Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4297–4302
Bioorganic & Medicinal Chemistry Letters

Synthesis and evaluation of pyrazolo[3,4-*b*]pyridine CDK1 inhibitors as anti-tumor agents

Ronghui Lin,* Peter J. Connolly, Yanhua Lu, George Chiu, Shengjian Li, Yang Yu, Shenlin Huang, Xun Li, Stuart L. Emanuel, Steven A. Middleton, Robert H. Gruninger, Mary Adams, Angel R. Fuentes-Pesquera and Lee M. Greenberger

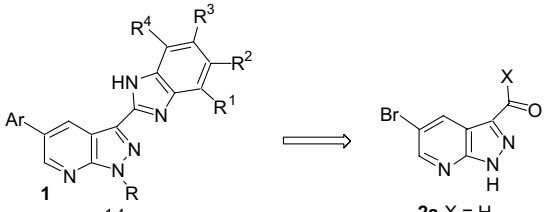
Johnson & Johnson Pharmaceutical Research & Development L.L.C., 1000 Route 202, PO Box 300, Raritan, NJ 08869, USA

Received 7 April 2007; revised 7 May 2007; accepted 9 May 2007
Available online 16 May 2007

Abstract—A series of 3,5-disubstituted pyrazolo[3,4-*b*]pyridine cyclin-dependent kinase (CDK) inhibitors was synthesized. These compounds showed potent and selective CDK inhibitory activities and inhibited in vitro cellular proliferation in cultured human tumor cells. Selected compounds were evaluated in an in vivo tumor xenograft model. The synthesis and biological evaluation of these pyrazolo[3,4-*b*]pyridines and related compounds are reported.

Cyclin-dependent kinases (CDKs), such as CDK1, CDK2, and CDK4, constitute a class of serine–threonine protein kinases that plays an important role in regulation of the cell cycle.[1] Abnormal CDK control of the cell cycle has been strongly linked to the molecular pathology of cancer. CDKs have thus become attractive therapeutic targets for cancer therapy.[2] The CDKs regulate cell cycle progression through complexes with their corresponding cyclin partners such as cyclin A, B, D, and E. For example, CDK1 associated with cyclin B regulates the cell cycle at the G2 and mitosis (cell division) phases. CDK1 inhibitors could block mitosis entry and arrest cell growth, and therefore may be useful therapeutic agents with potentially fewer side effects than conventional cytotoxic drugs targeting DNA synthesis.

Several CDK inhibitors have entered clinical evaluation for the treatment of cancer.[3] These include flavopiridol, 7-hydroxystaurosporine (UCN-01), roscovitine (CYC202), BMS-387032 (SNS-032),[4] PD0332991,[5] and R547[6]. In our program to develop CDK inhibitors as anti-cancer agents, we recently reported that 1-acyl-1*H*-[1,2,4]triazole-3,5-diamine analogs[7] and 2-amino-3-benzoyl-6-anilinopyridine analogs[8] are novel anti-cancer CDK inhibitors and anti-proliferative agents. To discover structurally different CDK1 inhibitors with improved pharmacokinetic and solubility properties, we have designed, synthesized, and evaluated several new series of compounds.[9] Herein we report the synthesis of 3,5-disubstituted pyrazolopyridine analogs (**1**) and their biological evaluation as inhibitors of CDK activity and tumor cell proliferation.



1a, R,R$^{1\text{-}4}$ = H
Ar = isoquinolin-4-yl
1b, R,R$^{2\text{-}4}$ = H, R^{1}= Me
Ar = isoquinolin-4-yl

A convergent approach to synthesize the designed compounds (**1**) was envisioned to employ core intermediates such as 5-bromo-pyrazolo[3,4-*b*]pyridine-3-carboxaldehyde (**2a**) or 5-bromo-pyrazolo[3,4-*b*]pyridine-3-carboxylic acid or its ester (**2b** or **2c**). Key steps for the synthesis would involve (1) a benzimidazole ring formation by cyclization of the 3-carboxaldehyde or 3-carboxylic acid group of **2a** and **2c** with a substituted

Keywords: Pyrazolo[3,4-*b*]pyridine; Cyclin dependent kinase inhibitor.
* Corresponding author. Tel.: +1 908 704 5268; fax: +1 908 526 6465; e-mail: Ronghui.Lin@yahoo.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.05.029

