A New Binding Mode for Inhibitors of Src Reminiscent of Gleevec in Abl

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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

❖ There is significant evidence that Src is disregulated in various human tumors and increased Src activity is observed in metastatic tumors, particularly in colon and breast tumors. Src kinase has been considered a therapeutic target for cancer, diabetes, rheumatoid arthritis, autoimmune diseases, stroke and numerous diseases of the eye, *i.e.* AMD, DME, and DR

❖ Canonical binding modes for ATP-competitive inhibitors of Src have been established with a number of molecules

❖ TargeGen has designed and optimized a novel series of pyrimidines that are used to target Src by exploiting an induced pocket between the C-helix and the DFG portion of the activation loop as predicted by modeling

❖ This induced fit is reminiscent of the binding of Imatinib (Gleevec) in Abl, which shows a distinct conformation of the C-helix and the activation loop. While these structures provide clues and confirm that such a pocket may be induced, a model must be made that is not based exactly on Abl, or on activated Src, or on inactivated Hck or Src – but has the requisite features to suitably explain the data

❖ The focus in this poster will be the work carried out to obtain potent inhibitors of Src and the proposal of an alternative mode of binding not commonly found with well established ATP competitive inhibitors of Src. The design, SAR and novel binding mode will be presented

Crystal structures of Gleevec and PD 173955: Canonical binding in kinase active site

❖ Gleevec and PD 173955 show distinct binding modes in Abl with an alteration in the activation loop position

❖Abl and Src have similar ATP binding pockets

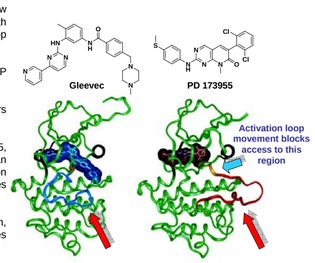
❖ All ATP-competitive inhibitors of Src are also inhibitors of Abl

❖ When bound with PD 173955, the Abl activation loop is in an extended conformation characteristic of protein kinases in a fully active state

❖ In this active site conformation, the hydrophobic pocket leaves little room for large substituents

❖ Unlike Abl, Src had shown no evidence of the capability to bind structures with altered positions of the activation loop

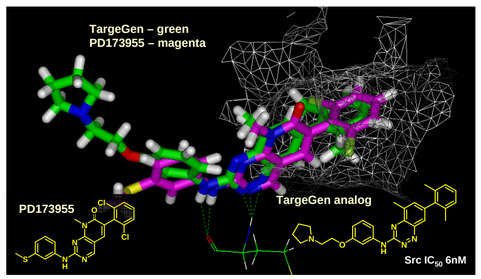
❖ The TargeGen benzotriazine inhibitors of Src and Abl are ATP competitive



Note activation loop movement from inactive to active form of kinase

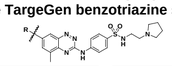
Cancer Research 62, 4236-4243, August 1, 2002

TargeGen benzotriazine series: inhibitors that bind to activated Src

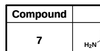
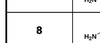
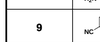
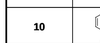
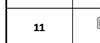


❖TargeGen benzotriazines share similar binding modes as PD 173955 in Src, and in Abl kinase as well

There is a lack of tolerance for larger groups in the hydrophobic pocket in the TargeGen benzotriazine series in Src



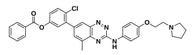
Compound	R	Src IC_{50} (nm)
1		>10,000
2		10,000
3		10,000
4		2,900
5		631
6		353

Compound	R	Src IC_{50} (nm)
7		282
8		51
9		25
10		14
11		9

❖ At the time of this work, there were no crystal structures or evidence of binding modes tolerant of large groups in the back end of the Src hydrophobic pocket

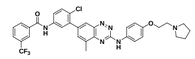
Leveraging findings into a novel TargeGen series

From benzotriazine ester…



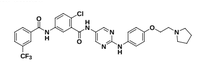
Src
>10,000 nM

…to benzotriazine amide…



Src
135 nM

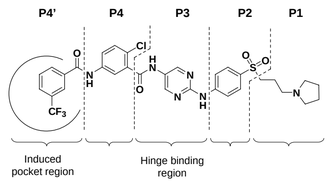
…to pyrimidine amide



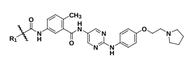
Src
1.2 nM

❖ Suggests a requisite set of interactions for obtaining Src activity

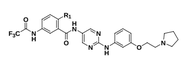
Design – P4’ extended template regions



Enzymatic data of P4’ groups



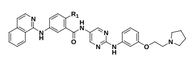
R_1	Src
	>10,000 nM
	3.3 nM



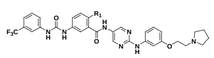
R_1	Src
Cl	63 nM
CH_3	51 nM

❖ Suggests an amide (donor and acceptor) and a hydrophobic moiety with certain spatial requirements is necessary for activity

