Design, Syntheses and SAR of Inhibitors Targeting the T315I-ABL Mutation

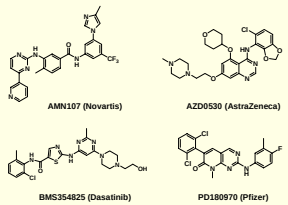
Jianguo Cao[1], Kathy Barrett[2], Richard M. Fine[3], Colleen Gritzen[1], John Hood[2], Jason Kang[3], Dan Lohse[2], Chi Ching Mak[1], Andrew McPherson[1], Glenn Noronha[1], Ved P. Pathak[1], Joel Renick[1], Richard Soll[1], Ute Splittgerber[1], Binqi Zeng[1], Hong Zhu[2]

TargeGen Inc., 9380 Judicial Drive, San Diego, CA 92121. (1) Medicinal Chemistry, TargeGen Inc., (2) In-vitro Biology, TargeGen Inc., (3) Discovery Partners International, Inc. and BioPredict, Inc.

Introduction

- Imatinib (Gleevec, Novartis AG) is frontline therapy for all phases of CML in patients. Unfortunately, resistance to imatinib has been reported in patients with CML. While resistance mechanisms are often complex, careful analyses has shown that 50-90% of all imatinib resistance arises from mutations in Bcr-Abl. These mutations are mostly clustered in specific regions that may be correlated with the binding of imatinib in Bcr-Abl
- Resistance to several of the mutations is overcome to a limited extent by increasing the dose of imatinib, or by using alternative inhibitors that present themselves differently to the protein and thus show varying degrees of effectiveness against many of the mutations
- Notable among these inhibitors is AMN-107 (Figure **1**), a Novartis compound that is based on Imatinib. AMN-107 works reasonably well against many of the mutations. This compound is currently in the clinic to treat Gleevec refractory CML. AMN-107 does not work at all against the T315I gatekeeper mutation, the most frequently occurring mutation
- On account of the similarity between Src and Abl in the kinase domain and the fact that all Src kinase domain inhibitors are also inhibitors of Abl, several inhibitors have emerged that are dual Src/Abl inhibitors (Examples in Figure **1**). Most of these compounds work very well against many of the mutations
- None of these compounds show any efficacy against the T315I mutation

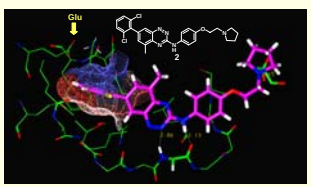
Figure 1. Differentiated Chemo-types That Are Effective Against Bcr-Abl Mutations That Cause Resistance to Imatinib



AMN107 (Novartis) | AZD0530 (AstraZeneca) | BMS354825 (Dasatinib) | PD180970 (Pfizer)

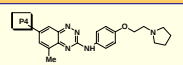
- Except AMN-107, the other examples are dual Src and Abl inhibitors
- Remarkable among them is Dasatinib (BMS), a highly potent Src/Abl inhibitor. Dasatinib has shown efficacy against all Gleevec mutations except the T315I mutation, and has just been submitted for registration for Gleevec refractory CML
- None of the dual Src/Abl inhibitors are active against the gatekeeper T315I mutation.
- There is current unmet need for a T315I abl mutant inhibitor
- At TargeGen, we have developed a Src-inhibitor program (See posters MEDI-**64**, **65**, **66**) using an internally designed and optimized benzotriazine core structure, and we have adapted and optimized this series for T315I Abl inhibition

Figure 2. TargeGen Benzotriazine Binding Mode in Active Src



The minimized binding mode of a representative compound from this series in a homology model of active Src, built from the crystal structure of active Lck. The dichloro phenyl moiety is orthogonal and deep within the hydrophobic pocket. This series shares a similar binding mode in Abl, on account of the strong similarity in the kinase domains of Src and Abl.

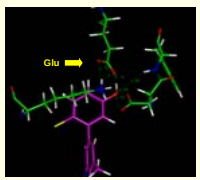
Table 1. Src Kinase Inhibition with Varying P4



Compounds	P4	Src (IC50, nM)	Compounds	P4	Src (IC50, nM)
1		7	4		25
2		11	5		788
3		35	6		1.3

- 2,6-Disubstitued phenyl compounds (**1, 2**) give rise to highly potent inhibitions for Src (Table **1**). This substitution pattern has been well established even in several other series (PD180970, Dasatinib)
- Surprisingly we found that mono-substitution at the 2-position is well tolerated (**3**). Orthogonality is not necessarily reinforced by di-substitution (**2** vs **3**). This is also true for the 2-Me analog (Src, 10 nM)
- Certain 2,5-disubstitutions are tolerated (**4**) but not others (**5**)
- The active conformation of Src presents a glutamic acid (Glu 310) from the C-helix with an unique binding opportunity deep within the hydrophobic pocket (Figure **2**). This Glu (310) is part of the Asp-Lys-Glu triad that is a part of the Src activation mechanism.
- We decide to capitalize on this unique opportunity by making use of a donor group on the phenyl ring. Molecular modeling (Figure **3**) predicted that a donor located on the meta position of the phenyl ring would interact optimally with Glu (310)
- Using this design strategy, the resulting *meta* donor compound (**6**) provides at least a 20 times increase in Src activity (**6** vs **3**) (Table **1**)

