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Synthesis and CDK2 kinase inhibitory activity of 7/7′-azaindirubin derivatives

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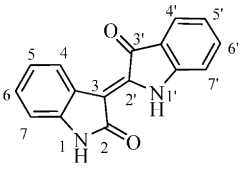
Abstract

A series of novel 7′-azaindirubin (**1a**–**g**) and 7-azaindirubin (**2a**, **2c**, **2e** and **2f**) derivatives were designed and synthesized. Their structures were characterized by ^{1}H NMR and MS spectroscopy as well as by elemental analysis. Their inhibitory properties against CDK2/cylinA were evaluated *in vitro*. In contrast to indirubin, some of the described azaindirubins emerged as potent inhibitors of CDK2/cylinA and compound **2b** had more potent activity. Biological tests also showed that nitrogen atom at 7-position of azaindirubin was more beneficial to enhance the kinase inhibitory activity.

Keywords: 7′-Azaindirubin; 7-Azaindirubin; Synthesis; CDK2/cyclinA; Inhibition

Dangui Longhui Wan which consisting of 11 herbal medicines is one of the traditional Chinese medicine recipe. It has been used for the treatment of chronic myelocytic leukaemia (CML) [1]. The clinical activity of the complex mixture was found to be associated with one ingredient, Qing Dai. Further investigations revealed that the antileukaemic principle was traced to minor constituent indirubin **I**, a red colored 2,3′-bisindole isomer [2,3].

Indirubin and its analogues have been reported to selectively inhibit cyclin-dependent kinases (CDKs), a family of key cell cycle regulators [4], by competing with ATP for binding to the catalytic site of the kinase and block cell proliferation in the late-G1 and G2/M phases of the cell cycle [5]. Indirubins are also potent ATP competitive inhibitors of glycogen synthase kinase-3β [6], which is one of the evolutionarily closest enzymes to the CDK family [7].



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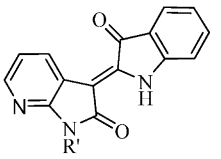

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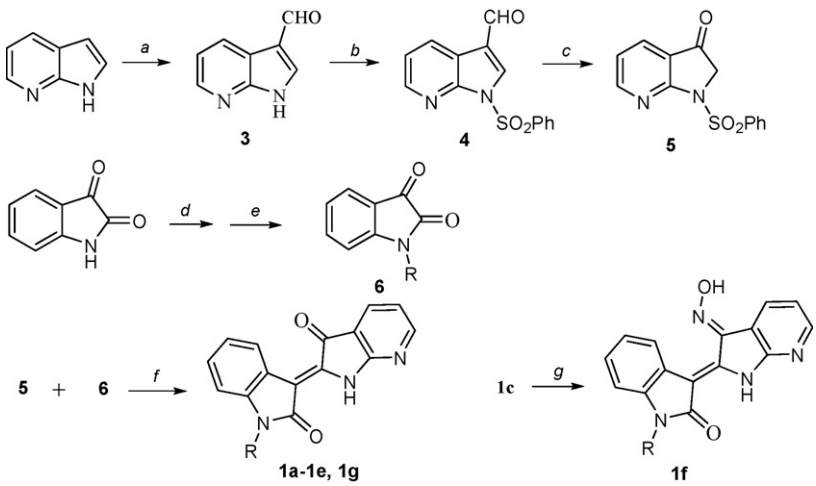


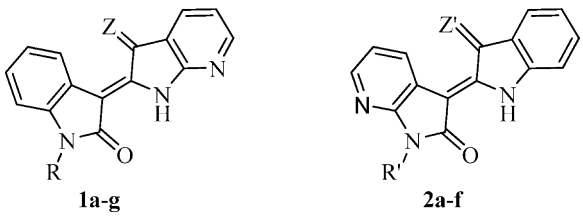
Scheme 1. Synthetic route of compounds **1a–g**: (a) $(CH_2)_6N_4$, 33% HOAc, reflux, 3 h, 47.7%; (b) $PhSO_2Cl$, pyridine, r.t., overnight, 48.9%; (c) *m*-CPBA, CH_2Cl_2, 0 °C then r.t., overnight, 25.8%; (d) NaH, DMF, r.t., 0.5 h; (e) RX, r.t., 5 h, 70–75%; (f) Et_3N, C_2H_5OH, reflux, 3 h, 30–40%; (g) $NH_2OH{\cdot}HCl$, KOH, C_2H_5OH, r.t., 0.5 h, 90.2%.

However, most indirubin derivatives show poor solubility, low absorption and present gastrointestinal toxicity. Numerous indirubin analogues have hereby been synthesized to optimize this promising kinase inhibition scaffold and/or to improve the pharmacological characteristic [8–10]. These prompted us to develop a hybrid structure, which we designed, synthesized and named azaindirubin analogues bearing one azaindole units to keep the basic indirubin skeleton, enhance the possibility of the hydrogen bond with CDKs and their solubility. We have recently reported a series of 7-azaindirubin derivatives **III** and assayed their antitumor activity, most of prepared compounds showed potent inhibitory effects on DU145 cell lines [11]. In this paper, novel 7′-azaindirubin derivatives and some 7-azaindirubins were designed and synthesized. These compounds were manufactured from 7-azaindole which is commercially available. Their inhibitory properties against CDK2/cylinA were evaluated *in vitro* because CDK2 inhibitors have anti-proliferative properties [12].

