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Arylphthalazines as potent, and orally bioavailable inhibitors of VEGFR-2

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ABSTRACT

A series of arylphthalazine derivatives were synthesized and evaluated as antagonists of VEGF receptor II (VEGFR-2). IM-094482 **57**, which was prepared in two steps from commercially available starting materials, was found to be a potent inhibitor of VEGFR-2 in enzymatic, cellular and mitogenic assays (comparable activity to ZD-6474). Additionally, **57** inhibited the related receptor, VEGF receptor I (VEGFR-1), and showed excellent exposure when dosed orally to female CD-1 mice.

1. Introduction

Tumor-induced angiogenesis is the term that describes the induction of blood vessels from existing vasculature to the site of a solid tumor, critical for tumor growth and dissemination.[1,2] Under hypoxic conditions, tumors induce angiogenesis by secreting vascular endothelial growth factor (VEGF). Endothelial cells lining the walls of neighboring blood vessels bind VEGF to cell surface VEGF Receptor 2 (also known as KDR; murine version known as Flk-1), triggering a signaling cascade which leads to endothelial cell proliferation, migration and increasing vascular permeability in the tumor milieu.[3,4] A positive feedback loop develops where growing tumors secrete VEGF, which in turn leads to higher levels of angiogenesis, allowing further tumor growth. Severing the feedback loop by inhibiting VEGF Receptor 2 (VEGFR-2) signaling would restrict blood vessel development to the tumor. Moreover, inhibiting blood vessel formation has the advantage of targeting a stable endothelial cell, rather than a genetically unstable tumor cell, which may become resistant to small-molecule therapies. Additionally, angiogenesis in the adult is restricted to wound healing, menstruation and ovulation, predicting diminished side-effects compared to commonly used proliferation inhibitors that can also target dividing cells in normal tissues. The popularity of this approach is seen by the numerous VEGFR-2 small-molecule inhibitors in clinical use, or in clinical trials, and the use of biologics in the clinical setting.[5–9] VEGFR-2 is a membrane-bound tyrosine kinase receptor requiring ligand binding and dimerization for transphosphorylation of the receptors and subsequent substrates.[10] Small-molecule inhibitors have been developed to the adenosine-tri-phosphate (ATP) binding pocket of the kinase region, retarding phosphorylation of downstream elements, resulting in attenuation of VEGF signaling. Of VEGFR-2 inhibitors, the most advanced are SU11248 **1** (Sutent®), which has been approved by the Federal Drug Administration (FDA) for treatment of gastrointestinal stromal tumor and advanced renal cell carcinoma,[11] BAY 43-9006 **2** (Nexavar®),[12] which is approved for renal cell carcinoma and unresectable hepatocellular carcinoma, PTK-787 **3** (valatanib)[13] and ZD-6474 **4** (Zactima®),[14] both of which are currently in late-phase clinical trials. In addition, a plethora of other small-molecules, exemplified by compounds **5**–**19** have been detailed as potent inhibitors of VEGFR-2 (Fig. 1).[15–29]

Our own investigations into inhibitors of VEGFR-2 have yielded a series of arylphthalzines having good in vitro potency against isolated VEGFR-2 enzyme (compound **20** and series **21**).[30,31] In this account we detail further results for this series of molecules, and show that they possess favorable cellular potency and

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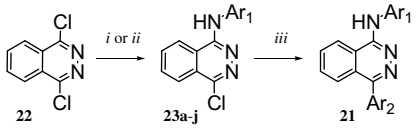
pharmacokinetic profiles which would be suitable for their further development as drug candidates.

2. Chemistry

The synthesis of the final 4-anilino-1-arylphthalazine derivatives was readily accomplished in two, operationally-simple steps, from commercially available 1,4-dichlorophthalazine (Scheme 1). Thus, 1,4-dichlorophthalazine **22** was first reacted with an aniline to provide the mono-displaced adducts **23a–j**. Suzuki coupling of these 4-anilino-1-chlorophthalazine intermediates was realized using K_2CO_3 as base, in a mixture of 1,4-dioxane and water as solvent under microwave heating, to yield compounds of general structure **21**. This reaction usually proceeded in 10 min to 1 h of heating at 100–110 °C. Interestingly, when the Suzuki reaction was attempted using identical conditions, except employing conventional oil-bath heating instead of a microwave-based approach, the reaction took substantially more time, and proceeded in significantly lower yields. The general protocol of mono-displacement with an amine, and Suzuki



Scheme 1. Two-step preparation of final compounds. Reagents and conditions: (i) Ar_1NH_2, Et_3N, *n*-BuOH, 100 °C. (ii) Ar_1NH_2, EtOH, reflux. (iii) $Pd(Ph_3P)_2Cl_2$, K_2CO_3, $Ar_2B(OH)_2$, H_2O, 1,4-dioxane, 100–110 °C, microwave irradiation.

reaction of 1,4-dichlorophthalazine, to prepare unsymmetrical 1,4-disubstituted phthalazines as detailed in Scheme 1, has also been accomplished in a previous study of 5-HT_3 inhibitors.[32] Compounds **24**, **43**, **44**, **54**, **56** to **65** and **67** to **68** were synthesized using the procedure detailed in Scheme 1. An alternative protocol, in which the order of Suzuki coupling and amine displacement was reversed, was employed for the synthesis of compound **66** (see Section 6). All other compounds, although designed by ourselves, were provided by contract research organizations.[33]

