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# Novel Pyrrolyllactone and Pyrrolyllactam Indolinones as Potent Cyclin-Dependent Kinase 2 Inhibitors

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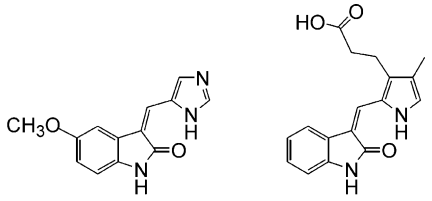
**Abstract**—Cyclin-dependent kinases (CDKs) are essential in the control of cell cycle progression. Inhibition of CDKs represents a new approach for pharmacological intervention in the treatment of a variety of proliferative diseases, especially cancer. Based on the crystal structure of CDK2 in complex with an imidazole indolinone compound **1** (SU9516), lead optimization through modeling, synthesis, and SAR studies has led to the discovery of a novel series of pyrrolyllactone and pyrrolyllactam indolinones as potent CDK2 inhibitors.


Cyclin-dependent kinases (CDKs) play important roles in cell cycle regulation.[1–4] CDKs activate host proteins through phosphorylation of serine or threonine residues using adenosine triphosphate (ATP) as a phosphate donor. Inhibitors of CDKs are anticipated to possess therapeutic potential in the treatment of proliferative disorders, including cancer.[1–9] Over the past few years, a large variety of small-molecule inhibitors have been reported in the literature which target the ATP-binding site of CDKs (see reviews in refs 5–9 and references cited therein). Some inhibitors have been co-crystallized with CDK2. These crystallographic investigations have provided a wealth of structural information for the design of inhibitors of CDKs. In the study reported here, we chose CDK2 as a target since both cyclin E/CDK2 and cyclin A/CDK2 complexes were suggested to play an important role in cell cycle regulation.[5] We have previously reported **1** (SU9516, Fig. 1), an imidazole indolinone compound identified as CDK2 inhibitor by screening of collections of synthetic organic molecules.[10] Subsequently, this compound was successfully co-crystallized with CDK2.[10] The crystal structure revealed that the binding mode of **1** in CDK2 is similar to SU5402 bound to FGFR1. SU5402 is a pyrrole indolinone compound **2** (Fig. 1) that has been co-crystallized with FGFR1 previously.[11]




SU9516, **1** SU5402, **2**

**Figure 1.** Chemical structures of SU9516 (**1**) and SU5402 (**2**).

As part of a continuing effort to optimize the indolinone scaffold in order to increase the CDK2 inhibitory activity, structure-based design and modeling studies have been performed using the co-crystal structure of CDK2 in complex with **1** (Fig. 2). The design considerations resulting from these studies are summarized in Figure 3. Like SU5402,[11] the oxindole (C=O)NH moiety and the pyrrole or imidazole NH are essential for binding. They make three hydrogen bonds with the backbone motif in the kinase hinge region. Appropriate substituents at the 4-, 5-, 3′-, and 4′-positions of the indolinone core are favorable for CDK2 inhibition, as shown in Figure 3. The 5′-position is, however, not suitable for substitution since it is very close to the peptide backbone of His84 and Gln85. Substitutions are not tolerated at either the 6- or 7-positions because they are close to Phe80 (Fig. 3). The 5′-steric exclusion of indolinone is unique to CDK2, while generally 5′-methylpyrrole indolinones such as SU5416,[12] SU6668,[13] and tetrahydroindole indolinones[14] are favorable for the inhibition of VEGF-

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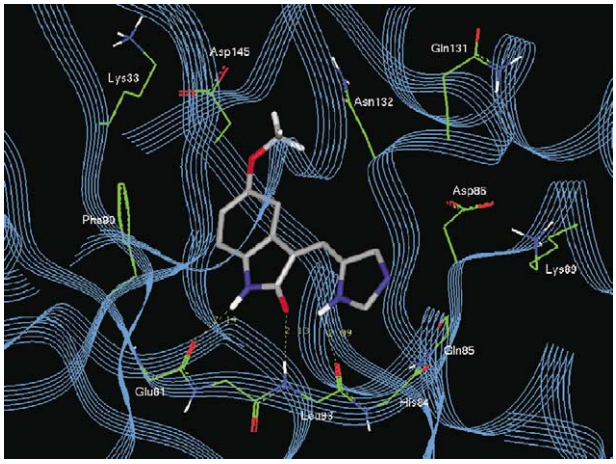



**Figure 2.** Co-crystal structure of CDK2 in complex with **1**. Color coding: oxygen, red; nitrogen, dark blue; carbons in CDK2, green; carbons in **1**, gray; hydrogen, white. Non-polar hydrogens are generally omitted for clarity. Light blue ribbon represents the backbone of CDK2.

