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# (1,2,3-Triazol-4-yl)benzenamines: Synthesis and activity against VEGF receptors 1 and 2

Alexander S. Kiselyov [a,*], Marina Semenova [b], Victor V. Semenov [c]

[a] deCODE, 2501 Davey Road, Woodridge, IL 60517, USA
[b] Institute of Developmental Biology, RAS, 26 Vavilov Str., 119334 Moscow, Russia
[c] Zelinsky Institute of Organic Chemistry, RAS, 47 Leninsky Prospect, 117913 Moscow, Russia

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ABSTRACT

Derivatives of (1,2,3-triazol-4-yl)benzenamines are described as potent and ATP-competitive inhibitors of vascular endothelial growth factor receptors I and II (VEGFR-1/2). A number of compounds exhibited VEGFR-2 and VEGFR-1 inhibitory activity comparable to that of Vatalanib™ in both HTRF enzymatic and cellular assays.



Anti-angiogenesis agents that target malignant vasculature are of considerable interest due to their perceived potential to target tumor resistance towards chemo- and radiotherapy.[1–3] Vascular endothelial growth factors (VEGFs) and their respective family of receptor tyrosine kinases (VEGFRs) are key proteins modulating angiogenesis, the formation of new vasculature from an existing vascular network.[4–10] These include VEGFR-1 (Flt-1) and VEGFR-2 (Kinase Insert Domain Receptor (KDR) or flk1).[10–13] VEGFR-2 is the major positive signal transducer for endothelial cell proliferation and differentiation.[11] There has been considerable evidence, including clinical observations, that the abnormal angiogenesis is implicated in a number of diseases including rheumatoid arthritis, inflammation, cancer, degenerative eye conditions and others.[12,13] Several molecules have been successfully used for sequestering VEGF leading to a signal blockade *via* VEGF receptors and, subsequently to an inhibition of malignant angiogenesis. One of the agents reportedly working *via* this mechanism is Avastin™.[14] A number of small-molecule inhibitors that affect VEGF/VEGFR signaling by directly competing with the intracellular ATP binding site of the respective intracellular kinase domain have been reported. These include Vatalanib™ (**A**),[15] BAY579352 (**B**),[16] and the isosteric anthranyl amide derivatives **C**[17] and AMG-706 (**D**).[18] Intramolecular hydrogen bonding in **C** and **D** was suggested[17–19] to be responsible for the optimal spatial orientation of the pharmacophore pieces (Fig. 1).[20]

The essential pharmacophore elements for the VEGFR-2 activity of phthalazine-based molecules and their analogues (**A-D**) include[20] (i) [6,6]fused (or related) aromatic system; (ii) *para*- or 3,4-*di*-substituted aniline function in position 1 of the phthalazine core; (iii) hydrogen bond acceptor (Lewis' base: lone pair(s) of a nitrogen- or oxygen atom(s)) attached to position 4 via an appropriate linker (aryl or fused aryl group). In this communication, we expand upon our initial findings[19,20] and disclose potent inhibitors of VEGFR-2 kinase based on (1,2,3-triazol-4-yl)benzenamines. We reasoned that this template could provide for the both proper pharmacophore arrangement and favorable in vitro/cellular potency of the resulting molecules.

A series of (1,2,3-triazol-4-yl)benzenamines **3–28** (Scheme 1, Table 1) were synthesized as described in Scheme 1.[21] Reaction sequence involved the initial formation of enamines **1** from the respective *o*-nitrotoluene derivatives (R = H, Cl, $CF_3$) in a 79–92% yield. Pharmacophore group $Ar^1$ was introduced by the 1,3-dipolar cycloaddition reaction of **1** with a series of aryl azides in MeCN at reflux to afford the key intermediate nitroaryl 1,2,3-triazoles **2** in a 56–84% isolated yields. Compounds **2** were further converted to anilines followed by the reductive amination of these in order to introduce the $Ar^2$ group. Using this methodology, we have prepared a library of 25 derivatives **3–28** (51–72% yields from **2**, 24–52% overall yields from nitrotoluenes).

* Corresponding author. Tel.: +1 630 783 4900.
*E-mail address:* akiselyov@decode.com (A.S. Kiselyov).

