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Synthesis and biological study of 2-amino-4-aryl-5-chloropyrimidine analogues as inhibitors of VEGFR-2 and cyclin dependent kinase 1 (CDK1)

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Abstract—The series of 2-amino-4-aryl-5-chloropyrimidines was discovered to be potent for both VEGFR-2 and CDK1. Described here are the chemistry for analogue synthesis, SAR study, and its kinase selectivity prolifing. The full rat PK data and in vivo efficacy study are also included.

Angiogenesis, the formation of new capillaries from the endothelium of an existing vascular network, plays a crucial role in tumor growth. Solid tumors cannot grow larger than several cubic millimeters until they establish a blood supply because cells must be within 100–200 μm of a blood vessel to survive.[1] The molecular mechanisms underlying angiogenesis have been studied and are well established due to the pharmaceutical potential of antiangiogenesis as cancer therapy. Although several growth factors play important role in angiogenesis, vascular endothelial growth factor (VEGF) and its tyrosine kinase receptor (VEGFR-2) are of particular interest because of the magnitude of their effects and the potential for therapeutic application.[2] Inhibition of VEGF activity or VEGFR-2 kinase has been shown to suppress tumor angiogenesis and tumor growth in tumor xenograft studies. FDA approval of the anti-VEGF antibody bevacizumab for the treatment of colorectal cancer provides valuable proof-of-concept in a clinical setting.[3] Recently, two small molecule inhibitors of VEGFR-2 kinase, sorafenib (BAY-43-9006)[4] and sunitinib (SU-11248),[5] were approved by the FDA for renal and/or gastrointestinal cancer. Numerous other small molecules have progressed to the clinical evaluation stage.[6,7] Cyclin dependent kinase 1 (CDK1) is a member of a family of serine/theronine kinases that plays an important function in regulation of the cell cycle.[8] Because abnormal CDK control of the cell cycle has been linked to the molecular pathology of cancer, attention has focused on CDKs as potential targets for cancer therapy.[9] CDK1 is an especially attractive target due to its crucial role in regulating the cell cycle at the G2 and mitosis stages. Although inhibition of CDK has yet to be validated in cancer patients, several CDK1 inhibitors have progressed into clinical trials, among them flavopiridol, UCN-01, CYC202, and SNS-032 (BMS-387032).[10]

We recently reported the in vitro anti-angiogenic and in vivo anti-tumor activity of a 5-cyanopyrimidine derivative that is a potent, selective inhibitor of VEGFR-2 kinase.[11] In this communication, we disclose a related series (Fig. 1) of 2-amino-4-aryl-5-chloropyrimidines

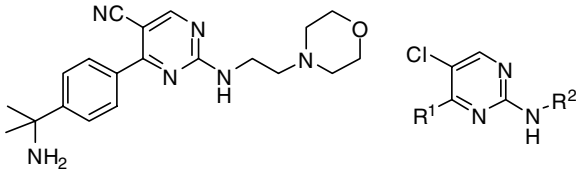

Figure 1.

Keywords: CDK1; VEGFR-2; 2-Amino-4-aryl-5-chloropyrimidine.
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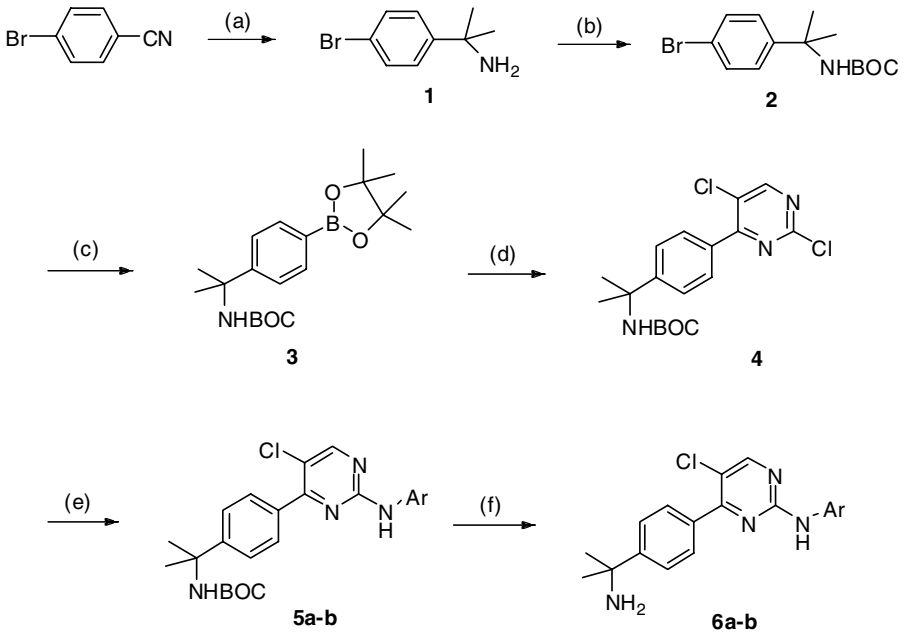
that are potent inhibitors of VEGFR-2 and CDK1.[12] This series also displays antiproliferative activity against cancer cells and therefore could more effectively inhibit tumor growth. Herein we describe the synthesis, initial structure–activity relationships (SAR), and PK properties of this novel series.

