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Design, synthesis, and evaluation of 3,4-disubstituted pyrazole analogues as anti-tumor CDK inhibitors

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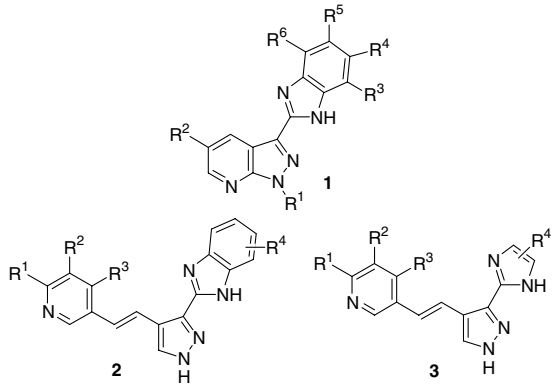
Abstract—Two series of 3,4-disubstituted pyrazole analogues, 3-(benzimidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**2**) and 3-(imidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**3**), were synthesized as novel cyclin-dependent kinase (CDK) inhibitors. Representative compounds showed potent and selective CDK inhibitory activities and inhibited in vitro cellular proliferation in various human tumor cells. The design, synthesis, and preliminary biological evaluation of these pyrazole compounds are reported.

Cyclin-dependent kinases are members of a family of serine–threonine protein kinases responsible for regulation of the eukaryotic cell cycle.[1] The conjunction of CDK subunits with their corresponding cyclin regulatory subunits results in a subset of kinase complexes with specific functions in the process of cell division. The regulated activation of these complexes guides cells through the cell cycle and ensures the accurate execution of cell division. Cell proliferation occurs in distinct phases, which are each controlled by different CDKs coupled with a corresponding cyclin partner. Cancer is well known as a disease of aberrant cellular proliferation and most cancer cells exhibit deregulation of CDKs. As a result, CDK inhibitors have been intensively pursued as potential anti-cancer agents.[2] A number of CDK inhibitors,[2,3] such as flavopiridol, 7-hydroxystaurosporine (UCN-01), roscovitine (CYC202), BMS-387032 (SNS-032),[4] PD0332991,[5] and R547,[6] have been studied in clinical trials for the treatment of cancer.

In our program to develop CDK inhibitors as anti-cancer agents, we have recently reported 1-acyl-1*H*-[1,2,4]triazole-3,5-diamine analogues and 2-amino-3-benzoyl-6-anilinopyridine analogues that are novel anti-cancer CDK inhibitors and anti-proliferative agents.[7,8] More recently, we reported that 3-benzimidazol-2-yl pyrazolo[3,4-*b*]pyridine analogues (**1**) were novel potent CDK inhibitors and anti-proliferative agents.[9] To discover structurally different CDK inhibitors with improved physicochemical, pharmacokinetic, and solubility properties, we have synthesized and evaluated two related series of 3,4-disubstituted pyrazole analogues, in particular, 3-(benzimidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**2**) and 3-(imidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**3**), as novel CDK inhibitors. Herein we report their design, synthesis, and preliminary biological evaluation.



Several properties of the 3-benzimidazoyl pyrazolo[3,4-*b*] pyridine analogues (**1**), including aqueous solubility and oral bioavailability, were targeted for improvement. To address these areas, a structural modification was

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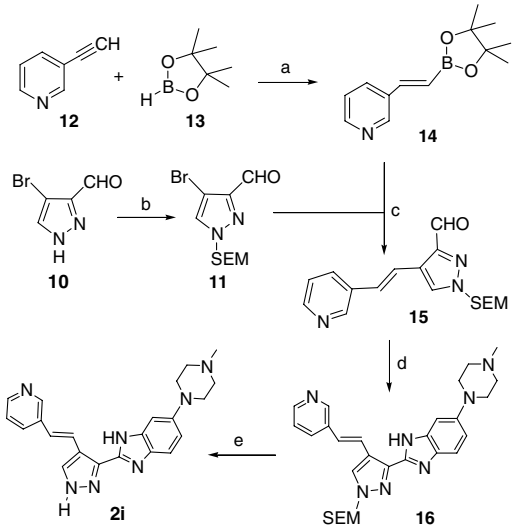
proposed to replace both bicyclic rings with monocycles while retaining necessary binding elements of the pharmacophore. Specifically, SAR of the previous 3-benzimidazoyl pyrazolo[3,4-*b*] pyridine series (**1**) revealed that pyrazole, imidazole, and pyridinyl or isoquinoyl groups were critical for CDK inhibitory activity. Thus, we envisioned using an (*E*)-4-vinyl pyrazole moiety to replace the core bicyclic pyrazolopyridine, and also using imidazole to replace the benzimidazole, as shown in structures **2** and **3**.

