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Synthesis and biological evaluation of 3,5-diaminoindazoles as cyclin-dependent kinase inhibitors

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Abstract—A novel series of 3,5-diaminoindazoles were prepared and found to be CDK inhibitors. Potent inhibitors against CDK1 and CDK2 were obtained by introduction of $1\lambda^6$-isothiazolidine-1,1-dioxide at 5-position of indazole. Anti-proliferative activities of compounds were evaluated using EJ, HCT116, SW620, and A549 cancer cell lines.

Cyclin-dependent kinases (CDKs) are serine/threonine kinases which control the proliferation of eukaryotic cell.[1] Activities of CDKs are regulated precisely by multiple mechanisms. CDKs are activated by cyclin binding and phosphorylation while deactivated by either removal of cyclin or binding with CDK inhibitors (CDKIs) such as Cip/Kip and INK families.[2] Deregulation of CDKs by abnormal high expression of cyclin and/or downregulation of CDKIs have been found in various human tumors.[3] These results prompted the search for the inhibitors of CDKs as a possible candidate for the development of anti-cancer chemotherapy.[4,5] Researches have been mainly focused on the searching for ATP competitive inhibitors.

The efforts to find new scaffold for the inhibitor of CDK2 were started with the investigation of X-ray structure of CDK2.[6] The docking study with the ATP binding pocket of CDK2 resulted 3-aminopyrazole as an initial hit. It was expected to form three hydrogen bonds with hinge region of CDK2. However, 3-aminopyrazole itself did not show inhibitory activity against CDK2 up to 100 μM. Based on the X-ray structure of CDK2, 3,5-diaminoindazole was designed to provide hydrophobicity to 3-aminopyrazole while not bumping into the gate-keeper residue (Phe80) of the enzyme.[7] During the progress of research, 3-aminoindazole[8] and 3-aminopyrazole[9] derivatives were reported as potent kinase inhibitors including CDK2. Also, three hydrogen bonding interactions between pyrazole and CDK2 were confirmed by the crystal structure determination.[9]

The preparation of 3-aminoindazole **5** is outlined in Scheme 1. Commercially available 5-nitroanthranilonitrile was reacted with hydroxylamine to provide *N*-hydroxyamidine **2**. It was converted to 3-aminoindazole by the reaction of **2** with ethyl phenylacetate via the formation of [1,2,4]-oxadiazole.[10] The reduction of nitro group was followed by the derivatization of amino group which provided 3,5-diaminoindazole **5**.[11]

The inhibitory activities of indazoles showed dependency on the substituents at positions 3 and 5 (Table 1). Phenylacetyl type substituents at position 3 showed better inhibitory activity against CDK2 than alkanoyl or benzoyl (data not shown). Enzymatic inhibitory activity depends largely on the substituents at position 5. Bulky substituents were favored but too flexible substituents were not allowed (**5a**, **b** vs **5c**, **d**). $1\lambda^6$-Isothiazolidine-1,1-dione at position 5, not coplanar to the plane of the indazole,[13] provided high potency.

Keywords: CDK inhibitor; 3,5-Diaminoindazole; Isothiazolidine-1,1-dioxide.
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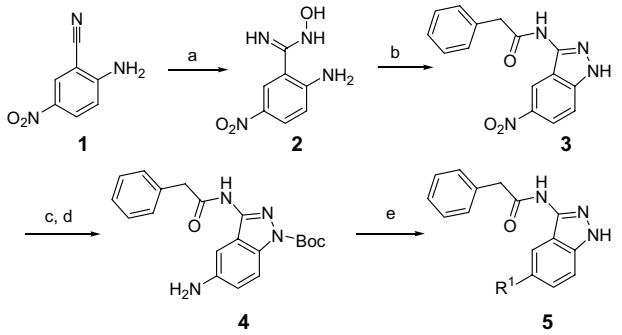
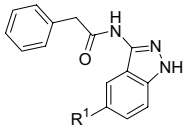


Scheme 1. General synthetic route for 3,5-diaminoindazole. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, 80% aqueous EtOH, reflux, 95%; (b) 1—NaH, $C_6H_5CH_2CO_2Et$, THF–DMF (5:2) mixture, 48%; (c) $(Boc)_2O$, NaOH, THF–H_2O (6:1) mixture, quantitative; (d) Pd/C, H_2, MeOH, quantitative; (e) **5a,b,c,d**: RCHO, $NaBH(AcO)_3$, DCE–DMF, yields were 20–30%, **5e,f**: 1—$Br(CH_2)_nCOCl$ (n = 3, 4), pyridine; 2—NaH, THF, yields in two steps were 41% (**5e**) and 34% (**5f**), **5g**: 1—$CbzNHCH_2CO_2H$, EDC, HOBt, DMF; 2—$(n\text{-}Bu)_4NF$, THF, 80% yield in two steps,[12] **5h**: 1—$ClCH_2CH_2CH_2SO_2Cl$, pyridine, DCM, quantitative; 2—NaH, DMF, quantitative; (e) sat. HCl in EtOAc, quantitative.

