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Arylphthalazines. Part 2: 1-(Isoquinolin-5-yl)-4-arylamino phthalazines as potent inhibitors of VEGF receptors I and II

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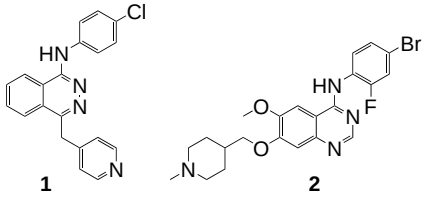
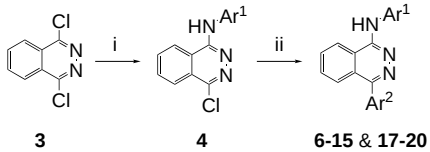
Abstract—A novel class of 1-(isoquinolin-5-yl)-4-arylamino-phthalazines is described as inhibitors of vascular endothelial growth factor receptor II (VEGFR-2). Many compounds display VEGFR-2 inhibitory activity with an IC_{50} as low as 0.017 μM in an HTRF enzymatic assay. The compounds also inhibit VEGFR-1, a related tyrosine kinase.

Vascular endothelial growth factor (VEGF) has been considered to play a major role in angiogenesis, the formation of new vasculature from an existing vascular network.[1] Angiogenic processes have been implicated in a number of disease states such as rheumatoid arthritis, inflammation, cancer, and degenerative eye conditions and modulators of angiogenesis are emerging as powerful clinical tools in oncology and ophthalmology.[2,3] One approach for inhibiting or reducing angiogenesis involves retarding, modulating or inhibiting the effects of VEGF binding at the VEGF receptor 2 (VEGFR-2; also known as the kinase insert domain receptor or KDR), a receptor tyrosine kinase expressed on vascular endothelial cells. Multiple reports have detailed small-molecule inhibitors of VEGFR-2 by binding to the adenosine triphosphate (ATP) site of its intracellular kinase domain resulting in diminished VEGF signal transduction, and a number of such compounds, exemplified by PTK 787 (**1**) and ZD 6474 (**2**), have entered clinical testing against various cancers.[4,5] In a previous communication, we detailed a series of 1-aryl-4-arylamino phthalazines as inhibitors of VEGFR.[6] In this Letter, we expand upon our previous findings and disclose a series of 1-(isoquinolin-5-yl)-4-arylamino phthalazines as potent inhibitors of VEGFR-2. In addition, we also show that these compounds inhibit VEGFR-1, a related receptor tyrosine kinase.



The desired arylphthalazines were synthesized by a two-step process as described in Schemes 1 and 2. Thus, 1,4-dichlorophthalazine (**3**) was allowed to react with an



Scheme 1. Reagents and conditions: (i) Ar^1NH_2, Et_3N, ^nBuOH, 100 °C or Ar^1NH_2, EtOH, reflux; (ii) $Ar^2B(OH)_2$ or $Ar^2B(OR)_2$, K_2CO_3, $PdCl_2(Ph_3P)_2$, 1,4-dioxane, H_2O, microwave, 100 °C.

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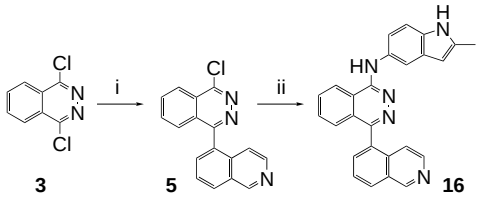


Scheme 2. Reagents and conditions: (i) isoquinolin-5-boronic acid, K_2CO_3, $PdCl_2(Ph_3P)_2$, 1,4-dioxane, H_2O, microwave, 100 °C; (ii) 5-amino-2-methylindole, EtOH, 100 °C, microwave.

