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Synthesis and evaluation of heteroaryl-ketone derivatives as a novel class of VEGFR-2 inhibitors

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ABSTRACT

We have discovered novel inhibitors of VEGFR-2 kinase with low nanomolar potency in both enzymatic and cell-based assays. Active series are heteroaryl-ketone compounds containing a central aromatic ring with either an indazolyl or indolyl keto group in the *ortho* orientation to the benzylic amine group (Fig. 1). The best compounds were demonstrated to be inactive against a small select panel of tyrosine and serine/threonine kinases with the exception of VEGFR-1 kinase, a close family member. In addition, the lead candidate **8** displayed acceptable exposure levels when administered orally to mice.

Angiogenesis, or formation of new blood vessels, is a highly complex process that involves proliferation, migration, and tissue infiltration by capillary endothelial cells from pre-existing blood vessels. It is an important physiological process involved in embryonic development,[1,2] follicular growth, and wound healing as well as in pathological conditions such as tumor growth.[3–6] Vascular endothelial growth factor (VEGF),[7,8] an endothelial cell-specific mitogen, is the primary regulator of angiogenesis *in vivo* and it mediates its biological effect through high-affinity VEGF tyrosine kinase receptors, which are expressed on the surface of endothelial cells.[9] VEGF binds with high affinity to VEGFR-2 (also known as Kinase Domain Region (KDR)[7,10,11] to induce activation of the angiogenic signaling pathway. Murine gene knockouts of VEGFR-2 pr VEGF have led to embryonic lethality, a result of disorganized vascular endothelial cells, indicating the pivotal role of VEGF in angiogenesis.[12–14] Additionally, several antagonists and inhibitors currently in preclinical and clinical development have shown tumor regression concomitantly with inhibited angiogenesis.[15,16] Therefore, a direct inhibition of the kinase activity of VEGFR-2 should result in the reduction of angiogenesis and the subsequent suppression of tumor growth.

During screening of the ImClone compound collection, we identified a heteroaryl-ketone compound **4** that demonstrated promising inhibition of VEGFR-2. We were encouraged to pursue the hit by the precedent set by Manley et al. in which anthranilamide-based inhibitors recapitulated the potency and binding mode of PTK787.[17] Promising biological data for compound **4** prompted us to proceed with the structure–activity analysis of the series, the results of which are presented in this letter. The heteroaryl-ketone series is a novel entry in this class of inhibitors which seek to exploit a similar mode of binding to the kinase domain of VEGFR-2. One of the key structural features of the heteroaryl-ketone series was the *ortho* substitution of the central aromatic ring with a ketone moiety and an anilinyl NH group (Fig. 1). We speculated that the resulting intramolecular hydrogen bond would consequently hold the shape of the molecule into a pseudo-bicyclic ring system.

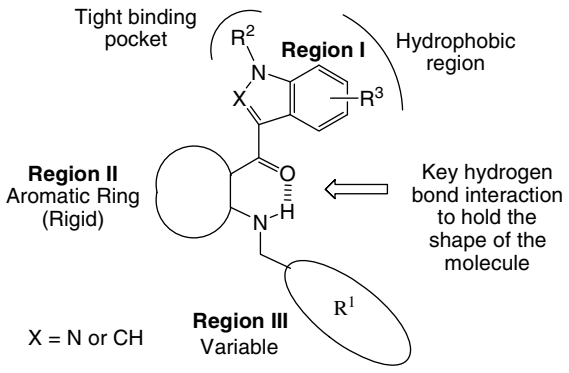

Figure 1. Pharmacophore hypothesis for the mode of binding of heteroaryl-ketones within the ATP-binding pocket of VEGFR-2.

