Comparative Biochemical Kinase Activity Analysis Identifies Rivoceranib as a Highly Selective VEGFR-2 Inhibitor

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BACKGROUND

- Vascular endothelial growth factor receptor 2 (VEGFR-2) is a key regulator of tumor angiogenesis that is highly expressed in several tumor types and is a known target for anti-cancer therapy.[1-3]
- The clinical use of VEGFR-2 inhibitors has been challenged by limited efficacy and various side effects, potentially due to the low selectivity of these tyrosine kinase inhibitors (TKIs) for VEGFR-2.[4-6] Thus, potent VEGFR-2 inhibitors with improved selectivity are needed.
- Rivoceranib is an oral tyrosine kinase inhibitor (TKI) that potently and selectively inhibits VEGFR-2.[7,8]
- A comparison of the potency and selectivity of VEGFR-2 inhibitors can provide a rationale for selecting a specific TKI for anticancer therapy in the clinic.
- Here, we performed head-to-head biochemical analyses of rivoceranib and 10 FDA-approved TKIs with known activity against VEGFR-2. We compared their activity against a panel of 270 kinases. This comparison serves as a potential basis for clinical decision-making.

METHODS

BINDING ASSAY

- A panel of 10 FDA-approved kinase inhibitors with known activity against VEGFR-2 ("reference inhibitors") plus the investigational VEGFR-2 inhibitor rivoceranib were evaluated in this study (**Table 1**).
- The affinity of rivoceranib for VEGFR-2 was determined by surface plasmon resonance (SPR) using biotinylated VEGFR-2 and Biacore T200.[9]
- Biotinylated recombinant cytoplasmic domain of VEGFR-2, encompassing amino acid residue 790 to 1356 (C-terminus), was purchased from Carna Biosciences, Inc.
- The same cytoplasmic domain fragment of VEGFR-2 was used to determine the inhibitory potency (IC_{50}) of rivoceranib in an enzyme activity assay using a mobility shift assay (MSA).

Table 1: Small Molecule Kinase Inhibitors with Activity Against VEGFR-2 Included in the Current Study

Inhibitor	
Axitinib	Sorafenib
Cabozantinib	Sunitinib
Lenvatinib	Tivozanib
Nintedanib	Vandetanib
Pazopanib	Regorafenib
Rivoceranib	

ENZYME ACTIVITY ASSAYS

- To compare the selectivity of rivoceranib and the 10 reference inhibitors, we performed either IMAP assays or off-chip MSA to assess the residual kinase activity of a panel of 270 kinases in the presence of 160 nM or 1600 nM rivoceranib or 1000 nM of reference inhibitor.
- For VEGFR-2, the same cytoplasmic domain was used as in the SPR binding assay. For dose-response and determination of the half-maximum inhibitory concentration (IC_{50}), rivoceranib was tested in duplicate 10-point dilution series.
- For the 10 reference inhibitors, the VEGFR-2 IC_{50} values and percent inhibition at 1000 nM were previously determined in identical assays.[10,11]

KINOME TREE BIOCHEMICAL SELECTIVITY

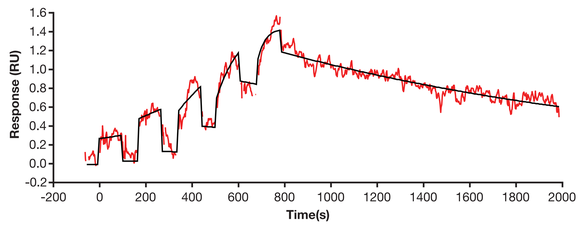
- To further understand the selectivity of rivoceranib compared to the reference inhibitors on an individual kinase basis, we generated kinome trees displaying the percent inhibition of each kinase in the presence of each inhibitor. These percent inhibition values were grouped into four categories (> 95%; > 90% & ≤95%; > 50% & ≤ 90%; and ≤ 50%) and mapped to corresponding nodes on the kinome tree.