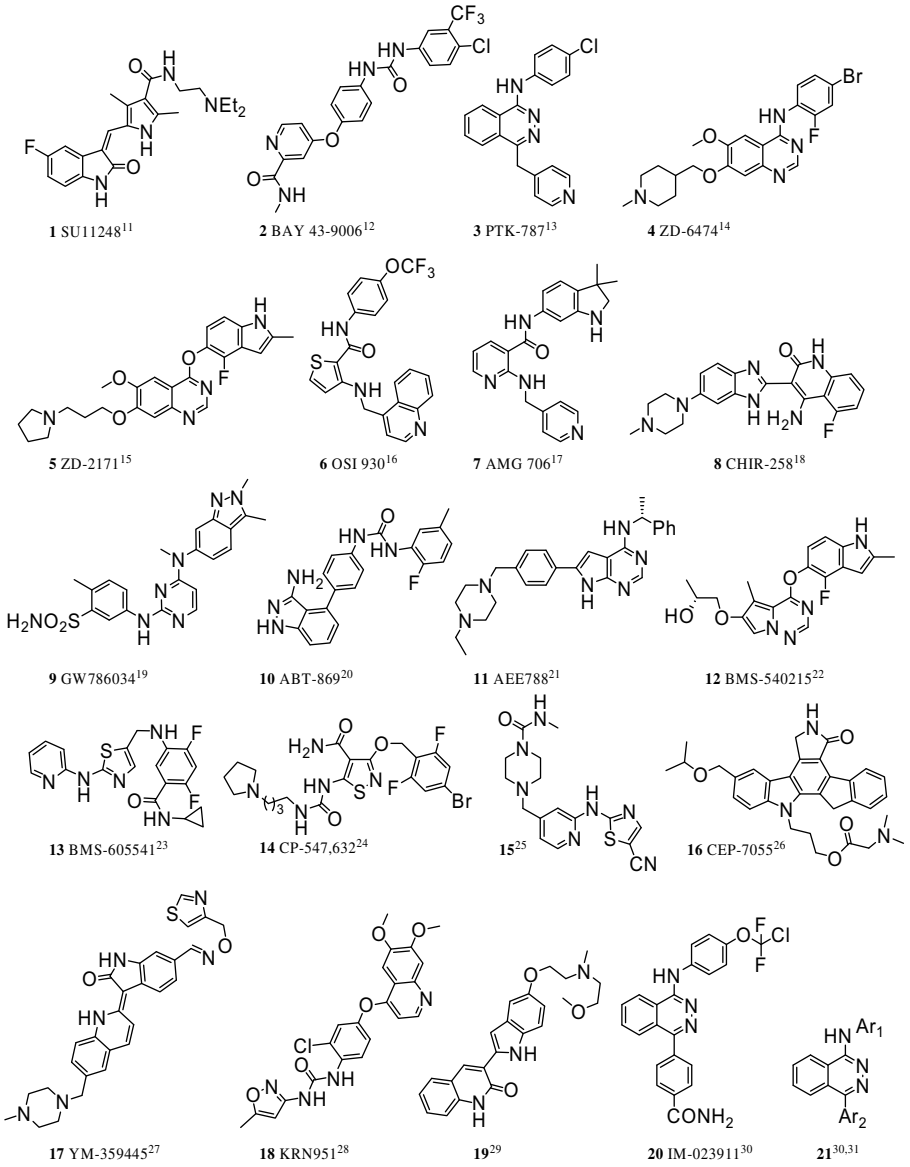


Figure 1. Select literature VEGFR-2 inhibitors.

3. Biology

Compounds were evaluated against isolated VEGFR-2 kinase in an enzymatic assay to assess their ability to inhibit tyrosine phosphorylation. Inhibition was determined by measuring the phosphorylation level of biotinylated poly-Glu-Ala-Tyr peptide (pGAT-biotin, CIS Bio International) upon exposure to VEGFR-2 and 2 μM ATP at room temperature for 45 min. Addition of a Europium-cryptate labeled anti-phosphotyrosine antibody and measurement of the resulting homogenous time-resolved fluorescence (HTRF) allowed quantification of inhibition upon exposure to various compound concentrations. IC_{50} values were determined from logarithmic concentration-inhibition curves (at least eight points) and reported as means of at least two separate experiments. A literature VEGFR-2 inhibitor, ZD-6474 **4**, was included in each set of experiments to ensure quality control. Select compounds were then evaluated in a cellular assay against a chimeric construct containing the extracellular portion of FGFR-1, and the intracellular (kinase) portion of VEGFR-2, transiently expressed in human embryonic kidney (HEK 293) cells. Measurement of tyrosine phosphorylation of the chimeric receptor by an enzyme-linked immunosorbent assay (ELISA) provided quantification of ED_{50} values. The ability of compounds to block native human VEGFR-2 signaling was assessed by evaluation in a mitogenic assay employing human umbilical vein endothelial cells (HUVEC), and measurement of 3H-thymidine incorporation after stimulation with VEGF. Finally, preliminary pharmacokinetic experiments were performed by quantifying the exposure of compound when dosed via oral (per os; po), or *intra-peritoneal* (ip) routes to female CD-1 mice.

4. Results and discussion

Screening of our corporate compound collection afforded the arylphthalazine IM-023911 **20** as a hit against VEGFR-2, with an enzymatic IC_{50} of 0.19 μM, and an ED_{50} of 1.6 μM in a cellular assay of VEGFR-2 inhibition. Although this compared somewhat unfavorably to the performance of ZD-6474 **4** in these assays (IC_{50} = 45 nM; ED_{50} = 0.15 μM), it was reasoned that **20** provided a reasonable starting point for a medicinal chemistry program, and that further exploration of the arylphthalazine class of compounds was warranted. Since we were aware of the work of other researchers who had demonstrated that compounds based around a phthalazine core could yield molecules suitable for clinical development,[13,34] we were confident that **20** could be optimized rapidly to afford similarly potent and drug-like compounds. Thus, we began an effort to explore the structure–activity relationships, both around the 1-anilino group, and also the 4-aryl moiety.

4.1. SAR around the 1-anilino group

Preliminary work began by investigating the structure–activity relationships about the 1-anilino group in the amide series. Compound **20** is somewhat unique since it possesses a 4-(chlorodifluoromethoxy) group attached to an anilino phenyl ring. The use of such a group has not been detailed extensively in the medicinal chemistry literature, although its application in agrochemicals seems to be more advanced.[35] Kinetic studies indicated that compound **20** was a reversible inhibitor with no apparent covalent modification of the 4-(chlorodifluoromethoxy) substituent. Thus, we began a search for complementary analogues to the OCF_2Cl substituent. As can be seen from Table 1 a number of compounds exhibited good potency against isolated VEGFR-2 enzyme. Of note were compounds that contained a hydrophobic substituent at the 4-position of the anilino phenyl ring. For example, the *tert*-butyl and bromo analogues (entries 2 and 3) gave the most active compounds. Chloro, methoxy, methyl and carbomethoxy substituents were also tolerated at the 4-position (entries 3–7), although the activity of these compounds was significantly less than parent compound **20**. Benzyloxy, phenoxy, sulfonamide or amide substituents gave rise to inactive compounds (entries 8–11). Movement of favorable substituents from the 4-position to the 3-position of the phenyl ring usually resulted in a moderate decrease in enzymatic activity (entries 12–14); similar results were observed when the 3,4-dichloro analogue **38** was tested (entry 16). The importance of substitution on the phenyl ring was confirmed by removing the 4-(chlorodifluoromethoxy) substituent in **20** to give an unsubstituted analogue **39** (entry 17), which was observed to be totally inactive. Cellular potencies for the compounds are also displayed in Table 1. Although some molecules exhibited good cellular activity (e.g., entries 2, 13 and 14), most cellular potencies had diminished activity relative to that seen in the isolated enzymatic assay. The reason behind this reduction in potency was not investigated, although a number of explanations could be offered (e.g., cell penetration). We also believed that low solubility of the compounds was hindering a reliable measurement of cellular activity.

The importance of an anilino nitrogen was confirmed by synthesis of oxygen and sulfur-linked analogues (Table 2, entries 1 and 2). Other non-anilino compounds were also inactive (results not shown).