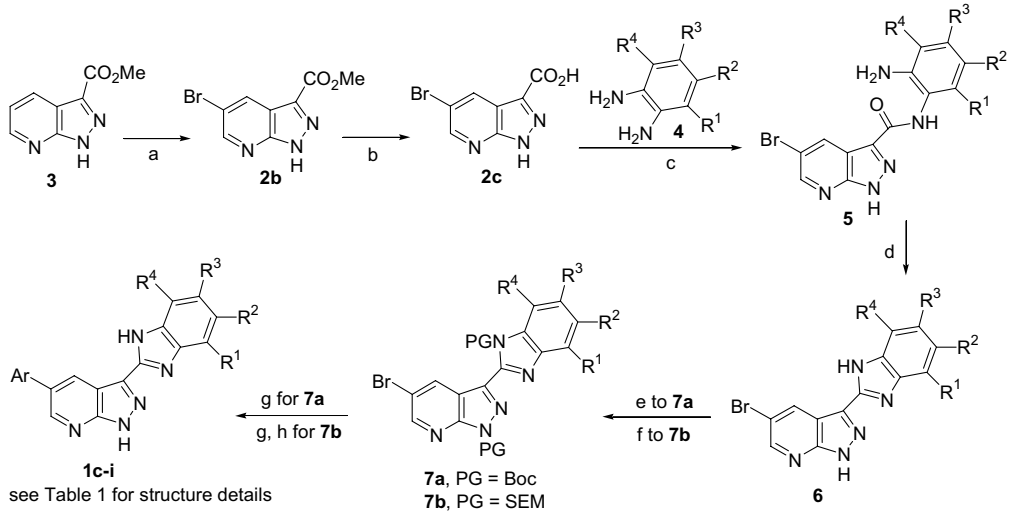
1,2-benzene diamine, and (2) arylation at the 5-position of the resultant 5-bromo-3-benzimidazolyl-pyrazolopyridine, via Suzuki or Stille coupling.

A multi-step approach to prepare 4-[3-(1*H*-benzimidazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl]-isoquinoline (**1a**) through 5-bromo-pyrazolo[3,4-*b*]pyridine-3-carboxaldehyde (**2a**) has been recently reported.[9a] This approach employed benzimidazole ring formation by treating 3-carboxaldehyde compound **2a** and a 1,2-benzene diamine with sulfur(0), followed by Stille coupling with 4-trimethylstannylisoquinoline. 4-[3-(4-Methyl-1*H*-benzimidazol-2-yl)-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl]-isoquinoline (**1b**) was similarly prepared according to these procedures. However, poor reproducibility of this approach prompted us to explore an alternative way through 5-bromo-pyrazolo[3,4-*b*]pyridine-3-carboxylic acid (**2b**) or its ester (**2c**).

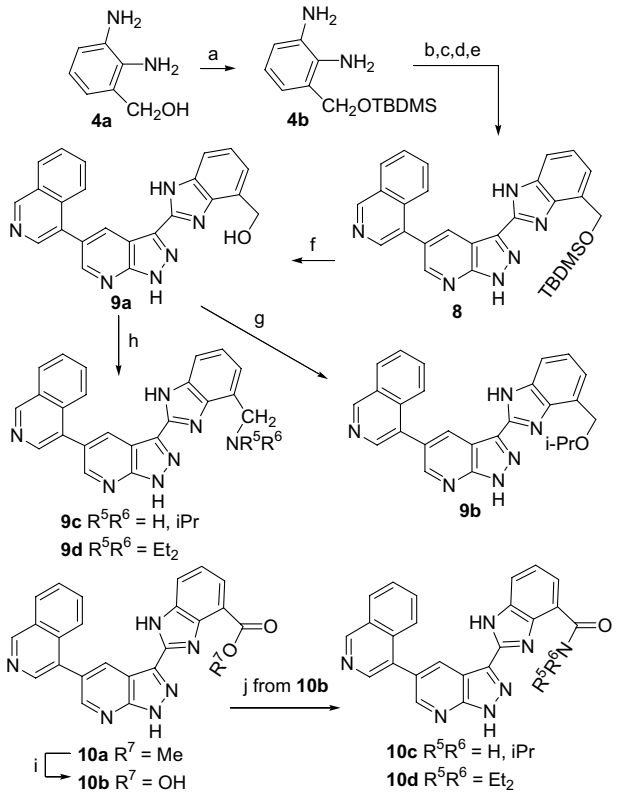
As outlined in Scheme 1, bromination of commercially available pyrazolo[3,4-*b*]pyridine-3-carboxylic acid methyl ester (**3**) afforded intermediate **2b** in 43% yield; **2b** was then hydrolyzed to 5-bromo-pyrazolo[3,4-*b*]pyridine-3-carboxylic acid **2c** quantitatively. Heterocyclization of 3-carboxylic acid group of **2c** with a substituted 1,2-benzene diamine (**4**) gave the desired 5-bromo-3-benzimidazolyl-pyrazolopyridine (**6**) in good yield, via a two-step sequence: amide formation to **5**, followed by treatment with glacial acetic acid. Next, arylation at the 5-position of the resultant **6** could be achieved either via Stille coupling with an organotin reagent, or more preferably via Suzuki coupling with a heteroaryl boronic acid or boronate. Efficient Suzuki or Stille coupling on the pyrazol[3,4-*b*]pyridine substrates usually required protection on pyrazole ring of **6** by a Boc or SEM group giving **7a** or **7b** correspondingly. For unsymmetric substitution patterns on the benzimidazole ring, **7a** or **7b** could be a mixture of isomers. The Suzuki coupling offered better commercial availability and low toxicity of the boron reagents compared to the organotin reagents. The Boc protection protocol for the Suzuki coupling was convenient for its readily useable crude Boc protected intermediate and simultaneous in situ Boc deprotection under Suzuki reaction conditions. In cases where the Boc group of some substrates was too labile and fell off, thus leading to poor Suzuki reaction yield, SEM protection would be an alternative choice. A series of target compounds **1c–i** was prepared by this general approach.

