

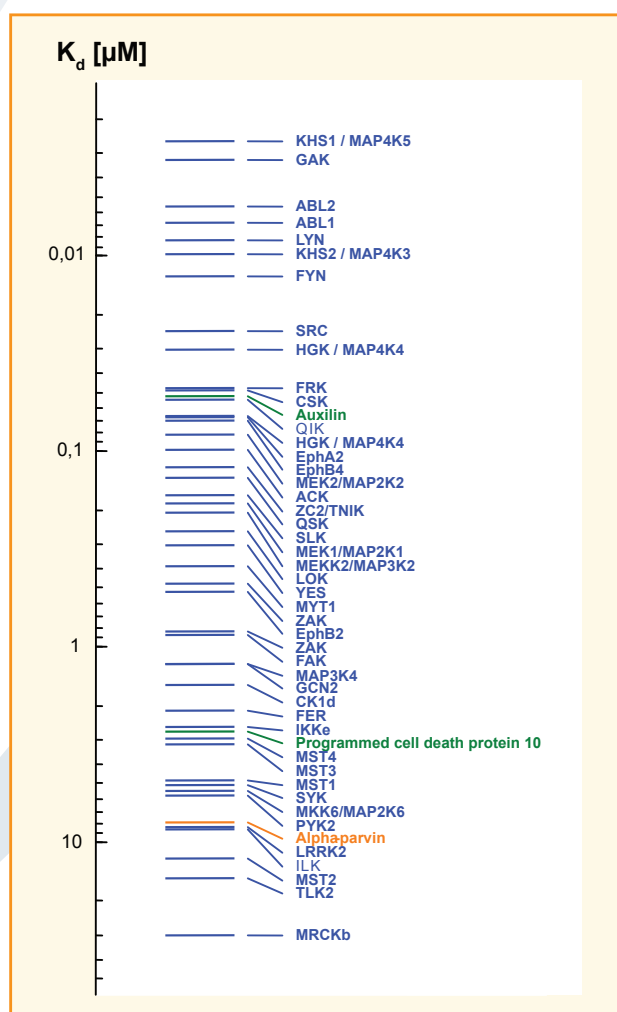
## KinAffinity® facilitates rapid and effective profiling of kinase inhibitors in cells or tissue

Numerous small molecule kinase inhibitors are currently being developed as promising candidates in drug discovery programs aimed at treating devastating diseases such as cancer, inflammation, autoimmune diseases or neurological disorders. However, drug development approaches face significant challenges, including unacceptable safety profiles due to poor inhibitor selectivity.

To address this, KINAXO's **KinAffinity®** technology meets important requirements for thorough, reliable selectivity analysis under physiological conditions, since it reveals a kinase inhibitor's quantitative target profile from a cell's or tissue's endogenously expressed kinome.

In the present study, we employed **KinAffinity®** to identify and quantitatively measure the protein target interactions of the multi-specific kinase inhibitor Bosutinib (SKI-606), as well as the marketed class II inhibitors Sorafenib (Nexavar®) and Imatinib (Gleevec®). This not only provided comprehensive insights into the compounds' selectivity profiles, but also impressively demonstrated how **KinAffinity®** can be applied to a broad range of kinase inhibitors with different binding modes.

