Nanomolar Inhibitors of the Transcription Factor STAT5b with High Selectivity over STAT5a

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Angew. Chem. Int. Ed., **2015**, *54*, 4758–4763.

Wipf Group Current Literature

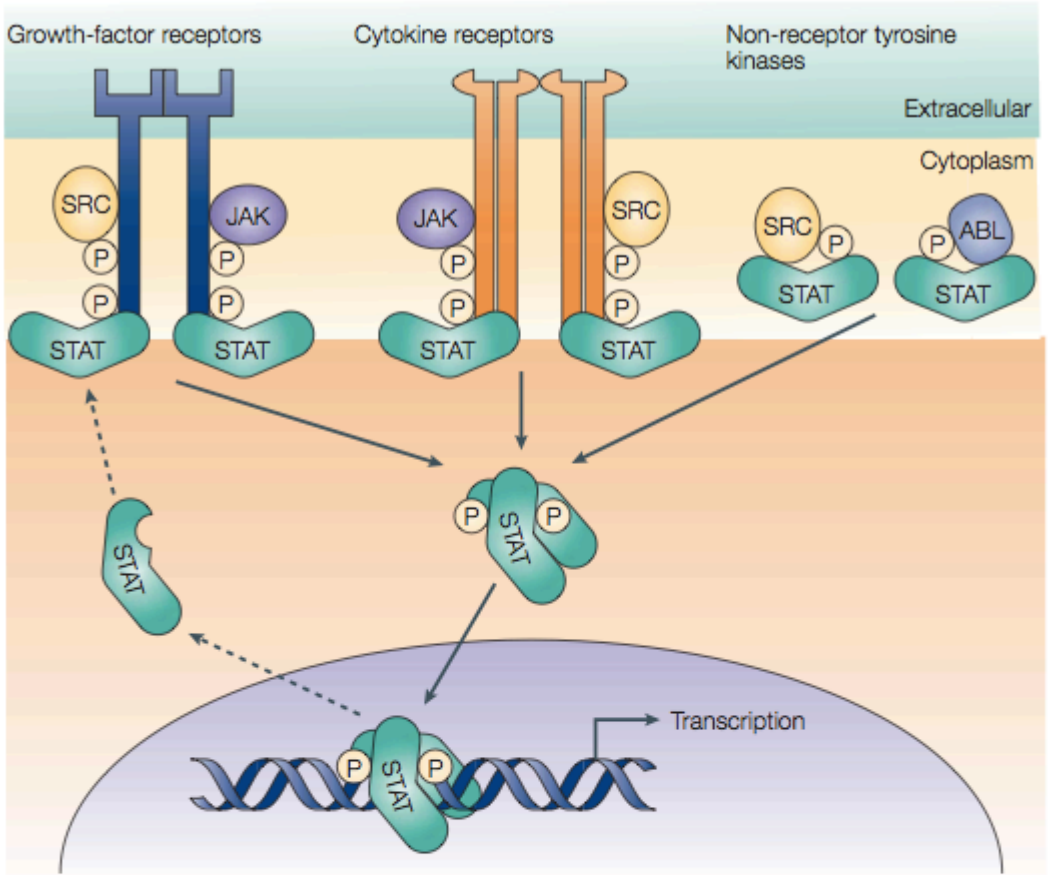
Chaemin Lim

05/02/2015

The STAT family of proteins

- ***Signal transducers and activators of transcription (STATs)***

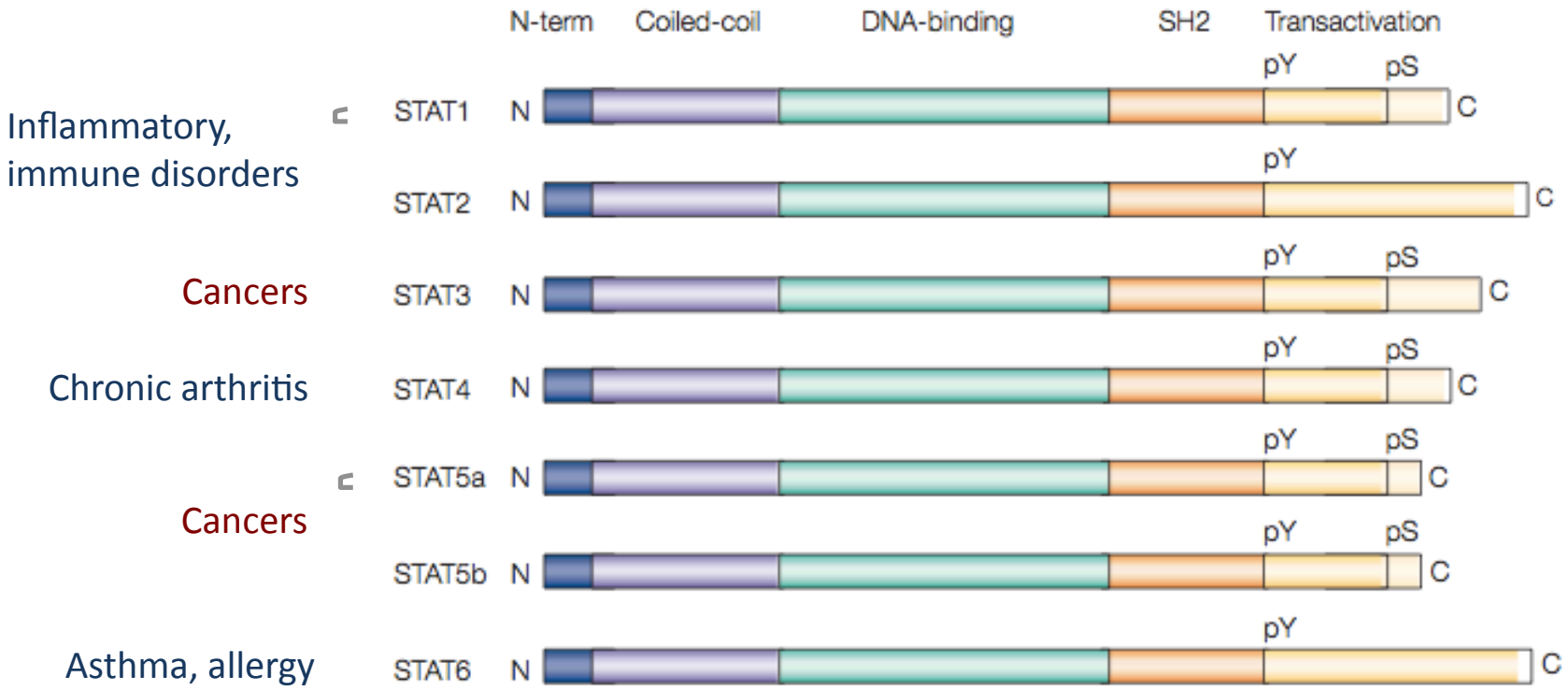
