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Preparation of novel aza-1,7-annulated indoles and their conversion to potent indolocarbazole kinase inhibitors

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Abstract—The synthesis of novel aza-1,7-annulated indoles was achieved and these were converted to indolocarbazoles that proved to be potent kinase inhibitors. These compounds were also evaluated in a human colon carcinoma cell line and proved to be good antiproliferative agents.

The complex machinery of the cell cycle is controlled by a plethora of kinases that in turn regulate tumor suppressor genes and transcription factors. The cyclins and cyclin-dependent kinases, in particular, cyclinD1/cdk4 and cyclinE or A/cdk2 play an essential role in the transition of cells from G1 to S phase. Several tumors, such as colon, rectum, lung, and breast have been found to have major alterations in this pathway.[1] Several structurally diverse molecular classes have been designed and discovered as ATP competitive inhibitors of the cyclin-dependent kinases.[2] The 1,7-annulated indolocarbazole series of analogues (Fig. 1) derived from the natural product Staurosporine was found to be effective at inhibiting cyclinD1/cdk4 and arresting cells in the G1 phase of the cell cycle. These derivatives possessed potent antiproliferative activity in the HCT-116 colon carcinoma cell line, commensurate with their in vitro enzyme inhibition activity.[3]

Follow-up efforts on this series led to the preparation of the 1,7-aza-annnulated carbazoles described herein. It

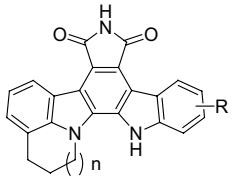

Figure 1.

was hoped that the aza-annulated carbazoles would provide additional binding interactions within the ATP active site of the enzyme and also allow for an additional handle to possibly improve the solubility of the series. The preparation of the desired glyoxylate esters and acetamide indoles was accomplished in an efficient manner in 5–8 steps from a common starting material.[4] Treatment of 7-formylindole **1** with ethanolamine under reductive amination conditions followed by protection of the resulting secondary amine with Boc anhydride gave intermediate **3**. Mesylation of the primary alcohol produced a good leaving group that readily underwent displacement by the indole nitrogen when subjected to NaH in DMF at 0 °C, thus producing the key tetrahydrodiazepinoindole **5**.

Preparation of the indole-3-glyoxylate ester **6** and its condensation with indole-3-acetamide was accomplished

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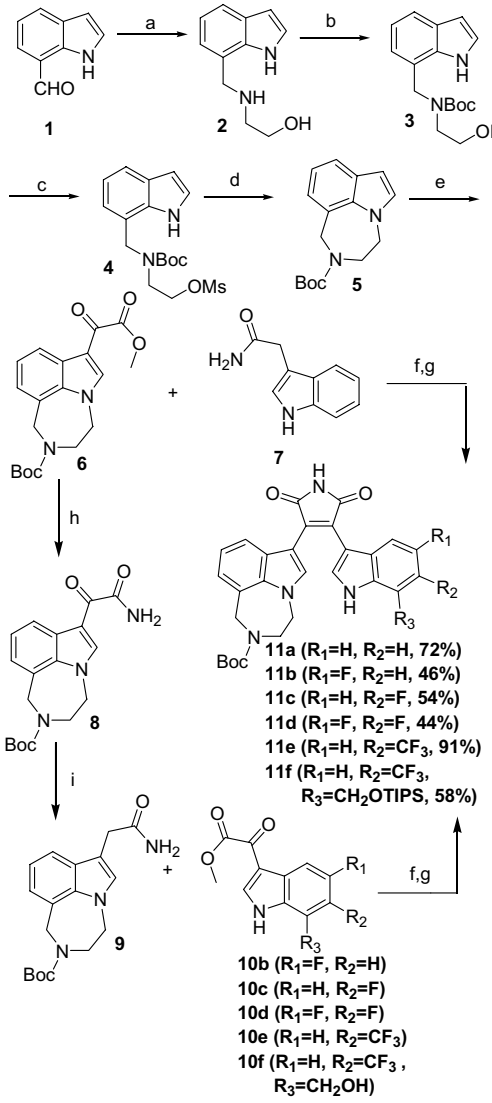
using previously described methods to provide the desired bis-indolylmaleimide **11a**.[5] Exploring the SAR around the indole ring was a little more challenging in that it was necessary to prepare acetamide **9** since the intermediates **10b–f** were only available as the glyoxylate esters (Scheme 1).[6]

Although efficient conditions to prepare the acetamide indoles of the all carbon annulated series had been reported,[3a] it was still to be confirmed that the reaction conditions would be compatible with the *t*-butoxycarbamate moiety of **8**. Treatment of **6** with ammonium hydroxide gave the corresponding amide in quantitative yield. Surprisingly, ketoamide **8** readily underwent reduction to **9** under the same previously reported reaction conditions of 10% Pd/C in the presence of sodium hypophosphite but instead of refluxing conditions the temperature was reduced to 65 °C to maintain the integrity of the carbamate.[7] Condensation with the variously substituted glyoxylate esters **10b–f** provided bis-indolylmaleimides **11b–f**.



