

Optimization of a novel Src inhibitor series to obtain sub-nM compounds

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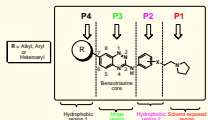


Introduction

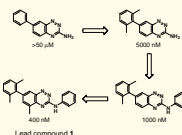
Src is one of the three ubiquitously expressed Src family kinases and is the best known cellular kinase. There is significant evidence that Src is overexpressed in various human tumors and increased Src activity is observed in metastatic tumors, particularly in colon and breast tumors. Increased Src activity has also been found in VEGF-mediated increase in vascular permeability as well as in bone disorders. As a result, Src kinase has been considered a therapeutic target for cancer, osteoporosis, stroke and myocardial infarction.

TargeGen has designed and optimized a novel series of benzotriazines that are used to target Src. The focus in this poster will be the work carried out to obtain sub-nM inhibitors of Src via elaboration of the R group (Figure 1), using a variety of substituted and unsubstituted alkyl, aryl and heteroaryl groups. Presented herein are details for the design (for synthesis; see poster MEDI 64) of the inhibitors with the R group deep within the Src hydrophobic pocket, the resulting SAR, and the optimization that led to highly potent sub-nM Src inhibitors from this series.

Figure 1: Design - template regions



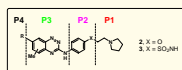
The benzotriazine series – early SAR for Src



Earlier work based on 1 indicated that optimization of the P2-P1 regions (see Figure 1) plays a crucial role in obtaining low nM inhibition for Src (please see poster MEDI 66)

Src activity can be improved or maintained with small hydrophobic or unchanged hydrophilic substituents introduced at position 5 or 6 of the benzotriazine core (P3 region) (e.g. methyl group in 1)

While keeping the P2-P1 section on the right hand side constant as in scaffolds 2 and 3, modification of the R group in the P4 region as described below has led to highly potent sub-nM Src inhibitors



All following P4 modified compounds fall into 3 major categories based on the resulting SAR:

- Hydrophobic aryl moieties with mono- or di- substitution (Category A)
- Hetero-aromatic or alkyl moieties (Category B)
- Aryl moieties with appropriate donor groups that are additionally mono-, di- or tri- substituted (Category C)

SAR of Category A compounds (Tables 1-4)

- The aryl group in the P4 region is crucial for the activity of compound. Simple phenyl ring increases the activity to nM inhibition (compare Table 1: 7 with Table 2: 13 and Table 4: 28 with 31)
- Compounds with or without a methyl substitution on the core exhibit similar potency (compare 5 with 9)
- With 2- or 2,6- substitution, Cl or Me (15-17), potency increases by at least 10 fold when compared to an unsubstituted phenyl group (13)
- The P4 binding pocket is not tolerant to groups at 2-position larger in size than a Cl or Me (29 and 30)
- The P4 binding pocket is less tolerant to hydrophilic groups (32 and 33)
- With 4-F, 5-F or 5-CN in the aryl group, 2,4- or 2,5- disubstituted compounds maintain good activity (e.g. 4, 6 and 25)
- With methoxy in 5-position (11 and 18), however, is not tolerated

Table 1: Enzymatic data of Category A compounds containing scaffold 2 with a 6-methyl or des-methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
4	H	26	7	H	>10,000
5	Me	12	8	Me	30
6	H	7	9	Me	15

- R group in P4 region is essential to the activity of the inhibitors (see 7-9)
- Methyl group at C6 in the benzotriazine core is not crucial to the activity (5 vs. 9)

Table 2: Enzymatic data of Category A compounds containing scaffold 2 with a 5-methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
10	H	1,200	14	H	208
11	Me	778	15	Me	35
12	H	505	16	Me	11
13	H	341	17	H	7

- Inhibitors with 2,6-disubstituted (Cl or Me) aryl groups, exhibit low nM activity (compare 13 with 15-17)

Table 3: Enzymatic data of Category A compounds containing scaffold 3 with a 5-methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
18	H	10,000	23	H	282
19	Me	10,000	24	Me	51
20	H	2,900	25	Me	25
21	H	631	26	H	14
22	H	353	27	H	9

- meta-Substitution in aryl group is only tolerant to small group such as CN (25)
- Inhibitors with donor aryl have moderate activity (21-24)

Table 4: Enzymatic data of Category A compounds containing scaffold 3 with a 6-methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
28	H	>10,000	32	H	92
29	Me	5,000	33	Me	72
30	H	2,216	34	H	20
31	Me	315	35	Me	12

- meta-Substitution in aryl group is only tolerant to small group (compare 30 with 35)
- ortho-Substitution is not tolerant to large or hydrophilic groups (see 29, 32 and 33)

SAR of Category B compounds (Tables 5 and 6)

- In general, the hydrophobic P4 region does not tolerate hetero-aromatics. Please compare the activities in the following examples: (a) Table 5: 36 with Table 3: 24; (b) Table 5: 37 or 38 with Table 2: 13; (c) Table 5: 39 with Table 2: 17; and (d) Table 5: 40 with Table 1: 6
- The P4 region also does not tolerate alkyl groups (see 48, 49 and 51)
- Of all the compounds with hetero-aromatics in P4 region, compounds 41 and 42 exhibit the best inhibitory activity at ~50 nM

