Validation of novel binding interaction for tyrosine kinases via crystal structures of low nM Src inhibitors

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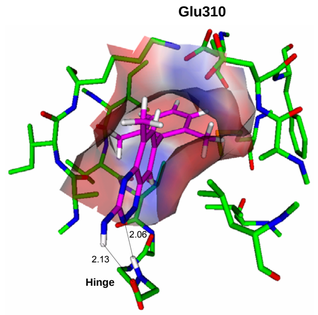
Introduction

- Src is the prototype member of the Src-family of tyrosine kinases, which comprises the highly homologous proteins Src, Yes, Fyn, Hck, Blk, Brk, Fgr, Frk, Srm, Yrk, and Lck.
- Src has been considered a therapeutic target for cancer, diabetes, rheumatoid arthritis, autoimmune diseases, stroke, myocardial infarction, osteoporosis and numerous diseases of the eye.
- Gene knockout experiments and clinical data suggest that inhibition of some members of the Src family might have potential therapeutic benefit.
- We report here a uniquely designed series based on molecular modeling that utilizes a hitherto unexploited interaction with the Glu310 residue deep within the hydrophobic pocket of active Src.
- Such Glu residues, as in the case of the Src Glu310, are part of the activation mechanisms of kinases.
- In this poster we present the selective targeting of the Glu310 residue of active Src through the construction of a model of activated Src and the design of a novel inhibitor series to interact with Glu310, along with validation of the approach via x-ray crystallography.

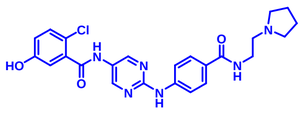
Figure 1: The hydrophobic pocket of Src showing the position of the Glu310 residue



After constructing the model of activated Src, we asked ourselves a simple structure based design question – Would we be able to selectively exploit the activation mechanism and interact with the Glu310 residue in Src by utilizing a rationally designed donor group?

- Interactions with this residue are relatively unexploited. Thus interaction with this Glu residue would provide a very unique method to selectively target kinases in the activated form, and kinases that are constitutively activated since this might have implications for targeting certain disease states.
- Most inhibitor series that target Src utilize small hydrophobic moieties in the hydrophobic pocket – chloro- and methyl- substituted phenyl groups are most commonly used.
- Targeting the active conformation indicates that the molecule would not have an interaction with Glu310 in the inactive conformation. The Glu on the C-helix would not be properly positioned for such an interaction.
- Glu310 is a residue on the C-helix that moves in to interact with Lys295 on activation in members of the Src family.
- We have designed and synthesized a novel series of molecules precisely to target this Glu310 in active Src, and obtained a crystal structure with active Src to validate this binding mode and specifically demonstrate the interaction with this residue.

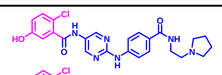
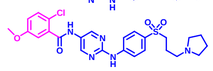
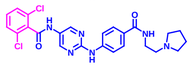
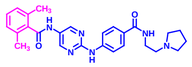
Figure 2: A representative of the series



Compound 1

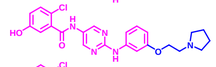
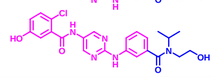
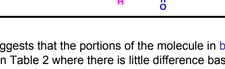
Src IC_{50} 7nM

Table 1: SAR

#	Structure	Src IC_{50} (nM)
1		7
2		10,000
3		60
4		117

- Replacing methoxy with hydroxy gives a one thousand fold more potent inhibitor (compound **2** vs. **1**).
- Changing from 2-chloro-5-hydroxyl to 2,6-dichloro (**3**) or 2,6-dimethyls (**4**) drops the activity ten to twenty-fold. The limited SAR provided here highlights the interaction with the glutamic acid.
- Simply increasing hydrophobic contacts alone did not bring the activity to the level of a donor interaction in the back of the pocket.

Table 2: SAR continued

#	Structure	Src IC_{50} (nM)
5		10
6		21
7		27
8		21
9		19

- The data suggests that the portions of the molecule in blue are solvent exposed as seen in the SAR in Table 2 where there is little difference based on the solublizing group.
- To confirm the binding mode predicted by the modeling, and specifically to validate the glutamic acid interaction, compound **1** was co-crystallized with active Src.

Figure 3: Crystal structure of compound 1 with active Src

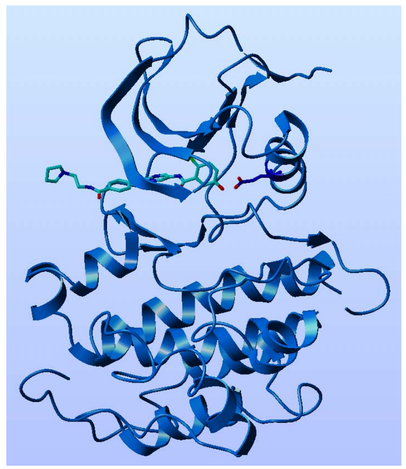
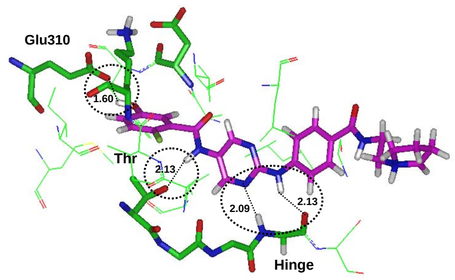


Figure 4: Key interactions in the active Src crystal structure with compound 1



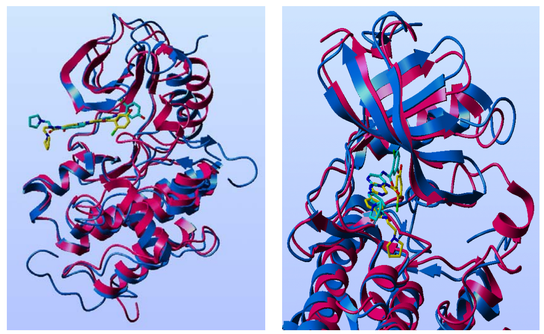
- Three main sets of interactions shown: Glu 310-Phenol (1.60 Å); gatekeeper Thr–NH (2.13 Å) and hinge donor acceptor interactions (2.09 Å and 2.13 Å).
- The crystal structure and the SAR obtained with active Src validate the design strategy of using a donor deep within the hydrophobic pocket to target the Glu310.
- The Glu310 is on the C-helix that moves in toward the hydrophobic pocket of Src on activation.