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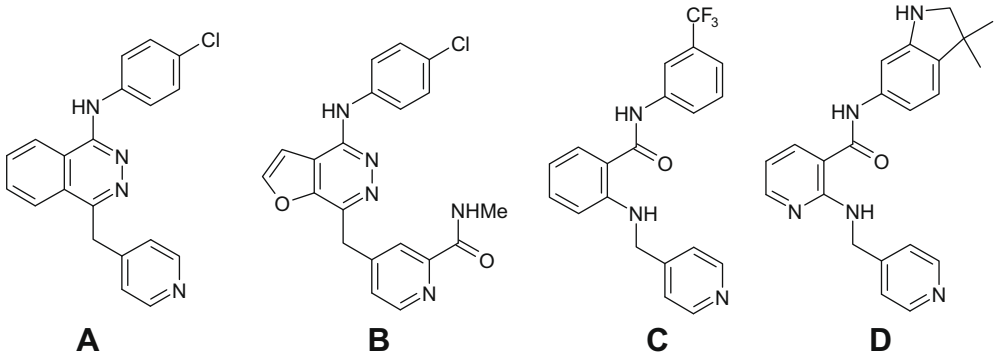
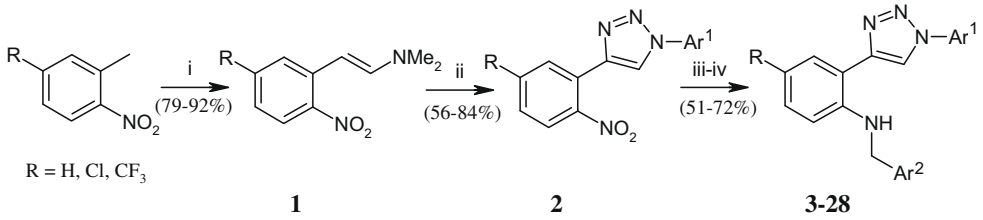



**Figure 1.** Selected Inhibitors of VEGFR-2.




**Scheme 1.** Synthesis of (1,2,3-triazol-4-yl)benzenamines **3**–**28**. Reagents and conditions: (i) $Me_2NCH(OMe)_2$, DMF, reflux, 40 h; (ii) $Ar^1N_3$, MeCN, reflux, 48 h; (iii) Fe, AcOH aq, reflux, 6 h; (iv) $Ar^2CHO$, *i*PrOH, reflux, 5 min; $NaBH_4$, *i*PrOH.

Compounds (**3–28**, Table 1) were first tested in vitro against VEGFR-2[22] measuring their ability to inhibit phosphorylation of a biotinylated-polypeptide substrate (p-GAT, CIS Bio International) in a homogenous time-resolved fluorescence (HTRF) assay at an ATP concentration of 2 μM. The results were reported as a 50% inhibition concentration value ($IC_{50}$). VEGFR-2 inhibitors Vatalanib™ and **C** (Fig. 1) were used as internal standards.

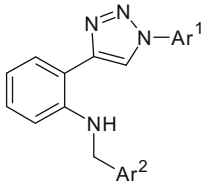
As can be seen from Table 1, several (1,2,3-triazol-4-yl)benzenamines exhibited good inhibitory activity against VEGFR-2 (**bolded** entries).[22] By varying substituents at both $Ar^1$ and $Ar^2$, it was possible to modulate compounds' potency against the enzyme. Notably, 5-substitution at the core benzene ring in **3–28** (e.g., R = 5-Cl, 5-$CF_3$-) was not tolerated. In studying SAR of the ***triazole*** pharmacophore ($Ar^1$), we found that compounds featuring *meta*-substituted $Ar^1$, as exemplified by *m*-Cl (**3**), *m*-$CF_3$- (**6**), *m*-$OCF_3$ (**9**), *m*-*i*Pr (**13**) displayed sound potency against VEGFR-2 with the $IC_{50}$ values of 51–110 nM. *Para*-substitution in this portion of the molecule was tolerated, however the respective analogues were >4-fold less active against the enzyme (Table 1, compare **3** and **10**, **6** and **11**, **9** and **12**). In order to further explore steric requirements for the $Ar^1$ substituent, we prepared a series of (1,2,3-triazol-4-yl)benzenamines featuring both heterocyclic (Table 1, **16**, **17**) and disubstituted $Ar^1$ functionalities (**18**–**22**). 2-(Quinolinyl) (**16**) and 5-(indazolyl) (**17**) $Ar^1$ groups displayed deleterious effect on the overall activity of resulting molecules against the kinase. Derivatives featuring 3,4-disubstituted aryl groups featured somewhat reduced potency when compared to the respective mono-substituted *meta*-derivatives (compare **6**, **18** and **21**). These facts suggest presence of a relatively tight hydrophobic pocket in the binding site of VEGFR-2 (vide supra).