In order to diversify physicochemical properties and improve ADME properties of target compounds, the benzimidazole ring was further derivatized with suitable functional groups such as hydroxy and carboxylic acid. Thus, following the general synthetic approach as shown in Scheme 2, compounds **9a–d** were first prepared from 3-hydroxymethyl-benzene-1,2-diamine (**4a**) and **2c**. The hydroxyl group of **9a** was treated with mesyl chloride to give corresponding mesylate, which was then displaced with isopropanol to give **9b** in 17% yield and with isopropyl amine and diethyl amine to give **9c** and **9d** correspondingly. Similarly, compound **10a** was prepared from 2,3-diamino-benzoic acid methyl ester and compound **2c** via the similar multi-step protocol. Subsequent hydrolysis of **10a** gave acid **10b**. The carboxylic acid group of compound **10b** was converted to amide analogs **10c** and **10d** in a straightforward manner by coupling with isopropyl amine and diethyl amine in 32% and 46% yield, respectively (Scheme 2).

To evaluate the effect of the pyrazole NH on activity, a methyl group was introduced. As shown in Scheme 3, 1*H*-1-methyl-pyrazolo[3,4-*b*]pyridine analog **12** was prepared from 3-methoxymethyl-benzene-1,2-diamine (**4c**) and **2e**, which itself was prepared by methylation of **2b** to **2d** and then saponification.



Scheme 1. General synthetic approach to 3-(1*H*-benzimidazol-2-yl)-5-heteroaryl-pyrazolo[3,4-*b*]pyridine analogs. Reagents and conditions: (a) Br_2, NaOAc, HOAc, 115 °C, sealed flask, 43%; (b) NaOH, H_2O, MeOH, reflux, 4 h, 100%; (c) HATU, DIPEA, DMF, 57–90%; (d) glacial HOAc, 80–120 °C, 3–4 h, 55–87%; (e) $(Boc)_2O$, DMAP, Et_3N, THF, 91–100%; (f) SEM-Cl, NaH, THF, 56–96%; (g) heteroaryl boronic acid or boronate, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, H_2O, 90 °C, overnight, 11–91%; (h) 1:1 4 M HCl/EtOH, 38–97%.

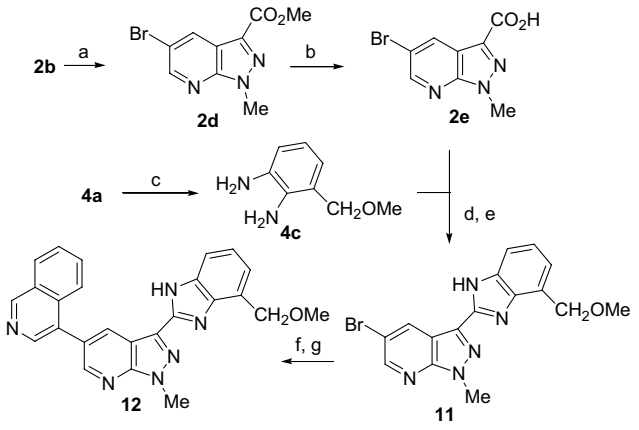


Scheme 2. Derivatization at 3-benzimidazol-2-yl group of pyrazolo[3,4-*b*]pyridine analogs. Reagents and conditions: (a) TBDMS-Cl, 95%; (b) **2c**, HATU, DIPEA, DMF, 61%; (c) glacial HOAc, 80 °C, 3.5 h, 88%; (d) $(Boc)_2O$, DMAP, Et_3N, THF, 100%; (e) 4-isoquinoline boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, H_2O, 90 °C, overnight, 63%; (f) TBAF, THF, 72%; (g) 1—MsCl, DIPEA, THF, 0 °C, 3 h; then 2—*i*-PrOH, NaH, rt, 2 h, 17%; (h) 1—MsCl, DIPEA, THF, 0 °C, 3 h; then 2—*i*-$PrNH_2$, or Et_2NH, rt, 2 h, 11–48%; (i) NaOH, H_2O, MeOH, 80 °C, 5 h, 83%; (j) *i*-$PrNH_2$, or Et_2NH, HATU, DIPEA, DMSO, 33–46%.