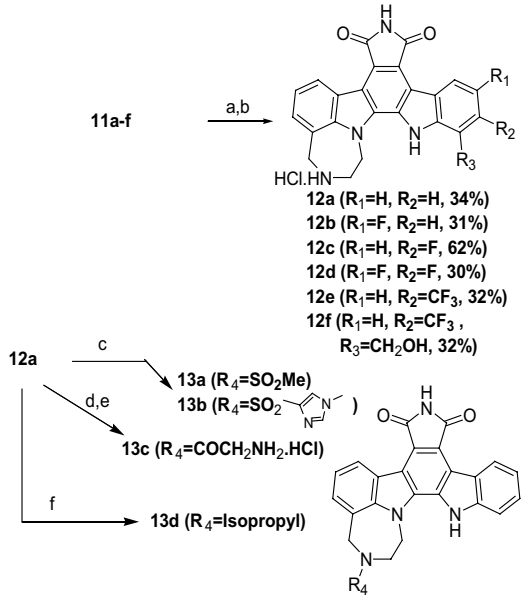
Scheme 1. Reagents and conditions: (a) ethanolamine, $NaBH(OAc)_3$, AcOH, DCE, 95%; (b) Boc_2O, DAMP, THF, 100%; (c) MsCl, TEA, CH_2Cl_2, 0 °C, 100%; (d) NaH, DMF, 0 °C, 97%; (e) oxalyl chloride, NaOMe, CH_2Cl_2, 91%; (f) KO*t*-Bu, THF; (g) concd HCl, 44–91%; (h) NH_4OH, THF, 0 °C to rt, 95%; (i) 10% Pd/C, NaH_2PO_2, dioxane, H_2O, 65 °C, 93%.

With the desired bis-indolylmaleimides **11a–f** in hand it was necessary to oxidize these to the indolocarbazoles **12a–f**, which was accomplished in good yield with $Pd(OAc)_2$ (Scheme 2).

Indolocarbazoles **12a–e** were very potent inhibitors of cyclinD1/cdk4 with activity ranging between 0.027 and 0.11 μM (Table 1). Although **12b** and **12d** showed some selectivity against cyclinE/cdk2, only **12d** and **12e** were 18 and 29-fold selective for cdk4 over PKA. Nevertheless, they were all equally potent in the HCT-116 cell line with activities ranging between 0.17 and 0.4 μM. Although **12f** was a very potent inhibitor of cdk4 it was not as active in the carcinoma cell line showing only 3.5 μM activity that might be due to reduced cell penetration.

It was hoped that substitution of the nitrogen of **12a** could potentially lead to more selective analogues and hence **13a–d** were prepared to explore this hypothesis. Although **13a** and **13b** were more selective for cyclinD1/cdk4, only **13a** had any cell activity despite the fact that **13b** was a potent inhibitor of cdk4. Amide **13c** was also plagued with the same issue of lacking cellular activity while having enzyme inhibitory activity. The sterically hindered tertiary amine **13d** was, on the other hand, 10 and 25-fold more selective for cdk4 over cdk2 and PKA, respectively, while maintaining potent activity in the cell based assay.

With these promising biological results, attention was focused on exploring the effect of ring size on the activity and selectivity of the aza series. The 8-membered aza-annulated indole **19** was therefore prepared again from



Scheme 2. Reagents and conditions: (a) $Pd(OAc)_2$, AcOH, 65 °C; (b) 4 M HCl, dioxane, CH_2Cl_2, 30–62%; (c) sulfonyl chloride, TEA, CH_2Cl_2, 50–75%; (d) EDCI, HOBT, DMF, Boc-glycine, 35%; (e) 4 M HCl, dioxane, CH_2Cl_2; (f) acetone, $NaBH(OAc)_3$, AcOH, DCE, 90%.

