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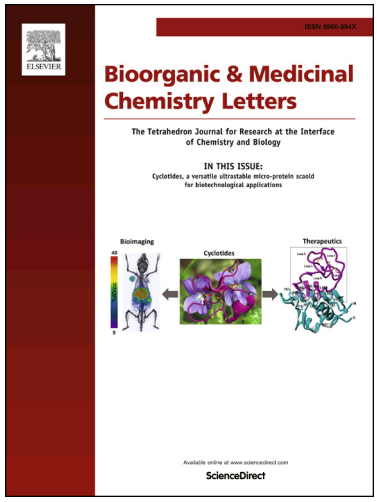

Design and synthesis of 4-(2,3-dihydro-1H-benzo[d]pyrrolo[1,2-a]imidazol-7-yl)-N-(5-(piperazin-1-ylmethyl)pyridine-2-yl)pyrimidin-2-amine as a highly potent and selective Cyclin-Dependent Kinases 4 and 6 Inhibitors and the discovery of structure-activity relationships

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ABSTRACT

Cyclin-dependent kinases 4/6 play an important role in regulation of cell cycle, and overexpress in a variety of cancers. Up to now, new CDK inhibitors still need to be developed due to its poor selectivity. Herein we report a novel series of 4-(2,3-dihydro-1H-benzo[d]pyrrolo[1,2-a]imidazole-7-yl)-N-(5-(piperazin-1-ylmethyl)pyridine-2-yl)pyrimidin-2-amine anologues as potent CDK 4/6 inhibitors based on LY2835219 (Abemaciclib). Compound **10d,** which exhibits approximate potency on CDK4/6 (IC_{50}=7.4/0.9 nM), has both good pharmacokinetic characters and high selectivity on CDK1 compared with LY2835219. Overall, Compound **10d** could be a promising candidate and a good starting point as anticancer drugs.

Cell cycle is a highly regulated process that leads to the transition from quiescence or cytokinesis to cell proliferation through its checkpoints ensures genome stability.[1] More importantly, cyclins and cyclin-dependent kinases (Cdks) play an important role in regulation of cell cycle.[2] In particular, D-type cyclins are overexpressed in tumor cells,[3] associated with Cdk4/6 to activate retinoblastoma protein (pRb) phosphorylation activity, which results in the release of E2F transcription factor and the activation of genes required for G1 phase to S phase transition.[4,5] Previous studies have shown that the CDK4/6-Rb-E2F pathway is disrupted in 90% of cancers.[6–9.]

CDK4/6 are critical regulators of cell cycle progression.[10] Surprisingly, genetic studies display that CDK4/6 are dispensable for the cell cycle.[11,12] Ablation of CDK4 kinase activity leads to complete tumor growth inhibition in CDK4/cyclin D1-dependent tumors.[13,14] Furthermore, genetic knock out experiments involving CDK4/6 in fibroblast cells are well tolerated due to compensation by CDK1.[15] Thus, it is suggested that a selective inhibitor of CDK4/6 may have a wider therapeutic window than pan-CDK inhibitors in cancer.

Kinase inhibitors can be classified into two types based on their modes of action: ATP-competitive inhibitors (I) and non-competitive inhibitors (II). Type I inhibitors bind to the ATP binding site through the formation of hydrogen bonds with the kinase “hinge” residues and hydrophobic interactions in surrounding the region occupied by the adenine ring of ATP. It is important to note that the recently reported CDK4/6 inhibitors (LY2835219, Palbociclib and Ribociclib) are all ATP competitive inhibitors.[10,16,17]

In the past few years, several small-molecule CDK inhibitors have been advanced to clinical trials and even approved for marketing (Palbociclib). More recently, another CDK 4/6 inhibitor, i.e. Ribociclib, has been used for the treatment of metastatic HR-positive, HER2-negative breast cancer combined with aromatase. LY2835219 is also a selective oral CDK4/6 inhibitor approved by the FDA recently (**Fig. 1**). In assessment,

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LY2835219 could selectively inhibit CDK4 and CDK6 with half maximal inhibitory concentration values of 2 and 10 nM,[10] respectively. However, its selectivity towards CDK1 is not good enough compared with Palbociclib and Ribociclib, which may bring more side effects and toxicities.[18] Therefore, we performed scaffold modification and structure–activity relationship (SAR) investigations of LY2835219 and its analogues to discover novel selective CDK4/6 inhibitors with drug-like properties.