In the next series of experiments, we optimized the ***aniline*** substituent $Ar^2$ (Table 1, entries **3–8**, **23–28**). Similar to the earlier findings,[19,20] 4-pyridyl group was present in the most active molecules. 4-Quinolinyl derivatives (**26, 27**) displayed diminished activity against VEGFR-2, even when endowed with the optimized $Ar^1$ functionality. 4-Imidazole derivative **28** was inactive. The combined SAR data indicated that (i) substitution pattern on the $Ar^1$ group of the triazole (compare **3** and **10**, **6** and **11**, **9** and **12**) and ii) nature of the aniline substituent $Ar^2$ (compare **3, 4** and **5**, **23–28**) were critical to the VEGFR-2 inhibitory activity of these new series. In addition, 5-substitution of the central aromatic core was not tolerated.

Compounds **3–28** were also tested in HTRF format against VEGFR-1.[22] The results in Table 1 suggest that all VEGFR-2 active (1,2,3-triazol-4-yl)benzenamine derivatives (e.g., **3**, **6**, **9**, **13**, **21, 26, 27**) consistently led to good activity against VEGFR-1 with the $IC_{50}$ values in 66–210 nM range for the most potent compounds. This outcome could be of benefit in the clinical setting as both receptors are reported to mediate VEGF signaling in the aberrant angiogenesis.[1–5,20] Further screening of **3–28** against a number of other receptor (IGF1R, InR, FGFR1, Flt3, EGFR, ErbB2, c-Met, Ron) and cytosolic (PKA, GSK3β, bcr-Abl, bcr-AblT315, Cdk1/2, Src, Auroras A and B, Plk1) kinases in HTRF format indicated no significant cross reactivity (PI < 30%, triplicate measurements) at a concentration of 10 μM.

In order to further expand the utility of triazole scaffold, we have prepared a series of 1*H*-1,2,3-triazole-4-carboxamides **31–34** as shown on Scheme 2. Specifically, amides of cyanoacetic acid (**29**) underwent cyclization with benzyl azide under basic conditions to furnish the key *N*-benzyltriazole intermediates **30**. *N*-Benzyl group in **30** was removed by hydrogenation over Pd/C followed by the reductive amination of resulting *N*H-triazoles to yield the targeted molecules **31–34** in a 24–47% overall yields. Disappointingly, these molecules did not display any significant activity against VEGFR-2 or VEGFR-1 kinases. For example, compounds **31** and **33** featuring optimized pharmacophores (Table 1, see compounds **3** and **6**) showed $IC_{50}$ values of ca. 5–7 μM against VEGFR-2 and were essentially inactive in the cell-based assay. Compounds **32** and **34** also did not show any activity against the kinases ($IC_{50}$ > 10 μM). We explained this outcome by the considerably different pharmacophore arrangement in the aryltriazole series exemplified by **3** and in 3,4-substituted triazoles (**31**–**34**).

**Table 1**
Activity of (1,2,3-triazol-4-yl)benzenamines **3–28** against VEGFR-2 and VEGFR-1 kinase in vitro and in cell-based assays.