Table 1. Enzyme activity (cyclinD1/cdk4 and cyclinE/cdk2) and cell based inhibition in HCT116 (colon) cell line for some prepared indolocarbazoles

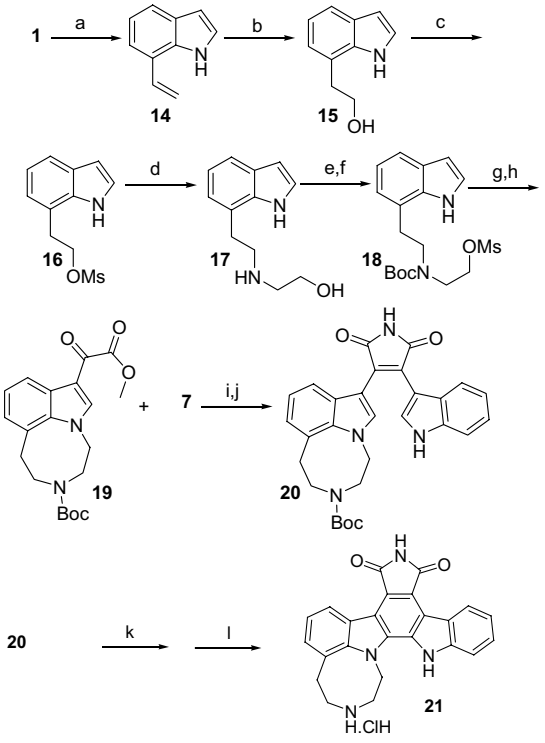
Compds	CyclinD1/cdk4 IC_{50} μM	CyclinE/cdk2 IC_{50} μM[a]	PKA IC_{50} μM[a]	Cytotoxicity HCT-116 IC_{50} μM
12a	0.027	0.038	0.16	0.31
12b	0.034	>0.2	0.2	0.26
12c	0.031	0.016	0.43	0.2
12d	0.11	>0.2	>2.0	0.4
12e	0.069	0.21	>2.0	0.17
12f	0.044	Nd	Nd	3.5
13a	0.053	>0.2	>2.0	1.6
13b	0.12	Nd	>2.0	>10
13c	0.10	Nd	>20	>10
13d	0.078	0.78	>2.0	0.64
21	0.16	Nd	2.7	0.039
28	0.084	0.2	0.04	0.097

[a] Nd = not determined.

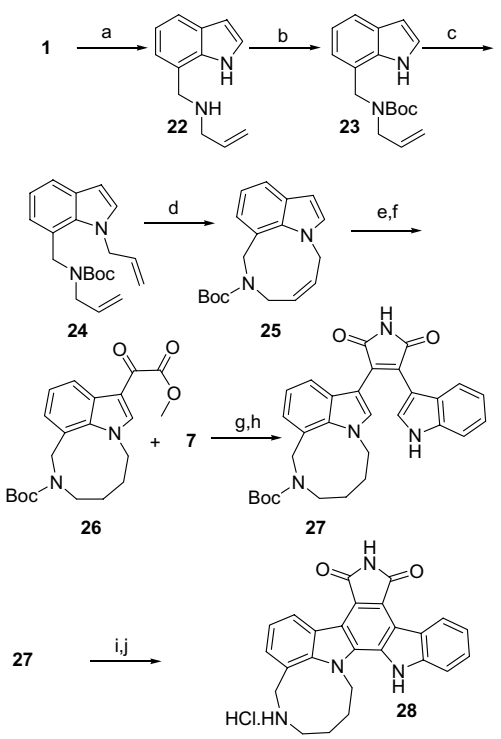
7-formylindole in eight total steps starting with a Wittig olefination,[8] followed by borane assisted homologation and oxidation to give alcohol **15**.[9] Mesylation of the primary alcohol and its subsequent displacement with ethanolamine provided the aminoalcohol **17**. All that remained to generate the desired ring was the protection of the amine as a *t*-butoxycarbamate and the mesylation of the primary alcohol setting the stage for the cyclization using previously described conditions thus providing 4,5,7,8-tetrahydro-[1,4]diazocino-[7,8,1-hi]indole-6-carboxylic acid *tert*-butyl ester, which was easily converted to glyoxylate **19**. Preparation of the final indolocarbazole **21** was accomplished under similar conditions used in the synthesis of **12a**–**e** (Scheme 3).

Evaluation of **12a** and **21** revealed that a 3–6-fold improvement in solubility was achieved with these aza-1,7-annulated analogues when compared to the simple all carbon parent compound (Fig. 1).

