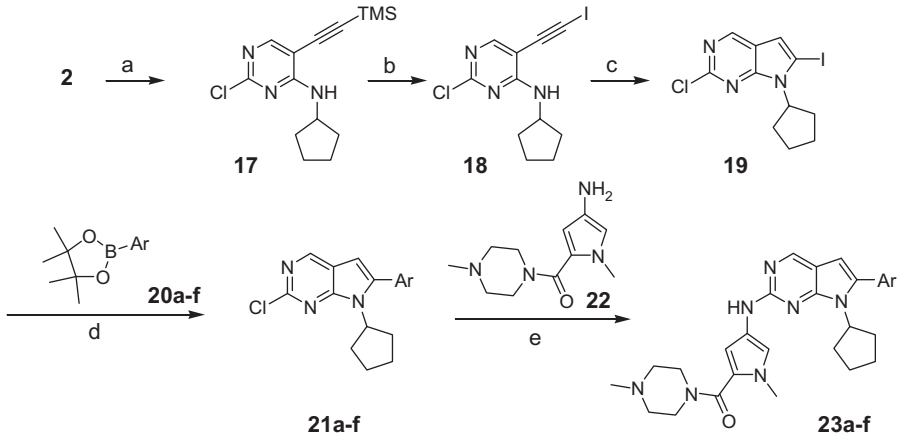


Figure. 4. (a) A pharmacophore for fragment search targeting K162; (b) one of the hits with pyrazole moiety.

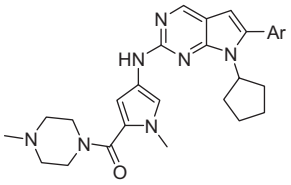
retained the high potency against AKA and AKB while increasing the IC_{50}s against CDK1 to the micromolar range. A positively charge secondary amine (**11**) reduced slightly the potency against AKA and AKB but had a more pronounced effect against CDK1. Finally, the negatively charge carboxylic acid (**10**) was not tolerated by any of the 3 kinases.

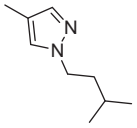
The binding mode of this class of pyrrolopyrimidine inhibitors was elucidated by co-crystallization of Aurora-A with compound **15** (Fig. 3). The compound forms two hydrogen bonds with hinge residue A213 and one hydrogen bond with R137. However, the oxygen of amide off C-2 position is 4.4 Å away from Nzeta of



Scheme 4. Reagents and conditions: (a) Trimethylsilylacetylene, CuI, dppfPdCl$_2$, DIEA, DMF, 40%; (b) NIS, AgNO$_3$, DMF, 68%; (c) TBAF 1M in THF, THF, 75%; (d) **20**, Pd(PPh$_3$)$_4$, K$_2$CO$_3$ 2M, DMF, 80 °C, 65%; (e) **22**, TsOH, DMF, 140 °C, MW, 48%.

Table 2
In vitro SAR of 2-heteroaryl-pyrrolopyrimidines[a]



Compound	Ar	AKA IC$_{50}$ (μM)	AKB IC$_{50}$ (μM)	CDK1 IC$_{50}$ (μM)	G2/M arrest (μM)	HCT116 IC$_{50}$ (μM)
9		0.002	0.004	0.028	0.03	0.06
23a		0.002	0.004	0.18	0.03	
23b		0.002	0.005	0.2	0.003	0.005
23c		0.002	0.004	0.33	0.03	0.11
23d		0.002	0.004	1.4	0.1	0.11
23e		0.005	0.004	1.7	1	0.59
23f		0.002	0.004	2	0.1	0.09

[a] The IC$_{50}$s are determined based on a curve with 6 data points. The G2/M arrest corresponds to the minimum concentration for G2/M versus G1 peak ratio over 2.

K162. In order to design more potent compounds through forming hydrogen bond with K162, a pharmacophore based approached was employed using MOE.[9] As shown in Figure 4a, the query pharmacophore consists of two link atoms off C2 position and one hydrogen bond acceptor, which can hydrogen bond with K162. One of the best hits is pyrazole (Fig. 4b). Thus, a set of 5-membered-ring heterocycle were proposed for synthesis.

For synthesizing the second set of pyrrolopyrimidine analogs, the intermediate **2** underwent a Sonogashira coupling with trimethylsilylacetylene followed by the treatment with NIS in presence of silver nitrate to give the iodoalkyne **18**. Cyclization of the latter and chemoselective Suzuki coupling with arylboronates **20a–f** led to **21a–f** which were coupled with the aminopyrazole **22** to produce the final compounds **23a–f** (Scheme 4).

The introduction of a small 5-membered heterocyclic ring on C2 maintained a strong potency against AKA and AKB independently of its substitution pattern (Table 2). On the other hand, only the oxazole analog **9** exhibited strong CDK1 potency. The other analogs with methyl substituents (compounds **23a–c**) had activities ranging from 0.18 to 0.33 μM; increasing further the size of the substituents (compounds **23d–f**) decreased further the CDK1 potencies to the micrometer range.

For this series, the cell potencies correlate better with the inhibition of Aurora A/B kinases than with the inhibition of CDK1 explaining why dialing out CDK1 activity does not reduce the cell potency dramatically.

In conclusion, this study demonstrated that the pyrazolopyrimidine to pyrrolopyrimidine core conversion gave rise to a new series of dual AKA/AKB inhibitors with tunable potency against CDK1. The weakest compounds against CDK1 remain to be screened against a large panel of kinases to assess their overall kinase selectivity profile.

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