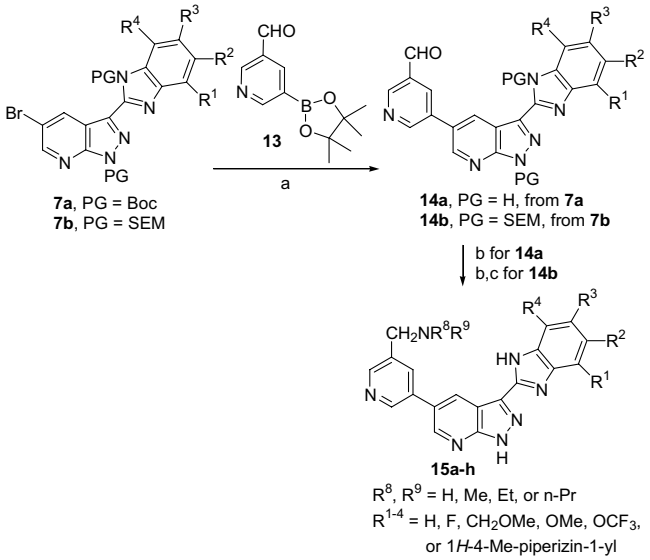
On the other hand, derivatization of the left side of the scaffold was directed toward introducing solubilizing groups onto the 5-pyridin-3-yl substituent. As exemplified in Scheme 4, the 5-bromo-3-benzimidazolyl-pyrazolopyridine, protected with a Boc (**7a**) or SEM (**7b**) group, was coupled with commercially available 5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridine-3-carboxaldehyde (**13**). Usually, Boc groups were simultaneously removed under Suzuki reaction conditions. The carboxaldehyde group of compounds **14a** and **14b** was efficiently converted to a series of target compounds with various amino side chains (**15a**–**h**) by reductive amination with primary or secondary amines, followed by subsequent deprotection in cases of SEM protection.

Alternatively, SEM-protected 5-bromo-3-benzimidazolyl-pyrazolopyridine **7b** was converted to 3-benzimidazolyl-pyrazolopyridine 5-boronate **16**, by treating with bis(pinacolato)diboron by the catalysis of 1,1′-bis(diphenylphosphino)ferrocenedichloro palladium(II) as depicted in Scheme 5. Coupling of intermediate 3-benzimidazolyl-pyrazolopyridine-5-boronate **16** with aryl halides is complementary to Suzuki coupling at the 5-position of 5-bromo-3-benzimidazolyl-pyrazolopyridines **7a** and **17b**. For example, 3,5-dibromo-4-methyl-pyridine (**17**) was formylated upon lithiation with *n*-BuLi and treatment with DMF to give compound **18**. Suzuki coupling of **16** with 3-bromo-pyridine- **18** gave pyridine-3-carboxaldehyde **19** in 69% yield. As before, reductive amination of the carboxaldehyde group of compound **18** with ethylamine gave the target compound having an amino side chain (**20**).

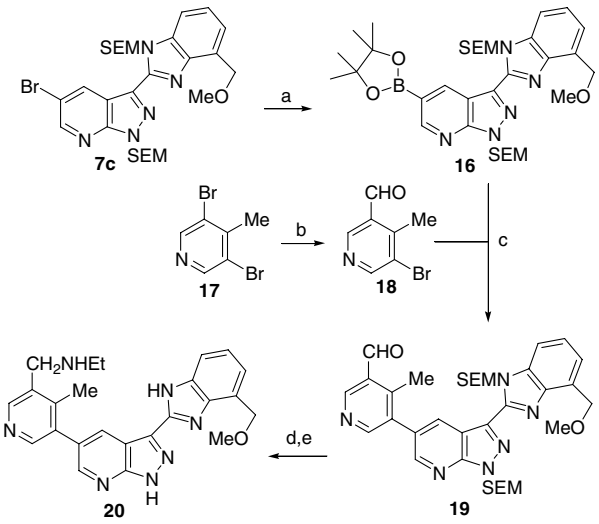
Table 1 lists the structures and inhibitory activities of the 3,5-disubstituted pyrazolo[3,4-*b*]pyridine analogs against CDK1 and four other protein kinase (VEGF-R2, HER2, Aurora-A, and RET kinase). The in vitro anti-proliferative activities in three human tumor cell



Scheme 3. Synthesis of 1*H*-1-methylated pyrazolo[3,4-*b*]pyridine analog **12**. Reagents and conditions: (a) MeI, NaH, THF, 97%; (b) NaOH, H_2O, MeOH, 90%; (c) MeI, NaH, THF, 34%; (d) HATU, DIPEA, DMF, 48%; (e) glacial HOAc, 80 °C, 3.5 h, 89%; (f) $(Boc)_2O$, DMAP, Et_3N, THF, 100%; (g) 4-isoquinoline boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane, H_2O, 90 °C, overnight, 48%.