Table 1. Structure and inhibitory activity for 3,5-diaminoindazoles against CDK2[a]



Compound	R^1	CDK2 IC_{50}[b] (μM)
4	NO_2	2.0
5a	C_2H_5NH	0.5
5b	$(C_2H_5)_2N$	0.2
5c	$(n\text{-}C_3H_7)_2N$	1.8
5d	$(n\text{-}C_4H_9)_2N$	>100
5e	$CO(CH_2)_3N$	0.17
5f	$CO(CH_2)_4N$	7.1
5g	$COCH_2NHCON$	2.0
5h	$SO_2(CH_2)_3N$	0.036

[a] The CDK2 inhibitory assays were performed as described in Ref. 14 [ATP] = 100 μM.

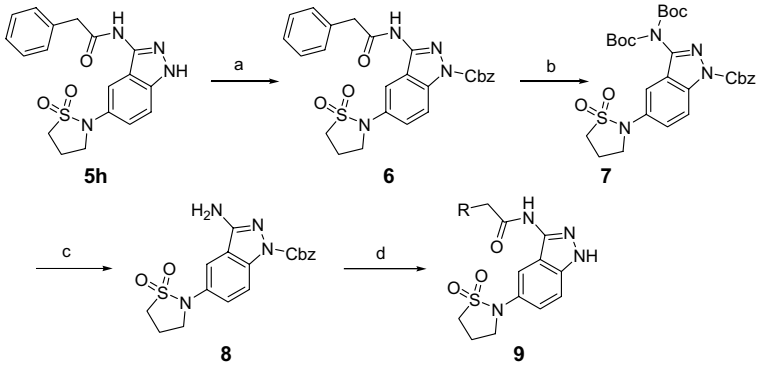
[b] Values are means of three experiments.

After $1\lambda^6$-isothiazolidine-1,1-dione at position 5 was found, the effect of substituents at position 3 had been examined. The modification of phenyl group was performed by acylation of **8** followed by deprotection (Scheme 2).[15] Phenylacetyl group was removed using regioselective cleavage of the Boc-imide.[16] In contrast to the references, Boc-imide formation, imide bond cleavage and di-Boc formation were performed in one step by using 1 equivalent of DMAP.[17]

The effect of substituent at phenyl ring on the inhibitory activity against CDK1 and CDK2 was not noticeable. Even hydroxy and amino group (**9j** and **9k**) which were expected to make hydrogen bond with the γ-carboxylate of Glu8 did not improve the potency at all (Fig. 1). In general, 3,5-diaminoindazole derivatives were about 2 order of magnitude more potent against CDK2 than CDK4 while selectivity between CDK1 and CDK2 was less than 10 (Table 2).

X-ray crystal structures revealed that chair-shaped 3,5-diaminoindazole compounds bind in the ATP pocket of CDK2 using three hydrogen bonds (Fig. 1), which are formed between aminoindazole moiety and the carbonyl group of Glu81, carbonyl and amide groups of Leu83.[18] One side of aminoindazole ring is packed against the side chain of Phe80, the gate-keeping residue. $1\lambda^6$-Isothiazolidine-1,1-dione at position 5 rotates approximately 120 deg compared to the aminoindazole ring, which makes its oxygen atom to form hydrogen bond with the side chain of Asp145 and its methylene moiety in close proximity of Gly13 and Val18. These additional interactions explain the high potency of $1\lambda^6$-isothiazolidine-1,1-dione compounds. The phenyl group makes an angle of 100 deg with respect to the aminoindazole group and is packed against the side chain of Ile10. In compound **9j**, the hydroxy group forms additional hydrogen bond with the side chain of Glu8. In compound **9n**, the side chain of Glu8 has an alternative conformation to accommodate the piperidine group.

Even though substituent at phenyl ring slightly affected the potency against CDKs, the anti-proliferative activity against several human cancer cell lines showed high



Scheme 2. General synthetic route for **9**. Reagents and conditions: (a) CbzCl, TEA, DCM, 94%; (b) $(Boc)_2O$, TEA, DMAP, DCM, quantitative; (c) sat. HCl in EtOAc, 47%; (d) 1—corresponding acyl chloride, THF, reflux; 2—2 N NaOH, yields in two steps were 40–70% except **9j**, **k**, **l**, **m**, **n**, **t** which were 10–20%.