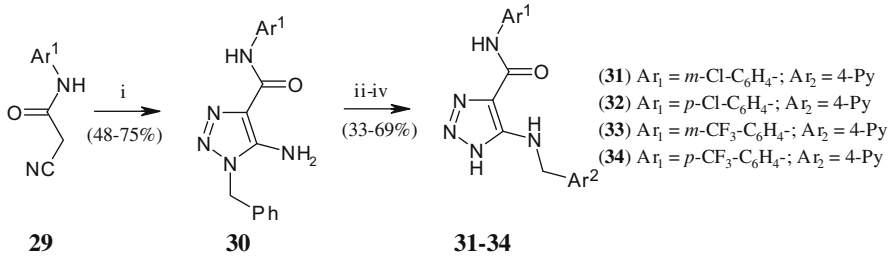


**3-28**

| Compound | $Ar^1$ | $Ar^2$ | VEGFR-2, enzymatic, $IC_{50}$[a] (μM) | VEGFR-1, enzymatic, $IC_{50}$[a] (μM) | VEGFR-2, cell-based ELISA, $IC_{50}$[a,b,c] (μM) |
|---|---|---|---|---|---|
| **A, PTK787 Vatalanib™** | | | 0.054 ± 0.006 (0.042 ± 0.003)[b] | 0.14 ± 0.02 (0.11 ± 0.03)[b] | 0.021 ± 0.03 (0.016 ± 0.001)[b] |
| **C** | | | 0.032 ± 0.005 (0.023 ± 0.006)[b] | 0.17 ± 0.05 (0.130 ± 0.081)[b] | 0.09 ± 0.01 (0.0012 ± 0.0002)[b] |
| **3** | **3-Cl-($C_6H_4$)** | **4-Pyridine** | **0.087 ± 0.011** | **0.13 ± 0.025** | **0.16 ± 0.05**[d] |
| **4** | 3-Cl-($C_6H_4$) | 3-Pyridine | 4.23 ± 0.51 | >10 | >10 |
| **5** | 3-Cl-($C_6H_4$) | 2-Pyridine | >10 | >10 | >10 |
| **6** | **3-$F_3C$-($C_6H_4$)** | **4-Pyridine** | **0.051 ± 0.05** | **0.066 ± 0.07** | **0.096 ± 0.14** |
| **7** | 3-$F_3C$($C_6H_4$) | 3-Pyridine | >10 | >10 | >10 |
| **8** | 3-$F_3C$($C_6H_4$) | 2-Pyridine | >10 | >10 | >10 |
| **9** | **3-$F_3CO$($C_6H_4$)** | **4-Pyridine** | **0.066 ± 0.005** | **0.072 ± 0.01** | **0.085 ± 0.02** |
| **10** | 4-Cl-($C_6H_4$) | 4-Pyridine | 0.39 ± 0.09 | 0.13 ± 0.025 | 0.27 ± 0.01[d] |
| **11** | 4-$F_3C$-($C_6H_4$) | 4-Pyridine | 0.59 ± 0.13 | 0.66 ± 0.12 | 1.92 ± 0.36 |
| **12** | 4-$F_3CO$($C_6H_4$) | 4-Pyridine | 1.22 ± 0.26 | 1.13 ± 0.19 | 3.11 ± 0.45 |
| **13** | **3-*i*Pr-($C_6H_4$)** | **4-Pyridine** | **0.11 ± 0.01** | **0.13 ± 0.03** | **0.15 ± 0.05** |
| **14** | 3-*t*Bu-($C_6H_4$) | 4-Pyridine | 0.27 ± 0.05 | 0.25 ± 0.08 | 0.33 ± 0.11 |
| **15** | 4-*t*Bu-($C_6H_4$) | 4-Pyridine | 0.61 ± 0.09 | 0.53 ± 0.07 | 1.63 ± 0.34 |
| **16** | 2-(Quinolinyl) | 4-Pyridine | 0.77 ± 0.18 | 0.64 ± 0.11 | 1.19 ± 0.22 |
| **17** | 5-(Indazolyl) | 4-Pyridine | 0.68 ± 0.14 | 0.65 ± 0.13 | 2.33 ± 0.28 |
| **18** | 3-$F_3C$,4-Cl($C_6H_4$) | 4-Pyridine | 0.44 ± 0.11 | 0.51 ± 0.10 | 0.78 ± 0.16 |
| **19** | 3-Cl,4-$F_3C$($C_6H_4$) | 4-Pyridine | 0.93 ± 0.18 | 1.13 ± 0.21 | 2.92 ± 0.44 |
| **20** | 3,4-di-Cl($C_6H_4$) | 4-Pyridine | 0.63 ± 0.15 | 0.65 ± 0.12 | 1.47 ± 0.27 |
| **21** | **4-Me-3-$F_3C$($C_6H_3$)** | **4-Pyridine** | **0.14 ± 0.03** | **0.11 ± 0.02** | **0.18 ± 0.03** |
| **22** | 3-Me-4-$F_3C$($C_6H_3$) | 4-Pyridine | 0.98 ± 0.21 | 0.86 ± 0.20 | 2.02 ± 0.35 |
| **23** | 3-Cl-($C_6H_4$) | 3,4-di-F-($C_6H_3$) | >10 | >10 | >10 |
| **24** | 3-Cl-($C_6H_4$) | 5-Piperonyl | >10 | >10 | >10 |
| **25** | 3-$CF_3$-($C_6H_4$) | 5-Piperonyl | >10 | >10 | >10 |
| **26** | **3-Cl-($C_6H_4$)** | **4-Quinolyl** | **0.19 ± 0.08** | **0.17 ± 0.02** | **0.27 ± 0.06** |
| **27** | **3-$F_3C$($C_6H_4$)** | **4-Quinolyl** | **0.16 ± 0.04** | **0.21 ± 0.05** | **0.31 ± 0.09** |
| **28** | 3-Cl-($C_6H_4$) | 4-Imidazolo | >10 | >10 | >10 |

