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1*H*-Pyrazolo[3,4-*b*]pyridine Inhibitors of Cyclin-Dependent Kinases: Highly Potent 2,6-Difluorophenacyl Analogues

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Abstract—Structure–activity studies of 1*H*-pyrazolo[3,4-*b*]pyridine **1** have resulted in the discovery of potent CDK1/CDK2 selective inhibitor **21h**, BMS-265246 (CDK1/cycB $IC_{50}=6$ nM, CDK2/cycE $IC_{50}=9$ nM). The 2,6-difluorophenyl substitution was critical for potent inhibitory activity. A solid state structure of **21j**, a close di-fluoro analogue, bound to CDK2 shows the inhibitor resides coincident with the ATP purine binding site and forms important H-bonds with Leu83 on the protein backbone.

Cyclin-dependent kinases (CDKs) are a family of protein kinases which along with their regulatory subunit cyclins play a key role in the growth, development, proliferation and death of eukaryotic cells and are responsible for insuring integrity in the coordination of events through the cell cycle.[1] Due to both their central role in the cell cycle and their misregulation in a number of cancers, CDKs have been implicated as contributing factors to the development of cancer. Consequently, oncology drug discovery programs have directed major efforts towards the identification of small molecule inhibitors of CDKs as potential therapeutic agents.[2] Our screening efforts recently revealed that pyrazolo[3,4-*b*]pyridines SQ-67563 (**1**) and SQ-67454 (**2**) as CDK inhibitors with relatively potent enzyme inhibitory activity and selectivity for the CDK family (Fig. 1).[3–5] Described herein are the synthesis and SAR of the 5-phenacyl substituent and the solid-state structure of an optimized inhibitor of this chemotype bound to CDK2.

Acyl analogues (Tables 1 and 2) were prepared via addition to key intermediate amide **9**. The overall synthetic route is shown in Scheme 1. Thus, neutralization of PMB-protected aminopyrazole **3**[5] and condensation with commercial acrylate **4** followed by pyrolysis in diphenyl ether solution (240 °C) afforded annulated pyrazole **5**. Treatment of **5** with neat phosphorous oxychloride gave 4-chloropyrazolopyridine **6**. Introduction of the 4-butyloxy substituent via the alkoxide followed by ester hydrolysis afforded acid **7**. Acid chloride coupling of **7** with *N*,*O*-dimethylhydroxylamine followed by removal of the PMB group with TFA yielded key intermediate **9**. In general, treatment of **9** with excess (5–10 equiv) of the appropriate organolithium afforded the desired 5-keto substituted analogues **10** and **21** (Tables 1 and 2).[6,7]

In contrast to organolithium reagents, addition of Grignard reagents to **9** gave competing substitution for the 4-butoxy group. Attempts to add aryllithiums to PMB-protected intermediate **8** also resulted in predominately substitution for the 4-butyloxy group. Amido analogues **10h** and **10i** were available from acid **7** as shown in Scheme 2 by generation of the acid chloride, addition of appropriate amine followed by removal of the PMB group with hot TFA.

The 5-thio analogues[4d] were prepared as shown in Scheme 3 from bicyclic pyridinol ester **5**. Hydrolysis of the ester gave acid **12** which upon heating smoothly decarboxylated to afford pyridinone **13**. Treatment of **13** with bromine gave aryl bromide **14**. The 4-butoxy group group was introduced by chlorination of **14** with

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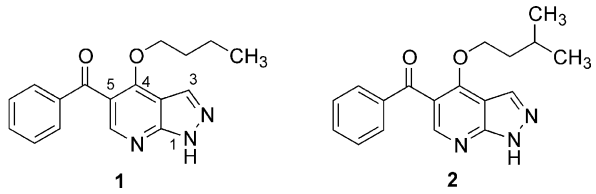
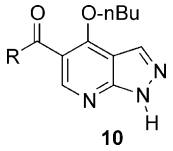


Figure 1. Structure of pyrazolo[3,4-*b*]pyridine screening hits SQ-67563 (**1**) and SQ-67454 (**2**).

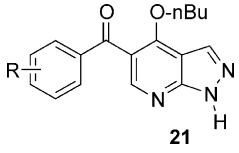
Table 1. SAR of pyrazolopyridine acyl substituent **10**



Compd	R Group	CDK1/cycB IC_{50}, μM[a]	CDK2/cycE IC_{50}, μM[a]
10a	Me	—	>1.0
10b	Cyclopropyl	—	2.7
10c	Benzyl	—	>1.0
1	Ph–	0.15	0.11
10d	3-Pyridyl	—	2.4
10e	2-Furanyl	0.28	0.18
10f	2-Thienyl	0.23	0.20
10g	2-Thiazolyl	0.45	0.21
10h	PhNH–	—	19
10i	EtNH–	—	8.1
10j	MeO–	—	>1.0

[a]See ref 8 for description of biological assays.

