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1,7-Annulated indolocarbazoles as cyclin-dependent kinase inhibitors

Rima S. Al-awar,[a,*] James E. Ray,[a] Kyle A. Hecker,[a] Jianping Huang,[a] Philip P. Waid,[a] Chuan Shih,[a] Harold B. Brooks,[b] Charles D. Spencer,[b] Scott A. Watkins,[b] Bharvin R. Patel,[b] Nancy B. Stamm,[b] Catherine A. Ogg,[b] Richard M. Schultz,[b] Eileen L. Considine,[b] Margaret M. Faul,[c] Kevin A. Sullivan,[c] Stanley P. Kolis,[c] John L. Grutsch[c] and Sajan Joseph[a]

[a]*Discovery Chemistry Research, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[b]*Cancer Research Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[c]*Chemical Process Research and Development, Eli Lilly and Company, Indianapolis, IN 46285, USA*

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Abstract—The synthesis and kinase inhibitory activity of a series of novel 1,7-annulated indolocarbazoles **6** and **16** is described. These compounds exhibited potent inhibitory activity against cyclin-dependent kinase 4 and good antiproliferative activity in a human colon carcinoma cell line.

The cyclin-dependent kinases (cdks) are important regulators of the cell cycle. The serine/threonine specific kinases, cdk2 and cdk4, play an essential role in the transition from the G1 to S phase. The enzyme activity of the cyclin-dependent kinases is controlled by their partners the cyclins, cdk4 has cyclin D1 as a partner whereas cdk2 is associated with both cyclin E and A in mid and late G1, respectively. Stimulation of cells by mitogens leads to induction of cyclin D1, which complexes with cdk4 and phosphorylates and hence inactivates the tumor suppressor gene pRb. The retinoblastoma protein (Rb) thus releases the E2F transcription factor, to which it normally binds, leading to cell cycle progression. This pathway is altered in many primary tumors such as: colon, rectum, lung, breast, and prostate. Therefore, a cyclin D1/cdk4 inhibitor will modulate the growth of Rb^+ tumors and serve as an effective chemotherapeutic agent.[1]

Many structural classes have been found to inhibit cyclin-dependent kinases,[2] including the flavones,[3] the paullones,[4] and several purine[5] based molecules, such as olomoucine and roscovotine (Fig. 1). Although not very selective, flavopiridol is undergoing clinical evaluation for the treatment of several tumors.[3] During our screening to identify novel inhibitors of cdk4, we discovered that indolocarbazole **1** (Fig. 2) was a potent inhibitor of both cdk2 and 4 ($IC_{50} = 0.54$ and 0.68 μM, respectively).[6] Therefore, we investigated this class of molecules, that have long been identified for their biological activity, as potential inhibitors of the cyclin-dependent kinases.[7] Our initial efforts were focused on the 1,7-annulated bisindolyl maleimides and their corresponding indolocarbazoles that are represented by analogues **6a**–**d** (Scheme 1).

Synthesis of the precursor bisindolyl maleimides **5**, was accomplished using previously described procedures[8]

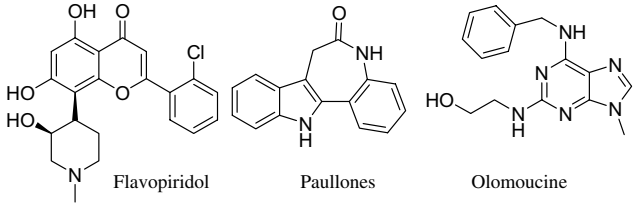

Figure 1.

* Corresponding author. E-mail: rima@lilly.com

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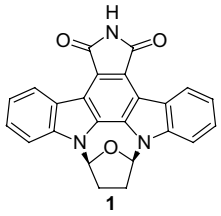
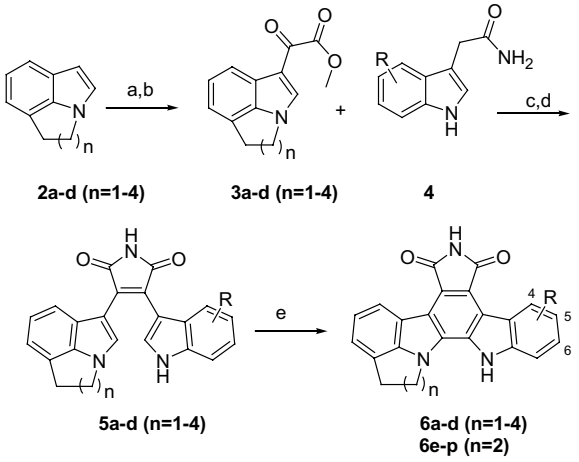
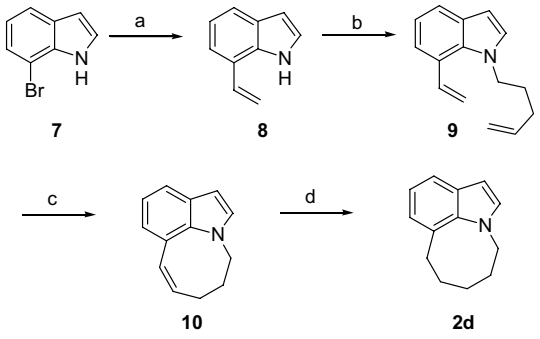


Figure 2.