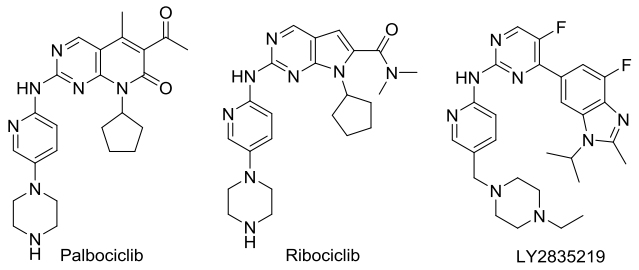


Fig. 1. Structure of Palbociclib, Ribociclib and LY2835219

According to the 2D interaction (**Fig. 2**),[19] some of these residues are critical structures for the CDK inhibition and selectivity. First, the introduction of the pyridine could enhance the CDK inhibition activity and selectivity over other kinases via interactions with the sidechain of hinge residue His100. Second, hydrogen bond between the ligands and the side chain of Lys43 also seems to contribute to a potent and selective CDK4/6 profile. Finally, the positively-charged piperazine ring of LY2835219 is stabilized by lying against a solvent-exposed ridge consisting of Asp104 and Thr107.[20] In CDK1/2/3/5, the residue analogous to CDK6-Thr107 is a lysine, which should lead to electrostatic repulsion between the piperazine and lysine, and thereby lower the CDK1/2/3/5 potency.

To preserve the activity and selectivity, the 1-ethylpiperazine ring with other scaffolds were modified, i.e. substituted piperazine or piperidine, which allowed for synthetic flexibility

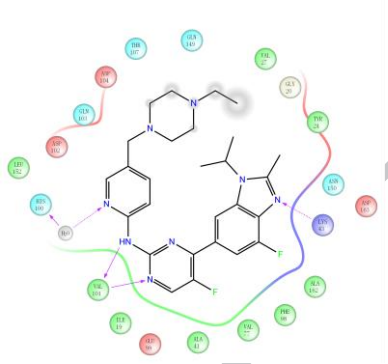
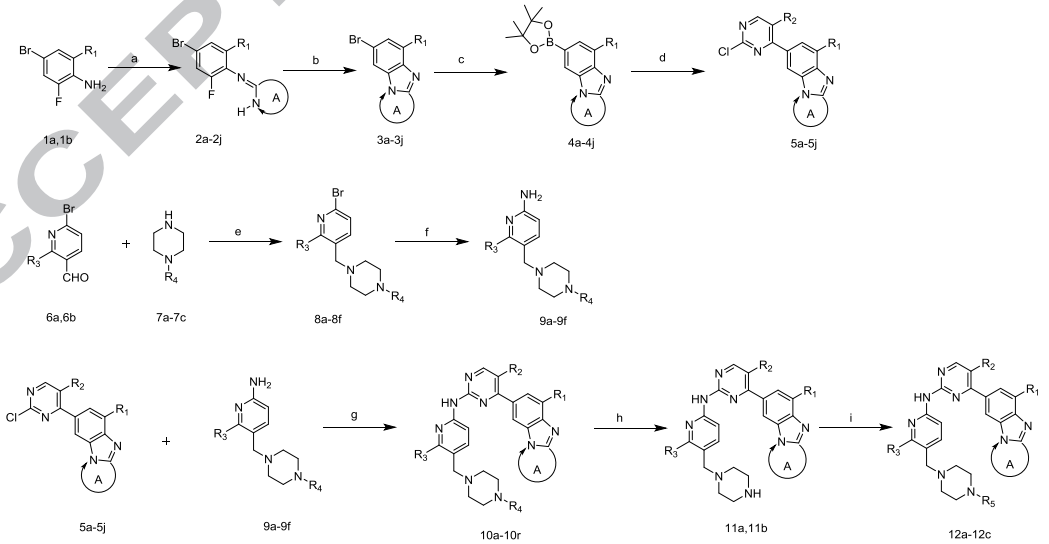


Fig. 2. 2D model of LY2835219 bound to the active site of CDK6

preclinical and also were the correct size for this position in structural modification. The pyridine ring and the imidazole were retained in our compounds, we attempted to add small groups to investigate the SAR surrounding the pyridine ring unit, and other naphthenic rings were incorporated into imidazole to investigate the inhibitory activities and selectivity, which we concluded this position might affect the molecule's selectivity for CDK1.[21, 22] In all, a series of compounds were designed, synthesized for CDK inhibitory activities evaluation.

The synthetic routes of the target compounds (**10a-10r**, **11a, 11b** and **12a-12c)** were outlined in **Scheme 1**. The key intermediate **5a-5j** were prepared from the commercially available 4-bromo-2-fluoroaniline (**1a-1b).** First, the starting material were treated with pyrrolidin-2-one to provide **2a-2j**, which was cyclized with Cs_2CO_3 and DMA at 180℃ to obtain the benzimidazole analogues (**3a-3j)**.

