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7-[1*H*-Indol-2-yl]-2,3-dihydro-isoindol-1-ones as dual Aurora-A/VEGF-R2 kinase inhibitors: Design, synthesis, and biological activity

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ABSTRACT

A novel series of 7-[1*H*-indol-2-yl]-2,3-dihydro-isoindol-1-ones designed to be inhibitors of VEGF-R2 kinase was synthesized and found to potently inhibit VEGF-R2 and Aurora-A kinases. The structure-based design, synthesis, and initial SAR of the series are discussed.

Vascular endothelial growth factor (VEGF) is an important regulator of vascular growth and permeability. Overexpression of VEGF in solid tumors has been shown to promote angiogenesis, thereby facilitating tumor growth. VEGF acts on VEGF-R2, a receptor tyrosine kinase present on the surface of endothelial cells. The inhibition of VEGF-R2 by small molecules is a validated therapeutic approach for treating cancer and has received much attention in the literature.[1] We have concentrated our efforts to find novel inhibitors of the VEGF receptor kinases. By coupling the computer modeling of virtual compounds with traditional medicinal chemistry principles, we designed a new kinase inhibitor scaffold **1** based on known kinase inhibitors (Fig. 1).[2] Initial modeling of our scaffold using a homology structure of VEGF-R2 overlaid with known VEGF-R2 inhibitors showed that key binding interactions were maintained. Kinase screening of early examples of this series revealed that the scaffold displayed inhibition of VEGF-R2 kinase and unexpected inhibition of Aurora-A kinase.

The Aurora kinases (Aurora-A, Aurora-B, and Aurora-C) are serine/threonine kinases that are essential for mitotic progression. Aurora-A kinase plays a role in spindle formation and organization

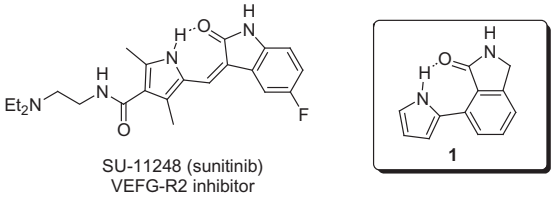

Figure 1. Pharmacophore **1** and known VEGF-R2 inhibitor SU-11248 (sunitinib). The dashed lines depict intramolecular hydrogen bonds.

of the centrosome and Aurora-B regulates chromosomal movement and cytokinesis. The biological function of Aurora-C is still not understood. The inhibition of Aurora kinases by a small molecule has been reported to induce apoptosis in a variety of cancer cell lines and suppress tumor growth in vivo.[3,4] The dual inhibition of VEGF-R2 and Aurora-A kinases is an attractive compound profile and may provide for enhanced antitumor activity toward a wide range of cancers.

In this letter, scaffold design, synthesis, and preliminary structure–activity relationships for the series are presented. Modeling of our scaffold using a homology structure of Aurora-A, and an X-ray structure of an analog in Aurora-A, have added to the understanding of the unexpected activity against Aurora-A, thereby helping to direct our future synthetic efforts.

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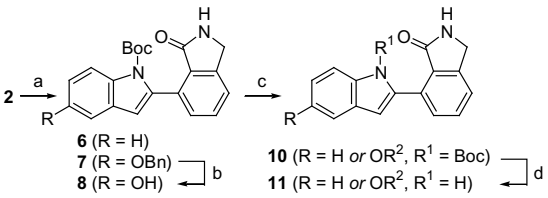
We observed that the VEGF-R2 kinase inhibitor SU-11248 (sunitinib) as well as other potent kinase inhibitors were capable of forming a pseudocycle via an intramolecular hydrogen bond (Fig. 1).[2,5,6] We felt that the intramolecular hydrogen bonding in these compounds held them in a flat, planar conformation that would facilitate binding to the hinge region in the active site of protein kinases. With this hypothesis in mind we proposed the novel pharmacophore **1** as a scaffold for the inhibition of protein kinases. Molecular modeling confirmed that in the minimum energy conformation, the pyrrole NH of pharmacophore **1** is well situated to participate in an intramolecular hydrogen bond with the isoindolinone carbonyl oxygen (Fig. 1).

The synthesis of compound **1** is shown in Scheme 1. Suzuki coupling of the known[7] 7-bromo-2,3-dihydro-isoindol-1-one (**2**) with commercially available 1-(*tert*-butoxycarbonyl)pyrrole-2-boronic acid afforded the Boc-protected core **3**. Thermolytic cleavage of the Boc-protecting group[8] cleanly afforded the proposed pharmacophore **1**. Additionally, the isoindolinone **3** was N-methylated with methyl iodide to give compound **4**. Subsequent thermal deprotection of **4** afforded the N-methylated analog **5**. Kinase data for these analogs are shown in Table 1.

