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Discovery of a class of diheteroaromatic amines as orally bioavailable CDK1/4/6 inhibitors

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ABSTRACT

The discovery of a class of diheteroaromatic amines based on LY2835219 as cyclin-dependent kinase (CDK1/4/6) inhibitors was described. The series was found to have much more improved CDK1 inhibition and potent in vitro anti-proliferative effects against cancer cell lines. The synthesis and structure–activity relationship studies of these compounds were reported. One promising compound was selected to evaluate as a novel lead compound after in *vitro* and in *vivo* profiling.

Cyclin-dependent kinases (CDKs), such as CDK1, CDK2, CDK4, CDK6, CDK7, CDK8, CDK9 and CDK11, are serine/threonine kinases that play important roles in the control of cell division and modulate transcription in response to several extra- and intracellular cues. The CDKs regulate cell cycle progression through complexes with their corresponding cyclin partners such as cyclin A, B, D and E. For example, CDK4 and CDK6 complex with cyclin D, and CDK2 complex with cyclin E or A sequentially phosphorylate retinoblastoma protein (pRb) to facilitate the G1/S progression.[1] Deregulation of the cell cycle is a hallmark of cancer. Indeed, genetic or epigenetic mutations in cyclin D/CDK4 and CDK6/pRb pathway are commonly observed in many types of human cancers, suggesting CDK4/ 6 may be an attractive target for development of anti-cancer drugs. Abnormal CDK4/6 control of the cell cycle has been strongly linked to the molecular pathology of cancer. Inhibition of CDK4/6 has been shown to inhibition of cancer cell proliferation and apoptosis. Thus CDK4/6 has become attractive therapeutic targets for cancer therapy.[2]

Several highly selective CDK4/6 inhibitors have entered into clinical trials for the treatment of cancer, including PD0332991 (Palbociclib),[3] LEE011 (Ribociclib)[4] and LY2835219[5] (Fig. 1). PD0332991 and LEE011 have been launched to the market as a CDK4/6 dual inhibitor to treat breast cancer. Lilly's CDK4/6 inhibitor LY2835219 is a late-stage clinical drug candidate shows strong single-agent activity in preliminary clinical study in metastatic breast cancer, while Palbociclib and Ribociclib show activity only in combination therapy.[6]

As is known that CDK1 can also bind to cyclin E or cyclin D in the absence of CDK4 in mediating cell cycle progression, suggesting that highly selective inhibition of CDK4/6-cyclin D complex may cause resistance to cancer cell.[7] Therefore addition of some CDK1 inhibition activity to CDK 4/6 inhibitors may be able to give us a useful treatment for CDK4/6 inhibitor resistance cancer therapy. Although CDK1 plays an important role in normal cell cycles and over-inhibition of the activity of CDK1 may cause some side effects, there is still no direct conclusive evidence for CDK1 inhibitor entered into clinical trials.[8] Instead, it was reported that CDK1 inhibitors can enhance cytotoxic effects of anti-tumor chemotherapeutic drugs such as taxol which is widely used clinically,[9] may be useful for mTOR inhibitor-resistant cancer treatment,[10] or could lead a robust synthetic lethal effect for KRAS/CDK1 interaction.[11] Herein, we report our hypothesis and efforts to discover novel CDK4/6 inhibitors with additional CDK1 inhibition activity.

We selected Lilly's compound (LY2835219) as a starting point. From the docking model of LY2835219 and CDK1 (Fig. 2), it was found that there were some water molecules involved in the pyridine backbone interaction with the protein. We presumed that if additional interaction could be formed between the pyridine group

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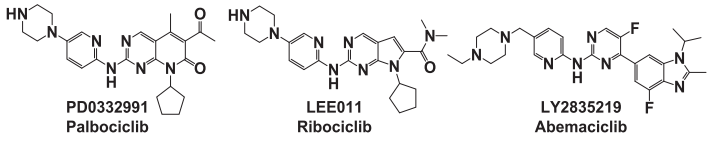


Fig. 1. Structures of known CDK4/6 inhibitors.

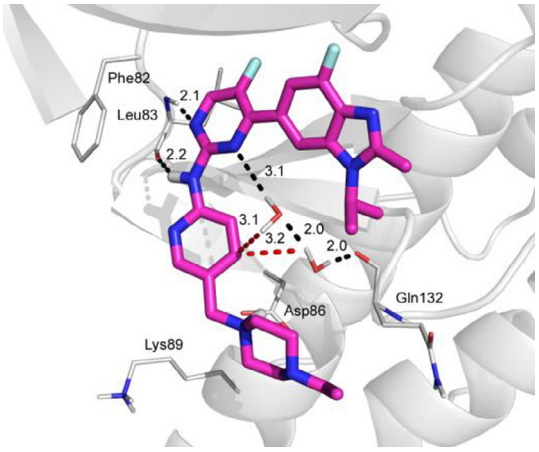
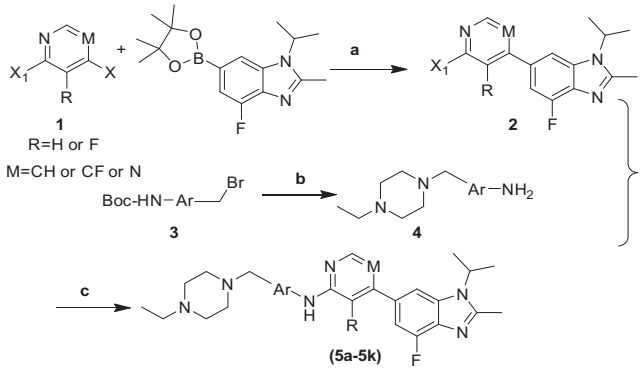


Fig. 2. Putative binding mode of LY2835219 (PDB code 5HQ0). LY2835219 was shown as magenta sticks. Hydrogen bonds (black dash) are labeled.