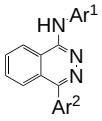
aniline to give the mono-displaced adduct (**4**),[7] which was then transformed to the desired final compound by way of a Suzuki reaction under microwave heating (Scheme 1). Alternatively, 1,4-dichlorophthalazine (**3**) could be reacted under our microwave Suzuki conditions to give the mono-arylated derivative (**5**). Displacement of the chloride in (**5**) with an aniline furnished the desired final product (Scheme 2). Although the synthesis outlined in Scheme 2 is more convergent, the higher yields encountered in Scheme 1 meant that the majority of analogues were synthesized in this fashion.

Fifteen compounds prepared by the above chemistries were tested against isolated VEGFR-2 by measuring the ability to inhibit phosphorylation of a biotinylated-polypeptide substrate (p-GAT, CIS Bio International) in a homogeneous time-resolved fluorescence (HTRF) assay at an ATP concentration of 2 μM. The results were reported as a 50% inhibition concentration value (IC_{50}), a literature VEGFR-2 inhibitor also being included as an internal standard for quality control (ZD 6474; $IC_{50} = 45 \pm 15$ nM).[8]

As can be seen from Table 1 isoquinoline-substituted phthalazines exhibited good to excellent inhibitory activity against VEGFR-2 (compounds **6**–**16**). By varying the substituents on the arylamino segment of the compound it was possible to modify the inhibitory activity against the enzyme. For instance, the *para*-chloro-, *tert*-butyl-, and *iso*-propyl derivatives (**6**–**8**) were highly active with IC_{50} values of 48, 75, and 17 nM, respectively. Such substituents have also been observed to be favorable in related phthalazine chemotypes.[4a] Interestingly, the (chlorodifluoro)methoxy group was also beneficial for VEGFR-2 inhibition, consistent with our previously reported findings (**9**; $IC_{50} = 79$ nM).[6] Other mono-substituted analogues resulted in mixed results. Whilst a methyl group was tolerated in the *meta*-position of the arylamino segment (**10**; $IC_{50} = 0.27$ μM), *ortho*-substitution of this group abolished enzymatic inhibition (**11**; $IC_{50} > 10$ μM). As shown by compounds (**13**–**16**) disubstitution also gave acceptable enzyme inhibition. Noteworthy are the tolerance of an *ortho*-fluoro group (**15**; $IC_{50} = 0.43$ μM), and ring fusion to yield a substituted indole analogue (**16**; $IC_{50} = 0.23$ μM). Related *ortho*-fluoro arylamino groups and substituted indole derivatives have been exemplified in other VEGFR-2 inhibitors such as ZD 6474 and ZD 2171.[5,9] Replacement of the 1-(isoquinolin-5-yl) moiety by 1-(quinolin-5-yl) resulted in a dramatic decrease in enzymatic inhibition, indicating that the position of the heteroatom in this aromatic substituent is of critical importance for effective binding to VEGFR-2 (compare **6**, $IC_{50} = 48$ nM to **17**, $IC_{50} > 10$ μM; or **7**, $IC_{50} = 75$ nM to **18**, $IC_{50} = 2$ μM). This result is in line with observations made with related phthalazine inhibitors of VEGFR-2.[4a]

A number of above mentioned analogues were also tested in an HTRF format against VEGFR-1, a receptor tyrosine kinase related to VEGFR-2. The results in Table 2 indicate that the compounds inhibit VEGFR-1 at a compound screening concentration of 10 μM. Our results are consistent with previous reports detailing other phthalazine derivatives as dual inhibitors of VEG-