The preparation of the target compounds **1a–g** was outlined in Scheme 1. 7-Azaindole-3-carboxaldehyde **3** was obtained from 7-azaindole in refluxing aqueous AcOH with hexamethylenetetramine according to the method described by Verbiscar [13]. The phenylsulfonyl protecting group on the indole nitrogen was introduced using pyridine as a base to give 1-phenylsulfonyl-3-formyl-7-azaindole (**4**). A Baeyer-Villiger oxidation was performed with compound **4** using *meta*-chloroperbenzoic acid (*m*-CPBA) [14]. 1-Phenylsulfonyl-7-azaindolinone **5** was synthesized as the major intermediate.

1-Alkylisatins **6** were achieved by reaction of the sodium hydride and indole-2,3-dione with the appropriate alkyl halide in DMF at room temperature. Coupling of compound **5** with compounds **6** led to 7′-azaindirubins **1a–e** and **1g** with simultaneous removal of phenylsulfonyl group which were in a (3*Z*)-form [15]. The corresponding oxime **1f** was prepared from the 7′-azaindirubin derivative **1c** with hydroxylamine in ethanol in a (3*Z*, 3′*E*)-form [16]. The structures of the newly synthesized compounds were given in Table 1 and characterized by MS, ^{1}H NMR and elemental analysis [17].

The synthesized compounds **1a–g** and 7-azaindirubin derivatives **2a–f** (their synthetic method see Ref. [11]) were evaluated for inhibition toward CDK2/cyclinA. Among **2a–f**, new compounds **2a**, **2c**, **2e** and **2f** were synthesized in the present study. Kinase inhibitor affinity measurement kit and related reagent were purchased. Experimental procedures were according to its manual with indirubin (**I**) as a positive control and the biological results were summarized in Table 1.



1a-g **2a-f**

Table 1
Structures and CDK2 kinase inhibitory activities of 7′-azaindirubins (**1a–g**) and 7-azaindirubins (**2a–f**).

Compd.	R	Z	Inhibition rate (%)[a]	IC_{50} (μmol/L)	Compd.	R′	Z′	Inhibition rate (%)[a]	IC_{50} (μmol/L)
1a	CH_3	O	11.5 ± 0.9	ND	**2a**	CH_3	O	9.1 ± 1.0	ND
1b	C_2H_5	O	10.5 ± 0.7	ND	**2b**	C_2H_5	O	80.4 ± 3.8	1.2 ± 0.4
1c	*i*-C_3H_7	O	10.5 ± 0.6	ND	**2c**	*i*-C_3H_7	O	51.9 ± 2.1	9.5 ± 1.2
1d	*n*-C_4H_9	O	10.5 ± 0.7	ND	**2d**	*n*-C_4H_9	O	10.4 ± 0.8	ND
1e	CH_2Ph	O	10.5 ± 0.5	ND	**2e**	CH_2Ph	O	11.3 ± 1.2	ND
1f	*i*-C_3H_7	NOH	10.6 ± 0.5	ND	**2f**	*i*-C_3H_7	NOH	11.2 ± 1.0	ND
1g	H	O	65.0 ± 2.3	8.8 ± 1.0	**I**			90.3 ± 3.5	2.4 ± 0.5

ND: not determined.
[a] Determined at a final concentration of 10^{-5} mol/L.

In the primary assay, we discovered that compound **1g**, **2b** and **2c** exhibited the higher potency for *in vitro* inhibition of CDK2/cylinA kinase (percent inhibition >50%). Comparison of CDK2 kinase inhibitory activity of compounds **1b** and **2b**, **1c** and **2c**, respectively, showed that nitrogen atom at 7-position was helpful to enhance CDK2 kinase inhibitory activity.

In summary, a series of novel 7′-azaindirubin (**1a–g**) and 7-azaindirubin (**2a**, **2c**, **2e** and **2f**) derivatives were synthesized and their inhibitory activity against CDK2/cylinA was evaluated. Compared to parent drug indirubin (**I**), compound **2b** had more potent activity. The investigation results showed that 7′-azaindirubin derivatives, as 7-azaindirubins, may be also brought to potential antineoplastic candidates for further studies. Moreover, being the first report of 7′-azaindirubin and 7-azaindirubin derivatives serving as CDKs inhibitors, these results demonstrated that the use of a 7-azaindole coupled with an indole strategy could effectively extend the anticancer compound libraries of indirubin family.

