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Bioorganic & Medicinal Chemistry Letters 17 (2007) 1243–1245
Bioorganic & Medicinal Chemistry Letters

Synthesis of 3-(1*H*-benzimidazol-2-yl)-5-isoquinolin-4-ylpyrazolo[1,2-*b*]pyridine, a potent cyclin dependent kinase 1 (CDK1) inhibitor

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Received 15 November 2006; revised 2 December 2006; accepted 4 December 2006
Available online 15 December 2006

Abstract—The novel compound 3-(1*H*-benzimidazol-2-yl)-5-isoquinolin-4-ylpyrazolo[1,2-*b*]pyridine was discovered to be a potent CDK1 inhibitor. Described here is the chemistry for its synthesis, including Pd(II) catalyzed Stille coupling reaction and sulfur(0) induced benzimidazole formation. Its effects on VEGFR-2 kinase activity and tumour cell proliferation are also described.

The cell division cycle is one of the most fundamental processes in biology that ensures the controlled production of subsequent generations of cells with defined biological function. Under normal growth conditions, cell proliferation is tightly regulated. This is achieved by a complex network of proto-oncogenes and tumour-suppressor genes that are components of various signal transduction pathways. Activation of a proto-oncogene and/or a loss of a tumour-suppressor gene can lead to the unregulated cell proliferation and to the accumulation of genetic errors which ultimately will result in the development of cancer.[1] In the eukaryotic cell cycle a key role is played by cyclin dependent kinases, which consist of regulatory subunits (such as CDK1, CDK2, CDK4, or CDK6) and catalytic subunits (such as cyclin A, B, D1, D2 or E). The coordinated activation of these complexes drives the cells through the cell cycle and ensures the fidelity of the process.[2] Each step in the cell cycle is regulated by a distinct and specific cyclin dependent kinase. For example, complexes of CDK4 and D-type cyclin govern the early G1 phase of the cell cycle, while the activity of the CDK2/cyclin E complex is rate limiting for the G1 to S-phase transition. The CDK2/cyclin A kinase is required for the progression through S-phase and the CDK1/cyclin B complex controls the entry into M-phase.[2] A key regulator of these transitions is CDK1, a universal intracellular factor which triggers the G2/M transition of the cell cycle in all organisms. Both biochemical and genetic evidences have shown that CDK1 is the primary activity required for a cell to enter mitosis in all eukaryotic cells. There is now strong evidence that CDKs, their substrates, and regulatory proteins are the target of genetic alterations in many human cancers.[3] Overexpression of CDK1 and CDK4 has been reported in a large variety of tumours.[4,5]

Developing small molecule CDK inhibitors for anticancer therapy has been the goal of intensive drug discovery efforts at many pharmaceutical companies. Several CDK inhibitors are currently in clinical trials including the 2-aminothiazole derivative BMS-387032 (SNS-032)[6] and the purine analogue (*R*)-roscovitine (CYC-202).[7] There are also numerous candidates in preclinical development.[8] Recently, the disubstituted indazole scaffold (**1a**) (Fig. 1) has emerged as a promising structural core for enzyme inhibitors with excellent potency against numerous kinase targets.[9] Part of our CDK effort was directed towards this series. In addition to focusing on a 3,5-arrangement of the R^1 and R^2 side chains, our strategy was to add one additional heteroatom to the central indazole ring,[10] by which we hoped to generate a novel scaffold with better potency and desirable PK properties. Though all the three unsubstituted carbons (C-4, C-6, C-7) of the indazole ring could potentially be replaced with nitrogen, only the chemistry for

Keywords: Pyrazolo[1,2-*b*]pyridine; Cyclin dependent kinase 1 inhibitor.
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doi:10.1016/j.bmcl.2006.12.031

