CRENOLANIB, A NOVEL TYPE I, MUTANT-SPECIFIC INHIBITOR OF CLASS III RECEPTOR TYROSINE KINASES, PREFERENTIALLY BINDS TO PHOSPHORYLATED KINASES

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CRENOLANIB BESYLATE (CP-868,596-26)

- Benzimidazole tyrosine kinase inhibitor (TKI) known to preferentially bind to class III receptor tyrosine kinases (RTKs) at nanomolar concentrations (Table 1)
- Has been evaluated in phase I[1] and phase Ib[2] trials in solid tumors and is currently being evaluated in a phase II trial in adult glioma, a phase II trial in gastrointestinal stromal tumors and a phase I trial in pediatric glioma.

RTK	Kd (nM)
FLT3	0.74
PDGFRB	2.1
PDGFRA	3.2
CFS1R	30
KIT	78

Table 1. Binding constants of crenolanib for the class III RTKs PDGFR, FLT3, KIT and CFS1R[3]. Crenolanib has nanomolar potency for these wild type kinases.

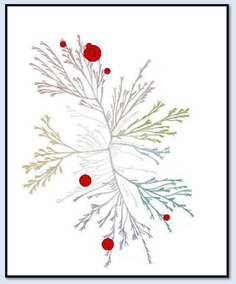


Figure 1. Selectivity profile of crenolanib. Crenolanib is highly specific for class III RTKs.[5]

COMPETITION BINDING ASSAY

The effect of ABL1 A-loop phosphorylation on crenolanib affinity was investigated in a competition binding assay. The relative affinities of TKIs for phosphorylated and non-phosphorylated ABL1 have been previously used to accurately distinguish type I from type II inhibitors. The KINOME*scan* assay combined a DNA-tagged kinase and an immobilized ligand with crenolanib. Crenolanib's ability to compete with the immobilized ligand was measured via quantitative PCR of the DNA tag (see Figure 1).[5]

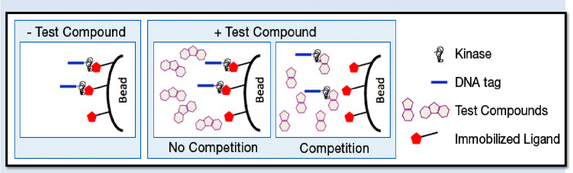


Figure 2. Mechanism of the KINOME*scan* competition binding assay. The assay combines a DNA-tagged kinase, an immobilized ligand and a test compound to determine the binding constant of the test compound for the kinase.[5]

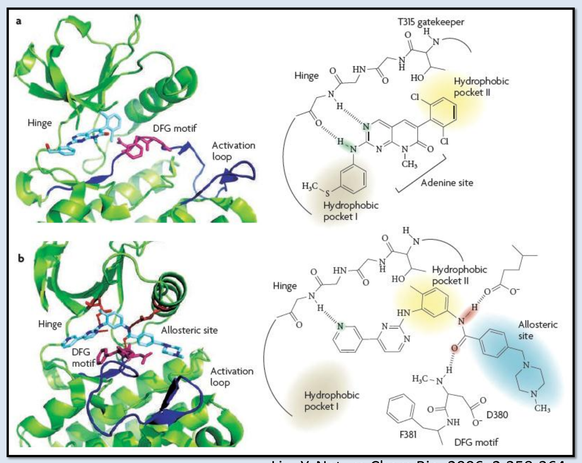
The binding constants (Kds) of crenolanib for FLT3 (wild-type and five mutant isoforms), PDGFRA, PDGFRB and KIT (wild-type and two mutant isoforms) were also determined using the KINOME*scan* KdELECT Assay (DiscoveRx, San Diego, CA).

TKIs CAN BE CLASSIFIED AS TYPE I OR TYPE II

Depending on the mechanism of binding to the kinase, tyrosine kinase inhibitors can be classified as type I or type II inhibitors. The characteristics of each type of inhibitor are listed in Table 2, below. A schematic of the kinase inhibitor binding modes is given in Figure 2, below. Resistant mutations to imatinib, sunitinib, quizartinib, and sorafenib are mutations that render the kinase domain of the RTK constitutively phosphorylated. Inhibitors that target the phosphorylated kinase thus may have potential use in treating diseases that harbor these mutations.

	Type I	Type II
Binding location	In and around ATP binding region	Hydrophobic site adjacent to ATP binding pocket
DFG conformation	DFG-out is not required	DFG-out is required
Phosphorylation	Bind to phosphorylated kinases	Bind to non-phosphorylated kinases
TKIs	Crenolanib, PIK-39	Imatinib, sorafenib, nilotinib

Table 2. Type I and type II TKIs differ in the conformation of the kinase they prefer to bind. Type I TKIs preferentially bind to phosphorylated "active" kinases while type II TKIs preferentially bind to non-phosphorylated "inactive" kinases.[4,6,7]



Liu, Y. Nature Chem Bio, 2006. 2:358-364

Figure 3. Type I TKIs (shown here as PD166326) prefer to bind to kinases in the active conformation (a) with the DFG motif in. Type II TKIs (shown here as imatinib) prefer to bind to kinases in inactive conformation (b) with the DFG motif out.