[a] $IC_{50}$ values were determined from the logarithmic concentration-inhibition point (10 points).[22] The important values are given as the mean of at least two duplicate experiments.
[b] Lit. $IC_{50}$ values, as measured at 8 μM ATP.[15,17]
[c] Lit. data correspond to the inhibition of VEGF-induced phosphorylation of VEGFR-2 in CHO cells.[15,17]
[d] Molecules with best enzymatic and cellular potencies against VEGFR-2 are bolded.[22,23]

We reasoned that proper alignment of the lower portion of the molecule, namely nitrogen atoms (Lewis' base, hydrogen bond acceptors) of 4-pyridyl or 4-quinolyl groups (Table 1, e.g., **3** and **30**) with the *Arg*1302 moiety in the ATP-binding pocket of VEGFR-2 is required for the activity. Docking of **3** into the cocrystal structure 2P2l of a biphenyl analogue **D** with VEGFR2 revealed that the 1,2,3-triazole function of **3** is accommodated in a well-defined binding pocket lined up with *Ala*866 and *Lys*868. This allows for a better fit of a *meta*-substituted aryl moiety in the hydrophobic pocket of the ATP-binding site of the kinase.[19,20] There is a possibility for it to displace a water molecule from the active site of kinase (Fig. 2a). In addition, we concluded that *N*-atoms of the triazole ring could make a contact with the backbone *N*H of Arg1046. These features are absent in the 1,2,3-triazole derivative **31**. Lack of activity for **31–34** likely results from the improper alignment of its amide substituent in the hydrophobic pocket of the kinase. Minimization studies suggest that the positioning of $Ar^1$ pharmacophore for **3** and **31** differs considerably (Fig. 2b). In addition,




**29** **30** **31-34**

**Scheme 2.** Reagents and conditions: (i) $PhCH_2N_3$, KOH, EtOH, 80 °C, 1 h; (ii) $H_2$, Pd/C, 80 °C, 4 h; (iii) $Ar^2CHO$, 4Å mol. sieves, benzene, 90 °C, 4 h; (iv) $NaBH_4$, *i*PrOH, 100 °C, 2 h.