: A family of transcription factors that transduce signals from the cell surface to the nucleus



Nat. Rev. Cancer **2004**, *4*, 97.

The STAT family of proteins

- Seven STAT family members identified to date
- All of the seven STAT proteins are thought to be associated with human disease
  : Promising therapeutic target



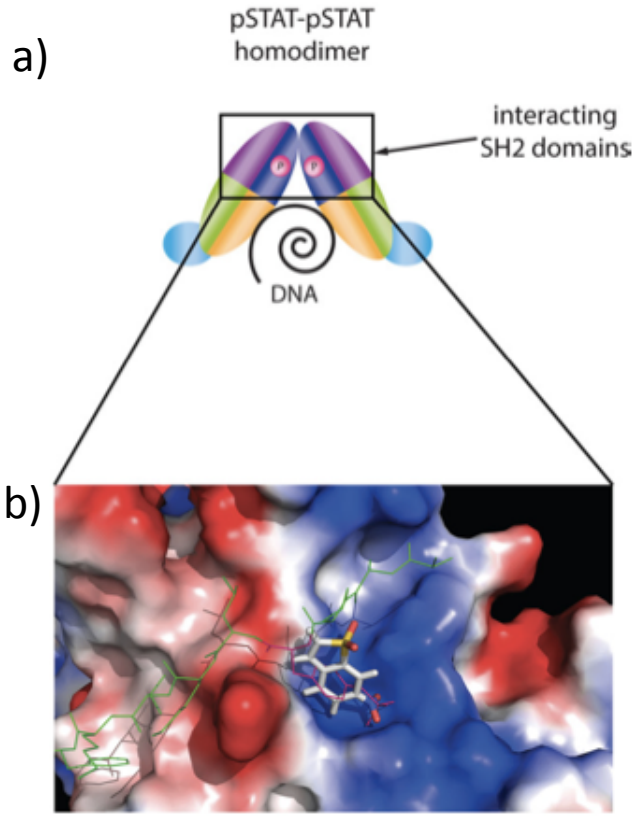
Nat. Rev. Cancer **2004**, *4*, 97.