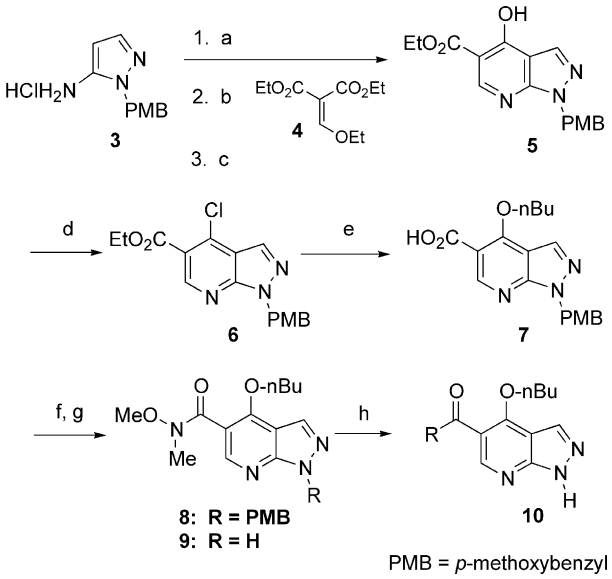
Table 2. SAR of pyrazolopyridine phenyl substitution



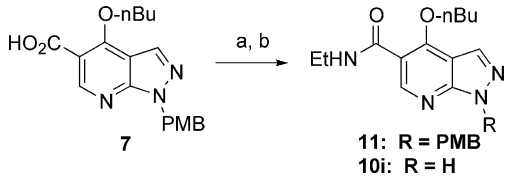
Compd	R Group(s)	CDK1/cycB IC_{50}, μM[a]	CDK2/cycE IC_{50}, μM[a]	CDK4/cycD IC_{50}, μM[a]
21a (1)	H	0.15	0.11	>25
21b	2-Me	0.41	0.73	—
21c	3-Me	—	0.66	—
21d	4-Me	—	0.21	—
21e	2-F	0.36	0.036	24
21f	2,6-Difluoro	0.032	0.064	21
21g	2,4,6-Trifluoro	0.092	0.036	0.69
21h	2,6-Difluoro-4-methyl	0.006	0.009	0.23
21i	2,6-Difluoro-4-chloro	0.013	0.027	0.31
21j	2,6-Difluoro-4-bromo	0.017	0.020	0.37
21k	2,6-Difluoro-4-methoxy	0.007	0.022	0.23
21l	2,4,6-Trimethyl	—	>1.00	—

[a]See ref 8 for description of biological assays.

$POCl_3$ followed by stirring the intermediate 4-chloro analogue **15** with sodium *n*-butoxide to give **16**. The 5-thio substituent was introduced by low temperature metal–halogen exchange of **16** and quenching the resulting anion with diphenyl disulfide to provide **17**. TFA removal of the PMB group afforded 5-thiophenyl analogue **18**. Oxidation of **18** with 1 equiv of mCPBA provided the racemic 5-sulfoxide **19** while oxidation with excess mCPBA gave and 5-sulfone analogue **20**.



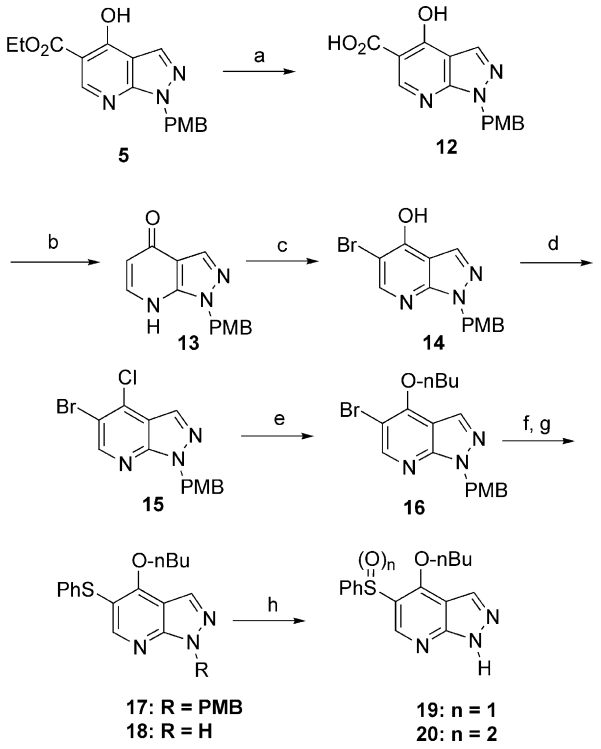
Scheme 1. Synthesis of 5-keto analogues of pyrazolopyridine **1**: (a) desalt; (b) 130 °C, 2.5 h; (c) Ph_2O, 240 °C, 1–2 h, 59% from **3**; (d) $POCl_3$, 120 °C 1 h, 66%; (e) *n*BuONa (3 equiv)/*n*BuOH, 65 °C, 3 h then H_2O added, 65 °C, 3 h, 100%; (f) $(COCl)_2$/cat DMF/CH_2Cl_2, 25 °C, then (MeO)NH(Me)HCl, Et_3N, 0–25 °C, 93%; (g) TFA, 65 °C, 2.5 h, 89%; (h) ArLi (5–10 equiv)/THF, −78 to 0 °C, 10–90%.



