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ortho-Substituted azoles as selective and dual inhibitors of VEGF receptors 1 and 2

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Abstract—We have developed a series of novel potent *ortho*-substituted azole derivatives active against kinases VEGFR-1 and VEGFR-2. Both specific and dual ATP-competitive inhibitors of VEGFR-2 were identified. Kinase activity and selectivity could be controlled by varying the arylamido substituents at the azole ring. The most specific molecule (**17**) displayed >10-fold selectivity for VEGFR-2 over VEGFR-1. Compound activities in enzymatic and cell-based assays were in the range of activities for reported clinical and development candidates (IC_{50} < 100 nM), including *Novartis*' PTK787 (*Vatalanib*)™. High permeability of active compounds across the Caco-2 cell monolayer (>30×10^{-5} cm/min) is indicative of their potential for intestinal absorption upon oral administration.

Angiogenesis, or formation of new blood vessels, is a highly complex process. Proliferation and migration of capillary endothelial cells from pre-existing blood vessels is followed by tissue infiltration and cell assembly into tubular structures, joining of newly formed tubular assemblies to closed-circuit vascular systems, and maturation of newly formed capillary vessels. Angiogenesis is involved in pathological conditions such as tumor growth and degenerative eye conditions.[1] A family of vascular endothelial growth factors (VEGFs),[2] endothelial cell-specific mitogens, has been implicated in regulation of angiogenesis in vivo. VEGFs mediate their biological effect through high-affinity VEGF receptors that are expressed on the endothelial cells. These include receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Kinase Insert Domain Receptor (KDR) or flk1).[3] Whereas VEGFR-1 functions are still under investigation, there is substantial evidence that KDR is a major mediator of vascular endothelial cell mitogenesis as well as angiogenesis and microvascular permeability. It is believed that a direct inhibition of the aberrant KDR kinase activity results in reduction of tumor angiogenesis and suppression of tumor growth. Potent, specific, and non-toxic inhibitors of angiogenesis are powerful clinical tools in oncology and ophthalmology.[3]

There are reports describing small-molecule inhibitors that affect VEGF/VEGFR signaling by directly competing with the ATP binding site of the respective intracellular kinase domain. This event leads to the inhibition of VEGFR phosphorylation and, ultimately, to the apoptotic death of the aberrant endothelial cells. Drug candidates that exhibit this mechanism of action include *Novartis*' PTK787 (*Vatalanib*,™ **A**) and *Astra-Zeneca's* ZD6474 (*Vandetanib*,™ **B**). These compounds are reported to be undergoing Phase III and Phase II clinical trials, respectively, for various oncology indications.[4,5] The pyridazine ring of phthalazine template in PTK787 **A** has been replaced with the isosteric anthranyl amide derivatives **C** and **D** (Fig. 1). Intramolecular hydrogen bonding was suggested to be responsible for the optimal spatial orientation of pharmacophore pieces, similar to the one of parent PTK787.[6]

It has been proposed that the essential pharmacophore elements for the VEGFR-2 activity of PTK787 type phthalazines and their analogs include (i) [6,6] fused (or related) aromatic system; (ii) *para*- or 3,4-*di*-substituted

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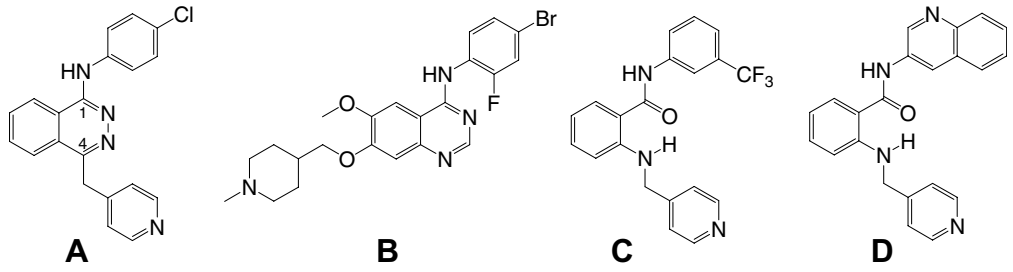

Figure 1. PTK787 (A), ZD6474 (B), and anthranyl amide derivatives (C, D).

aniline at position 1 of phthalazine core; (iii) hydrogen bond acceptor (Lewis' base: lone pair(s) of a nitrogen- or oxygen atom(s)) attached to the position 4 via an appropriate linker (aryl or fused aryl group).[4] In this communication, we expand upon our initial findings[7] and disclose potent inhibitors of VEGFR-2 kinase based on *ortho*-substituted azoles. We reasoned that these non-phthalazine templates could provide for a proper pharmacophore arrangement consistent with the model proposed for PTK787.[4,6]