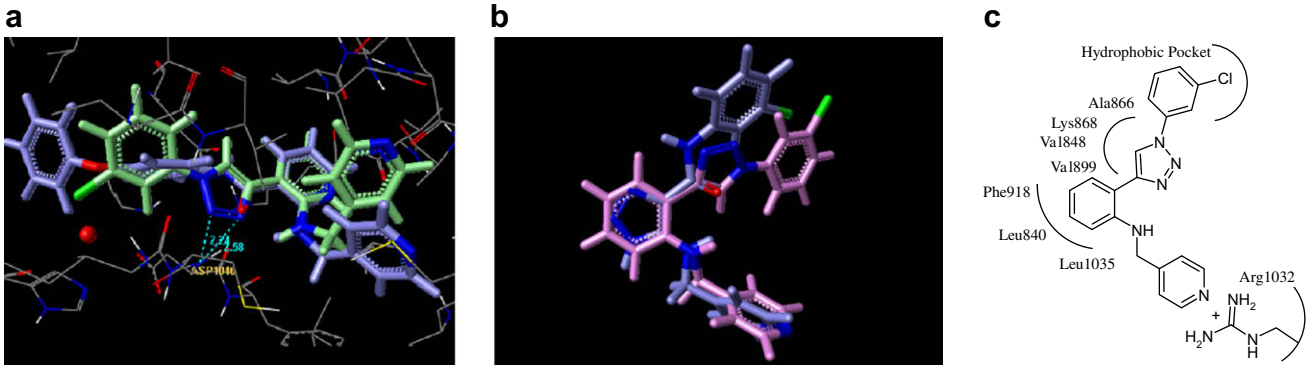



**Figure 2.** Structural overlap between (a) **3** (green) and biphenyl analogue of **D** (blue) from the 2P2 l co-crystal structure with VEGFR; (b) structural overlap of compounds **3** and **31**; (c) pharmacophore hypothesis for the mode of binding of (1,2,3-triazol-4-yl)benzenamine **3** within the ATP binding pocket of VEGFR-2. Central aromatic ring is surrounded by *Leu*840, *Val*848, *Val*899, *Phe*918 and *Leu*1035. The amide is in close proximity with the *Ala* 866 and *Lys*868, and pyridine nitrogen is near *Arg*1032.

**Table 2**
Compounds **3**, **6** and **13** are ATP-competitive inhibitors of VEGFR-2.

| Compound | $K_i$ at $IC_{50}$ (μM) | $K_i$ at $IC_{90}$ (μM) |
|---|---|---|
| **3** | 0.12 | 0.11 |
| **6** | 0.09 | 0.08 |
| **13** | 0.16 | 0.14 |

acidic proton of the pyrrole *N*H in the triazole ring of **31–34** may cause unfavorable interactions in the binding pocket lined up with *Leu*840, *Phe*918 and *Leu*1035 residues. Observed structure–activity relationship of the novel (1,2,3-triazol-4-yl)benzenamines **3–28** are in line with the proposed pharmacophore hypothesis (Fig. 2c).

Active in vitro inhibitors of VEGFR-2 were further characterized in a cell-based phosphorylation ELISA assay (Table 1).[23] In general, good in vitro-to-cell based activity correlation has been found for these compounds. In our hands, the best compounds displayed 85–310 nM activity in inhibiting cell-based phosphorylation of VEGFR-2. Further, competition assays were conducted for the selected molecules with varying concentration (0–100 μM) of ATP. Specifically, five different concentrations of $^{32}P$ ATP were incubated with VEGFR-2 along with varying concentrations (absence, $IC_{50}$, $IC_{90}$) of the inhibitors **3**, **6** and **13** for 45 min at RT. A double reciprocal graph of the degree of phosphorylation (1/cpm) against ATP-concentration (1/[ATP]) was plotted. The data were analyzed by a non-linear least-squares program to determine kinetic parameters using GraphPad software. Determined $K_i$ values for the three selected compounds are listed in Table 2. These data suggest an ATP-competitive mechanism for the interaction of these inhibitors with VEGFR-2.

In summary, we have described a series of (1,2,3-triazol-4-yl)benzenamines as potent inhibitors of both VEGFR-2 and VEGFR-1 receptors. Enzymatic and cellular activities of representative molecules are comparable to the clinical candidates Vatalanib™ and AMG-706. This outcome could be of benefit in the clinical setting as both receptors are reported to mediate VEGF signaling in the angiogenesis. Notably, 1*H*-1,2,3-triazole-4-carboxamide analogues did not show any appreciable activity against the kinases suggesting strict electronic and spatial demands for the proper alignment of ligand in a binding pocket of the enzymes.