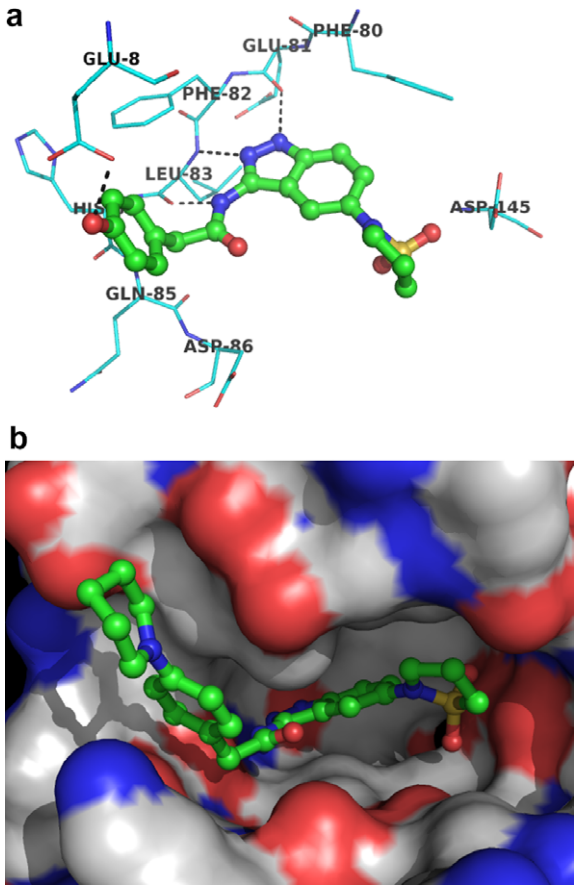


Figure 1. Crystal structure of compounds in complex with CDK2. (a) Complex structure of **9j** (green) with CDK2 reveals three hydrogen bonds with the CDK2 hinge region. (b) Surface representation of ATP binding pocket in complex with compound **9n** (green). The C, N, and O atoms are indicated as gray, blue, and red colors.

Table 2. Inhibitory activities of 3,5-diaminoindazoles against CDK1, CDK2, and CDK4

Compound	R	IC_{50}[a] (μM)		
		CDK1	CDK2	CDK4
5h	C_6H_5	0.08	0.036	3.7
9a	3-F–C_6H_4–	0.03	0.01	0.71
9b	4-F–C_6H_4–	0.05	0.02	3.0
9c	2-Cl–C_6H_4–	0.08	0.024	0.89
9d	3-Cl–C_6H_4–	0.034	0.01	1.05
9e	4-Cl–C_6H_4–	0.012	0.01	2.0
9f	3-Br–C_6H_4–	0.047	0.007	1.8
9g	4-Br–C_6H_4–	0.022	0.007	2.0
9h	3-CH_3–C_6H_4–	0.05	0.01	4.0
9i	4-CH_3–C_6H_4–	0.04	0.007	1.8
9j	4-HO–C_6H_4–	0.028	0.009	1.75
9k	4-NH_2–C_6H_4–	0.06	0.016	2.2
9l	4-$(CH_3)_2$N–C_6H_4–	0.028	0.01	0.21
9m	4-$(C_2H_5)_2$N–C_6H_4–	0.06	0.04	>1
9n	4-(1-Piperidinyl)-C_6H_4–	0.15	0.03	0.75
9o	4-CH_3S–C_6H_4–	0.04	0.01	1.3
9p	4-CH_3SO_2–C_6H_4–	0.2	0.022	1.4
9q	1-Naphthyl	0.067	0.02	3
9r	2-Naphthyl	0.04	0.009	1.6
9s	4-Biphenyl–	0.042	0.014	2.2
9t	4-(4-Pyridyl)-C_6H_4–	0.06	0.03	1.33

[a] Values are means of three experiments.