Scheme 4. Derivatization of the 5-pyridin-3-yl group of pyrazolo[3,4-*b*]pyridine analogs. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, dioxane, H_2O, 90 °C, overnight, 43–62%; (b) $MeNH_2$, $EtNH_2$, *n*-$PrNH_2$, or Et_2NH, $NaBH_4$ or $NaBH_3CN$, 28–99%; (c) 1:1 4 M HCl/EtOH, 38–86%.



Scheme 5. Synthesis and derivatization of 5-(2-methyl-pyridin-3-yl)-pyrazolo[3,4-*b*]pyridine analogs. Reagents and conditions: (a) bis(pinacolato)diboron, 1,1′-bis(diphenylphosphino)ferrocenedichloro palladium(II), 80 °C, overnight, 58%; (b) *n*-BuLi, −78 °C, then DMF; (c) $Pd(PPh_3)_4$, 90 °C, overnight, 69%; (d) $EtNH_2$, $NaBH_4$ or $NaBH_3CN$, 99%; (e) 1:1 4 M HCl/EtOH, 38%.

lines, HeLa (cervical carcinoma), HCT116 (colon carcinoma), and A375 (melanoma), are also shown.

Examination of substitution on the benzimidazole group reveals that a small methoxymethyl group as R^1 at the 4-position (**1c**) gave very favorable CDK1 potency (IC_{50} 5.6 nM), compared to the unsubstituted, methyl, hydroxymethyl, or larger isopropyloxymethyl substituted compounds (**1a**, **1b** and **9a**, **9b**). Small alkylaminomethyl or dialkylaminomethyl groups as R^1 (**9c** and **9d**) gave roughly comparable CDK1 potencies (IC_{50} = 13 and 1.8 nM, respectively). However, replacement of the methoxymethyl group as R^1 at the 4-position (**1c**) with methoxycarbonyl or carboxyl (**10a** and **10b**, $IC_{50} \sim 10$ and 0.54 μM correspondingly) reduced the CDK1 potency significantly. Still, converting the carboxylic acid to a primary or secondary amide (**10c** and **10d**) regained modest CDK1 potency (IC_{50} = 39 and 53 nM, respectively).

On the other hand, the effect of various heteroaryl groups on CDK1 potency seems drastic. For example, compound **1c** with the isoquinolin-3-yl group is much more potent than **1d** with the quinolin-3-yl group (IC_{50} = 5.6 nM vs 1.36 μM). Similarly, the pyridin-3-yl group in **1e** (IC_{50} = 5.6 nM) is much more favorable than the pyridin-4-yl group in **1f** (>1 μM) and pyrimidin-5-yl group in **1g** (1.28 μM). Large-sized substituents such as 4-methyl-piperazin-1-yl and morpholin-1-yl as R^2 at the 5-position of the benzimidazole group (compounds **1h**–**i**) are generally tolerant to CDK1 potency (IC_{50} 14 and 8.5 nM correspondingly).

The importance of the pyrazole NH group in the bicycle core was also remarkable as its methylation led to a substantial reduction on CDK1 activity (compound **12** vs compound **1c**, IC_{50} = 5.6 vs 120 nM).

Replacing the isoquinolin-3-yl group with the pyridin-3-yl group at the left side of the scaffold usually gave better solubility properties while retaining favorable CDK1 potency. Thus derivatization of the left side of the scaffold was focused to introduce solubilizing groups onto the pyridin-3-yl substituent. The most favorable groups among those studied were 5-(alkylaminomethyl)-pyridin-3-yls such as those in compounds **15a**–**h**, which all have single digit or sub-nM IC_{50} values against CDK1. Various substituents such as alkyl, halogen, alkoxyl, and dialkylamino groups on the benzimidazole ring on the right side were well tolerated. Interestingly, the 5-dialkylaminomethyl substituted pyridin-3-yl in compound **15h** (IC_{50} = 120 nM), instead of 5-monoalkylamino methyl groups such as those in compounds **15a**–**h**, was detrimental to CDK1 potency. Introduction of one extra methyl group at 4-position of the pyridine substituent also drastically reduced CDK1 potency (**20**, IC_{50} = 110 nM).