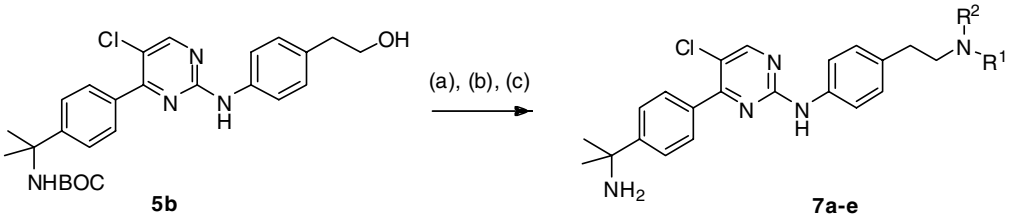
Our chemistry efforts began with an exploration of the 2-arylamino substituent on a subset of 4-(cumylamino)-5-chloropyrimidines. These derivatives were prepared using the procedure shown in Scheme 1, starting with addition of methylcerium chloride to 4-bromobenzonitrile to generate 4-bromocumylamine (**1**).[13] After protection of the amino group with Boc, bromide **2** was converted to boronate **3** using bis(pinacolato)diboron in the presence of Pd(II).[14] Compound **3** was then coupled with 2,4,5-trichloropyrimidine to afford the 4-aryl-2,5-dichloropyrimidine **4**. This step was highly regio-selective and proceeded in good yield (80%). Next, the 2-chloro substituent of compound **4** was displaced with various arylamines to produce 2-amino intermediates **5a**–**b**, which were in turn treated with HCl to afford final products **6a**–**b**. One of the intermediates (**5b**) was also used to generate analogues containing amino side chains; the chemistry is shown in Scheme 2. Compound **5b** was reacted with methanesulfonyl chloride, followed by displacement of the mesyl group with aliphatic amines. Finally, removal of the Boc protecting group with HCl generated compounds **7a**–**e**. To further expand SAR in the series, the cumylamino substituent at the 4-position was replaced by selected heterocylic groups. As indicated in Scheme 3, various arylboronates were coupled with 2,4,5-trichloropyrimidine to generate 4-aryl-2,5-dichloropyrimidines **8**.[15] Displacement of the 2-chloro of compounds **8** with *N*-substituted 4-(2-aminoethyl)phenylamines provided target molecules **9a**–**k**.

All compounds were evaluated for their ability to inhibit VEGFR-2 kinase, CDK1, and proliferation of three tumor cell lines: malignant melanoma (A375), cervical adenocarcinoma (HeLa), and colon carcinoma (HCT116).[11] Biological activities of the 4-(cumylamino) analogues are presented in Table 1. With the exception of the 3-bromo (**6a**) and morpholin-4-ylethyl (**7d**) derivatives, all of the cumylamino compounds were potent VEGFR-2 inhibitors, having IC_{50} values <0.1 μM. Neutral polar group (2-hydroxyethyl, **6b**) was compatible with good activity while basic substitution, incorporated to improve aqueous solubility, conferred the highest VEGFR-2 potency. Interestingly, the basicity of the terminal amino group seemed to correlate with potency, with the more basic methylamine (**7a**), dimethylamine (**7b**), and pyrrolidine (**7c**) analogues having the lowest IC_{50} values (0.019–0.032 μM), *N*-methylpiperazine (**7e**) having an intermediate value, and the least basic morpholine (**7e**) having the highest IC_{50}. CDK1 inhibition and cellular antiproliferative activity did not follow the same trend, however. CDK1 potency was 19- to 60-fold lower than VEGFR-2 for the amine-containing compounds, but only 3- to 10-fold lower for the neutral derivatives. Cellular antiproliferative activity was poor (IC_{50} > 1 μM) to moderate (IC_{50} 0.3–1 μM) with no obvious correlation to either VEGFR-2 or CDK1.