Table 3. Anti-proliferative activities of 3,5-diaminoindazole derivatives on different human cancer cell lines

Compound	Cytotoxicity[a] IC_{50} (μM)			
	EJ	HCT116	A549	SW620
5h	3.6	4.6	[b]	2.2
9e	0.93	1.23	1.5	0.93
9g	1.57	1.4	3.87	2.0
9i	1.67	1.77	2.57	1.56
9j	5.2	6.53	[b]	3.83
9l	[b]	0.83	0.35	[b]
9m	0.055	0.22	0.05	[b]
9n	0.98	1.2	1.1	[b]
9o	0.28	0.82	2.47	0.15
9p	35	48	[b]	[b]
9q	1.3	2.3	1.4	0.93
9r	0.12	0.31	0.09	0.08
9s	0.052	0.18	0.055	0.04
9t	1.05	2.5	1.4	[b]

[a] Exponentially growing cells were treated with test compounds at various concentrations for 48 h, and then the cell numbers were measured. The compound concentration with 50% growth inhibition activity was determined.[19] Values are means of three experiments.
[b] Not determined.

dependency on the type of substituents (Table 3). There were up to 2 order of magnitude differences in cell growth inhibitory activity between compounds which have similar enzymatic IC_{50} values. 3,5-Diaminoindazoles substituted by (4-diethylaminophenyl)acetyl **9m**, 2-naphtylacetyl **9r**, and 4-biphenylacetyl **9s** at 3-amino group potently inhibited the proliferation of human cancer cell lines such as EJ (bladder carcinoma), HCT116, SW620 (colon carcinoma), and A549 (lung carcinoma).

In summary, we have discovered a novel series of 3,5-diaminoindazole by structure based drug design, which is based on the initial hit obtained from the docking study. Introduction of $1\lambda^6$-isothiazolidine-1,1-dione at position 5 of indazole provided high potency against CDK1 and CDK2. It was found that cytotoxicity depended on the structure of substituent at 3-amino group. Optimization of 3,5-diaminoindazole afforded effective inhibitors against cyclin-dependent kinases 1 and 2 which had potent anti-proliferative activities on human cancer cell lines.

Acknowledgment

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15. All new compounds gave spectroscopic data according to the proposed structures, as indicated for **9r** and **9s**: **9r**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.90 (2H, p, J = 6.8), 2.99 (2H, t, J = 7.6), 3.17 (2H, t, J = 6.4), 3.47 (2H, s), 6.89 (1H, dd, J = 1.6, 9.0), 7.00–7.07 (3H, m), 7.10–7.11 (2H, m), 7.43–7.45 (4H, m), 10.28 (1H, s), 12.32 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.9, 42.8, 48.0, 48.2, 111.3, 114.9, 116.6, 122.7, 126.1, 126.6, 127.9, 128.0, 128.2, 128.3, 130.3, 132.3, 133.5, 134.2, 139.4, 140.7, 169.7; ESI-MS(*m*/*e*) = 487 [M+1]. **9s**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.91 (2H, p, J = 6.4), 3.00 (2H, t, J = 7.2), 3.21 (2H, t, J = 6.4), 3.34 (2H, s), 6.91–6.88 (2H, m), 6.98–7.04 (5H, m), 7.13 (1H, s), 7.20 (4H, t, J = 6.8), 10.24 (1H, s), 12.32 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6) δ 19.36, 42.75, 48.46, 48.61, 111.74, 115.40, 116.98, 123.09, 127.45, 127.54, 128.20, 129.80, 130.63, 130.71, 136.17, 139.38, 139.84, 140.84, 141.12, 170.11; ESI-MS (*m*/*e*) = 513 [M+1].
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17. Procedure for the synthesis of compound **7**. Compound **6** (5.2 g, 11 mmol) was dissolved in CH_2Cl_2 (100 ml). Then, DMAP (1.36 g, 11 mmol), TEA (1.55 ml, 11 mmol) and di-*tert*-butyldicarbonate (4.85 g, 22 mmol) were added at room temperature. After stirring for 30 min at room temperature, the solvent was evaporated and the resulting residue was purified by silica gel column chromatography using hexane–EtOAc (1:2) as eluent, to give compound **7** (6.4 g, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (18H, s), 2.61 (2H, m), 3.44 (2H, t, J = 7.6), 3.81 (2H, t, J = 6.4), 5.54 (2H, s), 7.31 (1H, d, J = 2), 7.45 (3H, m), 7.54 (2H, d, J = 7.2), 7.59 (2H, dd, J = 2, 9.2); ^{13}C NMR(100 MHz, $CDCl_3$) δ 18.8, 27.7, 47.3, 48.1, 69.7, 84.1, 109.7, 115.8, 122.8, 122.9, 128.7, 128.8, 128.9, 134.5, 134.6, 137.9, 145.6, 149.9, 150.2; ESI-MS (*m*/*e*) = 587 [M+1].
18. The coordinate has been deposited in the Protein Data Bank: PDB ID **2R64**.
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