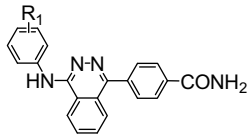
4.2. SAR around the 4-aryl group

Next, our attention focused on investigating SAR around the amide group (Table 3). Removal of the amide in lead structure **20** totally abolished activity at VEGFR-2 (entry 2). Next, alkylation of the amide was investigated. Small alkyl groups such as methyl, or hydroxyethyl, gave rise to active compounds (entries 3–5), whereas bulkier substituents such as *iso*-propyl, or *tert*-butyl, totally abolished activity (entries 6 and 7). A morpholinyl amide was also inactive (entry 8). Attempts to replace the amide were mostly unsuccessful. For instance, methyl, chloro, or methoxy substituents gave rise to inactive compounds. However, an ester group at the 4-position of the aryl ring was tolerated (entry 12), with an enzymatic IC_{50} = 0.63 μM, which suggested that a carbonyl group might be important for biological activity. Additionally, it was found that the amide could be moved from the 4-position to the 3-position of the aryl ring, to give compounds with acceptable enzymatic and cellular potencies (entries 13 and 14). Although these preliminary experiments suggested that compounds containing other amides might yield interesting inhibitors of VEGFR-2, it became apparent that molecules containing amides had severe limitations in terms of their aqueous solubility, which would likely make their further development challenging.[36]

4.3. Isoquinolin-5-yl derivatives

The activity observed with compound **54** prompted a more adventurous investigation to find alternative aryl groups that were potent VEGFR-2 inhibitors (Fig. 2). As the majority of arylphthalazines possessing an amide group appeared to be poorly soluble in aqueous media, first thoughts entertained the possibility of reducing the amide bond, to give rise to a structure such as **55** which would incorporate a free amine, and thus allow for salt formation in order to improve solubility. It was also reasoned that fusing the phenyl ring to the basic nitrogen in **55** might be beneficial as a conformational constraint, and this led to the idea that an isoquinolin-5-yl group might fit our needs in terms of aiding solubility, whilst minimizing conformational flexibility. As such, molecules such as **56** were designed and the similarity to PTK-787 **3** was noticed immediately. Testing of isoquinolin-5-yl compounds yielded

Table 1
Structure–activity relationships around 1-anilino group for lead structure **20**

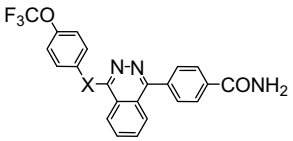


Entry	Compound	R_1	IC_{50} (μM) VEGFR-2[a]	ED_{50} (μM) VEGFR-2[b]
1	**20**	4-OCF_2Cl	0.19	1.6
2	**24**	4-^{t}Bu	0.078	0.21
3	**25**	4-Br	0.1	3.9
4	**26**	4-Cl	1.3	1.3
5	**27**	4-OMe	2.0	>10
6	**28**	4-Me	1.8	5.0
7	**29**	4-CO_2Me	2.7	>10
8	**30**	4-OBn	>10	—
9	**31**	4-OPh	>10	—
10	**32**	4-SO_2NHMe	>10	—
11	**33**	4-$CONH_2$	>10	—
12	**34**	3-^{t}Bu	0.47	1.2
13	**35**	3-Br	1.2	0.25
14	**36**	3-Cl	1.7	0.37
15	**37**	3-Me	1.6	—
16	**38**	3,4-Cl_2	0.84	—
17	**39**	H	>10	—

[a] Determined against isolated enzyme for at least two independent experiments (at least 8 points per experiment). Positive control, ZD-6474 **4** IC_{50} = 0.045 μM.
[b] At least two independent determinations against FGFR-1/VEGFR-2 chimeric receptor expressed in HEK-293 cells. Positive control, ZD-6474 **4** ED_{50} = 0.15 μM.

impressive enzymatic and cellular potencies in a number of instances (Table 4). Similar to the amide series, the most favorable isoquinolin-5-yl analogues were those that possessed a hydrophobic group at the 4-position of the anilino moiety. As such, the *tert*-butyl, chloro, (chlorodifluoromethoxy), and *iso*-propyl compounds gave rise to very active analogues with excellent inhibition against both isolated enzyme and a chimeric FGFR-1/VEGFR-2 receptor (entries 1–4). Indeed, IM-094482 **57** (entry 2), with an enzymatic IC_{50} of 48 nM and a cellular ED_{50} of 10 nM, performed comparably to the clinical compound ZD-6474 **4** in these assays (IC_{50} = 45 nM; ED_{50} = 0.15 μM). Interestingly, a hydrophilic morpholin-4-yl substituent gave rise to a moderately active compound when placed at the 4-position of the anilino phenyl ring (entry 5). Importantly, it was also observed that compounds in the isoquinolin-5-yl series seemed to show improved toleration of other substitution patterns when compared with their amide counterparts. For example, the 3,4-dichloro analogue **61** (entry 6) showed good enzymatic and cellular potency (0.11 μM and 70 nM, respectively). Additionally, the 4-chloro-3-trifluoromethyl and 2-fluoro-4-methyl compounds also displayed reasonable activities (entries 7 and 8). Nevertheless, compounds in the isoquinolin-5-yl series could be sensitive to the substitution pattern around the 4-anilino phenyl ring. This was neatly illustrated for the 2- and 3-methyl analogues **64** and **65** (entries 9 and 10), which showed contrasting activities (enzymatic and cellular potency >10 μM for **64** cf. enzymatic and cellular activities of 0.27 μM and 16 nM for **65**). The position of the nitrogen was also of crucial importance for VEGFR-2 activity (Table 5), with the corresponding quinolin-5-yl congeners showing diminished activity.

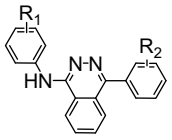
Table 2
Removal of anilino-NH



Entry	Compound	X	IC_{50} (μM) VEGFR-2[a]
1	**40**	O	>10
2	**41**	S	>10

[a] Determined against isolated enzyme for at least two independent experiments (at least 8 points per experiment). Positive control, ZD-6474 **4** IC_{50} = 0.045 μM.

Table 3
Initial structure–activity relationships around 4-aryl group in lead structure **20**



Entry	Compound	R_1	R_2	IC_{50} (μM)[a]	ED_{50} (μM)[b]
1	**20**	4-OCF_2Cl	4-$CONH_2$	0.19	1.6
2	**42**	4-OCF_2Cl	H	>10	—
3	**43**	4-Cl	4-CONHMe	3.1	—
4	**44**	4-^{t}Bu	4-CONHMe	0.13	0.27
5	**45**	4-OCF_2Cl	4-$CONH(CH_2)_2OH$	1.1	—
6	**46**	4-OCF_2Cl	4-$CONH^iPr$	>10	—
7	**47**	4-OCF_2Cl	4-$CONH^tBu$	>10	—
8	**48**	4-OCF_2Cl	4-$CON(CH_2)_4O$	4.9	—
9	**49**	4-OCF_2Cl	4-Me	>10	—
10	**50**	4-OCF_2Cl	3-Cl	>10	—
11	**51**	4-OCF_2Cl	4-OMe	>10	—
12	**52**	4-OCF_2Cl	4-CO_2Me	0.63	—
13	**53**	4-OCF_2Cl	3-$CONH_2$	4.1	—
14	**54**	4-^{t}Bu	3-$CONH_2$	0.40	0.90

[a] Determined against isolated VEGFR-2 enzyme for at least two independent experiments (at least 8 points per experiment). Positive control, ZD-6474 **4** IC_{50} = 0.045 μM
[b] At least two independent determinations against FGFR-1/VEGFR-2 chimeric receptor expressed in HEK-293 cells. Positive control, ZD-6474 **4** ED_{50} = 0.15 μM.