P4’ tolerates isoquinoline and ureas…

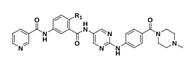


R_1	Src
CH_3	23 nM

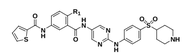


R_1	Src
CH_3	1.8 nM

…but is slightly less tolerant of pyridyl and thiophene groups

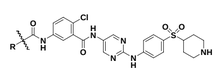


R_1	Src
Cl	93 nM



R_1	Src
Cl	38 nM

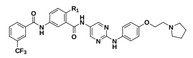
P4’ modifications: Aromatic ring sensitive to substitution



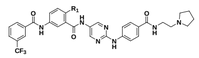
Compound	R	Src IC_{50} (nM)
13		1.8
14		4.2
15		18

Compound	R	Src IC_{50} (nM)
16		32
17		38

Enzymatic data of selected analogs: Chlorine vs. Methyl

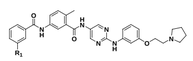


R_1	Src IC_{50} (nM)
Cl	1.2 nM
CH_3	3.3 nM

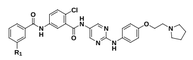


R_1	Src IC_{50} (nM)
Cl	1.4 nM
CH_3	1.7 nM

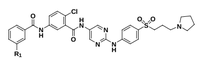
Enzymatic data of selected analogs: P4’ modifications



R_1	Src
CF_3	3.4 nM
H	17 nM



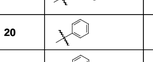
R_1	Src
CF_3	1.2 nM
F	3 nM

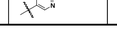


R_1	Src
CF_3	4.2 nM
CH_3	11 nM

❖ Electron withdrawing groups on P4’ ring increase activity

Enzymatic data of selected analogs: solubilizing groups not essential for potency



Compound	R_1	Src IC_{50} (nM)
18		1.2
19		2.1
20		4.5
21		2.8

SAR of P4’ analogs

1. P4’ region requires a minimum of a hydrophobic group and a donor/ acceptor interaction to maintain activity
   a. P4’ region is slightly less tolerant of heteroaromatic rings
   b. Hydrophobe generally superior to other groups in the P4’ region and much better than electron neutral or donating groups.
   c. Methyl or Chloro substituents on the P4 ring are equivalent
2. Size is well tolerated at the 5-position as evidenced by the urea and isoquinoline analogs
3. Solubilizing group may be eliminated leaving a simple phenyl ring and maintain activity

TargeGen analog in Src: A model to explain the data

❖ We proposed a model that utilizes a C-helix movement and an activation loop with a “DGF-out” conformation to explain our data

❖ Apparent induced pocket accommodates hydrophobic P4’ head group (arrow)

❖ This binding mode very reminiscent of Gleevec bound to Abl

Crystal structure of Gleevec in Abl kinase domain with docked TargeGen analog

❖ Gleevec and active site surface illustrating how it follows the shape of the ‘tunnel’ opened by activation loop movement

❖ This tunnel is normally closed in the activated form of Abl

❖ Very similar binding modes

Abl
200 nM

Gleevec

Abl
9.8 nM

TargeGen analog

C-Helix and DFG portion of activation loop move to accommodate TargeGen compounds

❖ C-Helix moves relative to its inactivated state to pick up Glu310 interaction

❖ DGF portion of activation loop flexes to allow room for P4’ amide

Recent supportive evidence: cocrystal structure of Lck and inhibitor

Lck
0.2 nM

2-aminoquinazoline analog†

❖ Inhibitor forces the protein to assume an extended “DGF-out” conformation

❖ C-Helix moves to maintain Glu 288 interaction

❖ Novel binding mode not seen in most Src-family kinase inhibitors

†Journal of Medicinal Chemistry (2006), doi:10.1021/jm0605482

Conclusions

❑ TargeGen has presented a novel series of potent Src inhibitors

❑ The proposed binding mode of these inhibitors is unique and unprecedented for Src

❑ Movement of the C-helix and the DFG portion of the activation loop are required in this binding model. This is unusual given that such structures were not known for Src although known for Abl

❑ We have utilized these structures to obtain low nM Src activity and have presented here the relevant SAR