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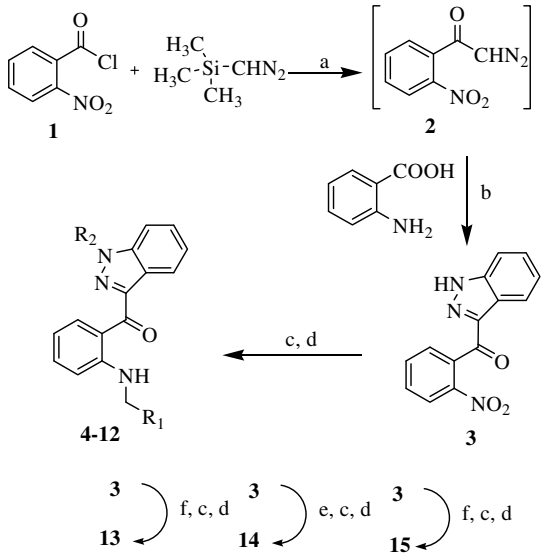
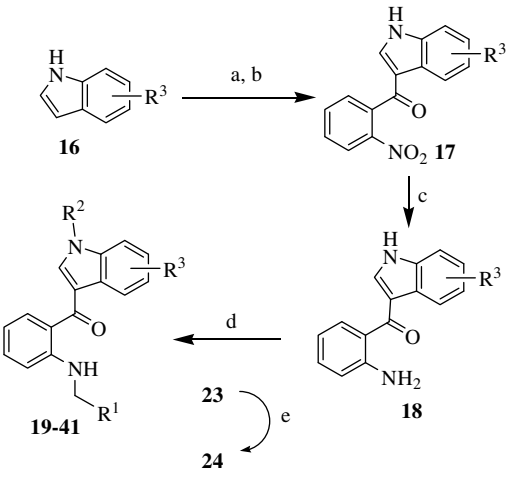
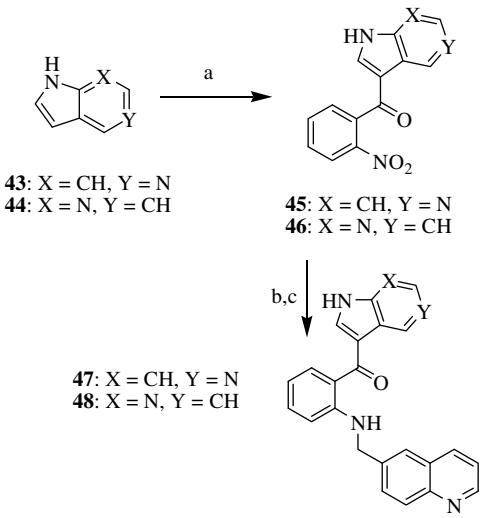


Scheme 1. Reagents and conditions: (a) Et_3N, Et_2O, 0 °C; (b) Isoamyl nitrite, CH_2Cl_2/Acetone; (c) H_2, Pd/C, MeOH or EtOAc; (d) R^1CHO, PhH, 100 °C then $Na(OAc)_3BH$; (e) 2-Methoxyethyl bromide, K_2CO_3, DMF; (f) CH_3I, K_2CO_3, DMF.



Scheme 2. Reagents and conditions: (a) 2-nitrobenzoyl chloride, EtMgBr, THF, −78 to 0 °C; (b) LiOH, MeOH, THF, rt; (c) H_2, 10%Pd/C, EtOAc or EtOH; (d) R^1CHO, PhH, 100 °C then $Na(OAc)_3BH$; (e) $(CH3)_2SO_4$, NaH, THF, 0 °C.



Scheme 3. Reagents and conditions: (a) **1**, $AlCl_3$, CH_2Cl_2; (b) H_2, Pd/C, THF, EtOH; (c) Quinoline-6-carboxaldehyde, PhH, AcOH then $Na(AcO)_3BH$.

The general preparation of the heteroaryl-ketone analogs is depicted in Scheme 1.[18] The initial strategy was to keep the top portion of the molecule as an indazole moiety and vary only the bottom portion in order to optimize the interactions in Region III. Treatment of 2-nitro benzoylchloride **1** with trimethylsilyl diazomethane gave the diazo intermediate **2** that was further reacted with anthranilic acid in the presence of isoamyl nitrite to give 2-nitro ketoindazole **3**. The amine moiety that was obtained via reduction of the nitro group was subjected to reductive amination with a variety of aldehydes to provide the final compounds **4–15** (Table 1). For the indole derivatives, the synthetic route was modified as shown in Schemes 2 and 3. A variety of Lewis acid based methods were pursued to introduce the 2-nitro acyl group in position 3 of indole ring system (Scheme 2). None of these methods produced the desired product in appreciable yields and often gave complex reaction mixtures. Reaction of the substituted indole **16** with the acid chloride in the presence of the Grignard reagent gave a 1,3-diacylated product which was subjected to mild hydrolysis conditions to achieve a quantitative yield of the desired mono-acylated product **17**. The nitro group was then reduced and the resulting amine moiety was subsequently subjected to reductive amination conditions to produce a series of final target compounds **19–41** (Table 2).

Furthermore, two azaindole analogs were also synthesized to explore heteroaromatic substitution patterns in Region I (Scheme 3). A modified Lewis-acid-catalyzed acylation worked well in this case to produce intermediates **45** and **46**.[19] The nitro group in the mono-acylated products was then reduced and subjected to reductive amination resulting in the desired compounds **47** and **48**.