As mentioned previously, compounds in the amide series did not seem to be particularly soluble in aqueous media.[36] Conversely, **57**, which was emerging as the most favored compound within the isoquinolin-5-yl sub-series, was prepared as a dihydrochloride salt by reaction with excess hydrogen chloride in 1,4-dioxane, to give rise to material with acceptable water solubility (ca. 1 mg/mL in deionized water).

4.4. Cross-screening

A number of compounds deemed to be of interest for further studies were cross-screened against a small panel of kinases, including the related receptor tyrosine kinases, VEGFR-1 and VEGFR-3. In addition, molecules were screened against c-Met, EGFR, IGF-1R, IR and FGF-R1. The results for this limited cross-screen indicated that compounds seemed to have significant inhibition against VEGFR-1, in addition to their inhibition of VEGFR-2 (Table 6). These results are in accordance with other studies on the inhibition of VEGF receptor tyrosine kinases with anilinophthalazine derivatives, such as PTK-787 **3**.[34] Additionally, compound **57** was tested against a wide panel of common receptors and transporters, with activity being observed against the dopamine transporter, adenosine receptor and histamine H2 receptor at a screening concentration of 10 μM.[37] In contrast to PTK-787, **57** did not inhibit the 3A4 isoform of cytochrome P450 (results not shown). However, some inhibition at the human i-hERG potassium channel was observed with **57** (ED_{50} 3 μM).

4.5. Activity in a HUVEC mitogenic assay

The ability of phthalazine compounds to inhibit VEGF-stimulated proliferation in human umbilical vein endothelial cells (HUVEC) was also measured. Unlike the cellular assay, which uses a

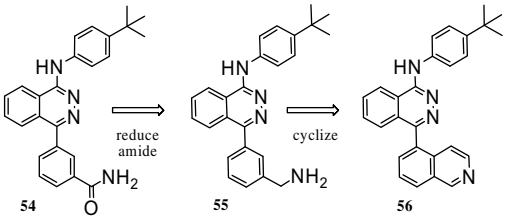


Figure 2. Modification of amide structures to produce isoquinolin-5-yl-phthalazines.

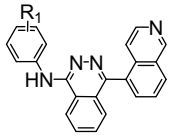
chimeric construct to assess potency, this functional experiment provides a measure of the compounds ability to block native VEGF-receptors in human tissue. Compounds **24** (IM-094261) and **57** (IM-094882) inhibited the growth of HUVECs with an ED_{50} of 0.25 and 0.21 μM, respectively (Table 7). In our hands, these compounds were approximately equipotent with ZD-6474 **4** (ED_{50} = 0.28 μM), but somewhat less potent than the more recent VEGFR-2 inhibitor, ZD-2171 **5** (ED_{50} = 0.012 μM).

4.6. Exposure studies in mice

Compounds **24** (mono-hydrochloride salt) and **57** (dihydrochloride salt) were selected for investigation of their systemic exposure following dosing to female CD-1 mice. The dosing vehicles were a DMSO-solution, 5% phosal solution and an ethanol:Tween 80:phosal (ETP) mixture. Compounds were dosed via the intraperotineal (ip) or oral (po) routes, and plasma was analyzed at the 1-h and 4-h timepoints. Initial results are shown in Figure 3. Compound **24** showed high exposure with all dosing solutions, when given by both ip and po routes. However, at 4-h post-dose compound levels had dropped significantly. This is not an unexpected result, as **24** contains a *tert*-butyl group which is potentially susceptible to metabolic oxidation. Conversely, compound **57** showed excellent exposure at both 1-h and 4-h timepoints. Of significance, was its oral exposure upon dosing in a 5% phosal solution (1 h 22.7 ± 2.6 μM; 4 h 18.8 ± 6.1 μM). ZD-2171 **5** was also included in these exposure studies and showed good exposure, although plasma levels were lower than for compound **57**. In order to gain a further understanding of the oral exposure with the lead phthalazine **57**, a 24-h experiment was undertaken in which **57** was dosed at 30 mg/kg in a 5% phosal mixture (target concentration ca. 3 mg/mL). Again ZD-2171 **5** was included as a comparison. As can be seen in Figure 4, **57** showed a rapid absorption, and high exposure in plasma (C_{max} 25.9 μM), that was sustained until about 8 h post-dose. Total compound exposure (0–24 h AUC) was also high at 891 μmol h.

The pharmacokinetic data for **57** appeared to be comparable to those for the related phthalazine, PTK-787 **3**,[34] which is currently in late-stage clinical trials. Since **57** also shows impressive enzymatic, cellular and functional (HUVEC) inhibition of VEGFR-2, the combined data support the continued evaluation of this compound as a potential anti-angiogenic.

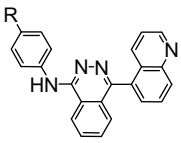
Table 4
Structure–activity relationships for isoquinolin-5-yl phthalamide series



Entry	Compound	R_1	IC_{50} (μM) VEGFR-2[a]	ED_{50} (μM) VEGFR-2[b]
1	**56**	4-^{t}Bu	0.075	—
2	**57**	4-Cl	0.048	0.01
3	**58**	4-OCF_2Cl	0.079	0.035
4	**59**	4-^{i}Pr	0.017	0.024
5	**60**	4-Morpholin-1-yl	0.39	0.88
6	**61**	3,4-Cl_2	0.11	0.07
7	**62**	4-Cl, 3-CF_3	0.12	0.28
8	**63**	2-F, 4-Me	0.43	0.35
9	**64**	2-Me	>10	>10
10	**65**	3-Me	0.27	0.016
11	**66**	2-Me-indol-5-yl	0.23	1.4

[a] Determined against isolated enzyme for at least two independent experiments (at least 8 points per experiment). Positive control, ZD-6474 **4** IC_{50} = 0.045 μM.
[b] At least two independent determinations against FGFR-1/VEGFR-2 chimeric receptor expressed in HEK-293 cells.

Table 5
Activity of quinolin-5-yl congeners



Entry	Compound	R	IC_{50} (μM) VEGFR-2[a]	ED_{50} (μM) VEGFR-2[b]
1	**67**	Cl	>10	>10
2	**68**	tBu	2.0	2.4

[a] Determined against isolated enzyme for at least two independent experiments (at least 8 points per experiment). Positive control, ZD-6474 **4** IC_{50} = 0.045 μM.
[b] At least two independent determinations against FGFR-1/VEGFR-2 chimeric receptor expressed in HEK-293 cells. Positive control, ZD-6474 **4** ED_{50} = 0.15 μM.