R2, FGF-R1, PDGF-Rβ, and some other kinases. Further pair-wise comparisons of 5′-H and 5′-methyl pyrrole indolinones clearly demonstrated that 5′-H analogues were significantly more potent in CDK2 inhibitory activity than the corresponding 5′-methyl analogues,[15] fully supporting the prediction of 5′-H preference of CDK2 based on modeling.

With the rationale discussed above, a novel pyrrolyllactone indolinone scaffold (**15a**–**n**) was designed and synthesized. In this scaffold, the lactone moiety can make additional hydrogen bonding and electrostatic interactions with the side chain of Lys89 in CDK2. Such interactions were absent in the co-crystal structure of CDK2 in complex with **1** (Fig. 2). Consistent with this hypothesis, a direct comparison of pyrrolyllactone indolinone **15a** with imidazole indolinone **1** in Table 1 revealed that the pyrrolyllactone compound is more potent and selective than the imidazole compound. With this promising result, the pyrrolyllactone indolinone scaffold was subsequently expanded to pyrrolyllactam indolinone (**16a**–**n**) since pyrrolyllactam was expected to have better pharmaceutical properties.

The general syntheses of pyrrolyllactone (**15a**–**n**) and pyrrolyllactam indolinones (**16a**–**n**) are illustrated in Schemes 1–3. The pyrrolyllactone (**5**) was prepared using commercially available valerolactone (**3**) that was treated with tosylmethyl isocyanide and followed with a Vilsmeier reaction to afford the corresponding pyrrolylaldehyde (**5**, Scheme 1). For the synthesis of pyrrolyllactam (**13**, Scheme 2), the key intermediate *t*-boc protected dihydro-pyridone **10** was synthesized from 2-piperidone (**6**) in four steps with an overall yield of 52%. The final indolinone compounds **15** and **16** were prepared via a condensation reaction (Scheme 3) of pyrrolaldehydes (**5**, **13**) in ethanol with the oxindoles **14** reported previously.[12–14] All new compounds were characterized by 300 MHz NMR and MS. The final products were purified by either re-crystallization or column chromatography.[16]

Pyrrolyllactone indolinones with various substituents at the 4-, 5-, and 6-positions in the oxindole core are shown in Table 2. Among the 5-substituted analogues (**15a**–**i**), 5-*N*-methylsulfonamide (**15b**) and 5-carboxyl (**15f**) compounds are the most potent and selective, consistent with their favorable interactions with the side chain of Lys33 (Fig. 3). In addition, 5-sulfonamide analogues (**15b**–**e**) are more potent than the 5-halo analogues (**15g**–**i**). Substitution at the 4-position was considered well tolerated from modeling study. Appropriate substituent at this position can further interact with the side chain of Gln131 or Asn132 (Fig. 3). In fact, compounds with a polar substituent at the 4-position (**15k**, **15l**) are significantly more potent than the 4-methyl analogue **15j** (Table 2). Compound **15l** is the most potent analogue among the three. In this compound, a piperidine was introduced to the 4-position based on modeling study of flavopiridol, the most

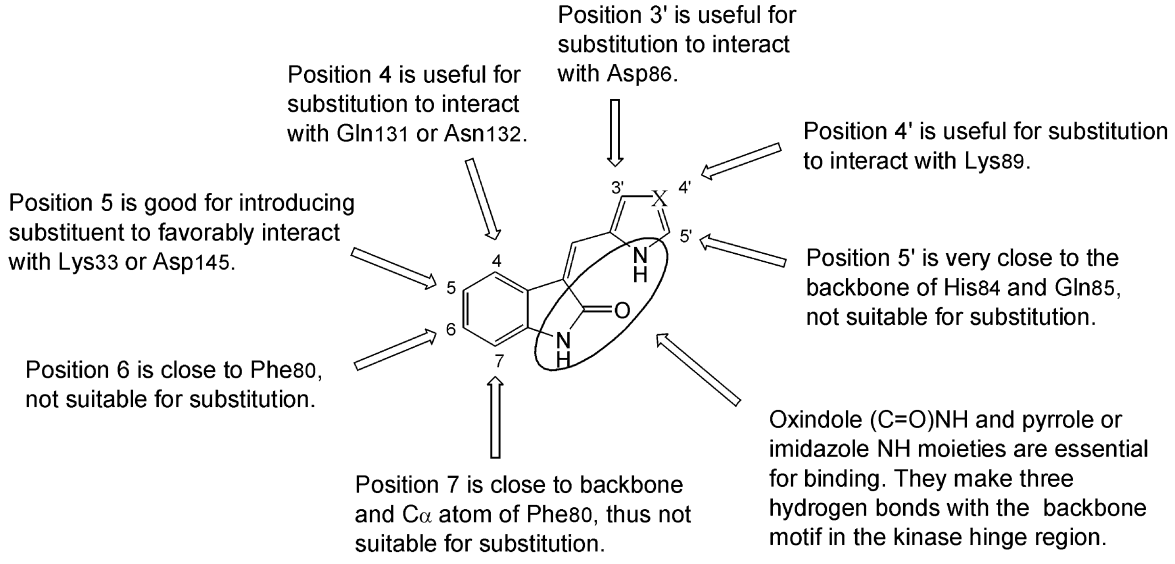
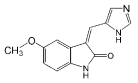
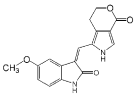
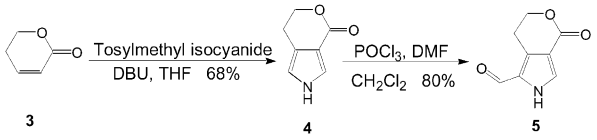