Comparison of CDK1 activity with the other four protein kinases tested, VEGF-R2, HER2, Aurora-A, and RET kinase, revealed that these pyrazolopyridine analogs are generally quite selective toward CDK1. Selectivity in favor of CDK1 inhibition versus the other kinase inhibitory activities was commonly seen in the range of 10–100-fold. In fact, many compounds showed IC_{50} values >10 μM or >100 μM for VEGF-R2, HER2, Aurora-A, and RET kinases, indicating no observed 50% inhibition at the highest dose tested.

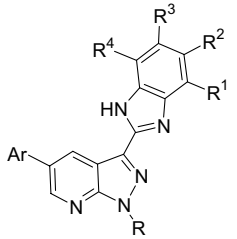
These pyrazolopyridine analogs also proved to be active in vitro as anti-proliferatives in three cultured human tumor cell lines, such as HeLa, HCT116, and A375. The lead CDK1 inhibitors showed potent inhibition of cell proliferation with IC_{50} values ranging from 10 to 500 nM among the tumor cell lines tested.

Preliminary pharmacokinetic studies were conducted in rats with selected compounds. Table 2 lists the pharmacokinetic data for compounds **1h** and **15e**. Pharmacokinetic parameters were determined after single oral (po) and intravenous (iv) doses in 10% Solutol® D5W and 20% HPβCD in water vehicle, respectively. Both compounds demonstrated poor oral availability (F%), low exposure (AUC), and high clearance in rats.

Selected compounds were also evaluated for their in vitro safety and ADME properties. They were tested for stability in human liver microsomes, inhibition of cloned cytochrome P450 enzymes, and inhibition of astemizole binding to hERG channel. Most of the lead compounds had human liver microsome stability with $t_{1/2}$ > 30 min and had IC_{50} values for cloned P450s and hERG generally >10 μM. Solubility was found to be around 12.5 mg/mL in 20% HPβCD for compounds **15b**–**g**.

The in vivo efficacy of selected compounds was examined in the A375 human melanoma cell xenograft model in nude mice, administered intraperitoneally (ip) at 25 mg/kg in 20% HPβCD, which is close to the maximal tolerated dose. Limited animal tolerance and poor anti-

Table 1. Structures, CDK1 and other protein kinase inhibitory activity, and cellular anti-proliferative activity on various tumor cells of the 3,5-disubstituted pyrazolo[3,4-*b*]pyridine analogs