5. Conclusion

In conclusion, this paper details a two-step synthesis of potent inhibitors of vascular endothelial growth factor receptor II (VEGFR-2), based around an arylphthalazine chemotype. Arylphthalazines containing an isoquinolin-5-yl group were particularly effective inhibitors of the VEGFR-2 kinase in enzymatic, cellular and mitogenic assays. Additionally, the lead compound from this series, **57** (IM-094482), was found to have high exposure in plasma when dosed orally to female CD-1 mice. The results detailed in this publication support the continued exploration of **57** as a research tool, and reinforce the attractiveness of phthalazine-containing compounds as kinase inhibitors.

6. Experimental

6.1. Enzymatic assay for determining inhibition of isolated VEGFR-2

Into a black 96-well Costar plate is added 2 μL/well of compound in DMSO (final concentration in the 50 μL kinase reaction is typically 1–10 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 VEGFR-2 enzyme is added to each well. After 5–10 min preincubation, the kinase reaction is initiated by addition of 10 μL of 10 μM ATP to reaction buffer, after which the plate is incubated at room temperature for 45 min. The reaction is stopped by the addition of 50 μL KF buffer (50 mM Hepes, pH 7.5, 0.5 M KF, 1 mg/mL BSA) containing 100 mM EDTA and 0.36 μg/mL PY20 K (Eu-cryptate labeled anti-phosphotyrosine antibody, CAS 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.

Table 6
Cross-screening of IM-094882 **57**

Entry	Compound	VEGFR-1[a]	VEGFR-3[a]	c-Met[a]	EGFR[a]	FGFR-1[a]	IGF-1R[a]	IR[a]	DT[b,c]	Adenosine[b]	H2[b]	*i*-hERG[d]
1	**57**	91	33	2	13	38	25	10	77	65	54	3.0

[a] Percentage inhibition at a drug screening concentration of 10 μM (average of at least two independent determinations). Inhibition of less than 40% at 10 μM is considered to be inactive in our hands.
[b] Percentage inhibition at a drug screening concentration of 10 μM (average of at least two independent determinations). Inhibition greater than 50% at 10 μM is considered to be of significance.
[c] DT, dopamine transporter.
[d] ED_{50} (μM).

Table 7
Activity of leading arylphthalazines and literature compounds in a mitogenic assay

Entry	Compound	ED_{50} (μM)[a]
1	**24** (IM-094261)	0.25
2	**57** (IM-094882)	0.21
3	**4** (ZD-6474)	0.28
4	**5** (ZD-2171)	0.012

[a] Determined for at least two independent experiments.

6.2. Cell-based assay for VEGFR-2 inhibition: Transfection of 293 cells with DNA expressing FGFR-1/VEGFR-2 chimera

A chimeric construct containing the extracellular portion of FGFR-1 and the intracellular portion of VEGFR-2 is transiently transfected into HEK-293 adenovirus-transfected kidney cells. DNA for transfection is diluted to a final concentration of 5 μg/ml DNA in serum-free medium and incubated at room temperature for 30 min. with 40 μl/ml of Lipofectamine 2000, also in serum-free media. Two hundreds and fifty microliters of the Lipofectamine and DNA mixture is added to HEK-293 cells suspended at 5×10^5 cells/ml. 200 μl/well is dispensed into a 96-well plate, with subsequent overnight incubation. Within 24 h, media are removed and 100 μl of media with 10% fetal bovine serum is added to the now adherent cells followed by an additional 24 h incubation. Target compounds are added to the individual wells with a final DMSO concentration of 0.1%. Cells are lysed by resuspension in 100 μl Lysis buffer (150 mM NaCl, 50 mM Hepes pH 7.5, 0.5% Triton X-100, 10 mM NaPPi, 50 mM NaF, 1 mM Na_3VO_4) and rocked for 1 h at 4 °C.

6.3. ELISA for detection of tyrosine-phosphorylated chimeric receptor

96-well ELISA plates are coated using 100 μl/well of 10 μg/ml of αFGFR1 antibody, and incubated overnight at 4 °C. αFGFR1 is prepared in a buffer made with 16 ml 0.2 M Na_2CO_3 and 34 ml 0.2 M $NaHCO_3$ with pH adjusted to 9.6. Concurrent with lysis of the transfected cells, αFGFR1 coated ELISA plates are washed three times with PBS + 0.1% Tween 20 and blocked by addition of 200 μl/well of 3% BSA in PBS for 1 h and washed again. Eighty microliters of lysate is then transferred to the coated and blocked wells and incubated for 1 h at 4 °C. The plates are washed three times with PBS + 0.1% Tween 20. To detect bound phosphorylated chimeric receptor, 100 μl of anti-phosphotyrosine antibodies (RC20:HRP, Transduction Laboratories) was added per well (final concentration 0.5 μg/ml in PBS) and incubated for 1 h. The plates are washed six times with PBS + 0.1% Tween 20. Enzymatic activity of HRP is detected by adding 50 μl/well of equal amounts of the Kirkegaard & Perry Laboratories (KPL) Substrate A and Substrate B. The reaction is stopped by addition of 50 μl/well of 0.1 N H_2SO_4 and absorbance is detected at 450 nm.

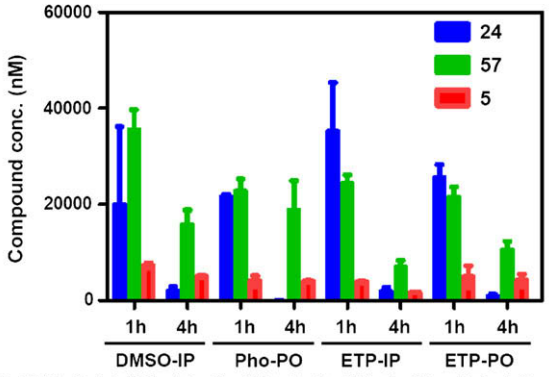


Figure 3. Preliminary exposure data for **24** (IM-094261), **57** (IM-094882) and ZD-2171 **5** in female CD-1 mice.

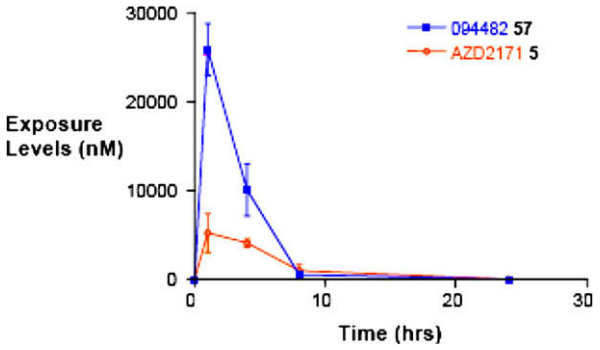


Figure 4. Oral exposure of **57** (30 mg/kg) and ZD-2171 **5** (30 mg/kg) in female CD-1 mice over 24 h using a 5% phosal formulation. Compound **57** (IM-094882) dosed orally to female CD-1 mice ($n = 3$) at 30 mg/kg in a 5% phosal solution (animals dosed at 10 mL/kg). C_{max} = 25.9 μM; 0–24 h AUC = 891 μmol h.