Gratifyingly, our proposed core **1** displayed promising activity against VEGF-R2 kinase (IC_{50} = 1.10 μM), and good activity against Aurora-A kinase (IC_{50} = 0.338 μM). It was established that the NH of the isoindolinone ring was important for kinase inhibition since the N-methylated analog **5** displayed very weak kinase activity. Additionally, the NH of the pyrrole ring was also found to be important for kinase activity since the *N*-Boc analog **3** was also only weakly active. At this point we considered **1** to be a solid hit for VEGF-R2 and Aurora-A kinase inhibition with surprisingly good potency, low molecular weight (198), and a straightforward synthesis. We then explored the SAR for the left hand pyrrole side of the pharmacophore **1**. The synthesis of a representative set of analogs is shown in Scheme 2.

The Suzuki coupling of **2** with 1-Boc-indole-2-boronic acid afforded **6** in good yield when Pd(dppf)Cl_2 was used as the catalyst. Similarly, coupling of **2** with 5-benzyloxy-1-Boc-indole-2-boronic acid afforded **7**. Removal of the benzyl group of **7** proceeded smoothly under mild hydrogenolysis conditions to yield the phenol **8**. Alkylation of the phenol with alkyl chlorides and Cs_2CO_3 in DMF at 50 °C occurred to give protected intermediates **10**. Final removal

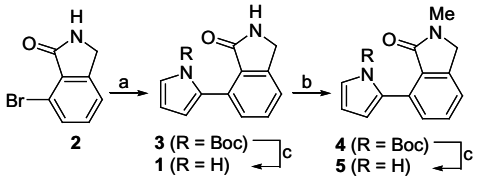


Scheme 2. Reagents and conditions: (a) 1-Boc-indole-2-boronic acid or 5-benzyloxy-1-Boc-indole-2-boronic acid, Pd(dppf)Cl_2, 2 N Na_2CO_3, THF (70–80%); (b) H_2 (balloon), 10% Pd/C, THF/MeOH, (100%); (c) Cs_2CO_3, DMF, 50 °C, R^2Cl, (65–83%); (d) TFA, DCM (95%) or 185 °C, neat (85–100%).

of the Boc group was achieved by either thermolytic cleavage or under acidic conditions using TFA in DCM at 25 °C to give the analogs **11**. Various indolyl analogs (**12c**, **12d**, **12f**–**12j**) were prepared using this general route (Scheme 2). Analogs **12a**, **12b**, and **12e** were synthesized from **12c** via an oxidation/reductive-amination sequence. The kinase data for representative analogs are summarized in Table 2.

Changing the pyrrole ring of pharmacophore **1** to an indole ring as in **12i** provided 10-fold better VEGF-R2 potency (IC_{50} = 144 nM) and a 3-fold increase in Aurora-A potency (IC_{50} = 100 nM). Molecular modeling suggested that substitution at the 5-position of the indole ring should be allowed as that position is solvent-exposed. Extension of the series through alkylation of the phenol **12h** afforded very potent inhibitors (**12a**–**12f**) of VEGF-R2 and Aurora-A. Generally, the VEGF-R2 potency tracked with the Aurora-A potency, with the most potent analogs having a pendant amine tethered to the indole ring by an alkyloxy linker. The length of the linker, either 2-carbon (**12d**) or 3-carbon (**12e**), did not have a significant effect on potency. All analogs of **12** were inactive against cyclin-dependent kinase—1 (CDK1, IC_{50} > 100 μM).

To continue variations of the left side of pharmacophore **1** we synthesized the carboxylic acid intermediate **18** (Scheme 3). Reaction of the commercially available 3-methyl phthalic anhydride (**13**) with MeOH and H_2SO_4 afforded an isomeric mixture of phthalic acid mono-esters. The isomeric mixture was converted to the bis methyl ester **14** with TMS diazomethane in toluene/MeOH. Treatment of **14** with NBS in CCl_4 using AIBN as a catalyst afforded the benzyl bromide **15**. Direct conversion of **15** to the isoindoli-



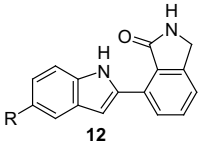
Scheme 1. Reagents and conditions: (a) Pd(OAc)$_2$, 2 N Na_2CO_3, P(*o*-tolyl)$_3$, 1-(*tert*-Butoxycarbonyl)pyrrole-2-boronic acid, DMF, (17%); (b) THF, NaH, MeI, (23%), (c) N_2, 185 °C, (73%).

