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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.
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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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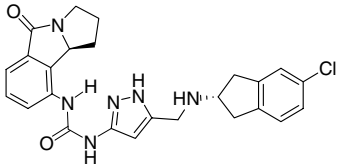
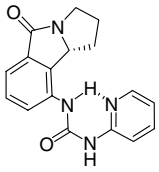
Table 1. IC_{50} values of compounds **1** and **2** against members of the CDK family (enzyme assay)

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

[a] ND, not determined.

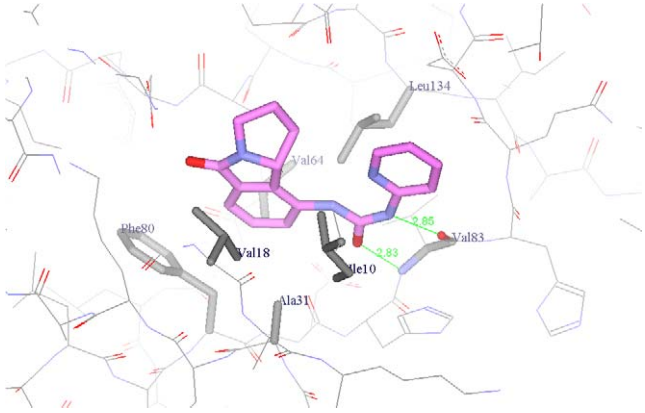
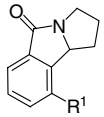
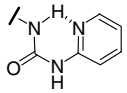
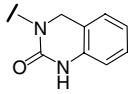
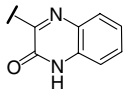


Figure 1. X-ray crystal structure of **2** in the ATP-binding site of CDK4 mimic CDK2.[1,9]

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]

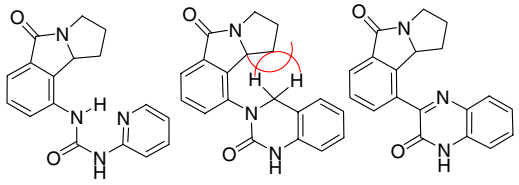
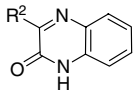
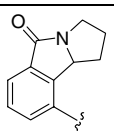
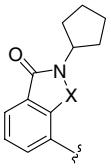


Figure 2. Structures of compounds **2**–**4** showing 3's steric hindrance.

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



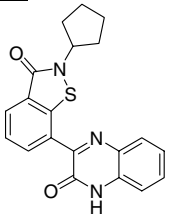
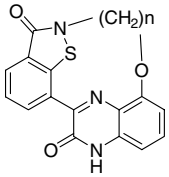
Compound	R^2	IC_{50} (nM)	Dihedral angle[a]
4		480	38.4
5 (X = CH_2)		170	34.7
6 (X = S)		6.0	1.5

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

chains of 4-6 carbon atoms to link the quinoxaline-2-one and the benzoisothiazol-1-one as shown in Table 4; these linkers fixed the dihedral angle and filled the void space around the ribose site of the ATP-binding pocket. CDK4-inhibitory activities were measured for compounds **7**-**9** (Table 4); compound **8**, which has a 5-carbon linker, showed the best activity in both assays.

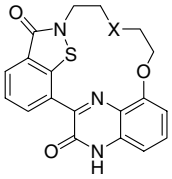
We further optimized compound **8** by inserting amines into the linker. Table 5 summarizes the results: compound **11**, which has *N*-methylamino functionality, showed little discrepancy between the two assays. However, it was insufficiently soluble in water for iv dosing so we went on to design more hydrophilic compounds such as **12**. Although its cellular activity was a little decreased compared with compound **11**, its solubility in aqueous saline solution was much improved (Table 6). This result encouraged us to further optimize the linker, and cellular potency was improved by the introduction of a pyrrolidine ring (compound **13**, Table 7). 3-D molecular modeling of compound **13** in the ATP-binding pocket of the CDK4 mimic CDK2 suggested that incorporation of a methyl group on the pyrrolidine ring would improve binding affinity to CDK4 by means of additional lipophilic interactions with Ala144, Leu134, and Asn132. Indeed, compound **14** was highly inhibitory against CDK4 in both the enzyme and the cell-based assay. As shown in Figure 3, the X-ray structure of compound **14** co-crystallized with

Table 4. IC_{50} values of compounds **6**–**9** against CDK4 as measured by the enzyme and the cellular assay[11]

Compound		IC_{50} (nM)	
		CDK4	E2F
6		6.0	310
7 (n = 4)		160	ND[a]
8 (n = 5)		3.6	30
9 (n = 6)		11	>100

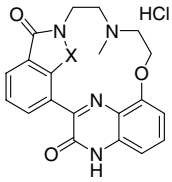
[a] ND, not determined.