Activity is critically dependent on phosphopeptide-SH2 domain interaction

Src homology 2 (SH2) domains as therapeutic target

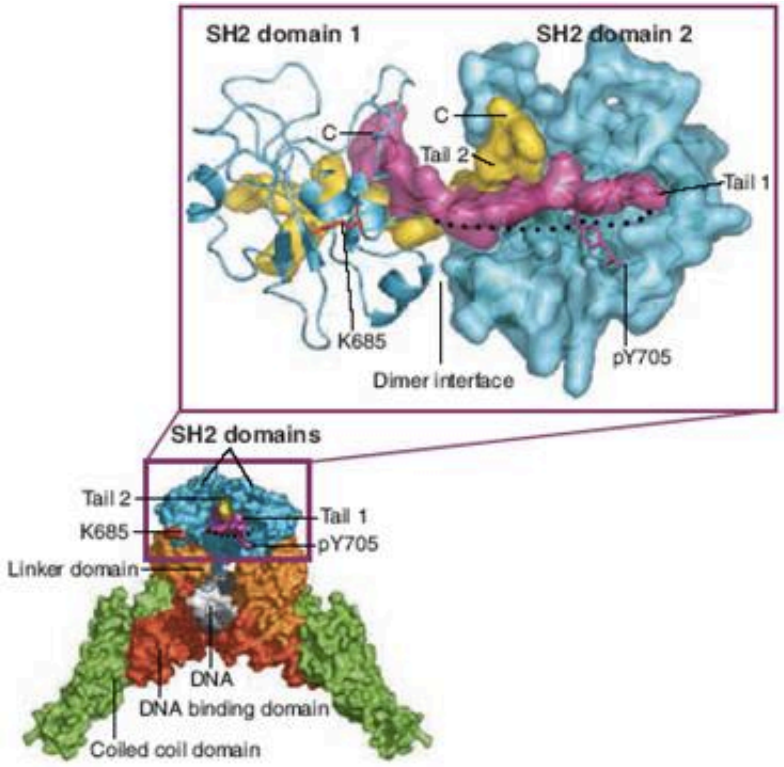
- SH2 domains: Key mediator of protein-protein interactions and mediate cell signaling

Figure 1. a) Homodimer of phosphorylated STAT binding to DNA.
b) STAT activity inhibition example.



STAT activity inhibition

Figure 2. The active binding site of the STAT3 protein



http://chemistry.berea.edu/~biochemistry/2011/cj/#Yua05

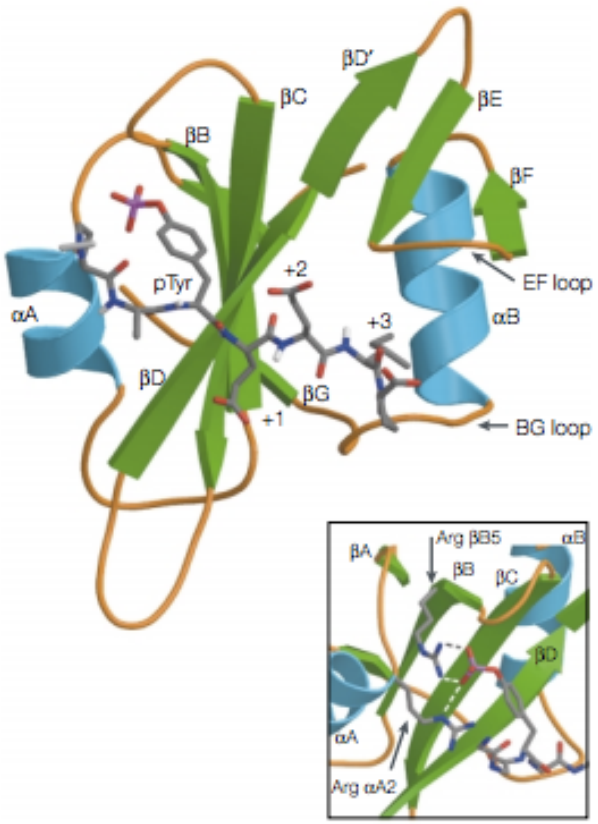
J. Comput. Sci. 2015, http://dx.doi.org/10.1016/j.jocs.2015.03.001