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[17] The data of selected compounds: **1d**. 136–138 °C; ESI-MS *m/z*: 320.2 $[M+H]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 0.97 (t, 3H, *J* = 7.32 Hz, CH_3), 1.38–1.43 (m, 2H, CH_2), 1.65–1.74 (m, 2H, CH_2), 3.83 (t, 2H, *J* = 7.29 Hz, N-CH_2), 6.89 (d, 1H, *J* = 7.78 Hz, 7-H), 6.99 (dd, 1H, *J* = 5.05, 7.49 Hz, 5′-H), 7.12 (t, 1H, *J* = 7.72 Hz, 5-H), 7.34 (t, 1H, *J* = 7.72 Hz, 6-H), 8.01 (dd, 1H, *J* = 0.90, 7.49 Hz, 6′-H), 8.44 (d, 1H, *J* = 3.62 Hz, 4-H), 8.91 (dd, 1H, *J* = 0.90, 7.80 Hz, 4′-H), 10.87 (s, 1H, N′-H); Anal. Calcd. for $C_{19}H_{17}N_3O_2$ (319.3) (%): C 71.46, H 5.37, N 13.16; Found: C 71.05, H 5.37, N 13.25. **1f**. mp: 275–276 °C; ESI-MS *m/z*: 321.2 $[M+H]^+$; ^1H NMR (300 MHz, DMSO-d6): δ 1.49 (d, 6H, *J* = 7.05 Hz, $2CH_3$), 4.70–4.76 (m, 1H, N-CH), 7.02–7.12 (m, 2H, Ar-H), 7.22–7.29 (m, 2H, Ar-H), 8.29–8.31 (m, 1H, Ar-H), 8.47 (d, 1H, *J* = 7.53 Hz, 5′-H), 8.71(d, 1H, *J* = 7.53 Hz, 4′-H), 11.84 (s, 1H, N′-H), 13.82 (s, 1H, NOH); Anal. Calcd. for $C_{18}H_{16}N_4O_2$ (320.3) (%): C 67.49, H 5.03, N 17.49; Found: C 67.40, H 5.05, N 17.27. **1g**. mp: 325–327 °C; ESI-MS *m/z*: 261.9 $[M-H]^-$; ^1H NMR (300 MHz, DMSO-d6): δ 6.92 (d, 1H, *J* = 7.75 Hz, 7-H), 7.02–7.13 (m, 2H, Ar-H), 7.30 (dd, 1H, *J* = 1.16, 7.69 Hz, Ar-H), 8.11 (dd, 1H, *J* = 1.55, 7.51 Hz, Ar-H), 8.49 (dd, 1H, *J* = 1.66, 5.02 Hz, Ar-H), 8.68 (d, 1H, *J* = 7.39 Hz, 4′-H), 10.78 (s, 1H, N′-H), 11.01 (s, 1H, N-H); Anal. Calcd. for $C_{15}H_9N_3O_2$ (263.2) (%): C 68.44, H 3.45, N 15.96; Found: C 68.12, H 3.16, N 16.24. **2c**. mp: 197–200 °C; ESI-MS *m/z*: 306.1 $[M+H]^+$, 328.0 $[M+Na]^+$, 304.2 $[M-H]^-$, $C_{18}H_{15}N_3O_2$ (Mr = 305.3); ^1H NMR (300 MHz, $CDCl_3$): δ 1.53 (d, 6H, *J* = 6.93 Hz, $2CH_3$), 4.80–4.83 (m, 1H, N-CH), 6.89–6.98 (m, 3H, Ar-

H), 7.45 (dt, 1H, J = 1.22, 7.55 Hz, 5′-H), 7.66 (d, 1H, J = 7.55 Hz, 4′-H), 8.10 (dd, 1H, J = 1.59, 5.19 Hz, 6-H), 8.93 (dd, 1H, J = 1.59, 7.68 Hz, 4-H), 10.42 (s, 1H, N′-H); Anal. Calcd. for $C_{18}H_{15}N_3O_2$ (305.3) (%): C 70.81, H 4.95, N 13.76; Found: C 70.92, H 5.14, N 13.68. **2e**. mp: 256–257 °C; ESI-MS m/z: 353.1 $[M+H]^+$, 376.1 $[M+Na]^+$, $C_{22}H_{15}N_3O_2$ (Mr = 353.3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.15 (s, 2H, N-CH_2), 6.95–6.98 (m, 1H, Ph-H), 7.02–7.04 (m, 1H, Ph-H), 7.06 (dd, 1H, J = 5.19, 7.67 Hz, 5-H), 7.26–7.31 (m, 3H, Ar-H), 7.47–7.50 (m, 2H, Ar-H), 7.52 (dd, 1H, J = 0.56, 1.24 Hz, 6′-H), 7.73 (d, 1H, J = 0.56 Hz, 4′-H), 8.20 (dd, 1H, J = 1.57, 5.20 Hz, 6-H), 8.99 (dd, 1H, J = 1.57, 7.67 Hz, 4-H), 10.41 (s, 1H, N′-H); Anal. Calcd. for $C_{22}H_{15}N_3O_2$ (353.3) (%): C 74.78, H 4.28, N 11.89; Found: C 74.62, H 4.14, N 11.68.