Structure based design of novel, single-digit nM benzotriazine Src inhibitors

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Introduction

Protein kinases are families of enzymes that catalyze the phosphorylation of specific residues in proteins

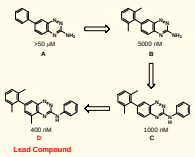
Src is a member of a larger family of cytoplasmic tyrosine kinases capable of communicating with a large number of different receptors. The c-Src proto-oncogene plays a major role in the development, growth, progression, and metastasis of a wide variety of human cancers

Src kinase modulates signal transduction through multiple oncogenic pathways, including EGFR, Her2/neu, PDGFR, FGFR, and VEGFR

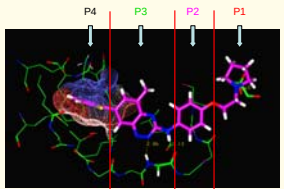
Since the activation and perhaps over-expression of Src has been implicated in cancer, osteoporosis, stroke, myocardial infarction, vascular leak, and bone disorders, a small molecule inhibitor of c-Src has potential in the treatment of several disease states

TargeGen has designed and optimized a novel series of benzotriazines that target Src. The series was optimized to obtain low–nM inhibitors via elaboration of the lead compound (**D**), using a variety of substituted and unsubstituted alkyl, aryl and heteroaryl groups. Presented herein are the details of the design of these inhibitors and the resulting SAR

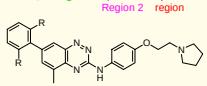
The benzotriazine series – early SAR in Src



Design - template regions



Representative compound (R = Cl)



Earlier work indicated that an aromatic moiety in the P4 region and a hinge binding moiety in the P3 region are both crucial for inhibition (Data presented at the 2005 ACS meeting in Washington DC, MEDI 408, summarized in part in Table 1a)

Initial campaign to increase Src inhibition held the P4 region constant as dimethylphenyl and modified moieties in the P1, P2 and P3 regions

Continuation of optimization for Src in the P4 region is described in MEDI 65

An overview of the synthetic strategies for all of these optimizations is described in MEDI 64

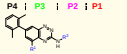
Initial exploration began in the P2 region

Assay conditions

IC_{50} Value Determinations: Our compounds were tested using the KinaseGlo assay. The test compound at concentrations ranging from 1 nM through 100 μM was treated with recombinant human c-Src (28 ng/well, Panvera-Invitrogen, Madison, WI), ATP (2 μM), and a tyrosine kinase substrate (PTK2, 250 μM, Promega, Corp, Madison, WI) in Src kinase reaction buffer (Upstate USA, Lake Placid, NY) at room temperature for 90 minutes. The residual ATP was determined using a luciferase-based assay (KinaseGlo, Promega Corp.) as a measure of kinase activity. The data from four wells was averaged and used to determine the IC_{50} using Prism software (GraphPad Software, San Diego, CA).

Table 1a: Enzymatic data of selected compounds with various moieties in the P2 region (Summary of 2005 ACS Washington DC MEDI 408)

P4 ; P3 ; P2 ; P1



Compound	R^1	R^2	Src IC_{50} (nM)	Compound	R^1	R^2	Src IC_{50} (nM)
1	H	H	2206	**8**	CH_3		400
2	CH_3	H	7400	**9**	CH_3		1000
3	CH_3		>72000	**10**	CH_3		433
4	CH_3		9000	**11**	CH_3		545
5	H		5800	**12**	CH_3		140
6	CH_3		1800	**13**	CH_3		244
7	H		1520	**14**	CH_3		341

SAR of compounds with various moieties in the P2 region (Table 1a)

1. Aromatic in P2 region is crucial for Src inhibition (**2** vs. **8** and **3-5**)
2. 5-CH_3 substitution on core gives 4x increase in potency (**7** vs. **8**)
3. Alkyl moieties in the P2 region are not well tolerated (**3-6**)
4. With 5-CH_3 on the core, phenyl group vs. H increases the activity to nM inhibition (**8** vs. **2**)
5. Simple substitution patterns on phenyl in P2 region make little difference to activity (**9-13**)
6. Electronics of the aryl group in the P2 region (Cl vs OMe) makes little difference to activity (**10** vs. **11**)
7. Heteroaromatics (**14**) in the P2 region are also well tolerated
8. Ethyl ether analog indicates preference for increased hydrophobe leading to solvent exposed region (**12** vs. **11**)

Table 1b: Enzymatic data of ether linked moieties in the P2-P1 regions

Compound	R^1	R^2	Src IC_{50} (nM)	Compound	R^1	R^2	Src IC_{50} (nM)
12	CH_3		140	**20**	CH_3		39
15	CH_3		36	**21**	CH_3		40
16	CH_3		33	**22**	CH_3		10
17	H		15	**23**	CH_3		6
18	CH_3		7				
19	CH_3		13				

SAR of ether linked moieties in the P2-P1 region (Table 1b)

1. With an amine-bearing moiety in the P1 region, potency increases by up to 20x (**12** vs. **18**)
2. Activities of meta vs. para phenyl substitutions in the P2 region are indistinguishable (**18** vs. **23** and **15** vs. **20**)
3. Chain length unimportant for potency (**18** vs. **19** and **20** vs. **21**)

Table 1c: Enzymatic data of sulfonyl linked moieties in the P2-P1 regions

Compound	R^1	R^2	Src IC_{50} (nM)	Compound	R^1	R^2	Src IC_{50} (nM)
24	CH_3		143	**31**	CH_3		137
25	CH_3		48	**32**	CH_3		128
26	CH_3		322	**33**	CH_3		9
27	CH_3		41	**34**	CH_3		26
28	CH_3		29				
29	CH_3		26				
30	CH_3		35				