SH2 domains: details…

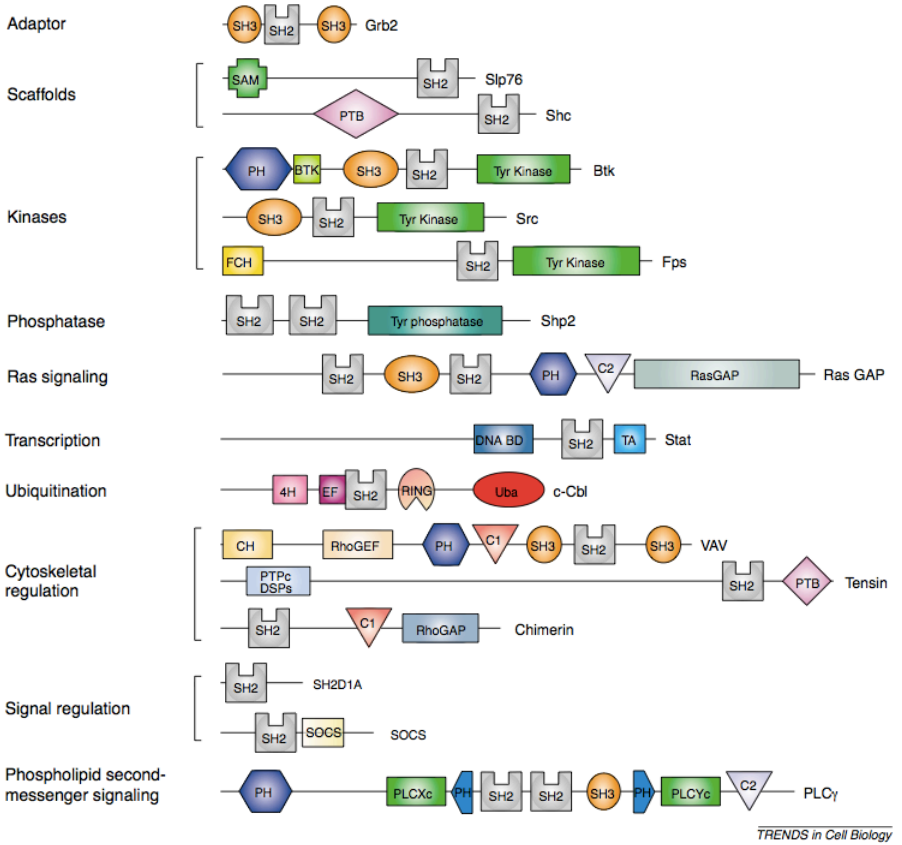
- Consist of about 100 amino acids that binds to specific phosphotyrosine (pY)-containing peptide motifs
- More than 120 different SH2 domains have been identified in over 110 human proteins

- Kinases, transcription factors, phosphatases, adaptor proteins, cytokine signaling suppressors and nucleotide exchange factors…

Selective targeting of SH2 domain in specific protein is challenging part



Nat. Mol. Cell Biol. **2002**, *3*, 177.



Trends in Cell Biology **2001**, *11*, 504.

SH2 doamins of STAT5

- A particularly challenging example is given by the SH2 domains of the transcription factors STAT5a and STAT5b.

STAT5a SH2 domain (amino acids 594-684)

```
ILGFVNKQQAHDLLINKPDGTFLLRFSDSEIGGITIAWKFDSPERNLWNLKPFTTRDFSIRSLADRLGDLSYLIYVFPDRPKDEVFSKYYT
ILGFVNKQQAHDLLINKPDGTFLLRFSDSEIGGITIAWKFDSQERMFWNLMPFTTRDFSIRSLADRLGDLNYLIYVFPDRPKDEVYSKYYT
```

STAT5b SH2 domain (amino acids 594-684)

93% identical on the amino acid level

Figure S1. Sequence comparison of the SH2 domains of human STAT5a and STAT5b.