Table 1
VEGF-R2, Aurora-A, and CDK1 kinase activity for **1**, **3**–**5**

Compound	VEGF-R2 IC_{50}[a] (μM)	Aurora-A IC_{50}[a] (μM)	CDK1 IC_{50}[a] (μM)
1	1.10	0.338	(63%)[b]
3	(46%)[b]	(25%)[b]	(<10%)[b]
4	>100	>100	>100
5	(<10%)[b]	(37%)[b]	(24%)[b]

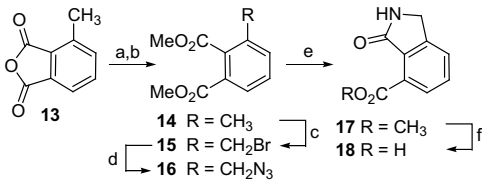
[a] IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.
[b] Values in parentheses indicate % inhibition at 100 μM.

Table 2
VEGF-R2, Aurora-A, and CDK1 kinase activity for **12**



Compound	R	VEGF-R2 IC_{50}[a] (μM)	Aurora-A IC_{50}[a] (μM)	CDK1 IC_{50}[a] (μM)
12a	$O(CH_2)_3$4-ethyl-piperazin-1-yl	0.014	0.044	>100
12b	$O(CH_2)_3$4-hydroxy-piperidin-1-yl	0.018	0.061	>100
12c	$O(CH_2)_3OH$	0.030	0.100	>100
12d	$O(CH_2)_2$piperidin-1-yl	0.056	0.200	>100
12e	$O(CH_2)_3$piperidin-1-yl	0.065	0.179	>100
12f	$O(CH_2)_2$morpholin-4-yl	0.074	0.135	>100
12g	OMe	0.111	0.070	>100
12h	OH	0.119	0.090	13.3
12i	H	0.144	0.100	>100
12j	$OCH_2C_6H_5$	11.26	21.23	>100

[a] IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >10 or >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.



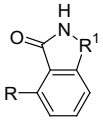
Scheme 3. Reagents and conditions: (a) MeOH, H_2SO_4, reflux; (b) $TMSCHN_2$, (95%); (c) NBS, AIBN, CCl_4, (80%); (d) NaN_3, DMF, 25 °C, (88%); (e) PPh_3, THF, H_2O, 25 °C, (52%); (f) LiOH, THF, MeOH, H_2O, 25 °C, (83%).

none **17** with concentrated NH_4OH in THF was problematic and afforded the amide **19d** due to unexpected reactivity of the aryl methylester **17** to concd NH_4OH.[9] This was overcome by first converting **15** into the benzyl azide **16** by treatment with sodium azide in DMF. The benzyl azide **16** was then reduced in situ to the benzyl amine, which cyclized to afford the isoindoline **17**. Standard hydrolysis of the ester group of **17** using LiOH in THF/MeOH/H_2O afforded the desired carboxylic acid **18**.

Starting from carboxylic acid **18**, series of analogs **19** were prepared (kinase data are shown in Table 3). The reaction of carboxylic acid **18** with 2-amino-6-methoxyaniline in refluxing 6N HCl afforded the benzimidazole **19a**. The kinase potencies of the 2-substituted benzimidazole analog **19a** and the 2-substituted indole analog **12g** were similar. Peptide coupling of **18** with 4-methoxyaniline under standard coupling conditions afforded **19b**. We were surprised that **19b**, an isostere of the potent indole **12g**, displayed lower potency against VEGF-R2 (IC_{50} = 5.94 μM) and reduced activity against Aurora-A (45% inhibition at 100 μM). Changing the connection on the indole ring to the 3-position, analog **19c**, resulted in a significant reduction of VEGF-R2 and Aurora-A inhibitory activity. The 3-substituted indole analog **19c** was synthesized from the corresponding boronic acid as detailed in Scheme 2. The imide analog **19g**, prepared from 4-iodopthalimide (**22**, synthesized as shown in Scheme 4) and 5-methoxy-1*H*-indol-2-boronic acid, was active at 0.1 μM but did not exhibit greater inhibition at higher concentrations, indicative of poor solubility under assay conditions.