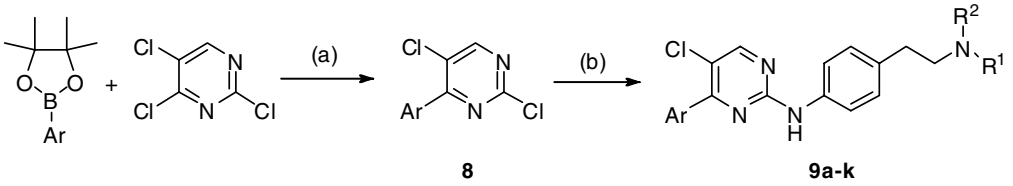
VEGFR-2 activity was found to be quite sensitive to the nature of the 4-substituent on the 5-chloropyrimidine core, as seen in Table 2. For example, thien-2-yl analogue **9a** was 8-fold less potent (IC_{50} = 0.16 μM) than



Scheme 1. Synthesis of compounds **6a**–**b**. Reagents: (a) $CeCl_3$, MeLi, THF, 95%; (b) $(Boc)_2O$, toluene, 96%; (c) KOAc, bis(pinacolato)diboron, $Pd(dppf)_2Cl_2$, THF, 82%; (d) 2,4,5-trichloropyrimidine, 1,2-dimethoxyethane, 80%; (e) $ArNH_2$, 2-methoxyethanol, 80–90%; (f) HCl, MeOH, 90–100%.

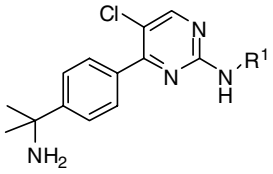


Scheme 2. Synthesis of compounds **7a**–**e**. Reagents: (a) MsCl, Et_3N, CH_2Cl_2; (b) HNR^1R^2, DMF; (c) HCl, MeOH, 60–80%.



Scheme 3. Synthesis of compounds **9a**–**k**. Reagents: (a) $Pd(PPh_3)_4$, 1,2-dimethoxyethane, 60–85%; (b) 4-[2-(*N*-R^1,R^2-amino)ethyl]aniline, 2-methoxyethanol, 60–80%.

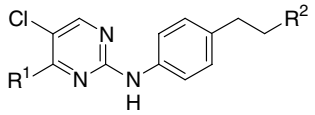
Table 1. Kinase and cellular antiproliferative activities of 4-(cumylamino) derivatives



Compound	R^1	Kinase inhibition (IC_{50}, μM)		Cell proliferation (IC_{50}, μM)		
		VEGFR-2	CDK1	A375	HCT116	HeLa
6a	3-Bromophenyl	0.348	1.040	2.610	3.690	3.350
6b	4-($HOCH_2CH_2$)phenyl	0.069	0.687	1.370	1.600	2.920
7a	4-($MeNHCH_2CH_2$)phenyl	0.033	1.060	0.175	0.885	0.542
7b	4-($Me_2NCH_2CH_2$)phenyl	0.019	1.140	0.163	1.680	0.500
7c	4-(Pyrrolidin-1-yl-CH_2CH_2)phenyl	0.026	0.808	0.247	0.359	0.339
7d	4-(Morpholin-4-yl-CH_2CH_2)phenyl	0.148	2.770	3.170	3.220	3.010
7e	4-(1-Me-piperazin-4-yl-CH_2CH_2)phenyl	0.076	1.610	0.868	1.300	0.811

the corresponding 4-cumylamino derivative **7b** and the 2,4-dimethylthiazol-5-yl analogue was 42-fold less potent (IC_{50} = 0.80 μM). At the other extreme, the indol-3-yl (**9c**, **9i**) and indol-6-yl (**9f**, **9j**, **9k**) analogues were extremely potent VEGFR-2 inhibitors (IC_{50} values ranged from 0.007 to 0.036 μM). Attachment at other positions of the indole was detrimental, however, with VEGFR-2 potency decreasing in order from indol-5-yl (**9e**) > indol-4-yl (**9d**) > indol-7-yl (**9g**). VEGFR-2 activity was significantly reduced by substituting the indol-3-yl bicyclic system with the isosteric indazol-3-yl moiety (**9h**, IC_{50} = 0.314 μM), suggesting an important binding role for the indole N–H. With the exception of compounds **9f**, **9j**, and**9k**, the 4-heteroaryl-5-chloropyrimidines were relatively non-selective with respect to CDK1, having VEGFR-2/CDK1 IC_{50} ratios less than 8. Notably, the weakly potent thiazole derivative **9b** was a better inhibitor of CDK1 than VEGFR-2. In contrast, **9f**, **9j**, and **9k** were 34- to 120-fold selective for VEGFR-2, with CDK1 IC_{50} values in excess of 1.2 μM. Cellular antiproliferative activities were generally modest, but the indol-3-yl derivative **9c** distinguished itself as a highly potent antiproliferative agent in all three tumor cell lines (IC_{50} values ranged from 0.050 to 0.084 μM). The other indo-3-yl derivative (**9i**), although equipotent against VEGFR-2, had much weaker antiproliferative activity, perhaps as a result of poorer membrane permeability due to the doubly ionizable piperazine group. To determine general kinase selectivity for the series, compound **9i** was evaluated in a panel of tyrosine and serine/threonine kinases.[16] Out of 100 kinases tested, **9i** exhibited >80% inhibition of 62 kinases at a concentration of 1 μM, indicating that the 2-arylamino-4-(indol-3-yl)-5-chloropyrimidine scaffold is a relatively promiscuous kinase pharmacophore. Indeed, examples of the non-chloro version of this scaffold have been reported to be inhibitors of protein kinase C and Bcr-abl kinase.[17]