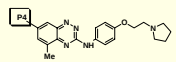
Figure 3. Donor Accesses the Glu (310) of the Glu-Lys Salt Bridge as Predicted by Src Homology-Based Molecular Modeling



- Donor binding has been validated via X-ray crystallography of a related TG inhibitor possessing a *meta* donor group co-crystallized in the active form Src kinase
- Having validated this concept of making an interaction deep within the hydrophobic pocket in Src, we designed T315I mutant Abl inhibitors based on the benzotriazine core
- The hypothesis is that obtaining this uniquely positioned donor interaction would provide a method to inhibit the gatekeeper T315I mutation on account of the additional interaction deep within the hydrophobic pocket. The postulated driving force would be strong binding interactions provided on both sides of the gatekeeper where the mutation occurs – with the Glutamic acid interaction in the back of the hydrophobic pocket being a unique, and to the best of our knowledge, unexploited interaction
- Compound **1** & **2** are dual Src/Abl inhibitors (Table **2**), but lack T315I ABL inhibition. This is similar to other series (PD180970, AZD0530, Dasatinib) with similar 2,6-disubstituted phenyl moieties in the hydrophobic pocket
- Extremely significant enhancement of potency toward T315I Abl with the *meta* positioned donor was observed (**6** vs **1** & **2**)
- The concept designed and validated in Src transfers well to Abl and makes a very striking difference in the case of T315I mutant Abl

The rest of this poster will describe our optimization of T315I Abl activity and the SAR we developed around this class of molecules

Table 2. Src, WT Abl & T315I Abl Kinase Inhibition with Varying P4



Compounds	P4	Src (IC50, nM)	Abl (Ki, nM)	Abl-T315I (Ki, nM)
1		7	11	4240
2		11	20	10300
6		1.6	1.3	55

SAR of the P4 Region (Tables 3-5)

Ki Value Determination

Ki values for TargeGen compounds were determined against Abl (Invitrogen) and the T315I mutant form of Abl (Upstate Cell Signaling Solutions). In white, flat-bottom, 96-well plates (Nunc) assays were run at room temperature at a final volume of 50 mL. Each well contained 40 mL of buffer consisting of 75 mM Tris buffer, pH 7.2 containing 95 mM MgCl2, 1.5 mM EGTA, 0.35 mM Triton X-100, 10 mM b-mercaptoethanol and an appropriate amount of Abl or T315I-Abl such that the assay was linear over 60 min. Varying amounts (1 to 100 mM) of Abltide substrate (Upstate Cell Signaling Solutions) in water, were added in the presence of a fixed varied amount of compound. The fixed varied concentrations of compounds were generated by adding the appropriate amount in 2.5 mL of DMSO; the DMSO present in each assay was constant at 5%. The reaction was initiated by the addition of ATP to a final concentration of 0.5 mM. After the reaction was allowed to proceed for 60 min, 50 mL of Kinase-Glo reagent (Promega) was added to terminate the reaction. This solution was then allowed to proceed for an additional 10 min to maximize the luminescence reaction. Values were then measured using an Ultra 384 instrument (Tecan, Charlotte NC, USA) set for luminosity measurements. A control reaction containing no peptide substrate was used for a zero point. Enzyme reaction rates were derived by calculating the difference between kinase catalyzed and uncatalyzed reactions at a specific compound concentration. *Ki* values were derived from rate data using the noncompetitive enzyme kinetics curve fitting capabilities of Prism (Version 4; GraphPad Software, San Diego CA, USA).

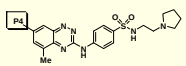
pKa of the donor group (Table 3)

- No significant activity change was observed toward WT Abl by changing the pKa of the donor group in the P4 hydrophobic pocket (**7**-**9**)
- However, donor group pKa has profound effects on T315I Abl. Strong inhibition was observed in compounds with lower pKa donors in the P4 (**7** vs **8** & **9**)

Position of the donor group (Table 4)

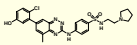
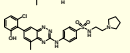
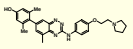
- By comparing to other positions, the donor group (OH) in the 5-position on the phenyl ring seems optimal for Abl and T315I Abl
- By changing the donor group to the 6-position, WT Abl activity is maintained, but T315I Abl activity decreases dramatically (**7** vs **10**)
- Moving the donor from the 5- to 4-position, apparently results in a decrease in both WT Abl and T315I Abl activity (**11**)