SAR of sulfonyl linked moieties in the P2-P1 region (Table 1c)

1. Tolerant of sulfonamide linkage in the P2-P1 region with para and meta substituted phenyl analogs possessing similar activities (**29** vs. **33** and **26** vs. **31**)
2. Cyclic amine moieties are well tolerated in the P1 region, though open chain analogs have up to 15x increase in potency (**31** vs. **33**)
3. Para substituted compounds show no preference between 2° and 3° cyclic amines in the P1 region (**31** vs. **32**)
4. Surprisingly, meta substituted compounds bearing 2° cyclic amine moieties in the P1 region have an almost 7x increase in potency relative to their 3° counterparts (**25** vs. **26**)
5. N-H of sulfonamides appears to play little role in Src binding
   A. Sulfone linkage in the P2-P1 region is indistinguishable from sulfonamide counterpart (**30** vs. **29**)
   B. N-H vs. N-CH_3 analogs show little change in potency (**33** vs. **34**)

Table 1d: Enzymatic data of amide linked moieties in the P2-P1 regions

Compound	R^1	R^2	Src IC_{50} (nM)	Compound	R^1	R^2	Src IC_{50} (nM)
35	CH_3		89	**38**	CH_3		15
36	CH_3		18	**39**	CH_3		16
37	CH_3		27	**40**	CH_3		6

SAR of amide linked moieties in the P2-P1 region (Table 1d)

1. Tolerant of acyclic amide linkage in the P2-P1 region with para and meta analogs giving similar activities (**36** vs. **39**)
2. Para aryl substituted cyclic amide analogs have a 6x increase in potency over their meta counterparts (**38** vs. **35**)
3. No difference in potency between para aryl substituted cyclic and acyclic analogs (**38** vs. **39**)
4. Little difference in potency between dimethyl amine and pyrolidine moieties in the P1 region (**39** vs. **40**)

Table 2: Enzymatic data of compounds with various heteroaromatics in the P2 region

Compound	R^2	Src IC_{50} (nM)	Compound	R^2	Src IC_{50} (nM)
41		77000	**47**		76
42		38	**48**		6
13		341	**49**		1500
43		118	**50**		94
44		232	**51**		29
45		>100000	**52**		15
46		12800			

SAR of compounds with various heteroaromatics in the P2 region (Table 2)

1. The six-membered ring pyridyl moieties in the P2 region afford a range of activities (compare **13**, **41-44**)
2. Fused ring systems in the P2 region are not well tolerated resulting in drastic loss of Src activity (**45** vs. **46**)
3. In general, heteroaromatic moieties in the P2 region are not well tolerated unless the compound also contains an amine moiety in the P1 region (left vs. right column)
4. Pyridone analogs in the P2 region are well tolerated and maintain similar activity to their phenyl counterparts (**48** vs. Table 1b: **23**)
5. Furan analogs in the P2 region are well tolerated, although a shortened carboxylic acid in the P1 region results in a significant loss in potency (**49** vs. **50**)
6. Thiazole analogs in the P2 region are well tolerated providing activity similar to their phenyl counterparts (**52** vs. Table 1d: **39**)

Table 3: Enzymatic data of analogs in the P3 region – 5-, and 6- positions on core

Compound	Structure	Src IC_{50} (nM)	Compound	Structure	Src IC_{50} (nM)
8		400	**58**		11
53		130	**59**		21
18		7	**51**		29
54		12	**60**		137
55		11	**33**		15
56		10	**61**		30
57		11	**62**		34

SAR of analogs in P3 region – 5- and 6- positions on core (Table 3)

1. While compounds with no solubilizing extension into the P1 region show a modest 3-4x activity enhancement with a dimethyl substitution on the core (**8** vs. **53**), analogs containing the extension show no differentiation between 5- and 6- methyl substitutions (compare **18**, **54-55**)
2. Similarly, 5-amino analogs with the ether linkage in the P2-P1 region are undifferentiated (**56-59**)
3. Similar 5-aminoacetyl substitution of the core with either the furan-amide or the phenyl-sulfonamide moieties in the P2-P1 region results in a modest 2-5x activity loss (**60** vs. **51** and **61** vs. **33**)
4. Further acylation of sulfonamide in the P2-P1 region (**62** vs. **61**) maintains activity similar to previous data (Table 1c: **33** and **34**)

Table 4: Enzymatic data of selected compounds containing a constant solubilizing moiety in the P1 region

Compound	R^1	R^2	Src IC_{50} (nM)	Compound	R^1	R^2	Src IC_{50} (nM)
18	CH_3		7	**51**	CH_3		29
33	CH_3		9	**52**	CH_3		15
38	CH_3		16				

SAR of the compounds containing a constant solubilizing moiety in the P1 region (Table 4)

1. Para linked aryl moieties in the P2 region bearing an amine in the P1 region have similar potency in Src (**18**, **33**, and **38**)
2. Keeping the amine in the P1 region constant, the aromatic moieties in the P2 region have similar potency

Highlights

An aryl or heteroaryl moiety in the P2 region is essential to the activity of TargeGen's benzotriazene inhibitors

A wide range of solvent exposed amines in the P1 region as well as linker chain lengths are well tolerated and can be optimized in conjunction with aromatic moieties in the P2 region to afford single digit nM Src inhibitors

Modifications at the 5- and 6- positions of the core in the P3 region have limited effect

Further optimization in the P4 region using the knowledge presented herein has allowed TargeGen to rapidly produce sub-nM Src inhibitors (please see poster MEDI 65)

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