6.4. Mitogenic assay in human umbilical vein endothelial cells (HUVEC)

HUVEC cells are seeded at 5×10^4 cells/ml into 96-well plates at 200 μl/well to obtain a final concentration of 1×10^4 cells/well in EGM media (Cambrex, New Jersey). Cells are allowed to adhere to the plate for a minimum of 4 h before changing to media deficient in EGF, VEGF and FGF but including 1% FBS. After allowing the cells to quiese for 72 h, the desired compound is incubated with the cells for 2 h at DMSO concentrations of 0.1%. HUVEC cells are stimulated with 5 ng/well solution of VEGF-165 for 3 days before adding 25 μl of 0.25 μCi/well of ^{3}H-Thymidine. Cells are harvested onto a mat after an additional 5 h incubation and washed three times with water. The radioactive mat is placed in scintillant and read on a beta-emitter counter.

6.5. Chemistry

6.5.1. General

All reagents were purchased from commercial suppliers and used without further purification. All NMR spectra were recorded

on a Brücker spectrometer and are quoted in ppm relative to a tetramethylsilane internal standard, or by referencing on the chemical shift of the deuterated solvent.

6.5.2. General procedure A for amine displacement. Preparation of (4-chlorophenyl)-(4-chlorophthalazin-1-yl)amine 23a

1,4-Dichlorophthalazine (1.0 g, 5.0 mmol), 4-chloroaniline (0.64 g, 5.0 mmol) and Et_3N (700 μL, 5.0 mmol) in n-butanol (15 mL) was heated to 100 °C in a sealed tube and stirred for 48 h. After allowing to cool, to room temperature, the mixture was concentrated in vacuo to leave a crude solid. The solid was triturated with Et_2O, filtered and the filter cake washed with H_2O and Et_2O to give the title compound (1.05 g, 72%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 10.50 (br s, 1 H), 8.81–8.94 (m, 1 H), 8.15–8.25 (m, 3H), 7.83 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 8.7 Hz, 2H). m/z = 290 (M+1 for ^{35}Cl).

6.5.3. (4-*tert*-Butylphenyl)-(4-chlorophthalazin-1-yl)amine 23b

Prepared using general procedure A. Yield 1.5 g (96%); ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.74 (br s, 1 H), 8.74–8.77 (m, 1H), 8.08–8.20 (m, 3H), 7.71 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.7 Hz, 2H), 1.34 (s, 9 H). m/z = 312 (M+1 for ^{35}Cl).

6.5.4. [4-(Chlorodifluoromethoxy)phenyl]-(4-chlorophthalazin-1-yl)amine 23c

Prepared using general procedure A. Yield 0.7 g (79%) as an impure solid; ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.83 (br s, 1H), 8.58–8.61 (1H, m), 7.91–8.17 (m, 3H), 7.72 (d, J = 9.0 Hz, 2H), 7.16 (d, J = 9.0 Hz, 2H). m/z = 336 (M+1 for ^{35}Cl).

6.5.5. (4-Chlorophthalazin-1-yl)-(2-fluoro-4-methylphenyl)amine 23d

Prepared using general procedure A. Isolated as an impure solid that was used directly in the next step.

6.5.6. General procedure B for amine displacement. Preparation of (4-chlorophthalazin-1-yl)-(4-morpholin-4-yl-phenyl)amine hydrochloride 23e

1,4-Dichlorophthalazine (1.0 g, 5.0 mmol) and 4-(4-morpholino)aniline (0.88 g, 5.0 mmol) in EtOH (10 mL) was heated to reflux and stirred for 72 h. After allowing to cool to room temperature the mixture was filtered to give the title compound as an impure solid that was used directly in the next step. ^{1}H NMR (300 MHz; DMSO-d_6) δ 11.50 (br s, 1H), 9.13 (d, J = 6.9 Hz, 1H), 8.20–8.29 (m, 3H), 7.59 (d, J = 8.1 Hz, 2H), 7.36 (d, J = 7.5 Hz, 2H), 3.88 (m, 4 H), 3.33 (m, 4H). m/z = 341 (M+1 for ^{35}Cl).

6.5.7. (4-Chlorophthalazin-1-yl)-(4-isopropylphenyl)amine hydrochloride 23f. Prepared using general procedure B

Isolated as an impure solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 11.50 (br s, 1H), 9.18 (d, J = 7.2 Hz, 1H), 8.18–8.29 (m, 3H), 7.57 (d, J = 8.1 Hz, 2H), 7.37 (t, J = 9.2 Hz, 2H), 2.87–3.02 (m, 1H), 1.24 (d, J = 6.9 Hz, 6H). m/z = 298 (M+1 for ^{35}Cl).

6.5.8. (4-Chlorophthalazin-1-yl)-(3,4-dichlorophenyl)amine hydrochloride 23g

Prepared using general procedure B. Isolated as an impure solid that was used directly in the next step.

6.5.9. (4-Chlorophthalazin-1-yl)-(4-chloro-3-trifluoromethylphenyl)amine hydrochloride 23h

Prepared using general procedure B. Isolated as an impure solid that was used directly in the next step.

6.5.10. (4-Chlorophthalazin-1-yl)-*m*-tolylamine hydrochloride 23i

Prepared using general procedure B. Isolated as an impure solid that was used directly in the next step.

6.5.11. (4-Chlorophthalazin-1-yl)-*o*-tolylamine hydrochloride 23j

Prepared using general procedure B. Isolated as an impure solid that was used directly in the next step.

6.5.12. General procedure for Suzuki coupling. Preparation of (4-Chlorophenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)amine 57

(4-Chlorophenyl)-(4-chlorophthalazin-1-yl)amine (200 mg, 0.68 mmol), 5-isoquinoline boronic acid (236 mg, 1.36 mmol) and palladium(II)chloride bis(triphenylphosphine) (24 mg, 0.034 mmol) were combined in a CEM microwave vial. 1,4-dioxane (4 mL) was added, followed by a solution of K_2CO_3 (192 mg, 1.36 mmol) in H_2O (1 mL). The vial was sealed (CEM microwave cap) then heated to 100 °C for 60 min under microwave irradiation (CEM microwave parameters: solvent choice = 1,4-dioxane; microwave power = 50 W; run time = 10 min; hold time = 60 min; stirring = on; cooling = off. Note: in previous studies this reaction takes less than 60 min—a prolonged reaction time was used to ensure complete reaction). After cooling to room temperature the mixture was poured in to EtOAc (50 mL) and H_2O (50 mL) and the aqueous and organic layers were partitioned. The aqueous layer was extracted with EtOAc (1× 50 mL) and the combined organic extracts were washed with brine (1× 50 mL), dried (Na_2SO_4), filtered and the mixture concentrated in vacuo to leave a crude solid. The solid was purified by column chromatography on silica gel (compound dry-loaded on to $MgSO_4$; Biotage Flash+ 25 M cartridge used) using EtOAc/hexanes (3:7–1:0) as an eluent gave a solid which was triturated with Et_2O and filtered to give the title compound (140 mg, 53%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.57 (s, 1H), 9.53 (s, 1H), 8.77 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 6.0 Hz, 1H), 8.39 (d, J = 7.8 Hz, 1H), 8.10–8.16 (m, 3H), 7.89–7.98 (m, 3H), 7.49 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 8.4 Hz, 1H), 7.36 (d, J = 5.7 Hz, 1H). m/z = 383 (M+1 for ^{35}Cl).