**Figure 3.** Design considerations for lead optimization. X is a carbon or nitrogen in the structure.

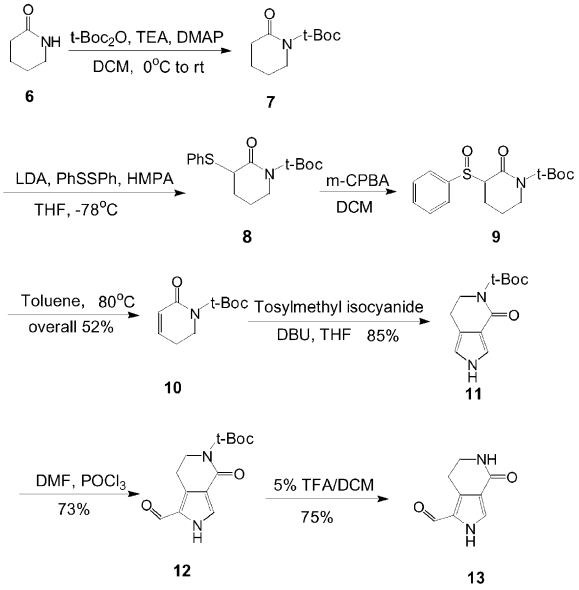
**Table 1.** Biochemical screening data[a] [$IC_{50}$ (μM)] for a direct comparison of pyrrollylactone indolinone **15a** with imidazole indolinone **1**

| Compd | Structure | CDK2 | FLK | PDGF-Rβ | SRC |
|---|---|---|---|---|---|
| **1** |  | 0.11 | 0.13 | 18 | 11 |
| **15a** |  | 0.040 | >20 | >20 | >20 |

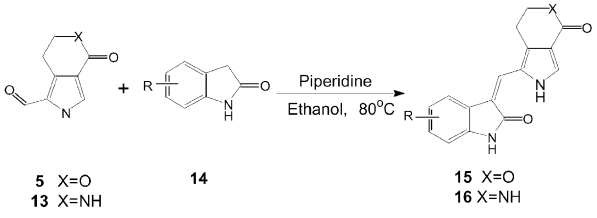
[a]Assay procedures are provided in ref 16. All experiments were conducted in duplicate.




**Scheme 1.** Synthesis of pyrrolyllactone.




**Scheme 2.** Synthesis of pyrrolyllactam.




**Scheme 3.** General synthesis of indolinones.

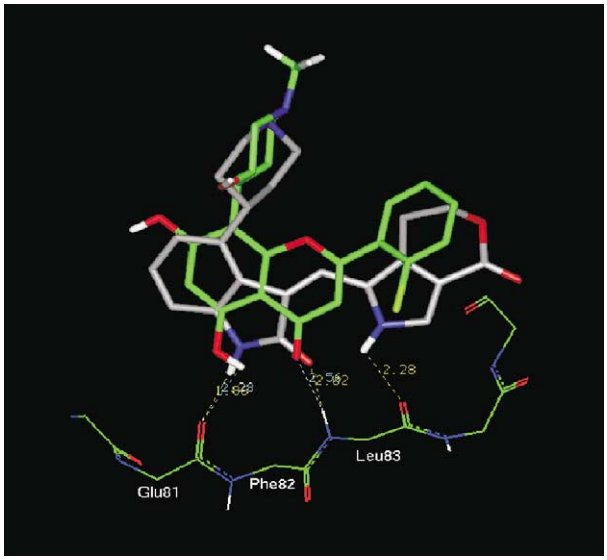


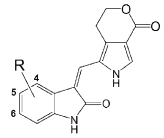

**Figure 4.** A comparison of **15l** with flavopiridol by docking in the ATP binding site of CDK2, suggesting that their piperidine moieties are in a similar position. Color coding: oxygen, red; nitrogen, dark blue; carbons in **15l**, gray; carbons in flavopiridol, green; chlorine, light green; hydrogen, white. Non-polar hydrogens are generally omitted for clarity.

advanced CDK inhibitor in clinical trials.[17] The piperidine moiety of **15l** was in a similar position to that of flavopiridol when the two molecules were docked in the ATP binding site of CDK2, as shown in Figure 4. The 6-position is not suitable for substitution (Fig. 3). As shown in Table 2, **15m** and **15n** are clearly less potent than the 4- or 5-substituted compounds **15a–l**.