To further validate this design strategy of selectively targeting active Src, we also obtained a crystal structure with an inactive Src family member.

Shown below are overlapped crystals structures of the same inhibitor, compound **1**, with active Src and inactive Hck illustrating the key differences in interactions.

Figure 5: Overlapped crystal structure of active Src & inactive Hck with compound 1

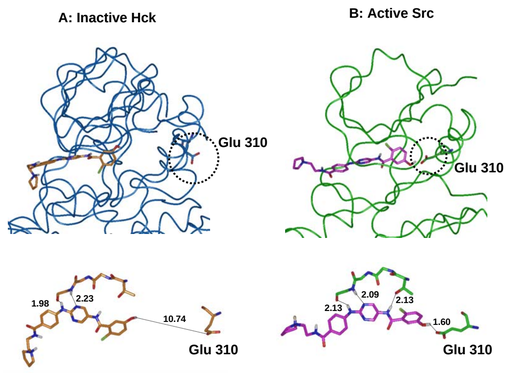
(Blue = Active Src; Red = Inactive Hck)



View A **View B**

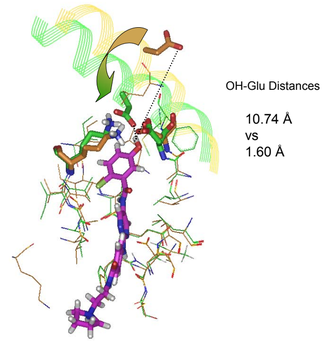
The crystal structure of compound **1** in both active and inactive kinases shows that the compound is bound in the ATP pocket in both cases.

Figure 6: More detailed views of compound 1 co-crystal structures in the Src kinases



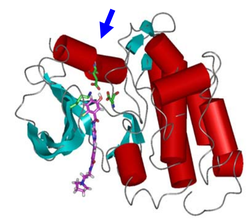
- Glu310 position shown (circles) – Detailed view of the key residues shown below the full structures.
- The donor (phenol) makes a strong hydrogen bond (1.53 Å) with the Glu310 only in the active kinase validating the design strategy.

Figure 7: C-helix movement from inactive to active crystal structures



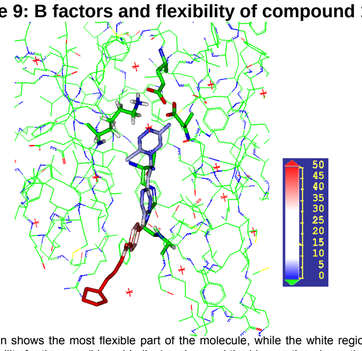
This shows the movement of Glu310 from another angle. The distance of Glu310 to the phenol OH on the molecule is 10.74 Å in inactive vs. 1.60 Å in active structures.

Figure 8: Kink at Glu310 in the activate Src co-crystal structure with compound 1



Detailed inspection of the C-helix reveals a kink precisely at the Glu310. Interestingly the interaction is sufficient enough to distort the helix, pulling the residue closer to interact with the ligand.

Figure 9: B factors and flexibility of compound 1



- The red region shows the most flexible part of the molecule, while the white region shows intermediate flexibility for the core (hinge binding) region, and the blue portion shows the regions of the molecule that demonstrate the tightest binding portions in the molecule.
- The B-factors indicate that the tightest binding is deep within the hydrophobic pocket, while the solvent exposed portion of the molecule has the highest B factors.

Highlights and Implications

- Presented here is a novel series of Src inhibitors designed to selectively target a residue (Glu310) deep within the hydrophobic pocket of Src. Low single digit nM inhibitors have been obtained.
- Compound **1** possesses a reasonably selective kinase profile (Src 0.007 μM; Yes 0.026 μM; Vegfr2 0.56 μM; EphB4 0.20 μM; Pdgfrβ 0.020 μM; and Fgfr1 20 μM) supporting the idea that although the Glu residue is conserved across kinases, different families of kinases have unique topologies, and therefore different structural requirements for inhibitors.
- A crystal structure that validates the binding mode of compound **1** in active Src has been obtained, and is presented here clearly illustrating a strong binding interaction with the targeted Glu310 residue.
- A crystal structure with inactive Src family (Hck) protein to demonstrate the binding mode in the inactive kinase has also been obtained.
- Targeting the active conformation indicates that the molecule would not have an interaction with Glu310 in the inactive conformation. Crystal structures obtained with active and inactive kinases provide evidence for this design strategy.
- Thus interaction with this residue that is part of the activation mechanism might provide a very unique method to selectively target kinases in the activated form and to target kinases that are constitutively activated, since this might have implications for targeting certain disease states.
- Directions and implications – We have adopted this approach of selective targeting as a design strategy to target other therapeutically important kinase targets with similarly untapped potential interactions deep within the hydrophobic pocket.

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