6.5.13. (4-Chlorophenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)amine dihydrochloride

A solution of HCl in 1,4-dioxane (4 M; 1 mL, excess) was added in one portion to a stirred suspension of (4-chloro-phenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)-amine (140 mg) in MeOH (20 mL) at room temperature (a solution develops). The mixture was concentrated in vacuo to leave a solid that was triturated with Et_2O and filtered to give the title compound (160 mg) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.97 (s, 1H), 9.24 (d, J = 8.4 Hz, 1H), 8.72 (d, J = 8.4 Hz, 1H), 8.63 (d, J = 6.3 Hz, 1H), 8.14–8.33 (m, 3H), 8.02–8.08 (m, 2H), 7.78 (d, J = 8.4 Hz, 2 H), 7.64 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.1 Hz, 1H).

6.5.14. (4-Chlorophenyl)-(4-quinolin-5-yl-phthalazin-1-yl)amine 67

Prepared using general procedure for Suzuki coupling. After workup the solid was triturated with Et_2O then purified by column chromatography on silica gel using EtOAc/hexanes (1:4–1:0) as an eluent. Recrystallization from MeOH gave the title compound (30 mg, 23%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.50 (s, 1H), 8.95 (s, dd, J = 3.9, 1.5 Hz, 1H), 8.71 (d, J = 8.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.81–8.10 (m, 6H), 7.37 (dd, J = 6.9, 0.9 Hz, 1H), 7.38–7.47 (m, 4H). m/z = 383 (M+1 for ^{35}Cl).

6.5.15. (4-*tert*-Butylphenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)amine 56

Prepared using general procedure for Suzuki coupling. Purified by column chromatography on silica gel using Et_2O/hexane (1:1–

1:0) as an eluent. The material was purified further by ion exchange chromatography (TsOH loaded on to SiO_2; Silicycle). The captured compound was washed with MeOH (6× 6 mL) then eluted with 2 M NH_3/MeOH. Trituration with Et_2O and filtration gave the title compound (40 mg, 29%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.35 (s, 1 H), 9.22 (s, 1H), 8.59 (d, J = 8.4 Hz, 1H), 8.27 (d, J = 5.7 Hz, 1H), 8.21 (d, J = 7.5 Hz, 1H), 7.87 (t, J = 7.7 Hz, 1H), 7.67–7.82 (m, 5 H), 7.28 (d, J = 8.4 Hz, 2 H), 7.18 (app. t, J = 8.7 Hz, 2H), 1.20 (s, 9H). m/z = 405 (M+1 for ^{35}Cl).

6.5.16. (4-*tert*-Butylphenyl)-(4-quinolin-5-yl-phthalazin-1-yl)amine 68

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the reaction mixture was filtered and the filter cake washed with Et_2O to give the title compound (100 mg, 72%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.32 (s, 1H), 8.95 (d, J = 3.9 Hz, 1H), 8.70 (d, J = 8.4 Hz, 1H), 8.21 (d, J = 8.1 Hz, 1H), 7.88–8.04 (m, 5H), 7.88 (t, J = 7.7 Hz, 1H), 7.73 (d, J = 6.9 Hz, 1H), 7.35–7.46 (m, 4H), 1.32 (s, 9H). m/z = 405 (M+1 for ^{35}Cl).

6.5.17. [4-(Chloro-difluoro-methoxy)-phenyl]-(4-isoquinolin-5-yl-phthalazin-1-yl)amine 58

Prepared using general procedure for Suzuki coupling. Purified by column chromatography on silica gel using Et_2O/hexane (1:1–1:0) as an eluent. The material was purified further by ion exchange chromatography (TsOH loaded on to SiO_2; Silicycle). The captured compound was washed with MeOH (6× 6 mL) then eluted with 2 M NH_3/MeOH. Trituration with Et_2O and filtration gave the title compound (60 mg, 39%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.58 (s, 1 H), 9.47 (s, 1H), 8.72 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 6.0 Hz, 1H), 8.39 (d, J = 7.8 Hz, 1H), 8.13 (d, J = 8.7 Hz, 2H), 8.03 (t, J = 7.8 Hz, 1H), 7.85–7.93 (m, 3 H), 7.36–7.41 (m, 3H), 7.30 (d, J = 6.0 Hz, 1H). m/z = 449 (M+1 for ^{35}Cl).

6.5.18. (4-Isopropylphenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)amine 59

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the reaction mixture was poured in to H_2O (20 mL), filtered and the filter cake washed with Et_2O (3× 5 mL) to give the title compound (75 mg, 57%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.59 (s, 1 H), 9.44 (s, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.36 (d, J = 6.0 Hz, 1H), 8.26 (d, J = 9.6 Hz, 1H), 7.96 (t, J = 7.5 Hz, 1 H), 7.76–7.91 (m, 5 H), 7.23–7.32 (m, 4H), 2.83–2.92 (m, 1H), 1.20 (d, J = 6.9 Hz, 6H). m/z = 391 (M+1).

6.5.19. (4-Isoquinolin-5-yl-phthalazin-1-yl)-(4-morpholin-4-yl-phenyl)-amine 60

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the mixture was allowed to stand overnight then filtered. The filter cake was washed with H_2O (1× 10 mL) and Et_2O (1× 10 mL) to give the title compound (130 mg, 88%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.46 (s, 1 H), 9.22 (s, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 6.0 Hz, 1H), 8.32 (d, J = 7.2 Hz, 1H), 7.97 (t, J = 7.7 Hz, 1H), 7.77–7.92 (m, 5H), 7.29–7.33 (m, 2H), 6.99 (d, J = 9.0 Hz, 2 H), 3.76–3.78 (m, 4 H), 3.10–3.12 (m, 4H). m/z = 434 (M+1).

6.5.20. (3,4-Dichloro-phenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)-amine 61

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the mixture was poured in to H_2O (20 mL), filtered and the filter cake washed with Et_2O (4× 5 mL). The filter cake was purified further by the following procedure. An ion exchange cartridge (TsOH loaded on to SiO_2; Silicycle) was washed with MeOH (6 mL). The filter cake was dissolved in MeOH, loaded on to the silica cartridge and the captured compound washed with MeOH (6× 6 mL). Elution with 2 M NH_3/MeOH gave the title compound (30 mg, 21%) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.65 (s, 1H), 9.48 (s, 1H), 8.71 (d, J = 8.4 Hz, 1H), 8.55 (s, 1H), 8.42 (d, J = 6.0 Hz, 1H), 8.35 (d, J = 7.8 Hz, 1H), 8.02–8.09 (m, 2H), 7.83–7.96 (m, 3H), 7.63 (d, J = 8.7 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.27 (d, J = 6.0 Hz, 1H). m/z = 417 (M+1 for ^{35}Cl).