CRENOLANIB HAS A GREATER AFFINITY FOR PHOSPHORYLATED KINASES

Kd of crenolanib for ABL1 and ABL(T315I)

Analysis of crenolanib's affinity for kinases ABL1 and ABL(T315I) demonstrated that the molecule exhibits the characteristic mechanism of a type I inhibitor. Crenolanib's binding constants for phosphorylated ABL1 and ABL(T315I) were 7- and 15-fold lower than its binding constants for non-phosphorylated ABL1 and ABL(T315I), respectively. Though crenolanib is not active against ABL, the molecule's significantly greater affinity for the phosphorylated kinase suggests that crenolanib may be a type I TKI.

Kinase Target	CP-868,596-26 (benzenesulfonate salt)
KINOMEscan Gene Symbol	Kd (nM)
ABL1(T315I)-nonphosphorylated	12000
ABL1(T315I)-phosphorylated	760
ABL1-nonphosphorylated	600
ABL1-phosphorylated	88

Table 3. The Kd of crenolanib for phosphorylated ABL1 is 7-fold lower than the Kd for non-phosphorylated ABL1. The Kd of crenolanib for phosphorylated ABL(T315I) is 15-fold lower than the Kd for non-phosphorylated ABL(T315I).[5]

Affinity of crenolanib for FLT3

Crenolanib's difference in binding affinities for the non-autoinhibited and autoinhibited states of FLT3 also indicate that the molecule functions as a type I inhibitor. As is shown in Figure 2, crenolanib has a Kd of 0.61nM for non-autoinhibited FLT3 and a Kd of 6.7nM for autoinhibited FLT3. Crenolanib thus has an approximately 10-fold affinity shift between the non-autoinhibited and autoinhibited states of FLT3. This value is within the range of affinity shifts reported for other type I TKIs and is far outside the range of 100- to 1000-fold affinity shifts reported for type II TKIs.

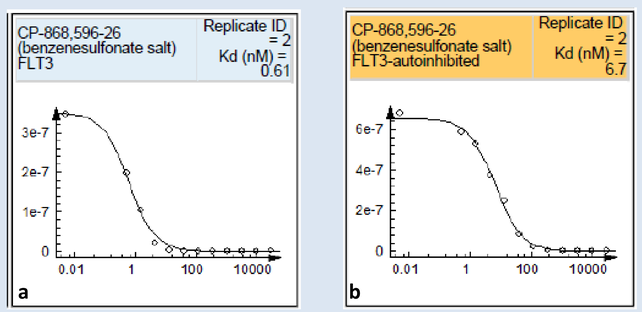


Figure 4. Crenolanib has a 10-fold affinity shift between the non-autoinhibited (a) and autoinhibited (b) states of FLT3.[5]

CRENOLANIB IS ACTIVE AGAINST CONSTITUTIVELY ACTIVE MUTATIONS

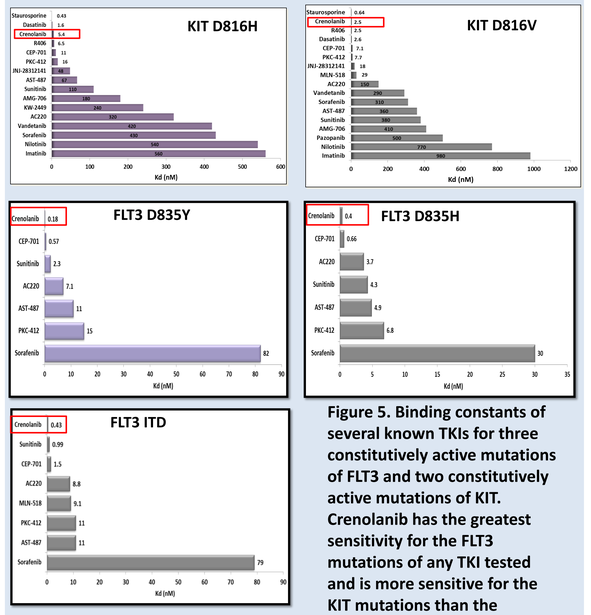


Figure 5. Binding constants of several known TKIs for three constitutively active mutations of FLT3 and two constitutively active mutations of KIT. Crenolanib has the greatest sensitivity for the FLT3 mutations of any TKI tested and is more sensitive for the KIT mutations than the majority of the TKIs tested. [4,5]

Crenolanib has high activity against constitutively active mutations in class III RTKs and is more active against these mutations than other known TKIs.

Table 4. Binding constants of crenolanib for constitutively active mutations in the class III RTKs FLT3 and KIT. Crenolanib has nanomolar potency for these mutated kinases.[5]

RTK Mutation	Kd (nM)
FLT3 ITD	0.43
FLT3 D835Y	0.18
FLT3 D835H	0.4
KIT D816H	5.4
KIT D816V	2.5

CONCLUSIONS

- Crenolanib is a unique chemotype, mutant-specific type I TKI
- Crenolanib's greater affinity for phosphorylated receptors makes it an ideal candidate for targeting indications that are driven by constitutively-active mutations of class III receptors

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