RESULTS

Rivoceranib Binds to VEGFR-2 with Low Nanomolar Affinity

- **Figure 1** shows a representative sensorgram of rivoceranib binding to VEGFR-2. The resulting sensorgram shows the rate of formation of a target-compound complex and the rate of its subsequent dissociation. The K_D was calculated from the association rate (k_a) and dissociation rate constant (k_d). Based on four replicates, a K_D of 2.08 nM was determined.

Figure 1: Binding Kinetics of Rivoceranib to VEGFR-2

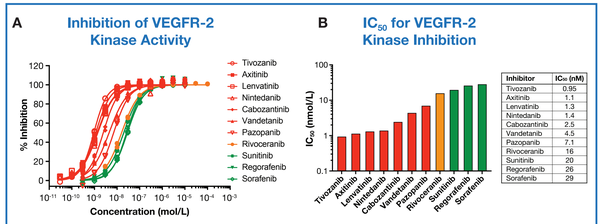


Representative sensorgram of a single-cycle kinetics experiment with rivoceranib and the cytoplasmic domain of VEGFR-2. The red line corresponds to the actual response and the black line to the response after fitting with a 1:1 model using Biacore T200 software. Binding of a compound is measured as a positive signal expressed in resonance units (RU). Dissociation of the compound results in a decrease of RU.

VEGFR-2 Kinase Inhibition of Rivoceranib is Comparable with Reference Inhibitors

- While some variation in potency of VEGFR-2 kinase activity inhibition was observed among compounds tested, rivoceranib activity against VEGFR-2 was within the range of the inhibition mediated by the reference inhibitors (**Figure 2A**).
- IC_{50} values calculated based on the concentration-percent inhibition curves ranged from 0.95 nM to 29 nM for the reference inhibitors, and the IC_{50} value for rivoceranib was 16 nM (**Figure 2B**).

Figure 2: Potency of Rivoceranib-Mediated VEGFR-2 Kinase Inhibition in Comparison with Reference Inhibitors



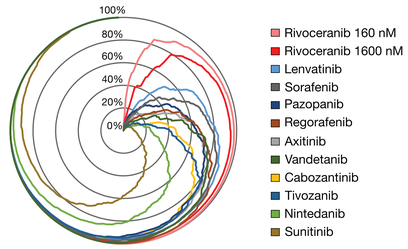
Inhibitor	IC_{50} (nM)
Tivozanib	0.95
Axitinib	1.1
Lenvatinib	1.3
Nintedanib	1.4
Cabozantinib	2.5
Vandetanib	4.5
Pazopanib	7.1
Rivoceranib	16
Sunitinib	20
Regorafenib	26
Sorafenib	29

Off-shift mobility shift assays (MSA) were performed to measure VEGFR-2 kinase activity in the presence of increasing concentrations of rivoceranib and 10 FDA-approved inhibitors. (**A**) Dose-response curves for extent of inhibition of VEGFR-2 kinase activity mediated by each inhibitor. Rivoceranib displayed in orange: inhibitors with greater potency displayed in red, and those with less potency displayed in green. (**B**) IC_{50} values obtained from dose-response curves of VEGFR-2 kinase inhibition mediated by each inhibitor. Inhibitors were ordered from most potent to least potent.

Rivoceranib Retains Greater Overall Activity of Non-targeted Kinases Compared with Reference Inhibitors

- Substantial differences in overall residual kinase activity were observed across the panel of 270 kinases among the 11 inhibitors. Among all inhibitors profiled, rivoceranib demonstrated the greatest residual kinase activity (at both 160 nM and 1600 nM) across the panel of kinases (**Figure 3**). Radar chart visualization of these results demonstrates substantial differences in the overall residual activity across the panel of kinases between inhibitors.

Figure 3: Residual Kinase Activity



The activity of 270 kinases was measured in the presence of a fixed concentration of each inhibitor (160 nM and 1600 nM rivoceranib, and 1000 nM for all reference inhibitors), and the percent inhibition values were calculated for each kinase + inhibitor pair. Residual kinase activity values were calculated as 100 - % inhibition and visualized via radar chart.