With the initial SAR beginning to take shape we evaluated the ability of some of the most promising analogs to inhibit aurora kinase in cells. Histone H3 is a direct downstream target of the Aurora kinases. It is involved in chromosomal condensation and

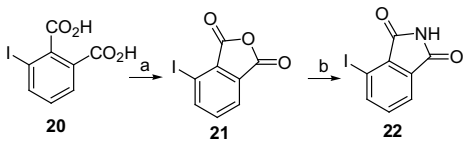
Table 3
VEGF-R2 and Aurora-A kinase activity for **19**



19

Compound	R	R^1	VEGF-R2 IC_{50}[a] (μM)	Aurora-A IC_{50}[a] (μM)
19a	5-methoxy-1*H*-benzoimidazol-2-yl	CH_2	0.142	0.205
19b	CONH(4-methoxy-phenyl)	CH_2	5.94	(45%)[b]
19c	1*H*-Indol-3-yl	CH_2	(50%)[b]	(60%)[b]
19d	$CONH_2$	CH_2	>100	14.34
19e	CO_2Me	CH_2	>100	>100
19f	CO_2H	CH_2	>100	>100
19g	5-Methoxy-1*H*-indol-2-yl	C=O	(35%)[c]	(30%)[b]

[a] IC_{50} data are the average of at least two separate experiments. IC_{50} values listed as >100 indicate no observed 50% inhibition at the highest dose tested, nor was an inhibition maximum observed.
[b] Values in parentheses indicate % inhibition at 100 μM.
[c] Values in parentheses indicate % inhibition at 0.1 μM.



Scheme 4. Reagents and conditions: (a) Ac_2O, reflux, (96%); (b) NH_2CONH_2, (85%).

phosphorylation on Serine 10 by Aurora B kinase is thought to be crucial for proper chromosomal alignment and cytokinesis. The inhibition of Aurora B kinase has been shown to reduce phosphorylation of H3 on Serine 10. Compound **12d** was shown to inhibit the phosphorylation of histone H3 at 2.5 μM (Fig. 2).

Polyploidy, the accumulation of cells with >4 N DNA content, and multinucleation are an expected phenotypic result of Aurora kinase inhibition.[10] Treatment of HeLa cells with 2.5 μM of **12d** for 72 h induced a large number of cells to endoreduplicate resulting in a significant population of cells with a greater than 4 N DNA content (Fig. 3).

To evaluate the ability of this class of compounds to effect tumor suppression in vivo we performed tumor xenograft studies using the A375 cell line in nude mice. When **12d** was dosed at 100 mg/kg ip, a 46% inhibition of tumor growth compared to ip vehicle control (P = 0.08) was observed (Fig. 4). No statistically significant inhibition was observed when **12d** was dosed 100 mg/kg po.

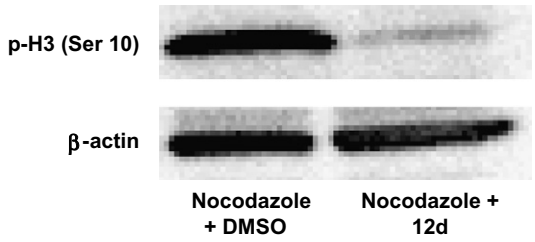


Figure 2. The level of p-H3 (Ser10) in lysates of HeLa cells synchronized in G2/M with Nocodazole and then treated for an additional 8 h with either Nocodazole and DMSO or Nocodazole and compound **12d**.

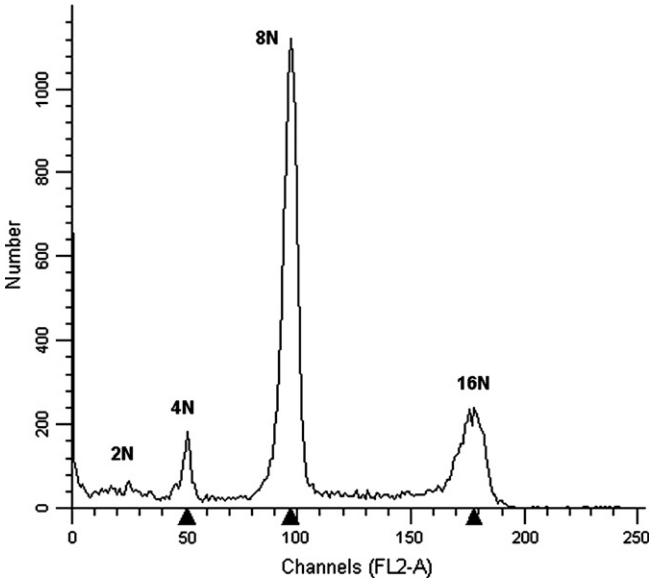


Figure 3. Polyploidy of HeLa cells upon treatment with 2.5 μM of **12d** for 72 h as determined by FACS anaylsis.