Table 1. Activity of arylphthalazines against VEGFR-2



Compound	Ar_1	Ar_2	Enzymatic, IC_{50} (μM)[a]
6	4-Cl Ph	Isoquinolin-5-yl	0.048 ± 0.06
7	4-*t*-Bu Ph	Isoquinolin-5-yl	0.075 ± 0.007
8	4-*i*-Pr Ph	Isoquinolin-5-yl	0.017 ± 0.03
9	4-OCF_2Cl Ph	Isoquinolin-5-yl	0.079 ± 0.009
10	3-Me Ph	Isoquinolin-5-yl	0.27 ± 0.03
11	2-Me Ph	Isoquinolin-5-yl	>10
12	4-Morpholin4-yl Ph	Isoquinolin-5-yl	0.39 ± 0.01
13	3,4-Cl_2 Ph	Isoquinolin-5-yl	0.11 ± 0.01
14	4-Cl-3-CF_3 Ph	Isoquinolin-5-yl	0.12 ± 0.02
15	2-F-4-Me Ph	Isoquinolin-5-yl	0.43 ± 0.07
16	2-Me-indol-5-yl	Isoquinolin-5-yl	0.23 ± 0.07
17	4-Cl Ph	Quinolin-5-yl	>10
18	4-*t*-Bu Ph	Quinolin-5-yl	2.0 ± 0.1
19	4-Cl Ph	Pyrazol-4-yl	>10
20	4-Cl Ph	(1-Me)-pyrazol-4-yl	>10

[a] IC_{50} values were determined from logarithmic concentration–inhibition curves (at least eight points) and are given as means of at least two separate experiments.

Table 2. Activity of arylphthalazines against VEGFR-1

Compound	% inhibition of VEGFR-1 at 10 μM[a]	% inhibition of VEGFR-2 at 10 μM[a]
6	91	95
7	86	100
8	91	97
9	88	100
10	89	90
11	60	59
12	54	88
13	84	90
14	92	97
15	92	88
16	57	92
17	40	35
18	31	63

[a] Average of $n = 3$.

FR-1 and VEGFR-2.[4a] Further screening of compounds (**6**–**18**) against a number of other kinases (c-Met, EGFR, Flt-3, IGF-1R, and InsR) in an HTRF format indicated little or no cross-reactivity at a screening concentration of 10 μM.[10]

In summary, we have described a number of 1-(isoquinolin-5-yl)-4-arylamino phthalazines as highly potent inhibitors of the VEGFR-2 receptor. These compounds are also inhibitors of the VEGFR-1 receptor. The analogues presented in this Letter are potentially useful in the treatment of conditions such as cancer and further details of their biological properties will be presented in due course.

Acknowledgment

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8. VEGFR tyrosine kinase inhibition is determined by measuring the phosphorylation level of poly-Glu-Ala-Tyr-biotin (pGAT-biotin) peptide in a homogeneous time-resolved fluorescence (HTRF) assay. Into a black 96-well Costar plate is added 2 μl/well of 25× compound in 100% DMSO (final concentration in the 50 μl kinase reaction is typically 1 nM to 10 μM). Next, 38 μl of reaction buffer (25 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 2 mM DTT, and 1 mg/ml BSA) containing 0.5 mmol pGAT-biotin and 3–4 ng KDR enzyme is added to each well. After 5–10 min preincubation, the kinase reaction is initiated by the addition of 10 μl of 10 μM ATP in reaction buffer, after which the plate is incubated at room temperature for 45 min. The reaction is stopped by addition of 50 μl KF buffer (50 mM Hepes, pH 7.5, 0.5 M KF, and 1 mg/ml BSA) containing 100 mM EDTA and 0.36 μg/ml PY20K (Eu-cryptate labeled anti-phosphotyrosine antibody, CIS Bio International). After 30 min, 100 μl of 10 nM SV-XL (modified APC-labeled streptavidin, CIS Bio International) in KF buffer is added, and after an additional 2 h incubation at room temperature, the plate is read in a RUBYstar HTRF Reader.
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10. For example, the percentage inhibition for compound (**6**) against these kinases at a screening concentration of 10 μM is as follows: c-Met, 2%; EGFR, 13%; FLT-3, 33%; IGF-1R, 25%; InsR, 10% (average of $n = 3$ or $n = 2$). A percentage inhibition of <40% at 10 μM is considered to be inactive in our hands.