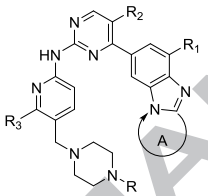


Scheme 1. Synthetic route for the target compounds. Reactions and conditions: (a) Toluene, pyrrolidin-2-one, TEA, $POCl_3$, reflux 3 h; (b) DMA, Cs_2CO_3, 180 °C,5 h; (c) Dioxane, Bis(pinacolato)diboron, Pd(dppf)Cl_2, AcOK, 100 °C, 6 h, 24.9%, three steps; (d) Dioxane/H_2O, pyrimidine, Pd(PPh_3)$_2Cl_2$, Na_2CO_3,100 °C, 3 h, 64%; (e) DCM, NaHB(OAc)$_3$, piperazine, RT, 5 h, 92%; (f) Toluene, 2-(Dicyclohexylphosphino)biphenyl, Pd_2(dba)$_3$, LiHMDS, 80 °C, 12 h, 61%; (g) Dioxane, Pd_2(dba)$_3$, XantPhos, Cs_2CO_3, 120 °C, 1 h, microwave, 12.4%; (h) DCM, TFA, RT, 3 h, 42%; (i) DMF, K_2CO_3, 80 °C,5 h, 14%.

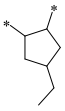
Second, compound **3a-3j** was converted to the corresponding heteroarylboronate ester (**4a-4j**) which underwent palladium catalyzed Suzuki reaction with 2,4-dichloropyrimidine to yield **5a-5j**, Compound **8a-8f** was prepared from substituted 6-bromonicotinaldehyde reductive amination with piperazine, catalyzed with $Pd_2(dba)_3$ and LiHMDS to give **9a-9f**. The target compounds **10a-10r** was obtained by utilizing Buchward coupling reaction of **5a-5j** and **9a-9f**. **10h**, **10i** and **10r** were deprotected to afford **11a-11c**, then docking other group to give target compounds **12a-12c**.

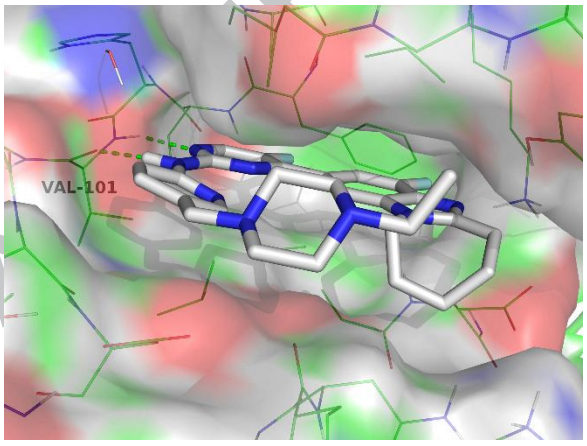
The novel LY2835219 analogue inhibitors (**10a-10r, 11a, 11b, 12a-12c**) showed good activities and achieved the initial goals. the structure-activity relationship was summarized in **Table 1**. When R^1 or R^2 was substituted with hydrogen (**10f** and **10g**) , they had slight impact to the CDK4/6 activities, however, it showed high inhibition to the CDK1. When an additional substituent methyl was drawn to the R^3 (**11b**, **10j**, **10k**, **10q),** the activities dropped largely, probably due to steric hindrance. R substituted compounds did not modulate CDK4/6 inhibitory activity significantly, however, CDK1's activity was deeply exchanged, which need further experiments to explain this phenomenon. The group A was substituted by bulkier aliphatic rings, like cyclopentyl, cyclohexyl, cycloheptyl (**10a**, **10b**, **10c)**, they had obvious effect on the CDK4/6 activity, when the group A substituted by methylcyclopentyl, ethylcyclopentyl or dimethylcyclopentyl analogues, and they had minor effect on the CDK4/6 activity, because they could tightly fit into the cavity. On the basis of Docking studies,[19] this effect did not derive from nonspecific lipophilicity but requires the specific steric demand available, In turn, unavailable spatial position also affected hydrogen bonding between the ligands and the side chain of Lys43(**Fig 3**).

Table 1. The structures and enzyme activity of compounds **10a-10r**, **11a**, **11b**, **12a-12c**.