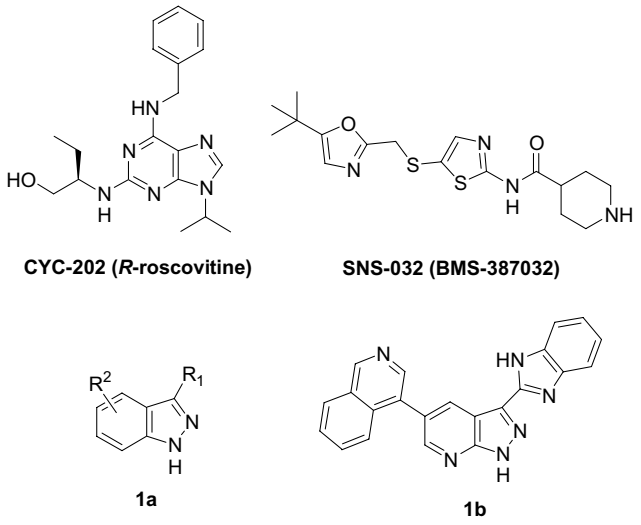


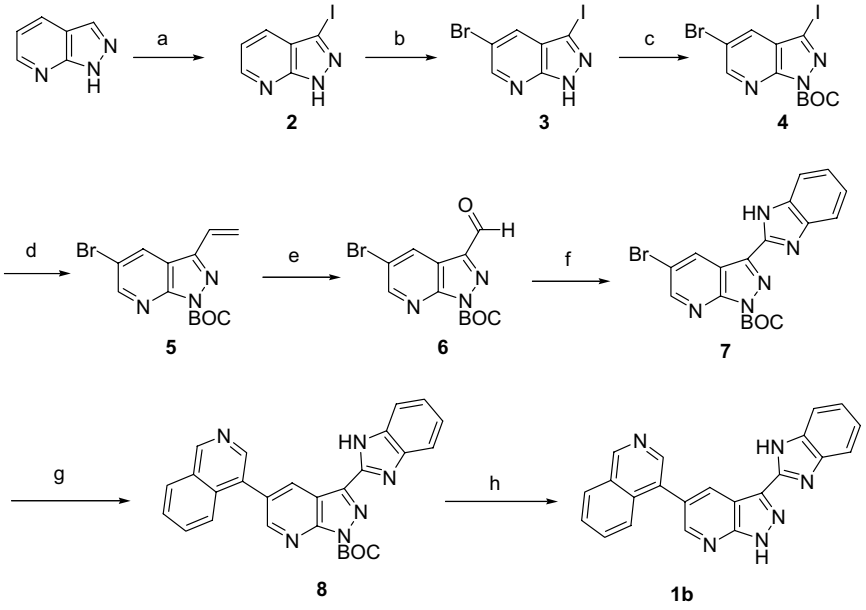
Figure 1.

pyrazolo[1,2-*b*]pyridine synthesis was successfully developed. Described here is the synthesis of our lead compound **1b** (Fig. 1). Its potency for CDK1 and other biological results are also presented.

The synthesis began with iodination of pyrazolo[1,2-*b*]pyridine[11] as shown in Scheme 1. Treatment of this intermediate with iodine in 1,4-dioxane afforded iodo compound **2**, which was subsequently reacted with bromine in acetic acid to generate bromide **3**.[12] Direct coupling of compound **3** with tributyl (vinyl)tin in the presence of Pd(II) failed to provide the desired product. Therefore, the N-1 was protected with BOC group to afford intermediate **4**, which was then coupled with tributyl(vinyl)tin to give compound **5** in 70% yield.[13] Careful temperature control in this step was necessary in order to avoid the coupling reaction of tributyl(vinyl)tin with the bromide-bearing pyridine ring. Next, ozonolysis of the vinyl group followed by reduction with dimethyl sulfide gave aldehyde **6**.[14] Direct conversion of iodo compound **4** to compound **6** was also explored by treating intermediate **4** with *tert*-butyl lithium, followed by addition of DMF. Though various conditions were studied, the desired product could only be generated in very low yield with the de-iodinated compound appearing as the major side product. Then, cyclization of carboxaldehyde **6** with 1,2-phenylenediamine in the presence of sulfur(0) generated the benzimidazole compound **7**.[15] Coupling of bi-aryl compound **7** with 4-(trimethylstannyl)isoquinoline gave tri-aryl compound **8** in 81% yield.[16] Last, the BOC group was removed with trifluoroacetic acid in dichloromethane to afford the final product **1b**.

Compound **1b** was tested for its ability to inhibit CDK1 activity, which was evaluated using CDK1 in complex with cyclin B to phosphorylate a histone-H1 biotinylated peptide substrate. Inhibition of CDK1 activity was measured by observing a reduced amount of ^{33}P-γ-ATP incorporated into the immobilized substrate in a Flashplate assay format. In the assay, this compound showed high potency against CDK1 with an IC_{50} of 23 nM.

Compound **1b** was also tested for its effect on the proliferation of several cultured human tumour cell lines as measured by the ability of cells to incorporate [^{14}C]thy-



Scheme 1. Synthesis of 3-(1*H*-benzimidazol-2-yl)-5-isoquinolin-4-ylpyrazolo[1,2-*b*]pyridine. Reagents and conditions: (a) I_2 (1 equiv), NaOH (1 equiv), 1,4-dioxane, 40 °C, 8 h, 90%; (b) Br_2 (2 equiv), acetic acid, 100 °C, 12 h, 52%; (c) $(BOC)_2O$ (1 equiv), DMAP (cat.), Et_3N (1 equiv), THF, 94%; (d) tributyl (vinyl)tin (1 equiv), $Pd(II)(PPh_3)_2Cl_2$ (cat.), THF, 80 °C, 12 h, 70%; (e) ozone, Me_2S, CH_2Cl_2, −78 °C, 50%; (f) sulfur(0) (1 equiv), 1,2-phenylenediamine (1 equiv), DMF, 90 °C, 12 h, 70%; (g) $Pd(II)(PPh_3)_2Cl_2$ (cat.), CuI (cat.), 4-(trimethylstannyl)isoquinonine (1 equiv), 80 °C, 12 h, THF, 81%; (h) TFA (5 equiv), DCM, 38%.

midine. In this assay, **1b** inhibited the proliferation of HeLa cervical adenocarcinoma, A375 malignant melanoma and HCT-116 colon carcinoma cells with IC_{50} values of 1.7, 0.87 and 0.55 μM, respectively. Finally, **1b** was evaluated for its ability to inhibit VEGFR-2 kinase, a receptor tyrosine kinase implicated in angiogenesis, another important mechanism for tumour progression.[17] Compound **1b** inhibited VEGFR-2 kinase with an IC_{50} value of 1.46 μM, revealing approximately 60-fold selectivity in favour of CDK1 inhibition.

In summary, a novel heterocyclic compound was discovered to be potent CDK1 inhibitor. In addition to its potential use for anticancer therapy, this CDK1 inhibitor may also be useful for the treatment of other indications such as restenosis, psoriasis, retinopathy and allopecia.[8] An efficient chemistry was developed for the synthesis, with key steps including Pd(II) catalyzed Stille coupling reaction and sulfur(0) induced benzimidazole formation. A detailed structure–activity relationship (SAR) study and pharmacokinetic properties for this series will be reported in due course.

Acknowledgments

We are grateful to Robert H. Gruninger and Angel Fuentes-Pesquera for running the kinase and cell proliferation assays.

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