A general approach to synthesize the target compounds **2** and **3** was designed to use commercially available 4-iodo-pyrazole-3-carboxylic acid (**4**) and 4-bromo-pyrazole-3-carboxaldehyde (**10**). Key steps for the synthesis involved benzimidazole or imidazole ring formation by cyclization of the 3-carboxaldehyde or 3-carboxylic acid group of **4** or **10**; and an arylvinylation at the 4-position of the pyrazole ring via stepwise Stille and Heck couplings or via one-step Suzuki coupling.

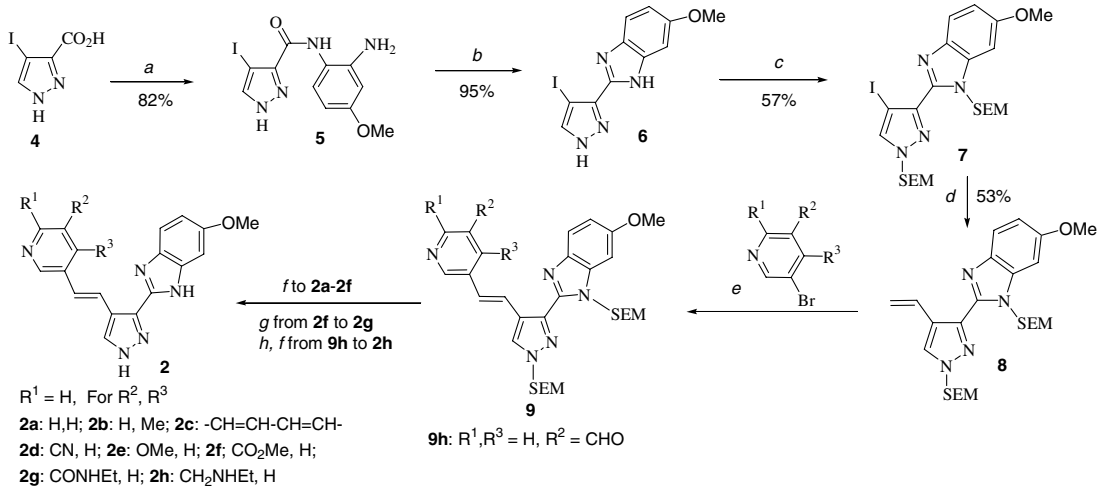
As outlined in Scheme 1, commercially available 4-iodo-pyrazole-3-carboxylic acid (**4**) was coupled with 4-methoxy-benzene-1,2-diamine to give amide **5**, followed by cyclization in glacial acetic acid. Efficient Suzuki or Stille coupling on the pyrazole substrates required *N*-protection on the pyrazole ring, accomplished using SEM chloride to provide **7**. Next, the vinylation at the 4-position of the SEM-protected **7** was achieved via Stille coupling with tributyl vinyl stannane. Then, 4-vinyl pyrazole **8** underwent Heck reaction with a heteroaryl bromide to give coupled compound **9**. Subsequent SEM-de-protection gave the targeted compound (**2**). For unsymmetric substitution patterns on the benzimidazole ring, **7**–**9** could be a mixture of two isomers (only one isomer shown in Scheme 1). A series of target analogues **2a**–**f** were prepared by this general approach. Finally, the carboxylic ester of compound **2f** was hydrolyzed and the resultant carboxylic acid was further coupled with ethylamine to form amide compound **2g**.

Alternatively, SEM-protected 3-benzimidazolyl-5-bromopyrazole **8** was coupled with commercially available 5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridine-3-carboxaldehyde to give compound **9h**, in 28% yield. The carboxaldehyde group of **9h** underwent a reductive amination with ethylamine and $NaBH_4$ in 55% yield, and consequent de-protection of the SEM protecting group gave compound **2h** in 72% yield.