Rivoceranib is the Most Selective Inhibitor of VEGFR-2 Kinase Activity Among the Tested Inhibitors

- Rivoceranib demonstrated >95% inhibition of VEGFR-2 at 160 nM and 1600 nM, with 54.7% to 99.5% inhibition of only 5 kinases (i.e., FLT1, FLT4, Ret, PDGFRβ, and Lyn) detected at both rivoceranib concentrations (**Figure 4**).
- Compared with rivoceranib, all reference inhibitors tested demonstrated activity against a broader array of kinases (**Figure 5**).

Figure 4: Rivoceranib-Mediated Inhibition Across Kinome Tree at 10-fold Dose Difference

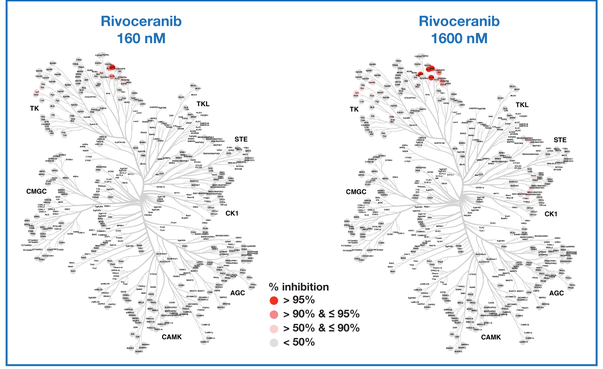
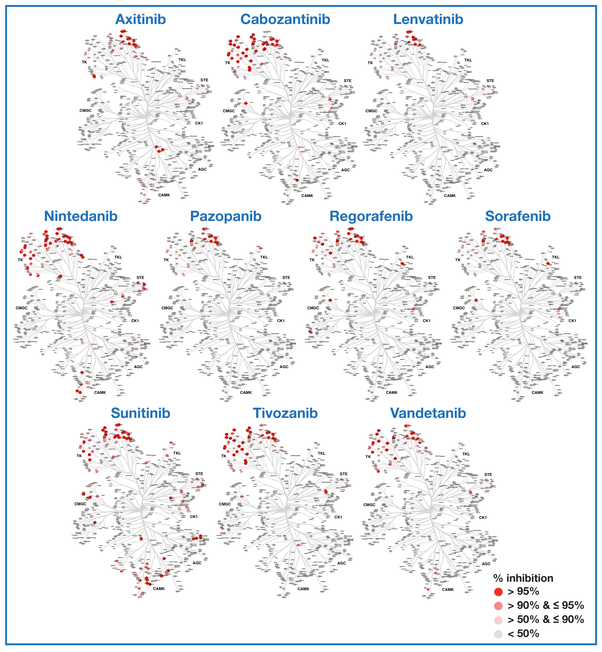


Figure 5: Comparison of Selectivity of All 10 FDA-Approved Inhibitors Included in this Study



CONCLUSIONS

- Rivoceranib was identified as the most selective inhibitor of VEGFR-2 in our analysis of the inhibitory profiles of rivoceranib and 10 reference inhibitors against a panel of 270 known kinases.
- Differences in selectivity among compounds within a similar range of VEGFR-2 kinase inhibition potency are clinically relevant, as toxicities associated with available VEGFR-2 inhibitors are thought to be due in part to their inhibitory effects against kinases outside of the VEGFR family.[12]
- With the increased selectivity seen with rivoceranib, more effective targeting of VEGFR-2 may be achieved due to an ability to deliver higher therapeutic doses with fewer off-target toxicities compared to other TKIs.
- This increased ability to reach higher drug concentrations would potentially result in greater anti-tumor efficacy as well as a capacity to achieve adequate concentrations of the drug in sites with limited drug penetration, such as brain metastases.[13]
- Furthermore, improved VEGFR-2 targeting has implications for combination therapy approaches, particularly for settings in which the toxicities associated with other TKIs have limited dosing or delivery of the agents.
- Rivoceranib, as the most selective inhibitor of VEGFR-2, represents an attractive option for improved VEGFR-2 targeting in cancer.

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