Table 5. IC_{50} values of compounds **8**, **10** and **11** against CDK4 as measured by the enzyme and the cellular assay[11]



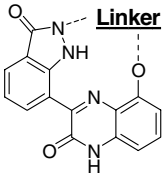
Compound	X	IC_{50} (nM)	
		CDK4	E2F
8	CH_2	3.6	30
10	NH	6.0	600
11	NMe	11	13

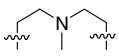
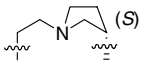
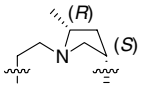
Table 6. IC_{50} values of compounds **11** and **12** against CDK4 and the solubility of their hydrochloride salts in saline



Compound	X	IC_{50} (nM)		Solubility (μg/ml)
		CDK4	E2F	in saline
11	S	11	13	20
12	NH	13	77	990

Table 7. IC_{50} values of compounds **12**–**14** as measured by the enzyme and the cellular assay[11]



Compound	Linker	IC_{50} (nM)	
		CDK4	E2F
12		13	77
13		29	27
14		6.4	3.6

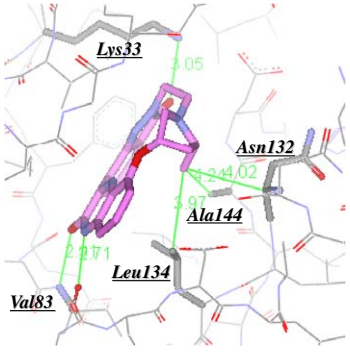


Figure 3. X-ray crystal structure of the CDK inhibitor **14** in the ATP-binding site of CDK2.[12]

the CDK4 mimic CDK2 shows its coplanar structure; it forms hydrogen bonds with Val83 like those of the lead compound **2**; there are additional hydrogen bonds with Lys33 in the salt-bridge region and hydrophobic interactions with Ala144, Leu134, and Asn132.[12]