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21. *Analytical data for selected molecules:2-(1-(3-Chlorophenyl)-1H-1,2,3-triazol-4-yl)-N-(pyridin-4-ylmethyl)benzenamine* (**3**): mp 174–175 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$, ppm: 4.28 (d, $J$ = 8.0 Hz, 2H), 6.50 (d, $J$ = 7.2 Hz, 1H), 6.61 (br s, exch $D_2O$, 1H, *N*H), 6.93 (m, 1H), 7.06 (d, $J$ = 7.6 Hz, 1H), 7.22 (m, 1H), 7.28 (d, J = 7.6 Hz, 1H), 7.35 (s, 1H), 7.40 (m, 1H), 7.46 (d, $J$ = 7.2 Hz, 2H), 7.94 (s, 1H), 8.55 (d, $J$ = 7.2 Hz, 2H), 8.73 (d, J = 7.2 Hz, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$, ppm: 45.8, 109.6, 113.7, 118.0, 124.3, 125.6, 127.9, 128.2, 128.5, 129.4, 129.8, 132.1, 134.2, 134.7, 146.4, 147.4, 148.7, 150.1; ESI MS (M+1): 363, (M-1): 361. Elemental analysis: Calcd for $C_{20}H_{16}ClN_5$: C, 66.39; H, 4.46; N, 19.36. Found: C, 66.17; H, 4.58, N, 19.13.
*N-(Pyridin-4-ylmethyl)-2-(1-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)benzenamine* (**6**): mp 196–197 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$, ppm: 4.31 (d, $J$ = 8.0 Hz, 2H), 6.48 (d, $J$ = 7.2 Hz, 1H), 6.58 (br s, exch $D_2O$, 1H, *N*H), 6.94 (m, 1H), 7.21 (m, 1H), 7.29 (d, $J$ = 8.0 Hz, 1H), 7.33 (s, 1H), 7.36–7.41 (m, 4H), 7.97 (s, 1H), 8.56 (d, $J$ = 7.6 Hz, 2H), 8.78 (d, $J$ = 7.2 Hz, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$, ppm: 46.1, 109.4, 117.9, 120.1, 124.0, 124.3, 125.3, 127.8, 128.6, 128.9, 129.1, 129.7, 130.7, 131.5, 133.4, 146.0, 147.9, 148.5, 150.1; ESI MS (M+1): 396, (M-1): 394. Elemental analysis: Calcd for $C_{21}H_{16}F_3N_5$: C, 63.79; H, 4.08; N, 17.71. Found: C, 63.52; H, 3.87, N, 17.49.2-*(1-(3-Chlorophenyl)-1H-1,2,3-triazol-4-yl)-N-(quinolin-4-ylmethyl)benzenamine* (**26**): mp 231–233 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$, ppm: 4.31 (d, $J$ = 7.6 Hz, 2H), 6.49 (d, $J$ = 7.2 Hz, 1H), 6.65 (br s, exch $D_2O$, 1H, *N*H), 6.91 (m, 1H), 7.03 (d, $J$ = 7.6 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.18 (m, 1H), 7.28–7.30 (m, 2H), 7.42 (m, 1H), 7.54 (m, 1H), 7.68 (m, 1H), 7.98 (d, $J$ = 7.6 Hz, 1H), 8.07 (s, 1H), 8.21 (d, $J$ = 7.2 Hz, 1H), 8.71 (d, $J$ = 7.2 Hz, 1H), 8.91 (d, $J$ = 7.2 Hz, 1H); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$, ppm: 44.5, 109.4, 113.8, 118.1, 121.8, 124.3, 125.2, 126.2, 126.9, 127.5, 128.3, 128.9, 128.3,129.1, 129.3, 129.8, 130.2, 130.6, 132.3, 134.2, 146.4, 146.6, 149.1, 150.8; ESI MS (M+1): 413, (M-1): 411; Elemental analysis: Calcd for $C_{24}H_{18}ClN_5$: C, 69.98; H, 4.40; N, 17.00. Found: C, 69.76; H, 4.28; N, 16.81.
*N-((1H-imidazol-4-yl)methyl)-2-(1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl)benzenamine* (**28**): mp 163–164 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$, ppm: 4.30 (d, $J$ = 7.6 Hz, 2H), 6.51 (d, $J$ = 7.2 Hz, 1H), 6.59 (br s, exch $D_2O$, 1H, *N*H), 6.77 (s, 1H), 6.90 (m, 1H), 7.08 (d, $J$ = 7.6 Hz, 1H), 7.21 (m, 1H), 7.29 (d, $J$ = 7.6 Hz, 1H), 7.32 (s, 1H), 7.43 (m, 1H), 7.48 (s, 1H), 8.12 (s, 1H), 8.73 (d, $J$ = 7.2 Hz, 1H), 12.9 (br s, exch $D_2O$, 1H, *N*H imidazole); $^{13}C$ NMR (100 MHz, DMSO-$d_6$) $\delta$, ppm: 43.9,

109.2, 113.6, 118.2, 120.2, 125.1, 127.6, 127.9, 128.5, 129.7, 130.2, 131.6, 132.2, 135.6, 134.4, 146.7, 148.1, 148.7; ESI MS (M+1): 352, (M-1): 350. Elemental analysis: Calcd for $C_{18}H_{15}ClN_6$: C, 61.63; H, 4.31; N, 23.96. Found: C, 61.38; H, 4.12, N, 23.69.