Our synthetic route to imidazole derivatives (**9**–**36**) is illustrated in Scheme 1. Nitration of *N*-methyl 5-chloroimidazole (**1**) with a 1:1 mixture of HNO_3/H_2SO_4 yielded the expected 4-nitro derivative in 91% yield. This was treated with KI/KCN (10-fold excess) mixture in EtOH at reflux for 12 h to result in the product of formal substitution of chlorine with nitrile (**2**, 69% yield). Product (**2**) was subsequently hydrolyzed to the corresponding acid followed by its conversion into acyl chloride and a coupling with a series of anilines leading to intermediates (**3**) in 39–67% yield over three-steps. Reduction of NO_2 functionality into a corresponding amine followed by its reaction with various aldehydes to furnish Schiff bases and their reduction with $NaBH_4$ in *i*-PrOH afforded the desired imidazoles (**9**–**36**) (59–75% yield over three steps). In the next series of experiments, we prepared pyrazole derivatives with a similar substituent arrangement (Scheme 2). Acid (**4**) was methylated into a mixture of two regioisomeric *N*-pyrazoles (ratio of 1-Me to 2-Me products was ca. 1:1). These compounds were separated by crystallization and nitrated with a 1:1 mixture of HNO_3 and H_2SO_4 (a respective scheme for 1-Me isomer is shown) to yield the corresponding 4-nitro-5-carboxy pyrazole (**5**) (77% yield for the three step sequence). Pyrazole (**5**) was then converted into a series of *ortho*-substituted derivatives (**37**–**60**) via the same sequence reported above for the synthesis of imidazoles. The desired pyrazoles were isolated in 37–56% yield starting from (**5**). Syntheses of the corresponding *ortho*-substituted isoxazole derivatives (**61**–**78**) were accomplished starting with ethyl cyanoacetate as reported in the literature (Scheme 3).[8] The targeted structures (**61**–**78**) were prepared in 41–63% overall yields starting from (**6**). Notably, all three protocols were amendable for scale up to produce multi-gram quantities of compounds (**9**–**78**).[9]

Compounds (**9**–**78**, Table 1) were tested in vitro against isolated VEGFR-2 kinase. Specifically, we measured their ability to inhibit phosphorylation of a biotinylated polypeptide substrate (*p*-GAT, *CIS Bio International*) in a homogeneous time-resolved fluorescence (HTRF) assay at ATP concentration of 2 μM. Literature-reported VEGFR-2 inhibitors **A**–**D** (Table 1) were included as internal standards for quality control.[7] As it is shown in Table 1, a number of azole β-amino acid amides exhibited a robust inhibitory activity against VEGFR-2. By varying both anilide substituents and aryl methylamino tailpiece, we modulated compound potency against the enzyme. Initial experiments in the imidazole series (entries **9**–**13**, Table 1) suggested that pyridin-4-ylmethylamino substituent yields the best activity with the enzymatic IC_{50} value of 650 nM against VEGFR-2 (**13**). The activity against VEGFR-1 followed the same trend with pyridin-4-ylmethylamino analog giving the lowest IC_{50} value in enzymatic assay. These results were somewhat disappointing since the corresponding 6-membered analog **C** in our internal enzymatic VEGFR-2 assay showed much better potency (IC_{50} = 32 nM). The respective analog (**24**) for compound **D** displayed enzymatic potency of 230 nM in enzymatic assay compared to 10-fold higher activity for the compound **D** (IC_{50} = 23 nM). We decided to further investigate the effect of aniline group on the compound activity within imidazole series. Substituents in the aniline ring were widely varied as well as the entire aniline moiety being replaced with bicyclic groups (**24**–**26**). It was observed that *t*-Bu group (**19**) gave the highest enzymatic activity (IC_{50} = 87 nM). Larger anilinic substituents led to the diminished potency against the enzyme (**21**–**23**, **49**–**51**, **70**–**72**). Specifically, *para*-phenyl-, phenoxy-, and benzyl-derivatives showed weak to no enzymatic activity. We speculated that these functions could not be properly accommodated in the tight hydrophobic pocket of VEGFR-2.[10]

Compounds (**9**–**78**) were also tested via HTRF format against VEGFR-1. The results in Table 1 indicate that many VEGFR-2 active ((pyridin-4-yl)ethyl)pyridines display good activity against VEGFR-1 as well. For the most potent compounds, the IC_{50} values were reaching 0.11 μM in the enzymatic assay. This outcome could be of benefit in the clinical setting as both receptors are reported to mediate VEGF signaling in angiogenesis.[11] One particular imidazole compound (**17**) yielded over 10-fold selectivity for the VEGFR-2 versus VEGFR-1 kinase providing us with a rare example of such selectivity between these closely related targets. Notably, a corresponding pyrazole analog also displayed some