The stepwise Stille and Heck coupling could be simplified with a one-step Suzuki coupling using a vinyl heteroaryl boronate. As shown in Scheme 2, hydroboration of commercially available 3-ethynyl-pyr-



Scheme 2. Synthetic approach toward 3-(1*H*-benzimidazol-2-yl)-4-[2-(pyridine-3-yl)-vinyl]-pyrazole analogues via Suzuki coupling. Reagents and conditions: (a) $RuHCl(CO)(PPh_3)$, 50 °C, toluene, 95%; (b) SEM-Cl, NaH, THF, 57%; (c) $Pd(dppf)Cl_2 \cdot DCM$, Na_2CO_3/H_2O, dioxane, 120 °C, 72%; (d) 4-(4-methyl-piperazin-1-yl)-benzene-1,2-diamine, S(0), DMF, 80 °C, 6 h, 50%; (e) 4 M HCl, EtOH, 70 °C, 6 h, 82%.



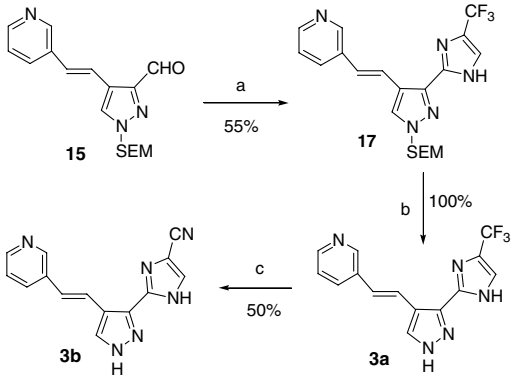
Scheme 1. General synthetic approach to 3-(1*H*-benzimidazol-2-yl)-4-[2-(heteroaryl)-vinyl]-pyrazole analogues. Reagents and conditions: (a) 4-methoxy-benzene-1,2-diamine, HATU, DIPEA, DMF, 82%; (b) glacial HOAc, 90 °C, 12 h, 95%; (c) SEM-Cl, NaH, THF, 57%; (d) tributyl vinyl stannane, $Pd(PPh_3)_4$, DMF, 72 °C, 10 h, 53%; (e) $Pd(dppf)Cl_2$.DCM, cat. BHT, DMF, 90 °C, 8 h, 11–28%; (f) 1:1 EtOH/4 M HCl, 80 °C, 12 h, 38–72%; (g) NaOH, MeOH; then $EtNH_2$, HATU, DIPEA, DMF, 45% for two steps; (h) $EtNH_2$, $NaBH_4$, 55%.

idine (**12**) with pinacolborane (**13**) in the presence of carbonylchlorohydridotris(triphenylphosphine) ruthenium (II), $RuHCl(CO)(PPh_3)$,[10] afforded (*E*)-2-(pyridine-3-yl)vinyl-boronate **14**. Suzuki coupling of **14** with SEM-protected 4-bromo-1*H*-pyrazole-3-carboxaldehyde (**11**, prepared from **10**) gave compound **15** in 72% yield. Benzimidazole ring formation was achieved by treating 3-carboxaldehyde **15** and 4-(4-methyl-piperazin-1-yl)-1,2-benzenediamine with sulfur (0) to give compound **16** in 50% yield. Subsequent de-protection of the SEM group in **16** gave compound **2i** in 82% yield.

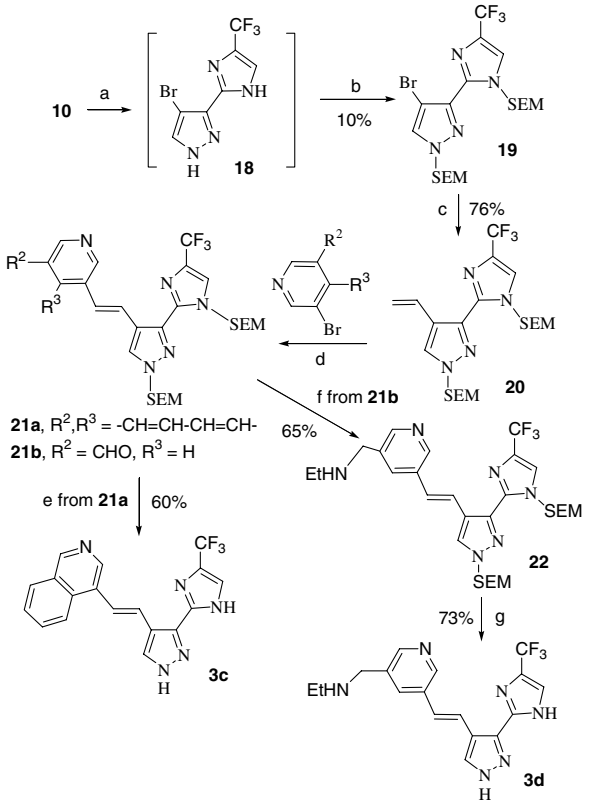
To further modify the structure of the benzimidazole series (**2**), the bicyclic benzimidazole ring was replaced with monocyclic imidazole as shown in Scheme 3. For example, following a literature procedure for imidazole ring formation,[11] aldehyde **15** was treated with dibromotrifluoroacetone, sodium acetate, and concentrated ammonium hydroxide, to give compound **17**; which upon de-protection produced target compound **3a**. Compound **3a** was further converted to compound **3b** with ammonium hydroxide in 50% yield.