Table 3. Donor pKa Affects WT Abl & T315I Abl Kinase Inhibition



Compounds	P4	pKa	Abl (Ki, nM)	T315I-Abl (Ki, nM)
7		9.5	3	3.4
8		17	24	1770
9		26	16	>2500

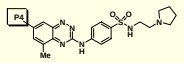
Table 4. Donor Position Affects WT Abl & T315I Abl Kinase Inhibition

Compounds	Structure	Abl (Ki, nM)	T315I-Abl (Ki, nM)
7		3	3.4
10		5	1620
11		458	9597

Substituents in addition to the meta donor (Table 5)

- In all of the discussion for Table **5**, the donor phenol is maintained at the 5-position
- Variations at the 2-position do not affect WT Abl activity (**7**, **12**-**13**)
- But 2-chloro seems optimal for T315I-Abl activity (**7** vs **12**-**13**)
- All multiple 2,6-disubstitutions are well tolerated for Abl, except for the 2,6-dichloro analog (**15**)
- Overall, 2-chloro-5-hydroxy seems optimal for T315I-Abl (**7** vs **12**-**16**)

Table 5: Substituents on P4 Affect WT Abl & T315I Abl Kinase Inhibition

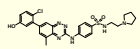
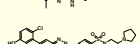
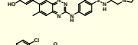
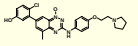
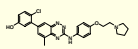


Compounds	P4	Abl (Ki, nM)	T315I-Abl (Ki, nM)
7		3	3.4
12		1	76
13			>50
14		1.3	570
15		27	50
16		3.4	51

SAR of the P3 Region (Table 6)

- Moving the methyl group from the 5- to the 6-position on the benzotriazine core does not change WT Abl activity, but decreases T315I Abl activity (**7** vs **17**, **6** vs **19**)
- Both WT Abl and T315I Abl are unaffected by the N-oxide on the benzotriazine core (**6** vs **18**)

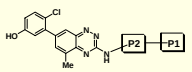
Table 6. Core (P3) Affects WT Abl & T315I Abl Kinase Inhibition

Compounds	Structure	Abl (Ki, nM)	T315I-Abl (Ki, nM)
7		3	3.4
17		2.4	183
18		2.2	87
6		1.3	55
19		1.2	284

SAR of the P1/P2 Region (Table 7)

- All compounds in Table **7** are very good WT Abl inhibitors. Among them, Compound **20**, **24**, **29**, and **37** stand out with picomolar inhibition against WT Abl
- The P1 moiety is crucial for T315I Abl activity (**26** vs **7**, **39** vs **40**)
- Compounds with *para* substituted P1 are more active towards T315I Abl than those that are *meta* substituted (**7** vs **32**)
- In general, compounds with good acceptors, such as amide, sulfonamide, sulfone are more active on T315I Abl than ethers, thioethers and alkyl linkages (**7**, **23**, **27**, **35** vs **6**, **20**, **21**, **28**). Sulfonamide and sulfone are better than amide in terms of T315I Abl activity (**27** vs **22**)
- The pyrrolidine moiety seems optimal as a solubilizing group (**27** vs **31**)
- T315I Abl activity decreases with more sterically hindered *N*-substituted groups on the sulfonamide (**27** vs **29** & **30**)
- Substitutions on the P2 phenyl ring cause a decrease in T315I Abl activity (**37** vs **27**)
- Changing the phenyl ring to a heteroaromatic ring decreases the T315I Abl activity dramatically (**38** vs **22**)

Table 7. P1/P2 Affects WT Abl & T315I Abl Kinase Inhibition



Compounds	P1/P2	Abl (Ki, nM)	T315I-Abl (Ki, nM)
6		1.3	55
20		0.062	26
21		5.0	1300
22		2.6	48
23		1.7	15
24		0.5	21
25		4.2	820
26		7.7	687
7		3	3.4
27		0.3	1.2
28		3.2	138
29		0.4	66
30		2.9	956
31		5.1	150
32		1.3	172
33		4.4	140
34		1.7	16
35		3.5	4.7
36		3.5	164
37		0.16	45
38		2.5	483
39		3.8	>10000
40		1.3	112

Conclusions and Outlook

- Having validated a structure-based design concept of making an interaction deep within the hydrophobic pocket in our dual Src/Abl inhibitors, we designed T315I mutant Abl inhibitors based on the proprietary benzotriazine core.
- The hypothesis is that obtaining a uniquely positioned donor interaction with the Glutamic acid deep within the hydrophobic pocket would provide a means to inhibit the gatekeeper T315I mutation on account of the key additional interaction.
- The concept designed and validated in Src apparently transfers well to Abl and more importantly to T315I mutant Abl.
- Promising initial biochemical potency was obtained and optimized in TargeGen's benzotriazine series.
- We are exploring the application of the concepts presented here to TargeGen's other Src and Abl scaffolds. We are also examining the application of these concepts to other kinases, and to other kinase inhibitors.

2006 ACS Spring National Meeting Atlanta, GA, March 26-30