Scheme 1. Reagents and conditions: (a) $(COCl)_2$, ether, −78 °C; (b) NaOMe, MeOH, 85–90%; (c) KO^{t}Bu, THF; (d) concd HCl, 60–90%; (e) $Pd(OAc)_2$, AcOH, Δ, 50–70%.

from the corresponding annulated indoles (**2a**,[9a] **2b**,[9b] **2c**,[9c] and **2d**). The eight-membered annulated indole **2d** was prepared from 7-bromo indole via Stille coupling with tributylvinyltin to give **8** followed by alkylation of the indole nitrogen with 5-bromopentene to generate di-alkene **9**. Terminal alkenes **9** underwent ring closing metathesis when subjected to the Grubbs catalyst (benzylidene-bis-(tricyclohexylphosphine)dichlororuthenium) to give intermediate **10**. Reduction of the alkene over hydrogen and platinum provided the saturated eight-membered ring indole **2d** (Scheme 2).



Scheme 2. Reagents and conditions: (a) tributylvinyltin, $PdPh_2Cl_2$, LiCl, PPh_3, DMF, 90 °C, 80%; (b) NaH, 5-bromopentene, DMF, 0 °C, 95%; (c) Grubbs catalyst (benzylidene-bis-(tricyclohexylphosphine)-dichlororuthenium), rt, CH_2Cl_2, 80%; (d) H_2, PtO_2, EtOH, 96%.

Preparation of the indole-3-glyoxylate esters of **2a**–**d** and their condensation with the corresponding indole-3-acetamides,[8] provided the desired bis-indolylmaleimides **5a**–**d**. Oxidation of **5a**–**d** to indolocarbazoles **6a**–**d** was performed in 50–70% yield using $Pd(OAc)_2$. Other conditions previously reported for this transformation (hυ with or without I_2 or Pd/C, DDQ with or without *p*-TsOH, $PdCl_2$, $Pd(O_2CCF_3)_2$, and $CuCl_2$) were less effective.

Indolocarbazoles **6a**–**d** were equally potent against cyclin D1/cdk4 and therefore the ring size did not appear to affect the activity (Table 1). All compounds were more selective for cdk4 versus cdk2 and equally potent in the HCT-116 cell line with the exception of **6c**, which was 3–5-fold less active. Evaluation of substitution on the nonannulated indole ring indicated that the 4- and 5-positions did not tolerate any substitution including a small fluoro group as demonstrated by analogues **6e**–**i**. With the exception of a methyl ester group, substitution at position 6, contrary to positions 4 and 5, was well tolerated. The fluoro (**6j**), chloro (**6k**), bromo (**6l**), and trifluoro methyl (**6l**) groups resulted in very potent inhibitors of cyclin D1/cdk4 with selectivity toward cyclin E/cdk2. In addition, they were equally effective at inducing apoptosis in the HCT-116 colon carcinoma cell line with activity ranging between 0.8 and 2.4 μM. The 3-pyridyl analogue **6o** ($IC_{50} = 0.16$ μM) was about 3-fold more potent than the 4-pyridyl (**6p**) ($IC_{50} = 0.59$ μM) in both the enzyme and cell based assays.

Table 1. Enzyme activity (cyclin D1/cdk4 and cyclin E/cdk2) and cell based inhibition in HCT116 (colon) cell line for some prepared indolocarbazoles

Compds	R	Cyclin D1/cdk4 IC_{50}, μM	Cyclin E/cdk2 IC_{50}, μM	Cytotoxicity HCT-116 IC_{50}, μM
6a	H	0.24	>2	1.02
6b	H	0.11	>1	1.6
6c	H	0.094	1.0	5.16
6d	H	0.12	>2	1.25
6e	4-F	>2	na	na
6f	5,6-Di F	>2	na	na
6g	5-F	>2	na	na
6h	5-Br	>2	na	na
6i	5-CO_2Me	>2	na	na
6j	6-F	0.42	>0.2	2.37
6k	6-Cl	0.11	>0.2	0.78
6l	6-Br	0.09	1.0	2.3
6m	6-CF_3	0.29	>2	1.48
6n	6-CO_2Me	>2	na	na
6o	6-(3-Pyridyl)	0.16	na	1.13
6p	6-(4-Pyridyl)	0.59	na	3.29
14	na	0.052	na	0.34
16a	NH_2·HCl	0.17	na	2.26
16b	Azetidine	0.075	na	0.23
16c	Pyrrolidine	0.05	na	0.17
16d	Piperidine	0.087	na	0.32
16e	Morpholine	0.075	na	0.37
16f	Thiomorpholine	0.09	na	0.6
16g	Piperazine	0.065	na	>10
16h	Diazepine	0.079	na	7.6