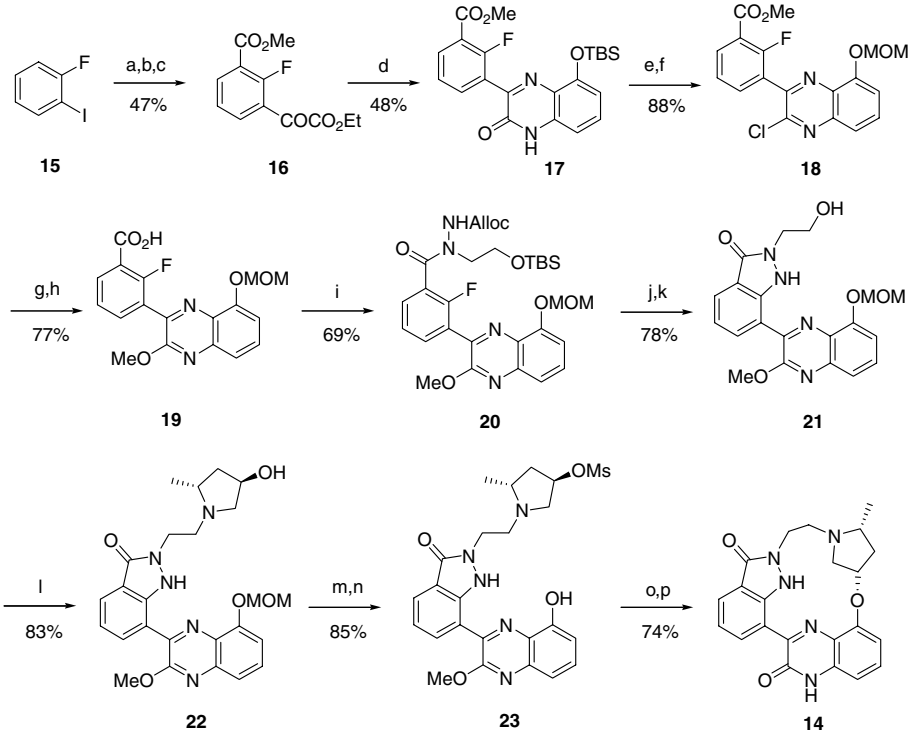
Compound **14** was synthesized as shown in Scheme 1. Deprotonation of the fluorine-adjacent position of 1-fluoro-2-iodobenzene, **15**, with LDA, followed by carbon dioxide trapping and then esterification of the carboxylic acid, led to methyl 2-fluoro-3-iodobenzoate. Iodine-magnesium exchange of methyl 2-fluoro-3-iodobenzoate according to Knochel's procedure[13] followed by reaction with chloroglyoxylic acid ethyl ester gave ketoester **16**. Compound **16** was coupled with 3-[(*tert*-butyldimethylsilyl)oxy]benzene-1,2-diamine[14] to yield quinoxaline-2-one, **17**. Activation of the 2-position of quinoxaline-2-one **17** via the corresponding 2-chloroquinoxaline using $SOCl_2$ was followed by replacement of the TBS group by a MOM group with TBAF to yield compound **18**. The addition of sodium methoxide at the 2-position of 2-chloroquinoxaline followed by hydrolysis with NaOH gave compound **19**. Allyl 2-[2-(*tert*-butyldimethylsilyloxy)ethyl]hydrazinecarboxlate[8] was condensed with compound **19** to yield compound **20**. Deprotection of the Alloc group of compound **20** in the presence of a palladium catalyst followed by cyclization to indazol-3-one under basic conditions gave compound **21**. Alcohol of compound **21** was mesylated and then aminated with (3*R*,5*R*)-5-methylpyrrolidin-3-ol[8] to yield compound **22**. The mesylation of compound **22** followed by deprotection of the MOM group with TFA at room temperature led to compound **23**. Macrocyclization of compound **23** under basic conditions followed by deprotection of the methyl group with TFA under reflux afforded compound **14**.[15]

The kinase-selectivity profile of compound **14** indicates that it has potent inhibitory activity against CDK1,2,4,6 as shown in Table 8 and is highly selective for the CDK family (IC_{50} > 1000 nM against 62 off-target kinases;[16]) it showed potent inhibitory activity against GSK3β (IC_{50} value = 13 nM). Compound **14** is available for iv administration and showed potent CDK-inhibitory activity in a preclinical animal model. Its biological profile will be reported separately.

In conclusion, structure-based drug design starting from the diarylurea scaffold of lead compound **2** led to the discovery of compound **14**, a potent CDK1,2,4,6 inhibitor that has a novel macrocyclic structure and low off-target activity.

Table 8. IC_{50} values of compound **14** against members of the CDK family

Compound	IC_{50} (nM)			
	CDK1	CDK2	CDK4	CDK6
14	1.0	3.4	6.4	12



Scheme 1. Reagents: (a) LDA, CO_2 gas, THF; (b) concd H_2SO_4, MeOH; (c) *i*-PrMgCl, chloroglyoxylic acid ethyl ester, THF; (d) 3-[(*tert*-butyldimethylsilyl)oxy]benzene-1,2-diamine,[14] AcOH, toluene; (e) $SOCl_2$, DMF; (f) MOMCl, TBAF, THF; (g) NaH, MeOH; (h) NaOH, THF, MeOH, H_2O; (i) allyl 2-[2-(*tert*-butyldimethylsilyloxy)ethyl]hydrazinecarboxylate,[8] DMC, Et_3N, $CHCl_3$; (j) $Pd(PPh_3)_4$, HCO_2H, Et_2NH, THF; (k) *N*,*N*-diisopropylethylamine, DMF; (l) MsCl, *N*,*N*-diisopropylethylamine, $CHCl_3$, (3*R*,5*R*)-5-methylpyrrolidin-3-ol;[8] (m) MsCl, *N*,*N*-diisopropylethylamine, $CHCl_3$; (n) TFA, H_2O; (o) K_2CO_3, DMF; (p) TFA, H_2O.