Similar to the results of pyrrolyllactone indolinones, among the 5-substituted pyrrolyllactam indolinones (**16a–i**) in Table 3, 5-sulfonamide (**16b**, **16e**) and 5-carboxyl (**16f**) analogues are more potent than the 5-halo analogues (**16g**, **16i**). Like **15l**, the 4-piperidine compound (**16l**) exhibits potent inhibitory activity. The 6-phenyl analogue (**16n**) is significantly less active than the 5- or 4-substitued analogues (**16a–l**). However, pyrrolyllactams are generally 2–10-fold less potent than the corresponding pyrrolyllactones, as seen in the pair-wise comparisons of **16a** versus **15a**, **16b** versus **15b**, **16e** versus **15e**, **16f** versus **15f**, **16g** versus **15g**, **16i** versus **15i**, **16k** versus **15k**, and **16l** versus **15l**. The better activity of pyrrolyllactone is attributed to its lactone moiety that makes favorable electrostatic interaction with the side chain of Lys89 (Fig. 3) in addition to hydrogen bonding. In pyrrolyllactam, the lactam amide NH is unfavorable in electrostatic interaction with Lys89, while the amide C=O can form hydrogen bonds with Lys89.

In summary, based on the crystal structure of CDK2 in complex with **1**, lead optimization through modeling, synthesis, and SAR studies has led to the discovery of a novel series of pyrrolyllactone and pyrrolyllactam indolinones as potent CDK2 inhibitors. The 4- and 5-substitutions in these indolinones are important to their CDK2 inhibitory activity, and pyrrolyllactone indolinones are generally more potent than pyrrolyllactam indolinones.

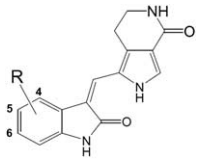
**Table 2.** Biochemical screening data[a] [$IC_{50}$ (μM)] of pyrrollylactone indolinones **15a–n**



| Compd | R | CDK2 | FLK | PDGF-Rβ | SRC |
|---|---|---|---|---|---|
| **15a** | 5-$OCH_3$ | 0.040 | >20 | >20 | >20 |
| **15b** | 5-$SO_2NHCH_3$ | 0.004 | 6.6 | >20 | >20 |
| **15c** | 5-$SO_2NH_2$ | 0.012 | 6.6 | >20 | 13 |
| **15d** | 5-$SO_2NH(_i$Pr) | 0.064 | 6.7 | >20 | >20 |
| **15e** | 5-$SO_2N(CH_3)_2$ | 0.033 | >20 | >20 | >20 |
| **15f** | 5-COOH | 0.004 | 6.2 | >20 | >20 |
| **15g** | 5-F | 0.082 | 10 | >20 | >20 |
| **15h** | 5-Cl | 0.088 | >20 | 2.1 | >20 |
| **15i** | 5-Br | 0.19 | 11 | 18 | >20 |
| **15j** | 4-$CH_3$ | 0.27 | >20 | >20 | >20 |
| **15k** | 4-$CH_2CH_2OH$ | 0.030 | 1.2 | >20 | >20 |
| **15l** |  | 0.009 | 0.49 | 1.1 | 1.1 |
| **15m** | 6-$OCH_3$ | 1.5 | >20 | 1.4 | >20 |
| **15n** | 6-Ph | >10 | 2.0 | 1.1 | 8.4 |

[a]Assay procedures are provided in ref 16. All experiments were conducted in duplicate.

**Table 3.** Biochemical screening data[a] [$IC_{50}$ (μM)] of pyrrollylactam indolinones **16a–n**



| Compd | R | CDK2 |
|---|---|---|
| **16a** | 5-$OCH_3$ | 0.25 |
| **16b** | 5-$SO_2NHCH_3$ | 0.009 |
| **16e** | 5-$SO_2N(CH_3)_2$ | 0.068 |
| **16f** | 5-COOH | 0.068 |
| **16g** | 5-F | 0.30 |
| **16i** | 5-Br | 0.53 |
| **16k** | 4-$CH_2CH_2OH$ | 0.34 |
| **16l** |  | 0.049 |
| **16n** | 6-Ph | 5.7 |

[a]Assay procedure is provided in ref 16. All experiments were conducted in duplicate.

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