Complementary to this approach, the imidazole series could be assembled from the right to left side as shown in Scheme 4. Imidazole ring formation from aldehyde **10** followed by SEM protection on both imidazole and pyrazole rings gave bromide **19**, which underwent Stille coupling with tributyl vinyl stannane to give compound **20**. The resultant 4-vinyl pyrazole **20** then proceeded via Heck reaction with a heteroaryl bromide to give the corresponding compound **21**. For example, Heck coupling of **20** with 4-bromoisoquinoline afforded **21a**, and subsequent SEM-de-protection of **21a** gave compound **3c**. Similarly, Heck coupling of **20** with 5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridine-3-carboxaldehyde to **21b** was followed by reductive amination of the resultant **21b** to give **22**, and subsequent de-protection gave compound **3d**.

Table 1 shows the inhibitory activities for the two series of 3,4-disubstituted pyrazole analogues against CDK1 and three other kinases (HER2, VEGF-R2, and Aurora-A kinases). Analogues in both series, the 3-(benzimidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**2**) and the 3-(imidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**3**), show modest to potent CDK1 inhibition, with IC_{50} values ranging from about 1 μM to single digit nM. In general, these two series are slightly less potent CDK1 inhibitors compared to the previously reported 3-benzimidazol-2-yl pyrazolo[3,4-*b*]pyridine analogues (**1**).[9] In the benzimidazole series (**2**), electron donating groups on the pyridine ring (e.g., compound **2e** with methoxy) seem to render more potent CDK1 activity compared to electron withdrawing groups (e.g., compounds **2d** with cyano and **2f** with methoxycarbonyl substituents). Also, in the benzimidazole series the monocyclic pyridine ring confers fivefold higher potency than isoquinoline bicycle (compound **2a** vs compound **2c**, IC_{50} = 0.033 vs 0.15 μM). On the other hand, in the imidazole series (**3**) the monocyclic pyridine ring leads to a fivefold drop in potency compared to the isoquinoline bicycle (compound **3a** vs compound **3c**, IC_{50} = 0.48 vs 0.11 μM). Similar to the parent pyrazolo[3,4-*b*]pyridine series (**1**), the 3-ethylamino methyl substituent in compound **2h** confers very favorable CDK1 potency (IC_{50} = 4.6 nM), compared to the unsubstituted (**2a**), methyl (**2b**), and other substituted compounds. Comparison of **2h** with corresponding amide compound **2g** is also striking (IC_{50} 0.0046 vs 0.11 μM). In addition,



Scheme 3. Synthesis of 3-(1*H*-imidazol-2-yl)-4-[2-(pyridine-3-yl)-vinyl]-pyrazole analogues. Reagents and conditions: (a) $CF_3COCHBr_2$, NaOAc, NH_4OH, EtOH, 55%; (b) 4 M HCl, EtOH, 80 °C, 16 h, 100%; (c) 5% NH_4OH/H_2O, 60 °C, 2 h, 50%.