Scheme 2. Synthesis of 5-amido analogues: (a) $(COCl)_2$/cat DMF/CH_2Cl_2, 25 °C then $EtNH_2/Et_3N$; (b) TFA, 65 °C, 2.5 h, 58% from **7**.

Table 1 shows the effect of replacement of the phenyl group in the 5-position on in vitro CDK inhibitory activity in a cell-free enzyme assay.[8] Thus, small alkyl groups (**10a**,**b**) or benzyl (**10c**) resulted in >10-fold loss of CDK2 potency. Replacement of the phenyl group with a pyridyl ring (**10d**), a small basic heterocycle, also resulted in a ~200-fold decrease in potency. In contrast, small neutral heterocycles, such as furan, thiophene and thiazole (**10e–g**), afforded analogues with CDK1 and CDK2 inhibitory potency comparable to phenyl analogue **1**. An amide or methyl ester functionality (**10h–j**) at the 5-position resulted in a >10 to 200-fold loss in CDK2 inhibitory potency. Interestingly, both racemic sulfoxide **19** and sulfone **20** were comparatively potent inhibitors of CDK2 with IC_{50} = 0.34 and 0.52 μM, respectively, indicating that a sulfone or sulfoxide may serve as a surrogate for the carbonyl group.

Shown in Table 2 is an examination of phenyl ring substituent effects on CDK inhibitory potency. Thus, substitution of a methyl group in the ortho or meta position of **1** (**21b**,**c**) gave a 6- to 7-fold loss in CDK2 inhibitory potency, while para methyl substitution (**21d**) resulted in only a modest 2-fold loss in CDK2 inhibitory potency relative to **1**. In contrast, mono- and di-*ortho* fluoro substitution (**21e** and **21f**) both resulted in 2- to 3-fold increases in CDK2 inhibitory potency relative to **1**. *Para*



Scheme 3. Synthesis of 5-thio analogues **18**–**20**: (a) aq NaOH/EtOH, 95 °C, 94%; (b) 230 °C, 15 min, 100%; (c) Br_2, EtOH, 0 °C, 97%; (d) $POCl_3$, 110 °C, 1 h, 95%; (e) *n*BuONa/*n*BuOH, 60 °C, 2 h, 86%; (f) *n*BuLi/THF, −78 °C, 30 min then PhSSPh, 29%; (g) TFA, 65 °C, 2.5 h, 62%; (h) mCPBA (1 equiv)/CH_2Cl_2, 0 °C, 74% for **19** or mCPBA(3 equiv)/CH_2Cl_2, 25 °C, 78% for **20**.

substitution coupled with 2,6-difluoro substitution (**21g**–**k**) afforded analogues that were ~3- to 12-fold more potent CDK2 inhibitors than **1**. In particular, the 2,6-difluoro-4-methylphenyl analogue **21h** exhibited CDK1 and CDK2 potency that was 25- and 11-fold more potent versus CDK1 and CDK2, respectively, than phenyl analogue **1** and represented the most potent CDK/CDK2 selective analogue from this chemotype. In contrast to **21h**, the 2,4,6-trimethylphenyl analogue **21l** was a very poor inhibitor of CDK2 indicating that the *ortho* fluoro substituents were a key for potent inhibitory activity. In an ovarian cancer cell line (A2780) inhibitor **21h** produced a cytotoxic effect with an $IC_{50} = 0.76$ μM.[8]

The three-dimensional structure of 2,6-difluoro analogue **21j** in complex with CDK2 was determined by X-ray crystallography. Crystals were obtained by incubating inhibitor **21j** (72 h) with crystalline protein in the absence of cyclin.[8] The crystal structure (Fig. 2) revealed that **21j** binds in the ATP-binding site as seen previously with **1** forming important hydrogen bonds between the pyrazolopyridine ring and Leu83.