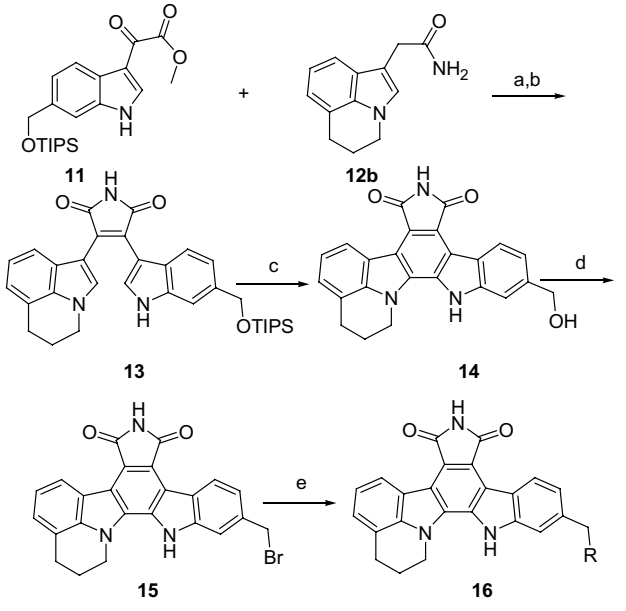
na = not applicable.

With the accessibility of position 6 well established, we turned our attention to utilizing this pocket to improve the biopharmaceutical properties of the six-membered indolocarbazole series. The synthesis of compounds **16** began with the condensation of glyoxylate ester **11**[10] and acetamide **12b**[11] to give the bis-indolylmaleimide **13**.

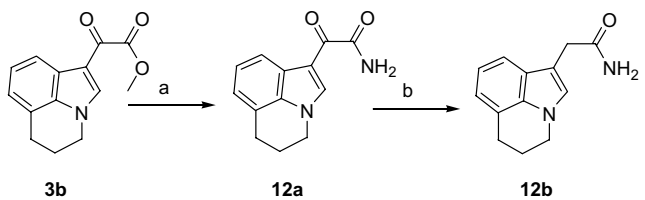
Indolocarbazole **14** was obtained under previously described oxidation conditions and was converted to benzyl bromide **15** using bromine and triphenyl phosphite. Subjecting bromide **15** to a variety of amines resulted in analogues **16a–h** with improved aqueous solubility (Scheme 3).

Acetamide **12b** was prepared from glyoxylate ester **3b** via keto amide **12a**. Treatment of glyoxylate ester **3b** with ammonium hydroxide gave amide **12a** in 85% yield, which was subsequently reduced to **12b** over 10% Pd/C in the presence of sodium hypophosphite under refluxing conditions (Scheme 4).[11]

The hydroxymethyl analogue **14** was about 3-fold ($IC_{50} = 0.052\,\mu M$) more potent than the amino methyl derivative **16a** ($IC_{50} = 0.17\,\mu M$). We found that derivatives **16b–h** were potent inhibitors of cdk4 with IC_{50}s ranging between 0.05 and 0.09 μM. This activity translated very well into the cytotoxicity assay with the exceptions of **16g** and **16h**. Although they inhibit the enzyme at 65 and 79 nM their activity in the HCT116 cell line was >10 and 7.6 μM, respectively. We speculated the diamine salts might have influenced the cell penetration abilities of these analogues and hence influenced their antiproliferative activities.



Scheme 3. Reagents and conditions: (a) KO^{t}Bu, THF; (b) concd HCl, 73%; (c) $Pd(OAc)_2$, AcOH, Δ, 81%; (d) $(PhO)_3P$, Br_2, pyridine, DMF, −15 °C to rt; (e) amine (R), NMP, rt, 55–95%.



Scheme 4. Reagents and conditions: (a) NH_4OH, THF, 0 °C to rt, 86%; (b) 10% Pd/C, NaH_2PO_2, dioxane, H_2O, Δ, 95%.

In conclusion, we have demonstrated that 1,7-annulated indoles are versatile building blocks for the preparation of indolocarbazoles that are potent inhibitors of cyclin D1/cdk4.

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