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Structure-based drug design of a highly potent CDK1,2,4,6 inhibitor with novel macrocyclic quinoxalin-2-one structure

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Abstract—The design of a novel series of cyclin-dependent kinase (CDK) inhibitors containing a macrocyclic quinoxaline-2-one is reported. Structure-based drug design and optimization from the starting point of diarylurea **2**, which we previously reported as a moderate CDK1,2,4,6 inhibitor [*J. Biol.Chem.* **2001**, *276*, 27548], led to the discovery of potent CDK1,2,4,6 inhibitor that were suitable for iv administration for in vivo study.

The cyclin-dependent kinase (CDK) protein family plays key roles in cell-cycle regulation in eukaryotic cells.[2] Orderly cell-cycle progression requires CDK activation, which is mainly controlled by expression of their activator subunit, cyclin. CDK1 (CDC2) complexed with cyclin B is key in G2/M phase progression. CDK4 and CDK6 complexed with cyclin D, and CDK2 complexed with cyclin E or A, sequentially phosphorylate retinoblastoma protein to facilitate G1/S progression. Retinoblastoma protein is a negative regulator of transcription factor E2F; when hyperphosphorylated, it releases E2F, which activates the transcription of genes whose products are critical for cell-cycle progression. Deregulation of the cell cycle is a hallmark of cancer;[3] indeed, genetic or epigenetic changes that lead to cyclin overexpression, or the absence or reduction of CDK-inhibitor proteins are commonly observed in human cancers. Consequently, CDK1,2,4, and 6 are attractive targets for new anticancer drugs. Recently the clinical progress of CDK1,2,4, and 6 inhibitors has been reviewed.[4]

We previously reported the diarylurea class of compounds, represented by compound **1** (Table 1), to be novel selective CDK4 inhibitors.[5,6] The pharmacological profile and value in cancer therapy of a CDK1,2,4,6 inhibitor are expected to be different from those of the CDK4-selective inhibitors; a potent CDK1,2,4,6 inhibitor with minimum off-target activity is desired. In the course of our structure–activity relationship study of the diarylurea class of compounds, we found that compound **2**, which lacks side chains on the pyridine ring, inhibited CDK1,2,4, and 6 to a similar degree (Table 1).[5,6] We took compound **2** as the starting point for the structure-based drug design of a potent CDK1,2,4,6 inhibitor.[7,8]

The X-ray crystal structure of the CDK4 mimic CDK2 bound with compound **2** (Fig. 1) revealed that the urea hydrogen and carbonyl group formed hydrogen bonds with Val83 in the ATP-binding hinge region of the protein; compound **2** was coplanar with an intramolecular hydrogen bond between pyridine and the urea hydrogen, as shown in Table 1.[1,9] Compound **2** was insufficiently potent so we hypothesized that conversion of the linear system to a ring to force coplanarity would improve potency.

The cyclic compounds that we synthesized were designed to form hydrogen bonds with Val83; their structures are shown in Table 2. Compound **3**, which had a cyclic urea structure, had no activity against CDK4; steric hindrance might make coplanarity difficult (Fig. 2). In contrast, the quinoxaline-2-one compound (**4**) could take a coplanar conformation and fit, like

Keywords: CDK inhibitor; CDK1; CDK2; CDK4; CDK6; X-ray; CDK4 mimic CDK4; E2F.
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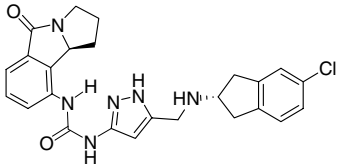
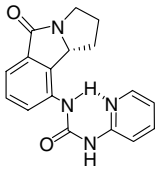
Table 1. IC_{50} values of compounds **1** and **2** against members of the CDK family (enzyme assay)

Compound	IC_{50} (nM)			
	CDK1	CDK2	CDK4	CDK6
1 	1800	440	2.3	ND[a]
2 	122	80	42	71

[a] ND, not determined.

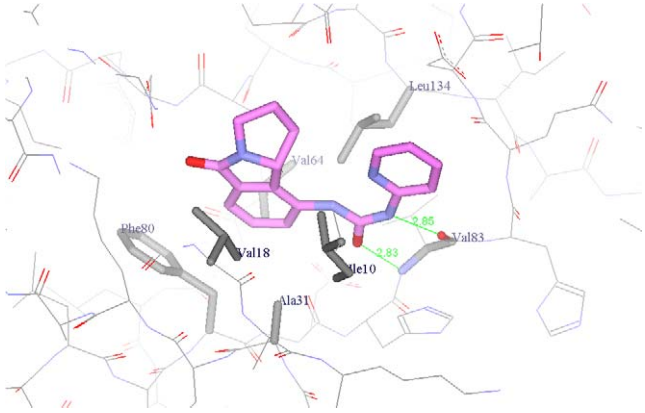
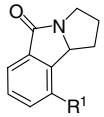
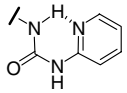
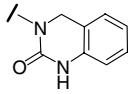
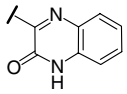


Figure 1. X-ray crystal structure of **2** in the ATP-binding site of CDK4 mimic CDK2.[1,9]

Table 2. Structures and IC_{50} values of compounds **2**–**4** against CDK4



Compound	R^1	IC_{50} (nM)
2		42
3		>10,000
4		480

compound **2**, in the ATP-binding pocket of CDK4; its CDK4-inhibitory activity was moderate.

We tried to improve the potency further by optimizing the isoindol-1-one moiety within the new quinoxalin-2-one template to provide a more favorable coplanar conformation in compounds **5** and **6**. As expected, they were better inhibitors of CDK4 (Table 3); compound **6** in particular showed high activity. The activities of compounds **4**-**6** correlate with their dihedral angles, which were optimized by a Gaussian method (HF/6-31G).[10]

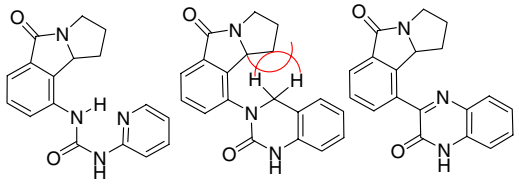
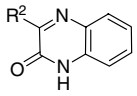
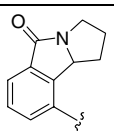
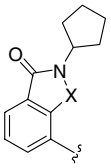


Figure 2. Structures of compounds **2**–**4** showing 3's steric hindrance.

Although the CDK-inhibitory activity of compound **6** was potent in the enzyme assay, its cellular potency was less good (IC_{50} = 310 nM; E2F assay).[11] We used

Table 3. IC_{50} values of compounds **4**–**6** against CDK4 and the dihedral angles between quinoxaline-2-one and benzoisothiazol-1-one



Compound	R^2	IC_{50} (nM)	Dihedral angle[a]
4		480	38.4
5 (X = CH_2)		170	34.7
6 (X = S)		6.0	1.5

[a] Data optimized by a Gaussian method (HF/6-31G).[10]