The first stage of exploring the SAR of heteroaryl-ketones was to modify Region III (Table 1). The pyridyl group in **4** gave an encouraging cell-based phosphorylation assay IC_{50} of 0.87 μM; however, the corresponding pyrazole derivative **5** turned out to be inactive. It was assumed that the nitrogen in a smaller group such as pyrazole was not within reach of the key interacting amino acid.[20] This hypothesis could also be confirmed via the presence of a larger indazole group (e.g., **6** and **7**) where the nitrogens in the indazole moieties are most likely picking up the key interaction resulting

Table 1
Modification of Region III in Indazole series

Compound	R^1	R^2	KDR-HTRF (IC_{50} μM)[a]	Cell-based phosphorylation (IC_{50} μM)[a]
4	4-Pyridyl	H	1.6 ± 0.4	0.87 ± 0.12
5	4-Pyrazolyl	H	>10	—
6	5-Indazolyl	H	0.80 ± 0.1	0.12 ± 0.3
7	6-Indazolyl	H	0.73 ± 0.19	0.074 ± 0.004
8	6-Quinolinyl	H	0.23 ± 0.03	0.13 ± 0.3
9	6-Isoquinolinyl	H	10.0 ± 2.0	—
10	4-Acetylaminophenyl	H	>10	—
11	piperonyl	H	>10	2.6 ± 0.3
12	5-Benzimidazolyl	H	3.0 ± 0.1	0.31 ± 0.05
13	6-Quinolinyl	CH_3	8.5 ± 1.5	>10 [b]
14	6-Indazolyl	2-Methoxy ethyl	0.56 ± 0.07	1.8 ± 0.5
15	6-Indazolyl	CH_3	0.25 ± 0.04	0.30 ± 0.08

[a] IC_{50} values were determined from the logarithmic concentration–inhibition point (at least eight points). The important key values are given as the mean of at least two duplicate experiments.

[b] Precipitation was observed for this compound under the assay conditions.

Table 2
Modifications of Region I and III in Indole series

Compound	R^1	R^2	R^3	KDR-HTRF (IC_{50} μM) [a]	Cell-based phosphorylation (IC_{50} μM) [a]
19	4-Pyridyl	H	H	9.0 ± 1.0	2.1 ± 0.7
20	5-Indazolyl	H	H	0.61[b]	0.24 ± 0.07
21	6-Indazolyl	H	H	0.14[b]	0.17 ± 0.01
22	6-Quinolinyl	H	H	2.0 ± 0.5	1.0 ± 0.3
23	6-Quinolinyl	H	5-CH_3	1.9 ± 0.15	0.16 ± 0.02
24	6-Quinolinyl	CH_3	5-CH_3	0.54[b]	0.18 ± 0.03
25	6-Quinolinyl	H	6-CH_3	0.94 ± 0.06	0.73 ± 0.15
26	6-Quinolinyl	H	7-CH_3	2.0[b]	0.70 ± 0.01
27	6-Quinolinyl	H	2-CH_3	>10	—
28	6-Quinolinyl	H	4-OCH_3	>10	—
29	6-Quinolinyl	H	5-OCH_3	1.9[b]	0.45 ± 0.15
30	6-Quinolinyl	H	6-OCH_3	1.9 ± 0.3	0.66 ± 0.11
31	6-Quinolinyl	H	5-F	5.9[b]	1.0 ± 0.2
32	6-Quinolinyl	H	6-F	1.9[b]	0.81 ± 0.02
33	6-Quinolinyl	H	5-Cl	3.9 ± 0.8	0.56 ± 0.03
34	6-Indazolyl	H	5-CH_3	0.87 ± 0.13	0.052 ± 0.015
35	6-Indazolyl	H	6-CH_3	0.25 ± 0.09	0.076 ± 0.005
36	6-Indazolyl	H	7-CH_3	0.95 ± 0.6	0.355 ± 0.05
37	6-Indazolyl	H	2-CH_3	>10	—
38	6-Indazolyl	H	5-F	1.8 ± 0.7	0.25 ± 0.03
39	6-Indazolyl	H	6-F	1.5 ± 0.5	0.25 ± 0.02
40	6-Indazolyl	H	5-Cl	1.1 ± 0.3	0.83 ± 0.06
41	6-Isoquinolinyl	H	H	>10	—
47	6-Quinolinyl	H	5-(N)	>10	—
48	6-Quinolinyl	H	7-(N)	7.8 ± 0.3	>10

[a] IC_{50} values were determined from the logarithmic concentration–inhibition point (at least eight points). The important values are given as the mean of at least two duplicate experiments.