With the successful preparation of the 8-membered aza-annulated indolocarbazole **21**, attention was focused on the synthesis of the 9-membered one. It was envisioned that ring closing metathesis[10] of the appropriate alkenes would yield the desired 9-membered aza annulated indole. Indeed, alkene **24** underwent ring closing metathesis when subjected to the Grubbs catalyst (benzylidene-bis-(tricyclohexyl-phosphine)dichlororuthenium) to give **25** in 61% yield. Alkene **25** was subsequently reduced over hydrogen and platinum and converted to the glyoxylate ester using oxalyl chloride followed by NaOMe to provide the saturated 9-membered ring indole **26** (Scheme 4). The precursor to the ring closing reaction was obtained in three steps starting with a reductive amination between 7-formylindole **1** and allylamine to give **22**. Protection of the amine and alkylation of the indole nitrogen with allylbromide provided the key intermediate **24** with the terminal alkenes set for ring closing metathesis (Fig. 2).



Scheme 3. Reagents and conditions: (a) $MePh_3PBr$, KO*t*-Bu, THF, 93%; (b) BH_3·THF, H_2O_2, NaOH, 56%; (c) MsCl, TEA, CH_2Cl_2, 98%; (d) ethanolamine, EtOH, Δ, 85%; (e) Boc_2O, DAMP, THF, 85%; (f) MsCl, TEA, CH_2Cl_2, 100%; (g) NaH, DMF, 0 °C, 79%; (h) oxalyl chloride, NaOMe, CH_2Cl_2, 83%; (i) KO*t*-Bu, THF; (j) concd HCl; (h) NH_4OH, THF, 0 °C to rt, 95%; (k) $Pd(OAc)_2$, AcOH, 65 °C; (l) 4 M HCl, dioxane, CH_2Cl_2.



Scheme 4. Reagents and conditions: (a) allylamine, $NaBH(OAc)_3$, AcOH, DCE, 82%; (b) Boc_2O, DAMP, THF, 100%; (c) NaH, allylbromide, DMF, 0 °C, 91%; (d) Grubbs catalyst (benzylidene-bis-(tricyclohexylphosphine)dichlororuthenium), Δ, CH_2Cl_2, 61%; (e) H_2, PtO_2, EtOH, 95%; (f) oxalyl chloride, NaOMe, CH_2Cl_2, 91%; (i) KO*t*-Bu, THF; (j) concd HCl, 43%; (k) $Pd(OAc)_2$, AcOH, 60 °C, 50%; (l) 4 M HCl, dioxane, CH_2Cl_2, 100%.

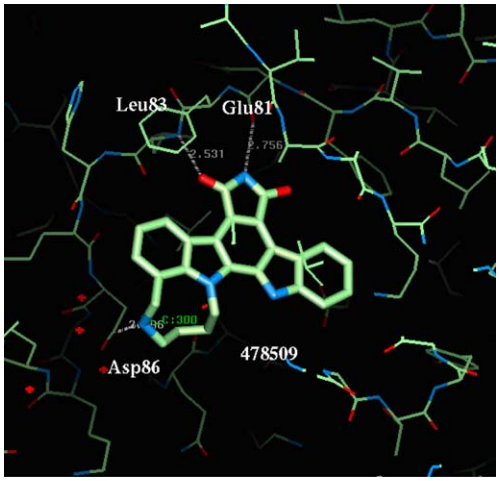


Figure 2. Key interactions of carbazole **28** bound to cdk2.

Condensation of **26** with acetamide indole **7** gave bis-indolylmaleimide **27**, which when oxidized over $Pd(OAc)_2$ and deprotected using hydrogen chloride treatment yielded indolocarbazole **28** as the hydrochloride salt.

Indolocarbazoles **12a**, **21**, and **28** were found to all be equally potent against cyclinD1/cdk4 and therefore the ring size did not appear to affect the activity (Table 1).

Although **21** was 17-fold more selective for cdk4 versus PKA, carbazole **28** was a 2-fold more potent inhibitor of PKA. Nevertheless, both compounds were equally cytotoxic when evaluated in the HCT-116 antiproliferation assay.

The indolocarbazole **28** was co-crystallized with cdk2 and was found not surprisingly to occupy the ATP binding pocket. Several key hydrogen bond interactions were identified whereby the N–H of the maleimide moiety was hydrogen bonding to the carbonyl of Glu81 (2.8 Å) and the carbonyl of the maleimide hydrogen bonded to the backbone amide group of Leu83 (2.5 Å). In addition to these expected interactions, the amine of the 9-membered ring also formed a hydrogen bond with Asp86 (2.7 Å), an amino acid that is highly conserved in many kinases including PKA, which shed further light on the reduced selectivity of **28**.

In conclusion, novel azaannulated indolocarbazoles of varying ring sizes were prepared from 7-formylindole and were readily converted to indolocarbazoles that are potent inhibitors of cyclinD1/cdk4.

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