Scheme 4. Synthetic approach to 3-(1*H*-imidazol-2-yl)-4[2-(heteroaryl)vinyl]-pyrazole analogues via Stille and Heck coupling. Reagents and conditions: (a) HATU, DIPEA, DMF; (b) SEM-Cl, NaH, THF, 10% for (a and b); (c) tributyl vinyl stannane, $Pd(PPh_3)_4$, DMF, 72 °C, 10 h, 76%. (d) $Pd(dppf)Cl_2 \cdot DCM$, cat. BHT, DMF, 90 °C, 8 h, 11–32%; (e) 1:1 EtOH/4 M HCl, 80 °C, 12 h, 60%; (f) $NaBH_4$, $EtNH_2$, 65%; (g) 1:1 EtOH/4 M HCl, 73%.

Table 1. CDK1 and other kinase inhibitory activities of the two series of 3,4-disubstituted pyrazole analogues (**2a–i**, **3a–d**)

Compound	IC_{50}[a] (μM)			
	CDK1/cyclin B	HER2	VEGF-R2	Aurora-A
2a	0.033	>10	0.97	0.51
2b	0.16	>10	0.76	0.55
2c	0.15	>100	0.61	0.53
2d	>1	>100	1.7	4.20
2e	0.047	12	0.65	0.40
2f	0.89	>100	1.28	>100
2g	0.11	37	1.35	>100
2h	0.0046	3.03	0.82	0.59
2i	0.029	9.3	0.29	>10
3a	0.48	>100	29	0.12
3b	0.70	>100	>10	>10
3c	0.11	32.73	3.06	0.30
3d	0.018	>100	~10	>10

[a] Values are means of at least two experiments and are rounded up to two significant figures. IC_{50} values listed as >10 or >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.

Table 2. In vitro cellular anti-proliferative activity of the two series of 3,4-disubstituted pyrazole analogues (**2a–i**, **3a–d**) on human tumor cells

Compound	IC_{50}[a] (μM)		
	A375	HCT-116	HeLa
2a	2.5	2.2	1.8
2b	4.3	3.2	2.8
2c	3.0	2.7	2.8
2e	0.91	0.75	0.89
2f	3.1	3.1	4.4
2g	3.6	2.5	3.7
2h	0.55	0.19	0.62
2i	0.86	0.19	0.46
3a	47	24	>10
3b	>10	70.7	>10
3c	5.6	2.9	3.6
3d	7.4	1.7	3.2

[a] Values are means of at least two experiments and are rounded up to two significant figures. IC_{50} values listed as >10 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.

examination of substitution on the benzimidazole group revealed that the bulky and polar 4-methyl-piperazine analogue **2i** has CDK1 potency comparable to the methoxy analogue **2e**.

Comparison of CDK1 activity with inhibition of the other three protein kinases, HER2, VEGF-R2, and Aurora-A kinase, revealed that pyrazole analogues **2** and **3** are generally selective toward CDK1. Selectivity toward CDK1 inhibition versus the receptor tyrosine kinases HER2 and VEGF-R2 was commonly seen in the range of 5- to 10-fold, while selectivity versus Aurora-A, which is a member of the same family of serine–threonine protein kinases as CDK1, was lower.

Table 2 shows the in vitro anti-proliferative activities in cultured human tumor cells for representative compounds. The selected compounds proved to be active in vitro as anti-proliferatives in various human tumor cell lines, such as HeLa (cervical carcinoma), HCT116 (colon carcinoma), and A375 (melanoma), although potencies were slightly lower than comparable compounds from the parent 3-benzimidazoy-2-yl pyrazolo[3,4-*b*]pyridine series (**1**). The lead CDK1 inhibitors showed potent cellular proliferative inhibition with IC_{50} values ranging from low μM to sub-μM among the tumor cell lines tested. Notable exceptions were compounds **3a** and **3b**, which were significantly less potent anti-proliferatives than other analogues.

In summary, we have discovered two novel series of 3,4-disubstituted pyrazole analogues, 3-(benzimidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**2**) and 3-(imidazol-2-yl)-4-[2-(pyridin-3-yl)-vinyl]-pyrazoles (**3**), that are effective cyclin-dependent kinase inhibitors. Key steps for the synthesis of these compounds employed the palladium-catalyzed Stille coupling and Heck reaction, or Suzuki coupling with activated aryl or heteroaryl halides, followed by benzimidazole/imidazole ring formation via a carboxylic acid or carboxaldehyde group on the pyrazole ring. Representative compounds showed potent CDK1 inhibitory activities and inhibited in vitro cellular proliferation in HeLa, HCT116, and A375 human tumor cell lines.

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