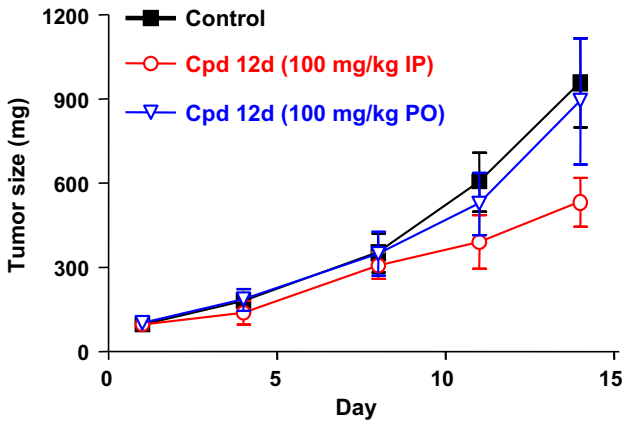


Figure 4. Tumor inhibition of **12d** in a A375 tumor xenograft model in nude mice. -■- vehicle control, -▼- **12d** 100 mg/kg po, -○- 100 mg/kg ip.

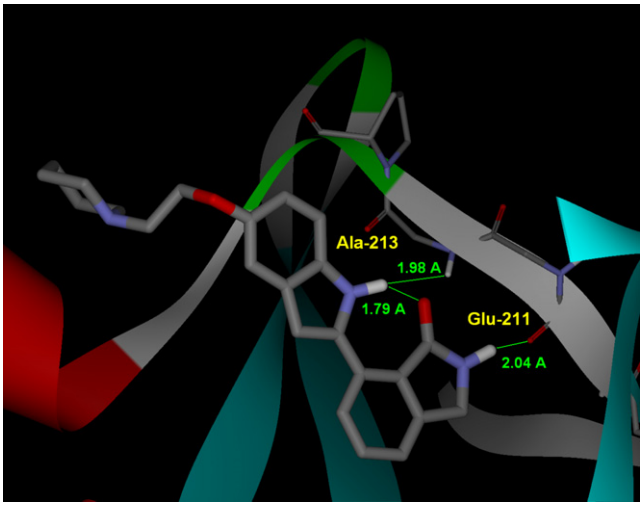


Figure 5. Model of compound **12f** docked into the ATP binding site of human Aurora-A kinase. The protein is depicted as a ribbon structure with backbone atoms of key residues 210–214 shown explicitly. Amino acids 138, 139, and 147–150 are hidden for clarity. Potential hydrogen bonds are shown in green.

Compound **12d** has a short half-life but is orally available in rat (po dose 10 mg/kg, $F = 20\%$, $C_{max} = 391$ ng/mL, $t_{1/2} = 0.9$ h). When 12d was dosed in nude mice at 10 mg/kg drug plasma levels of 154, 160, 41, and 12 ng/mL were observed for 0.5, 1, 2, and 4 h timepoints, respectively.

To investigate potential binding modes of compound **12f**, an energy-minimized structure was docked into the ATP-binding site of a published crystal structure of human Aurora-A kinase (Fig. 5).[11] In this model, the isoindolinone portion of **12f** occupies the same space in the active site as the amino pyrimidine of ATP. The backbone carbonyl of Glu-211 and the NH of Ala-213 in the hinge region form the characteristic dual H-bonds with **12f**. Additionally, the indole NH of compound **12f** forms an intramolecular H-bond with the C=O of the isoindolonone. The morpholine group is solvent-exposed and this region may provide a handle for modifying the physicochemical properties of the series.[12]

In summary, we have described the design, synthesis, and initial SAR development of 7-[1*H*-indol-2-yl]-2,3-dihydro-isoindol-1-ones as potent dual VEGF-R2 and Aurora-A kinase inhibitors that are orally available and display in vivo activity in a tumor xenograft model. The design of the progenitor of this series, pharmacophore **1**, was based on the hypothesis that an intramolecular hydrogen bond between the indole NH and the isoindolinone carbonyl O would hold the structure in a relatively flat conformation. Molecular modeling and a crystal structure of analog **12f** in Aurora-A kinase supported this type of intramolecular hydrogen bond and showed that **12f** binds in the ATP binding site. The dual activity of this series of kinase inhibitors provides a unique profile that may prove very potent for blocking the growth of cancer cells as it attacks two important pathways that drive proliferation of almost all cancers.

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12. A low-resolution (3.1 Å) crystal structure of compound **12f** with murine Aurora-A kinase was obtained, confirming the modeling results (data not shown).