6.5.21. (4-Chloro-3-trifluoromethylphenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)amine 62

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the reaction mixture was poured in to H_2O (50 mL) and filtered. The filter cake was purified further by preparative thin-layer chromatography to give the title compound (30 mg, 20%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.80 (s, 1H), 9.48 (s, 1H), 8.73 (d, J = 8.1 Hz, 1H), 8.69 (s, 1H), 8.33–8.47 (m, 3H), 8.06 (t, J = 7.7 Hz, 1H), 7.81–7.96 (m, 3H), 7.74 (d, J = 9.0 Hz, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.29 (d, J = 6.0 Hz, 1H). m/z = 451 (M+1 for ^{35}Cl).

6.5.22. (2-Fluoro-4-methylphenyl)-(4-isoquinolin-5-yl-phthalazin-1-yl)amine 63

Prepared using general procedure for Suzuki coupling. The reaction mixture was allowed to stand overnight then filtered. The filter cake was washed with H_2O (1× 10 mL) and Et_2O (1× 10 mL) to give the title compound (130 mg, 88%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.46 (s, 1H), 9.22 (s, 1H), 8.68 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 6.0 Hz, 1H), 8.32 (d, J = 7.2 Hz, 1H), 7.97 (t, J = 7.7 Hz, 1H), 7.77–7.92 (m, 5H), 7.29–7.33 (m, 2H), 6.99 (d, J = 9.0 Hz, 2H), 3.76–3.78 (m, 4H), 3.10–3.12 (m, 4H). m/z = 434 (M+1).

6.5.23. (4-Isoquinolin-5-yl-phthalazin-1-yl)-*o*-tolylamine 64

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the mixture was poured in to H_2O (50 mL) and filtered. The filter cake was purified further by ion exchange chromatography (TsOH loaded on to SiO_2; Silicycle). The captured compound was washed with MeOH (6× 6 mL) then eluted with 2 M NH_3/MeOH to give the title compound (50 mg, 41%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.45 (s, 1H), 9.12 (s, 1H), 8.63 (d, J = 8.1 Hz, 1H), 8.39 (d, J = 5.7 Hz, 1H), 8.31 (d, J = 7.5 Hz, 1H), 7.98 (t, J = 7.5 Hz, 1H), 7.79–7.91 (m, 3H), 7.46 (d, J = 7.5 Hz, 1H), 7.18–7.35 (m, 5H), 2.23 (s, 3H). m/z = 363 (M+1).

6.5.24. (4-Isoquinolin-5-yl-phthalazin-1-yl)-*m*-tolylamine 65

Prepared using general procedure for Suzuki coupling. After allowing to cool to room temperature the mixture was poured in to H_2O (20 mL), filtered and the filter cake washed with Et_2O (2× 5 mL). The filter cake was purified further by ion exchange chromatography (TsOH loaded on to SiO_2; Silicycle). The captured compound was washed with MeOH (6× 6 mL) then eluted with 2 M NH_3/MeOH to give the title compound (52 mg, 42%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.47 (s, 1H), 9.31 (s, 1H), 8.72 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 5.7 Hz, 1H), 8.33 (d, J = 7.5 Hz, 1H), 8.00 (t, J = 7.7 Hz, 1H), 7.79–7.94 (m, 5H), 7.25–7.36 (m, 3H), 6.89 (d, J = 7.2 Hz, 1H), 2.36 (s, 3H). m/z = 363 (M+1).

6.5.25. 1-Chloro-4-isoquinolin-5-yl-phthalazine

1,4-Dichlorophthalazine **22** (200 mg, 1.0 mmol), 5-isoquinoline boronic acid (173 mg, 1.0 mmol) and palladium(II) chloride bis(triphenylphosphine) (35 mg, 0.05 mmol) were combined in a CEM microwave vial. 1,4-Dioxane (4 mL) was added followed by a solution of K_2CO_3 (276 mg, 2.0 mmol) in H_2O (1 mL). The vessel was sealed (CEM microwave cap) and then heated to 100 °C for

30 min under microwave irradiation with stirring (CEM microwave parameters: solvent choice = 1,4-dioxane; power = 50 W; run time = 5 min; hold time = 30 min; stirring = on; cooling = off). The mixture was subjected to a further 30 min of heating at 100 °C under microwave irradiation. After allowing to cool to room temperature, the mixture was poured in to EtOAc (70 mL) and the mixture was washed with brine (1× 30 mL), dried (Na_2SO_4), filtered and the mixture concentrated in vacuo to leave a crude solid. The solid was purified by column chromatography on silica gel (compound dry-loaded on to silica) using EtOAc/hexanes (1:9–1:0) as an eluent to give the title compound (80 mg, 27%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 9.50 (s, 1H), 8.40–8.47 (m, 2H), 8.13 (dt, J = 8.3, 1.5 Hz, 1H), 7.90–8.04 (m, 2H), 7.52–7.65 (m, 3H), 7.30 (d, J = 5.7 Hz, 1H). m/z = 292 (M+1 for ^{35}Cl).

6.5.26. (4-Isoquinolin-5-yl-phthalazin-1-yl)-(2-methyl-1*H*-indol-5-yl)amine 66

1-Chloro-4-isoquinolin-5-yl-phthalazine (40 mg, 0.14 mmol), 5-amino-2-methylindole (20 mg, 0.14 mmol) and EtOH (2.5 mL) were combined and sealed in a CEM microwave vial. The mixture was heated to 100 °C for 120 min under microwave irradiation (CEM microwave parameters: solvent choice = ethanol; microwave power = 300 W; run time = 10 min; hold time = 120 min; stirring = on; cooling = off). After allowing to cool to room temperature the mixture was purified by preparative thin-layer chromatography using EtOAc as an eluent to give the title compound (25 mg, 45%) as a solid. ^{1}H NMR (300 MHz; DMSO-d_6) δ 10.62 (s, 1H), 9.22 (s, 1H), 9.01 (br s, 1H), 8.48 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 6.0 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 7.61–7.75 (m, 4H), 7.53 (t, J = 9.2 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 7.03–7.09 (m, 3H), 5.90 (s, 1H), 2.16 (s, 3H). m/z = 402 (M+1).

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36. Kinetic or thermodynamic solubilities of compounds were not measured. However, in several instances precipitation of amide compounds was noted in pH 7.4 buffer. This observation was interpreted as indicative of a need to improve the aqueous solubility of future molecules.
37. Cross-screening against more than 60 biological targets belonging to neurotransmitter, steroid, ion-channel, second messenger, prostaglandin, growth factor/hormone, brain/gut peptide and enzyme class performed by NovaScreen Biosciences Corporation. Inhibition of more than 50% at a drug screening concentration of 10 μM was deemed to be of significance.