Compound	Ar	R, R^1, R^2, R^3, R^4	IC_{50}[a] (μM)							
			CDK1/ cyclin B	VEGF-R2	HER2	Aurora-A	RET	HeLa	HCT-116	A375
1a	Isoquinolin-4-yl	H, H, H, H, H	0.023	1.46	0.14	ND	ND	1.7	0.55	0.87
1b	Isoquinolin-4-yl	H, Me, H, H, H	0.097	10	1	ND	ND	3.09	1.64	1.72
9a	Isoquinolin-4-yl	H, CH_2OH, H, H, H	0.041	>100	0.24	>10	>100	1.76	0.89	1.47
1c	Isoquinolin-4-yl	H, CH_2OMe, H, H, H	0.0056	0.46	0.085	0.32	>10	0.015	0.010	0.010
9b	Isoquinolin-4-yl	H, CH_2O(*i*-Pr), H, H, H	0.099	~0.1	>100	>1	>100	2.14	2.88	0.91
1d	Quinolin-3-yl	H, CH_2OMe, H, H, H	1.36	>100	>100	>100	>100	1.46	0.0032	0.011
1e	Pyridin-3-yl	H, CH_2OMe, H, H, H	0.029	0.16	>100	>1	1.1	0.65	0.019	0.033
1f	Pyridin-4-yl	H, CH_2OMe, H, H, H	>1.0	0.11	>100	>1	>100	3.09	0.062	0.023
1g	Pyrimidin-5-yl	H, CH_2OMe, H, H, H	1.28	0.11	>100	~10	>100	9.91	0.33	0.16
9c	Isoquinolin-4-yl	H, CH_2NH(*i*-Pr), H, H, H	0.013	~1	>100	3.62	>100	0.13	0.11	0.14
9d	Isoquinolin-4-yl	H, CH_2NEt_2, H, H, H	0.0018	1.55	0.089	1	1	0.029	0.031	0.047
1h	Isoquinolin-4-yl	H, H, (1*H*-4-Me-piperizin-1-yl), H, H	0.014	1.00	6.51	~1	~1	0.45	0.36	0.16
1i	Isoquinolin-4-yl	H, H, (1*H*-morpholin-1-yl), H, H	0.0085	1.07	>100	0.041	~1	0.40	0.37	0.30
10a	Isoquinolin-4-yl	H, CO_2Me, H, H, H	~10	~100	>100	~100	>100	10.5	8.01	7.38
10b	Isoquinolin-4-yl	H, CO_2H, H, H, H	0.54	>10	>100	>10	>100	>10	>10	>10
10c	Isoquinolin-4-yl	H, CONH(*i*-Pr), H, H, H	0.039	0.38	>10	>0.1	0.41	0.035	0.042	0.065
10d	Isoquinolin-4-yl	H, $CONEt_2$, H, H, H	0.053	~10	>10	~1	1.23	0.17	0.055	0.071
12	Isoquinolin-4-yl	Me, CH_2OMe, H, H, H	0.12	>100	>100	~100	>100	1.17	0.20	0.26
15a	5-($MeNHCH_2$)-pyridin-3-yl	H, CH_2OMe, H, H, H	0.0025	0.64	>100	>1	~1	0.46	0.31	0.27
15b	5-(*n*-$PrNHCH_2$)-pyridin-3-yl	H, CH_2OMe, H, H, H	0.0074	ND	>10	3.99	>10	0.26	0.27	0.25
15c	5-($EtNHCH_2$)-pyridin-3-yl	H, H, (1*H*-4-Me-piperizin-1-yl), H, H	0.0030	0.025	1.07	0.28	0.14	0.47	0.54	0.63
15d	5-($EtNHCH_2$)-pyridin-3-yl	H, H, F, F, H	0.0011	~0.1	3.60	>1	0.039	0.14	0.095	0.046
15e	5-($EtNHCH_2$)-pyridin-3-yl	H, H, F, H, H	0.0007	0.15	5.12	0.80	0.11	0.059	0.094	0.051
15f	5-($MeNHCH_2$)-pyridin-3-yl	H, H, OCF_3, H, H	0.009	>0.1	3.82	~1	>100	0.093	0.055	0.047
15g	5-($EtNHCH_2$)-pyridin-3-yl	H, H, OCH_3, H, H	0.0008	>0.1	4.17	0.36	~1	0.032	0.049	0.10
15h	5-(Me_2NCH_2)-pyridin-3-yl	H, CH_2OMe, H, H, H	0.12	1.60	>100	3.75	3.23	1.23	0.19	0.33
20	5-($EtNHCH_2$)-4-Me-pyridin-3-yl	H, CH_2OMe, H, H, H	0.11	4.67	>100	8.02	>10	2.07	0.44	0.12

[a] See Ref. 9b for descriptions of the kinase and cellular anti-proliferation assays. IC_{50} data are the average of at least two separate experiments and are rounded up to two significant figures. IC_{50} values listed as >1, or >10, or >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.

Table 2. Pharmacokinetics of the selected compounds in rats

Compound	**1h**	**15e**
% Oral bioavailability	4	8
Vehicle	10% Solutol® in D5W	20% HPβCD in water
$t_{1/2}$ (h), po	3.3	31.3
C_{max} (ng/mL), po	65	3.8
AUC (ng h/mL), po	173	150
Dose (mg/kg), po	10	10
$t_{1/2}$ (h), iv	0.73	16.1
Cmax (ng/mL), iv	1000	217
AUC (ng h/mL), iv	959	372
Clearance (mL/min kg)	34.9	93.6
Dose (mg/kg), iv	2	2

tumor activity were found in this preliminary efficacy model.

In summary, we have discovered a novel series of 3,5-disubstituted pyrazolo[3,4-*b*]pyridine analogs that are potent and selective cyclin-dependent kinase and cellular anti-proliferative inhibitors. The key steps for the synthesis employed benzimidazole ring formation by cyclization of the 3-carboxyl group of 5-bromo-pyrazolo-[3,4-*b*]pyridine-3-carboxylic acid with substituted 1,2-benzene diamines and arylation at the 5-position via Suzuki coupling. Representative compounds were potent inhibitors of in vitro cellular proliferation in HeLa, HCT116, and A375 human tumor cell lines. Selected compounds were evaluated for other biological properties including an in vivo tumor xenograft efficacy model.