Table 2. Kinase and cellular antiproliferative activities of 4-heteroaryl derivatives



Compound	R^1	R^2	Kinase inhibition (IC_{50}, μM)		Cell proliferation (IC_{50}, μM)		
			VEGFR-2	CDK1	A375	HCT116	HeLa
9a	2-thienyl	Me_2N	0.159	0.461	0.016	0.228	1.240
9b	2,4-dimethyl-thiazol-5-yl	Me_2N	0.797	0.439	0.399	0.391	0.431
9c	Indol-3-yl	Me_2N	0.007	0.048	0.050	0.084	0.075
9d	Indol-4-yl	Me_2N	0.216	0.540	0.210	0.084	0.292
9e	Indol-5-yl	Me_2N	0.103	0.847	0.030	0.321	0.596
9f	Indol-6-yl	Me_2N	0.015	1.770	0.336	0.446	0.472
9g	Indol-7-yl	Me_2N	0.455	0.334	0.774	0.470	1.290
9h	Indazol-3-yl	Me_2N	0.314	0.781	3.970	3.760	3.060
9i	Indol-3-yl	1-Me-piperazin-4-yl	0.007	0.18	3.930	3.050	3.330
9j	Indol-6-yl	Pyrrolidin-1-yl	0.016	1.54	1.970	1.680	0.973
9k	Indol-6-yl	1-Et-piperazin-4-yl	0.037	1.24	2.570	2.450	2.080

Table 3. Pharmacokinetic properties of compounds **6b**, **9j**, and **9k** in Sprague–Dawley rats

Compound	**6b**	**9j**	**9k**
Oral bioavailability	55%	70%	40%
Dose (mg/kg), po[a]	10	10	10
$t_{1/2}$ (h), po	4.5	2.6	4.6
C_{max} (μM), po	1.11	0.72	0.59
AUC (μM h), po	7.65	5.74	6.12
Dose (mg/kg), iv [b]	2	2	2
$t_{1/2}$ (h), iv	4.9	3.9	5.8
C_{max} (μM), iv	1.43	0.67	0.96
AUC (μM h), iv	2.77	1.63	3.06
Clearance (mL/min/kg)	34	51	25
V_{ss} (L/kg)	10.7	16.3	10.1

[a] Vehicle = 0.5% Methocel®.
[b] Vehicle = 10% Solutol®.

Pharmacokinetic parameters for several analogues were determined in male Sprague–Dawley rats. As shown in Table 3, compounds from the 4-(cumylamino) series (**6b**) and the 4-(indol-6-yl) series (**9j** and **9k**) were orally bioavailable. These derivatives achieved useful plasma concentrations (0.6–1.1 μM) and had reasonable plasma half-lives (2.6–4.6 h) after oral administration at 10 mg/kg, but also had high clearance values (25–51 mL/min/kg). Based on these results and their in vitro kinase and cell proliferation profiles, compounds **6b**, **9j**, and **9k** were evaluated for their ability to inhibit the growth of A375 xenograft implanted in the hind flank of nude mice. Unfortunately, all three compounds failed to demonstrate significant anti-tumor activity when dosed orally at 100 mg/kg for 28 days, although a trend toward tumor growth inhibition was observed. This outcome is surprising in light of the potent VEGFR-2 inhibition shown by all compounds and the robust antiproliferative activity exhibited by **6b**. It is possible that high clearance values indicate that the compounds are not resident in the tumor long enough to show the desired effect.

In summary, we identified a novel series of potent VEGFR-2 kinase inhibitors with CDK1 and antiproliferative activity. SAR at the 2- and 4-positions of the 5-chloropyrimidine core was studied, leading to the synthesis of many potent analogues having (2-aminoethyl)phenylamino at the 2-position and cumylamino, indol-3-yl, or indol-6-yl at the 4-position. Potent CDK1 inhibition and antiproliferative activity were found in several analogues, notably those having indo-3-yl at the 4-position. The mechanism for the antiproliferative activity did not appear to correlate with either VEGFR-2 or CDK1 inhibition. Several derivatives also displayed acceptable pharmacokinetic behavior in rat but were not orally active in an A375 xenograft experiment in nude mice.

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