Compd.	R^1	R^2	R^3	R	A	IC_{50} CDK1/D3 IC_{50} (nM)	IC_{50}(CDK4)/ IC_{50}(CDK6) IC_{50} (nM)
LY2835219	F	F	H	Et	-	56.0	1.7/7.8
10a	F	F	H	Et		1460.0	18/ 244.1
10b	F	F	H	Et		1535.0	6.8/45.6
10c	F	F	H	Et		1550.0	147.3/247.8
10d	F	F	H	Et		2670.0	7.4/0.9
10e	F	F	H	Me		353.9	0.8/4.0
10f	F	H	H	Et		610.0	1.0/17.4
10g	H	F	H	Et		733.0	1.72/15.8
11a	F	F	H	H		1365.0	0.6/3.7
11b	F	F	Me	H		2333.0	18.9/18.7
12a	F	F	H	2-fluoroethyl		1610.0	5.7/11.2

10j	F	F	Me	Et		1914.0	16.0/46.0
10k	F	F	Me	Me		2268.0	25.0/271.0
12b	F	F	H	Cyclopropyl		1874.0	9.7/19.0
10l	F	F	H	Et		1920.0	15.8/392.0
10m	F	F	H	Et		1880.0	49.7/339.0
10n	F	F	H	Et		2075.0	20.0/36.0
10o	F	F	H	Et		2189.0	27.0/28.0
10p	F	F	H	Me		2988.0	5.7/275.0
10q	F	F	Me	Et		2950.0	39.0/178.0
12c	F	F	H	i-Pr		3072.0	0.6/12.7
10s	F	F	H	Me		5050.0	10.0/2.4
10t	F	F	H	Et		5562.0	14.0/22.0
10u	F	H	H	Et		3363.0	17.6/752.0



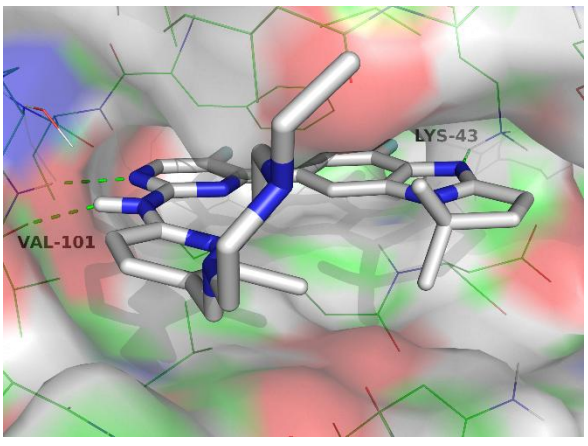


Fig. 3. Binding of compound **10c** and **10d** in CDK6 catalytic domain.

The above discussions have been validated by a docking study of compound **10c and 10d** into the catalytic domain of LY2835219 (PDB code: 5L2S), and the result is shown in **Figure 3**. [19] We found the dimethylcyclopentyl group of compound **10d** tightly fit into the catalytic domain which contributed to hydrogen bond between the nitrogen atoms of imidazole and the NH of Lys43. However, bulkier cycloheptyl of compound **10c** is tortuous in the cavity which results in the loss of the binding affinity to the kinase target. i.e, the losing of hydrogen bond with Lys43.

On the basis of low activity of CDK1 and high activity of CDK4/6, we chose **10d, 11b, 10n, 10o, 12c, 10s, 10t** to test cell activity (**Table 2**). Only **10d** exhibited good potency among these compounds. In addition, the hERG inhibitory of **10d** was also tested (**Table 3**), and compound **10d** showed low heart toxicity.

Table 2. The cell activity of compounds **10d, 11b, 10n, 10o, 12c, 10s, 10t**.

Compd.	LY2835219	10d	11b	10n	10o	12c	10s	10t
MDB-MA-231 IC_{50} (nM)	191	232	2126	5560	2108	1159	2630	2527

Table 3. The properties of hERG channel of **10d** and **LY2835219**.

Compd.	Amitriptyline (Positive Control)	LY2835219	10d
IC50 (μM)/hERG	3.29	10.90	6.38

Table 4. Pharmacokinetic parameters of rat (5mg/kg)

code	Cmax (ng/mL)	AUC (ng/Ml*h)	T1/2 (h)	MRT (h)	CL/F (ml/min/kg)	Vz/F (ml/kg)
LY2835219	312.0±33.0	3275.0±731.0	4.07±2.31	7.97±1.17	24.20±2.35	8533.0±1580.0
10d	343.0±67.0	3746.0±697.0	4.62±0.20	7.58±0.15	21.90±3.67	8751.0±1657.0

In summary, a novel series of substituted of LY2835219 derivatives were developed by changing A (**Table** 1) regions with the combination of potent activity on CDK4/6 and selectivity on CDK1, Compound **10d** also demonstrated good pharmacokinetic effects and druglike properties suitable for development (**Table 4**). Thus, the compound **10d** was worthwhile for deeper research as anticancer drugs. Further biological evaluation of these compounds is currently underway in our laboratory.

Acknowledgments

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