[b] Precipitation was observed for the compound under the enzymatic assay conditions hence the data for $n = 1$ are depicted. However, the corresponding cellular data are shown for $n = 2$.

in the observed activity. The substitution of these heterocyclic moieties with a carbonyl group (**10**) resulted in a negative outcome. The 6-Quinolynyl group as R^1 would position the basic nitrogen proximal to the 5- and 6-inadzolyl nitrogen atoms. Thus, the quinolinyl and indazolyl moieties were found to be optimized groups in Region III that resulted in nanomolar activity. Incorporation of a solubilizing chain (methoxyethyl group) into Region I led to enzymatically similarly potent compound **14**, but a significant loss of cellular potency was observed.

In the interest of synthetic tractability, we confined our exploration of Region I SAR to the indole derivatives (Table 2). The quinolinyl and indazolyl groups were maintained in Region III as they conferred superior potency compared to other moieties (Table 2). With the quinolinyl group in Region III, the 5-methylindole derivative **23** was found to be the most active in the cell-based assay with an $IC_{50} = 0.16$ μM. With the indazolyl in Region III, the 5- and 6-methyl compounds showed comparable activity in cellular assay (**34** and **35**). The loss of activity observed in analogs where the indole was substituted in the 2 position with a methyl group (**27** and **37**) could be attributed either to tight spacing in that region of the kinase active site or to dihedral angle alteration between the indole group and the central phenyl ring. However, the substitution of the free NH group in indole with a methyl group

Table 3
Correlation of VEGFR-1 and VEGFR-2-binding data

Compound	VEGFR-2 (IC_{50} μM)	VEGFR-1 (IC_{50} μM)
4	1.6	1.9
6	0.8	0.46
7	0.73	0.27
8	0.23	0.30

(e.g., **24**) resulted in a smaller change in activity as compared to the non-methylated compound **23** indicating that a small hydrophobic group is tolerable at that site. This notion is reiterated by the 7-methyl analogues **26** and **36** which retain activity. The azaindole analogs **47** and **48** were found to be inactive, suggesting that a nitrogen atom in the ring is not tolerated in the positions facing hydrophobic regions of the protein backbone (Fig. 1).[20] Overall, the SAR trend was similar in both the indazole and indole cases, where the 6-indazolyl group in Region III consistently gave slightly better activity than the corresponding quinolinyl group.

Characterization of the heteroaryl-ketones indicates that this series is highly selective for VEGF family receptors. When compounds **4** and **6**–**8** were screened against a panel of several tyrosine and serine/threonine kinases (EGFR, ErbB2, Raf-1, c-Met, IGF1-R, InsR, CDK2, and PKB), the only kinase besides VEGR-2 that was targeted was VEGFR-1, a close family member (Table 3). Moreover, inhibition of VEGFR-2 correlates well with that seen with VEGFR-1, indicating that this series would target both receptors involved in angiogenesis. ATP competition experiments demonstrated that this series acts as a direct and reversible competitor of ATP (data not shown). Representative compounds were not toxic to a pool of human hepatocytes or cytotoxic to NIH3T3 fibroblast cells ($GI_{50} > 100$ μM).

When **8** was administered to mice (interperitoneally, 30 mg/kg in a solution of 5% ethanol, 5% Tween-80, 5% PEG400 and 85% PBS), plasma concentration of 2.4 μM was present at 1 h post-injection; however, at 4 h compound concentration was below detection limits. Mass spectroscopy revealed that **8** was glucuronidated, presumably leading to its rapid elimination. Therefore, we explored indole compound **24** with an *N*-methyl indole moiety that should prevent the formation of glucuronidation adduct. When this compound was also dosed interperitoneally, plasma levels at the 1 h time point (2.9 μM) were similar to those seen with **8**. Notably, the plasma concentration of **24** was ~1.1 μM at the 4-h time point with no glucuronidation products detectable. Moreover, both analogues **8** and **24** demonstrated plasma exposure (~1.8 μM at 1 h) upon oral administration.

We have described the synthesis and biological activity of a novel series of potent VEGFR-2 inhibitors based on a heteroaryl-ketone scaffold. This series demonstrated potent cell-based inhibition of KDR autophosphorylation and selectivity against a group of tyrosine and serine/threonine kinases. Compounds bearing the 6-indazolyl group in Region III were slightly more potent compared to analogs with the corresponding quinolinyl group. However, the quinolinyl analog **8** showed oral exposure in mice. The most active compounds **7**, **34**, and **35** were comparable or more inhibitory to VEGFR-2 receptor than the standard clinical candidate ZD6474[21] (IC_{50} 100 nM) in our cellular phosphorylation assay.

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Supplementary data

Experimental data. This material is available free of charge via the Internet at http://pubs.acs.org. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.06.083.

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