## KinAffinity® reveals the full target spectrum of Bosutinib (SKI-606)



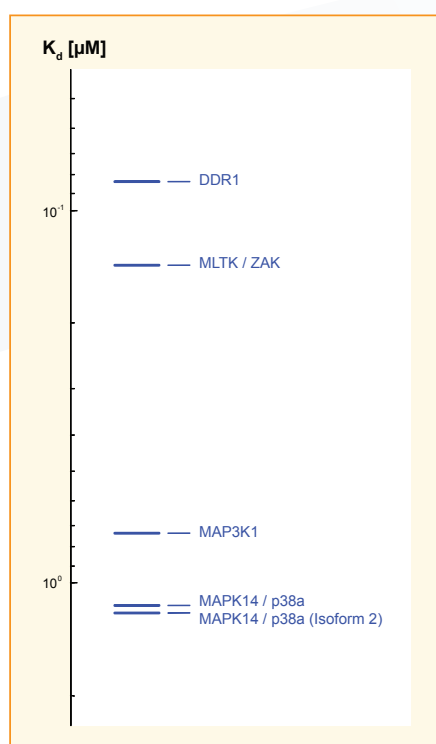
The target spectrum identified by **KinAffinity®** for Bosutinib in human prostate cancer cells (PC3) was highly consistent with published data, proving that **KinAffinity®** is a reliable technology for investigating a kinase inhibitor's target profile. To screen Bosutinib against the expressed kinome, **KinAffinity®** was employed to enrich the cellular kinases before performing competition assays using the free inhibitor. Quantitative mass spectrometric analysis then revealed the compound's complete target profile.

### Target Profile of Bosutinib in PC3 cells

Of 197 protein kinases enriched by **KinAffinity®** from PC3 cells, 45 were identified to be targeted by Bosutinib with a  $K_{d,free}$  value of up to 30  $\mu\text{M}$ . Depicted are kinase proteins with an affinity to Bosutinib of 10  $\mu\text{M}$  or better.

## KinAffinity® reveals the target spectrum of class II kinase inhibitors Sorafenib (Nexavar®) and Imatinib (Gleevec®)

Profiling of two approved drugs proved that **KinAffinity®** can be applied to determine the cellular selectivity of type II inhibitors that target the inactive kinase conformation. Today, many pharmaceutical and biotechnology companies are pursuing the development of type II inhibitors, since these inhibitors often exhibit distinct specificity for their targets with slow  $k_{off}$  rates. In traditional enzymatic assays, however, type II inhibitors are often overlooked due to their low affinity for active kinase targets.



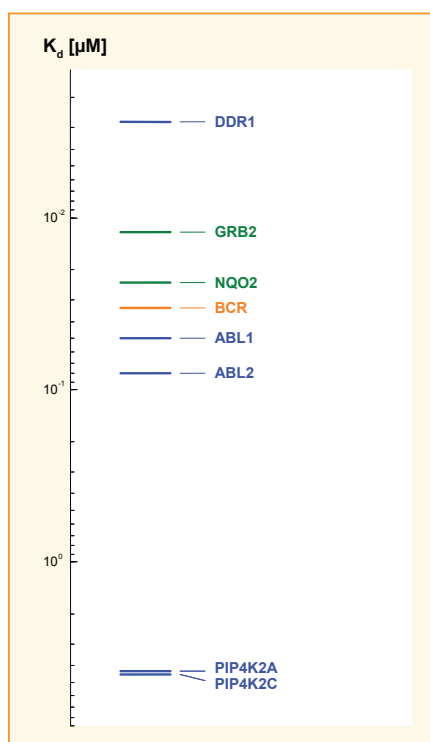
### Target profile of Sorafenib in PC3 cells

PC3 cells are sensitive to Sorafenib treatment, although the drug's clinical efficacy cannot be explained by inhibition of its reported main targets VEGFR, PDGFR- $\beta$ , or BRAF.

**KinAffinity®** reveals Sorafenib's quantitative kinase target spectrum in PC3 cells. The pro-apoptotic protein kinase MLTK, DDR1 and DDR2, as well as MAPK14/p38 $\alpha$ , a known suppressor of cell proliferation and tumorigenesis, are shown to bind Sorafenib with high affinities. These interactions shed some light on the inhibitor's cellular mode of action and might help in understanding its effect in PC3 cells.

### Target profile of Imatinib in K562 cells

Profiling in human chronic myelogenous leukemia (K562) cells revealed the inhibitor's quantitative target spectrum. Known Imatinib targets like DDR1, Abl2, and the BCR-Abl1 fusion protein constitutively expressed in K562 cells were confirmed by **KinAffinity®**. Other known Imatinib target proteins such as PDGFR were not expressed in these cells.



### References

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