References and notes

1. Harper, J. W.; Adams, P. D. *Chem. Rev.* **2001**, *101*, 2511.
2. (a) Sielecki, T. M.; Boylan, J. F.; Benfiled, P. A.; Trainor, G. T. *J. Med. Chem.* **2000**, *43*, 1; (b) Toogood, P. I. *Med. Res. Rev.* **2001**, *21*, 487; (c) Hirai, H.; Kawanishi, N.; Iwasawa, Y. *Curr. Top. Med. Chem.* **2005**, *5*, 167.
3. (a) Fischer, P. M.; Gianella-Borradori, A. *Expert Opin. Invest. Drugs* **2003**, *12*, 955; (b) Sausville, E. A. *Curr. Med. Chem. – Anti-cancer Agents* **2003**, *3*, 47; (c) Benson, C.; Kaye, S.; Workman, P.; Garrett, M.; Walton, M.; de Bono, J. *Br. J. Cancer* **2005**, *92*, 7.
4. Misra, R. N.; Xiao, H.; Kim, K. S.; Lu, S.; Han, W.; Barbosa, S. A.; Hunt, J. T.; Rawlins, D. B.; Shan, W.; Ahmed, S. J.; Qian, L.; Chen, B.; Zhao, R.; Bednarz, M. S.; Kellar, K. A.; Mulheron, J. G.; Batorsky, R.; Roongta, U.; Kamath, A.; Marathe, P.; Ranadive, S. A.; Sack, J. S.; Tokarski, J. S.; Pavletich, N. P.; Lee, F. Y. F.; Webster, K. R.; Kimball, S. D. *J. Med. Chem.* **2004**, *47*, 1719.
5. (a) Toogood, P. L.; Harvey, P. J.; Repine, J. T.; Sheehan, D. J.; VanderWel, S. N.; Zhou, H.; Keller, P. R.; McNamara, D. J.; Sherry, D.; Zhu, T.; Brodfuehrer, J.; Choi, C.; Barvian, M. R.; Fry, D. W. *J. Med. Chem.* **2005**, *48*, 2388; (b) Fry, D. W.; Harvey, P. J.; Keller, P. R.; Elliott, W. L.; Meade, M.; Trachet, E.; Albassam, M.; Zheng, X.; Leopold, W. R.; Pryer, N. K.; Toogood, P. L. *Mol. Cancer Ther.* **2004**, *3*, 1427.
6. Chu, X.; DePinto, W.; Bartkovitz, D.; So, S.; Vu, B. T.; Packman, K.; Lukacs, C.; Ding, Q.; Jiang, N.; Wang, K.; Goelzer, P.; Yin, X.; Smith, M. A.; Higgins, B. X.; Chen, Y.; Xiang, Q.; Moliterni, J.; Kaplan, G.; Graves, B.; Lovey, A.; Fotouhi, N. *J. Med. Chem.* **2006**, *49*, 6549.
7. (a) Lin, R.; Connolly, P. J.; Wetter, S.; Huang, S.; Emanuel, S.; Gruninger, R.; Middleton, S. *PCT Int. Appl.* **2002**, WO 2002057240; . *Chem. Abstr.* **2002**, *137*, 125160; (b) Emanuel, S. L.; Rugg, C.; Lin, R.; Connolly, P. J.; Napier, C.; Hollister, B.; Hall, C.; Middleton, S. *Proc. Am. Assoc. Can. Res.* **45**, 191. 95th Annual Meeting of the American Association for Cancer Research, Orlando, Florida, March 27–31, 2004; Abstract #833; (c) Emanuel, S.; Gruninger, R.; Lin, R.; Rugg, C.; Fuentes-Pesquera, A. R.; Connolly, P.; Wetter, S.; Hollister, B.; Kruger, W. W.; Napier, C.; Johnson, D.; Jolliffe, L.; Middleton, S. *Proc. Am. Assoc. Can. Res.* **44**, 162. 94th Annual Meeting of the American Association for Cancer Research, Washington, DC, July 11–14, 2003; Abstract #707.
8. (a) Lin, R.; Lu, Y.; Wetter, S. K.; Connolly, P. J.; Turchi, I. J.; Murray, W. V.; Emanuel, S. L.; Gruninger, R. H.; Fuentes-Pesquera, A.; Middleton, S. A.; Jolliffe, L. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2221; (b) Lin, R.; Wetter, S. K.; Lu, Y.; Connolly, P. J.; Emanuel, S.; Gruninger, R. H.; Middleton, S. A. *PCT Int. Appl.* **2005**, WO2005051387A1.
9. (a) Huang, S.; Lin, R.; Yu, Y.; Lu, Y.; Connolly, P. J.; Chiu, G.; Li, S.; Emanuel, S. L.; Middleton, S. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1243; (b) Chiu, G.; Li, S.; Connolly, P. J.; Middleton, S. A.; Emanuel, S. L.; Huang, S.; Lin, R.; Lu, Y. *PCT Int. Appl.* **2006**, WO2006130673A1; (c) Huang, S.; Connolly, P. J.; Lin, R.; Emanuel, S. L.; Middleton, S. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*(14), 3639; (d) Huang, S.; Li, R.; Connolly, P. J.; Emanuel, S.; Middleton, S. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4818.