Table 5: Enzymatic data of Category B compounds containing scaffold 2 with a 5- or 6- methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
36	H	>10,000	41	H	61
37	H	1,800	42	H	44
38	H	1,500			
39	H	207			
40	H	204			

- Not tolerant to any hetero-aromatics in P4 region

Table 6: Enzymatic data of Category B compounds containing scaffold 3 with a 5- or 6- methyl core

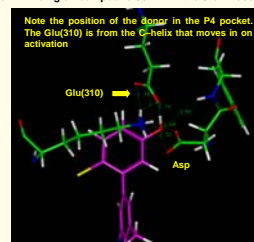
Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
43	H	>10,000	48	H	10,000
44	H	>10,000	49	H	10,000
45	H	10,000	50	H	8,000
46	H	10,000	51	H	1,772
47	H	144	52	H	1,730
			53	H	880

- Not tolerant to any hetero-aromatic or alkyl groups in P4 region

Design Strategy for Category C compounds

- 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. This Glu (310) is part of the Asp-Lys-Glu triad that is a part of the Src activation mechanism.
- We decided to capitalize on this unique opportunity by making use of a donor group on the phenyl ring. Molecular modeling (Figure 2) predicted that a donor located on the meta-position would interact optimally with Glu (310).

Figure 2: Binding of compound 56 within the Src kinase domain



SAR of Category C compounds (Tables 7-9)

- Interaction of meta-hydroxyl aryl group with the Glu residue within the hydrophobic pocket is extremely critical for the excellent activity of the compounds (compare Table 2: 11 with Table 7: 56 and see Figure 2)
- Compounds with less acidic aryl group exhibit ~13-170 fold less potent inhibitory activity (compare Table 7: 68 with amino compounds Table 3: 21-24)
- Compounds with ortho/para-hydroxyl aryl group are less potent (compare Table 7: 59 with 62 or Table 8: 65 with 68)

SAR of Category C compounds (Tables 7-9) (continued...)

- Again, compounds with 5- or 6- methyl substituted core exhibited similar activity (compare 56 with 62)
- Single digit nM or sub-nM activities are obtained for compounds with a meta-hydroxyl aryl group, 2- or 2,6- substituted with Me, F, Cl or Br (e.g. 67-74)
- Not tolerant to linker between meta-hydroxy aryl group and the core (compare 75 and 77), probably wrong orientation of the hydroxyl group

Table 7: Enzymatic data of Category C compounds containing scaffold 2 with a 5- or 6- methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
54	H	75	59	H	79
55	H	8	60	H	18
56	H	3.5	61	H	14
57	H	1.1	62	H	4.2
58	H	0.9			

- para-OH aryl groups in the P4 region are less favorable (see 54 and 59)
- meta-OH aryl groups with mono- or di- substitution in ortho positions provide inhibitors with single digit nM or sub-nM activity (56-58)

Table 8: Enzymatic data of Category C compounds containing scaffold 3 with a 5-methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
63	H	156	69	H	3.3
64	H	123	70	H	3.1
65	H	13	71	H	1.5
66	H	5.2	72	H	1
67	H	4.1	73	H	0.8
68	H	3.6	74	H	0.7

- para- or ortho- OH aryl groups provide less potent inhibitors (see 63 and 64)
- meta-OH aryl groups with mono- or di- substitution in ortho positions provide inhibitors with single digit nM or sub-nM activity (66-74)

Table 9: Enzymatic data of Category C compounds containing scaffold 3 with a 6-methyl core

Compound	R	Src IC ₅₀ (nM)	Compound	R	Src IC ₅₀ (nM)
75	H	10,000			
76	H	8.5			
77	H	1.6			

- Not tolerant to linker in between meta-hydroxyl aryl group and the core

Highlights

- A novel series of benzotriazine for Src inhibitors has been optimized from high μM to sub-nM inhibitory activity
- Aryl moiety in the P4 region, particularly with 2- or 2,6- substitutions (Cl or Me), is essential to the activity of TargeGen benzotriazine inhibitors
- Heteroaryl or alkyl moieties, in general, are not tolerated in the P4 region of TargeGen benzotriazine inhibitors
- Introduction of a meta-donor (e.g. OH) aryl group in the P4 region significantly enhances the activity. We postulate that this arises from a hydrogen bonding interaction from the donor OH and Glu (310) deep within the hydrophobic pocket of Src P4 region
- For compounds with a meta-hydroxyl aryl group, mono- or di- substitution in ortho position (e.g. Me, F, Cl or Br) enhance the potency to low- or sub- nM activity
- The design and optimization of the donor interaction with a conserved Glu residue within the hydrophobic pocket has led us to expand TargeGen oncology program to include Bcr-Abl inhibitors, in particular, targeting the T3151 mutant for CML (please see poster MEDI 251)

IC₅₀ Value Determinations: Our compounds were tested using the KinaseGlo assay. The test compound at concentrations ranging from 1nM 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 were averaged and used to determine the IC₅₀ using Prism software (GraphPad Software, San Diego, CA).