*N-(3-Chlorophenyl)-5-(pyridin-4-ylmethylamino)-1H-1,2,3-triazole-4-carboxamide* (**31**): mp 181–183 °C (decomp.); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$, ppm: 4.31 (d, $J$ = 8.0 Hz, 2H), 6.78 (br s, exch $D_2O$, 1H, *N*H), 7.13 (d, $J$ = 7.6 Hz, 1H), 7.37 (m, 1H), 7.45 (d, $J$ = 7.2 Hz, 2H), 7.78 (d, $J$ = 7.6 Hz, 1H), 7.98 (s, 1H), 8.65 (d, $J$ = 7.2 Hz, 2H), 10.20 (br s, exch $D_2O$, 1H, *N*H), 11.8 (s, exch $D_2O$, 1H, *N*H triazole); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$, ppm: 45.8, 119.6, 121.7, 124.4, 124.6, 124.9, 130.2, 133.9, 137.1, 142.9, 147.5, 150.2, 163.2; ESI MS (M + 1): 330, (M-1): 328; Elemental analysis: Calcd for $C_{15}H_{13}ClN_6O$: C, 54.80; H, 3.99; N, 25.56. Found: C, 54.59; H, 3.82, N, 25.38.

22. VEGFR tyrosine kinase inhibition is determined by measuring the phosphorylation level of poly-Glu-Ala-Tyr-biotin (pGAT-biotin) peptide in a Homogenous Time-Resolved Fluorescence (HTRF) assay. Into a black 96-well Costar plate is added 2 μl/well of 25X 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, 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 of 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, CIS bio international) is added and after an additional 2 h incubation at room temperature, the plate is read in a RUBYstar HTRF Reader.
23. Cell-based assay for VEGFR-2 inhibition:
(i) *Transfection of 293 cells with DNA expressing FGFR1/VEGFR-2 chimera*: A chimeric construct containing the extracellular portion of FGFR1 and the intracellular portion of VEGFR-2 was transiently transfected into 293 adenovirus-transfected kidney cells. DNA for transfection was diluted to a 5 μg/ml final concentration in a serum-free medium and incubated at room temperature for 30 min with 40 μl/mL of Lipofectamine 2000, also in serum-free media. 250 μl of the Lipofectamine/DNA mixture was added to 293 cells suspended at $5 \times 10^5$ cells/ml. Suspension (200 μl/well) was added to a 96-well plate and incubated overnight. Within 24 h, media was removed and 100 μl of media with 10% fetal bovine serum was added to the now adherent cells followed by an additional 24 h incubation. Test compounds were added to the individual wells (final DMSO concentration was 0.1%). Cells were lysed by re-suspension 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.
(ii) *ELISA for detection of tyrosine-phosphorylated chimeric receptor*: 96-well ELISA plates were coated using 100 μl/well of 10 μg/ml of αFGFR1 antibody, and incubated overnight at 4 °C. αFGFR1 was prepared in a buffer made with 16 ml of a 0.2 M $Na_2CO_3$ and 34 ml of a 0.2 M $NaHCO_3$ with pH adjusted to 9.6. Concurrent with lysis of the transfected cells, αFGFR1 coated ELISA plates were washed three times with PBS + 0.1% Tween 20, blocked by addition of 200 μl/well of a 3% BSA in PBS for 1 h and washed again. Eighty microliters lysate were then transferred to the coated and blocked wells and incubated for 1 h at 4 °C. The plates were washed three times with PBS + 0.1% Tween 20. To detect bound phosphorylated chimeric receptor, 100 μl/well of anti-phosphotyrosine antibodies (RC20:HRP, Transduction Laboratories) were added (final concentration 0.5 μg/ml in PBS) and incubated for 1 h. The plates were washed six times with PBS + 0.1% Tween 20. Enzymatic activity of HRP was detected by adding 50 μl/well of equal amounts of the Kirkegaard & Perry Laboratories (KPL) Substrate A and Substrate B. The reaction was stopped by addition of 50 μl/well of a 0.1 N $H_2SO_4$, absorbance was measured at 450 nm.