Scheme 1. General synthetic route of di-heteroaromatic amines **5a–5k**. Reagents and conditions: (a) $Pd(dppf)Cl_2$, Na_2CO_3 aq., DMF, 80 °C–120 °C, 45%–80%; (b) *N*-ethyl piperizine, DIPEA, DCM, rt; 4N HCl/dioxane, DCM, rt; 50%–90% for two steps; (c) $Pd_2(dba)_3$, X-Phos, K2CO3, DMF, 100 °C–120 °C, 30–65%.

of LY2835219 and the bridged waters, the potency of LY2835219 against CDK1 might be improved. Therefore we added nitrogen atom or changed the position of nitrogen atom of LY2835219 through scaffold-hopping and evaluated their biochemical and cellular activities. Herein we report our SAR results for the discovery a novel class of diheteroaromatic amines as CDK1/4/6 inhibitors.

First, we substituted various diaromatic amine groups for *N*-(pyridin-2-yl) pyrimidin-2-amine of LY2835219 by scaffold-hopping. A multi-step approach to synthesize the designed compounds would involve three key steps (Scheme 1): (a) Suzuki coupling of pyridinyl or pyrimidinyl halide (**1**) with boronic acid pinacoester; (b) *N*-ethyl piperazine substitution with aromatic methyl bromide **(3)** and then removal of the Boc group with 4 N HCl in dioxane; (c) Buchwald coupling of halopyridinyl or halopyrimidinyl 4-fluoro-1-isopropyl-2-methyl-1H-benzo[*d*]imidazole (**2**) with *N*-ethyl piperadinyl-aromatic amine (**4**).

The replacement of *N*-(pyridin-2-yl) pyrimidin-2-amine group in LY2835219 with different diheteroaromatic amines resulted in a new series of potent CDK4/6 inhibitors with further improvement CDK1 inhibition. Good to excellent enzymatic potency against CDK4/6 and CDK1, as well as the cell proliferation inhibition, were observed for various diheteroaromatic amine substituted analogues.

As shown in Table 1, compound **5a**-**5e** with (*N*-(pyridin-2-yl) pyrimidin-4-amine (**5a**), di(pyridin-2-yl)amine (**5b**), *N*-(pyrazin-2-yl)pyrimidin-4-amine (**5c**) and *N*-(pyridin-2-yl)pyrazin-2-amine (**5d**, **5e**) groups showed similar CDK4/6 inhibition activity with IC_{50} from 1 nM to 5 nM, but significant increase in enzymatic potency against CDK1, especially for compound **5d** and **5e** with CDK1 inhibition IC_{50} for 35.8 nM and 44.0 nM respectively. However, compound **5f**-**5i** with *N*-(pyridin-2-yl) pyridazin-3-amine (**5f**, **5i**), *N*-(pyrimidin-4-yl) pyridazin-3-amine (**5g**), *N*-(pyrimidin-4-yl) pyrimidin-2- amine (**5h**) groups showed a little decrease of CDK4 inhibition activity as well as the CDK1 inhibition activity. Compound **5j** with *N*-(pyrimidin-5-yl) pyrimidin-4-amine group completely lost its activity against CDK4. Unexpectedly, introduction of a fluorine atom to the pyrimidine group of compound **5c** resulted in more than 100 fold of CDK4 potency decrease comparison with compound **5c** (1.5 nM) and **5k** (283.4 nM). Comparison of compound **5a** (411.9 nM) with **5c** (135.5 nM), **5g** (2429.5 nM) and **5h** (20225.0 nM), all these compounds had the same aminopyrimidine hinge binding group and the only difference was the additional nitrogen atom in the pyridine group. It was clear that the additional nitrogen to the para-position of pyridine group was optimal for the CDK1 activity. The result could be also found between the compound **5b** (300.9 nM), **5d** (35.8 nM) and **5f** (800.9 nM). All these data showed that both *N*-(pyrazin-2-yl) pyrimidin-4-amine and *N*-(pyridin-2-yl) pyrazin-2-amine replacement were more suitable for remaining CDK4/6 activities with additional CDK1 potency.

As compounds **5c**, **5d** and **5e** were all having good inhibition potency against CDK1/4/6, we further characterized the metabolic stabilities of compound **5c** and **5e** to determine which scaffold (*N*-(pyrazin-2-yl) pyrimidin-4-amine **5c** or *N*-(pyridin-2-yl) pyrazin-2-amine **5e**) was more suitable to further lead optimization. PK parameters such as AUC, C_{max}, V_{ss} and Clearance rate were characterized after *iv* dosing of compound **5c** and **5e** in male ICR mice. From Table 2, it was found that compound **5c** had lower clearance and higher C_{max}/AUC than those of compound **5e**. It indicated that the scaffold of compound **5c** (*N*-(pyrazin-2-yl) pyrimidin-4-amine) was metabolically more stable than that of compound **5e**, because of the pyridine group in compound **5e** may be a liability to in *vivo* oxidation.

To better understand the mechanism of inhibition, molecular docking was used to analyze the putative binding mode of the designed compound with CDK1. The crystal structure of CDK1 in complex with an ATP-competitive inhibitor (PDB code 5HQ0) was selected for the docking studies. As depicted in Fig. 3, the para-position of pyridine in compound **5c** was 3.1 Å and 3.2 Å away from the two crystal waters, respectively. One of the nitrogen atom in pyrazine group formed water-mediated interaction with Gln132, which helped to improve the CDK1 inhibitory activity. As we expected, compound **5c** displayed more than 3-fold increase against CDK1 over compound **5a**, as well as the compound **5d** over **5b**. Meanwhile, the weak CDK1 inhibition activities of compound