The 4-butoxy substituent extends into the space occupied by the ribose of ATP and does not appear to form any specific contacts with the protein while the pendent difluorophenyl ring lies buried within the protein, stacking with Phe80. An overlay of inhibitors **1** and **21j** clearly show similar binding modes (see Fig. 2, bottom).

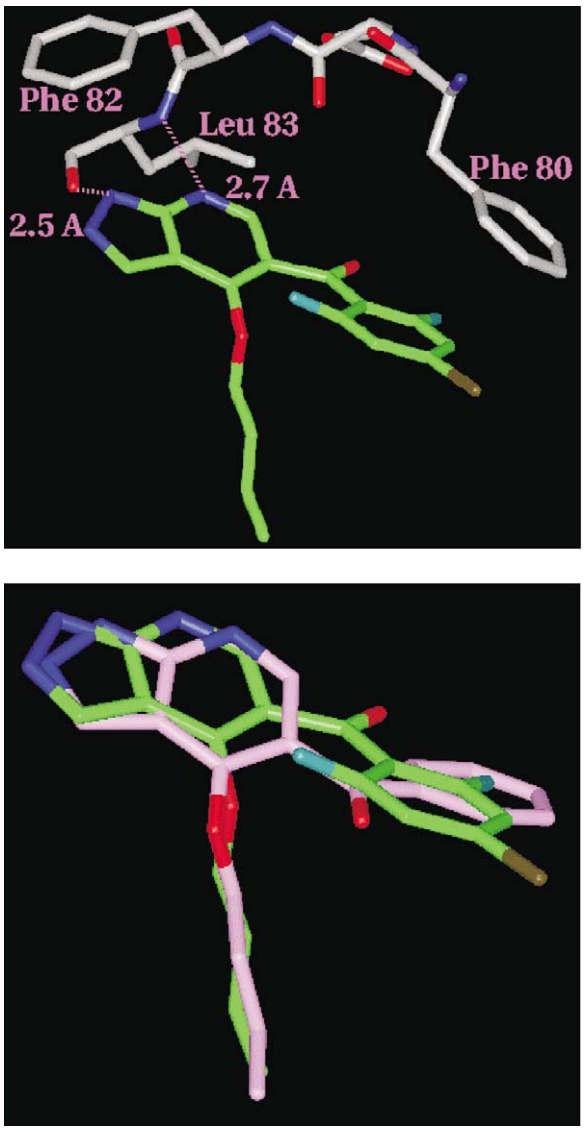
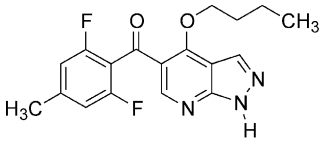


Figure 2. Top: Solid-state structure of pyrazolopyridine **21j** bound in the ATP-pocket of CDK2 (no cyclin). The inhibitor carbon atoms are colored green, the nitrogen atoms are colored blue and oxygen atoms are colored red. The protein carbons are colored gray. Hydrogen bonds are shown by the magenta dotted lines. Bottom: Overlay of compound **1** (magenta) and **21j** (green) bound to CDK2 showing location of 5-phenacyl substituent. The nitrogen atoms are colored blue, the oxygen atoms are colored red, the fluorine atoms are colored slate and the bromine atom is colored in brown.

In this arrangement small, flat pendent aryl groups would be expected to bind optimally to the protein while larger groups would create unfavorable steric interactions with Phe80. This is consistent with the SAR shown in Table 1 in which benzyl (**10c**) and amide substitution (**10h** and **10i**) showed diminished activity. Interestingly, although the pendent phenyl rings of **1** and **21j** occupy nearly the same region in space the carbonyl groups appear to be directed orthogonally. The contrasting orientation of the carbonyl group indicates that it is unlikely that the carbonyl oxygen is involved in a H-bonding interaction. The 2,6-difluorophenyl ring lies nearly coplanar with the carbonyl group (torsional angle = 7.4°) which may provide an explanation for the

reduced activity of the 2,4,6-trimethylphenyl analogue **21l** which requires significant skewing of the phenyl and carbonyl groups.

In summary, SAR studies of pyrazolopyridine **1** have resulted in the discovery of potent, CDK1/CDK2



BMS-265246 (21h)

CDK1/cycB IC_{50} = 0.006 μM

CDK2/cycE IC_{50} = 0.009 μM

CDK4/cycD IC_{50} = 0.230 μM

A2780 Cytotox IC_{50} = 0.76 μM

selective inhibitor **21h** (BMS-265246). The 2,6-difluorophenyl substitution pattern was critical for potent inhibitory activity.

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