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References and notes

1. Ikuta, M.; Kamata, K.; Fukasawa, K.; Honma, T.; Machida, T.; Hirai, H.; Suzuki-Takahashi, I.; Hayama, T.; Nishimura, S. *J. Biol. Chem.* **2001**, *276*, 27548.
2. (a) Sherr, C. J. *Science* **1996**, *274*, 1672; (b) Sherr, C. J. *Cancer Res.* **2000**, *60*, 3689; (c) Pines, J. *Adv. Cancer Res.* **1995**, *66*, 181; (d) Sherr, C. J.; Roberts, J. M. *Genes Dev.* **1999**, *13*, 1501; (e) Dyson, N. *Genes Dev.* **1998**, *12*, 2245.
3. Hall, M.; Peters, G. *Adv. Cancer Res.* **1996**, *68*, 67.
4. Misra, R. N. *Drugs Future* **2006**, *31*, 43.
5. Honma, T.; Hayashi, K.; Aoyama, T.; Hashimoto, N.; Machida, T.; Fukasawa, K.; Iwama, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Iwasawa, Y.; Hayama, T.; Nishimura, S.; Morishima, H. *J. Med. Chem.* **2001**, *44*, 4615.
6. Honma, T.; Yoshizumi, T.; Hashimoto, N.; Hayashi, K.; Kawanishi, N.; Fukasawa, K.; Takaki, T.; Ikeura, C.; Ikuta, M.; Suzuki-Takahashi, I.; Hayama, T.; Nishimura, S.; Morishima, H. *J. Med. Chem.* **2001**, *44*, 4628.
7. Hayama, T.; Kawanishi, N.; Takaki, T. WO 2002002550.
8. Hirai, H.; Kawanishi, N.; Hirose, M.; Sugimoto, T.; Kamijyo, K.; Shibata, J.; Masutani, K. WO 2004039809.
9. The X-ray coordinate has been deposited with the Protein Data Bank, as entry 1GII.
10. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, Revision C.02, Gaussian Inc.: Wallingford CT, 2004.
11. Cellular potency of the CDK inhibitor was determined by the E2F-dependent transcription assay described elsewhere (manuscript in preparation). Briefly, human glioma cell line T98G, stably containing a CDC6 promoter gene which contains E2F binding sites, was used: it was synchronously cultured from G1 to S phase by relief from contact growth inhibition, and induced E2F transcription activity was determined by SEAP reporter gene under control of the CDC6 promoter. Cells were cultured in the presence of CDK inhibitor, and inhibition of E2F reporter activity was determined.
12. The X-ray coordinate has been deposited with the Protein Data Bank, as entry 1DS1.
13. Knochel, P.; Dohle, W.; Gommermann, N.; Kneisel, F. F.; Kopp, F.; Korn, T.; Sapountzis, I.; Vu, V. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 4302.
14. Ueno, Y.; Noguchi, T.; Hirota, K.; Sawada, N.; Umezome, T. WO 2003106418.
15. ^{1}H NMR (300 MHz, DMSO-*d*) δ (ppm) 0.68 (m, 3H), 1.34 (m, 1H), 2.00–3.10 (m, 5H), 3.40–4.00 (m, 2H), 4.46 (m, 1H), 5.24 (br s, 1H), 6.94 (m, 2H), 7.14 (m, 1H), 7.48 (m, 1H), 7.83 (d, J = 8.4 Hz, 1H), 9.43 (m, 1H), 12.20 (br s, 1H), 12.60 (br s, 1H). MS (ESI+): *m*/*z* 404 $(M+H)^+$.
16. Compound **14** was tested against a wider panel of kinases. Notably, it had $IC_{50} > 1$ μM against Abl, Arg, Aurora-A, Axl, Blk, Bmx, CaMKIV, Chk1, Chk2, c-RAF, CSK, ERK1, ERK2, KDR, Flt-1, FGFR1, FGFR2, FGFR3, IGF-1R, IKKα, IKKβ, JNK1α1, JNK2α2, JNK3, Lyn, MAPK1, MAPK2, MAPKAP-K2, MEK1, MKK4, MKK6, MKK7β, p70S6K, PAK2, PDGFRα, PDGFRβ, PDK1, PKA, PKBα, PKBβ, PKγ, PKCα, PKCβII, PKCγ, PKC*δ*, PKCε, PKCη, PKCι, PKCμ, PKD2, PRAK, PRK2, ROCK-II, Rsk2, SAPK2a, SAPK2b, SAPK3, SAPK4, SGK, Syk, Tie-2 and ZAP-70.