- Antisense oligonucleotides directed against STAT5b, but not STAT5a, inhibited tumor cell growth in mice.

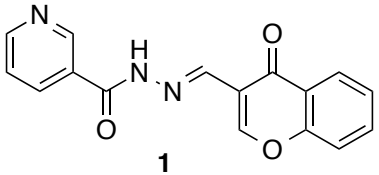
- In contrast to the well-explored STAT3 SH2 domain, little work has been done on developing an inhibitor of the STAT5 isoform.

  - STAT5's important role in cancer has only recently delineated
  - Limited STAT5 structural data

Reported STAT5 SH2 domain inhibitors

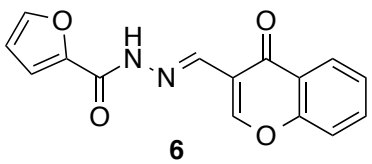
I. Chromone-based small molecule inhibitors: first report of nonpeptidic small molecules inhibitors of STAT5)

Berg *et al. ChemBioChem* **2008**, *9*, 723.



1

IC_{50} = 47 μM
(against the SH2 domain of STAT5b)

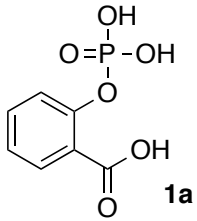


6

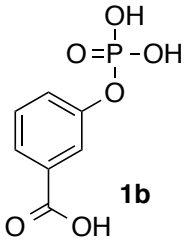
IC_{50} = 56 μM
(against the SH2 domain of STAT5b)

*II. Screening from known bioactive molecules: Fosfosal (**1a**, salicylic acid phosphate prodrug)*

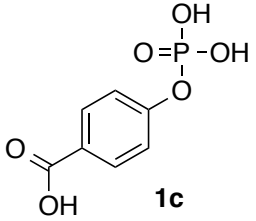
Berg *et al. ACS Chem. Biol.* **2011**, *6*, 1008.



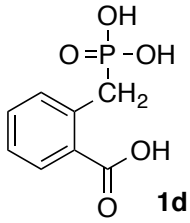
1a



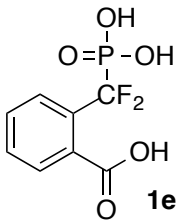
1b



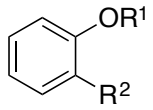
1c



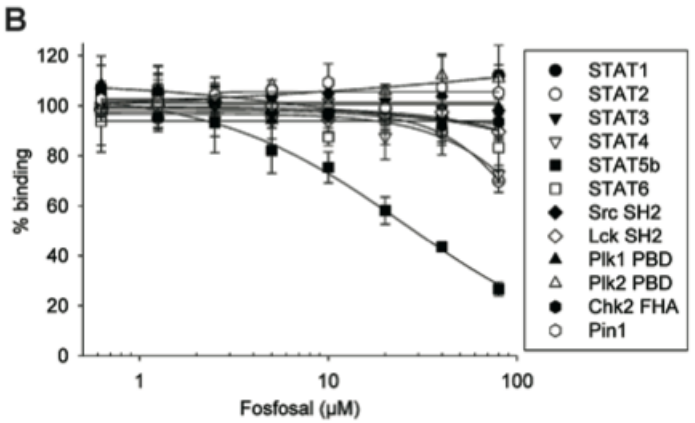
1d



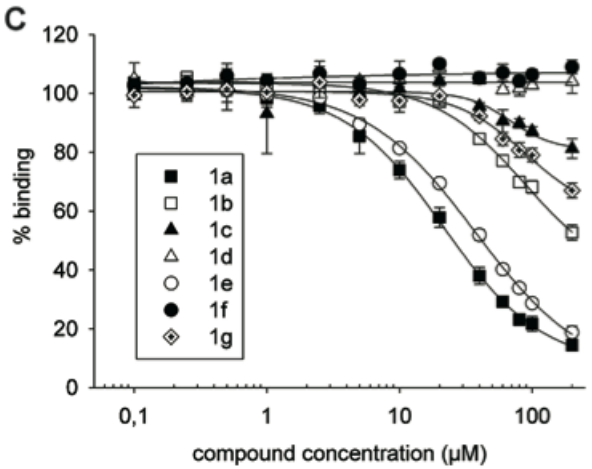
1e



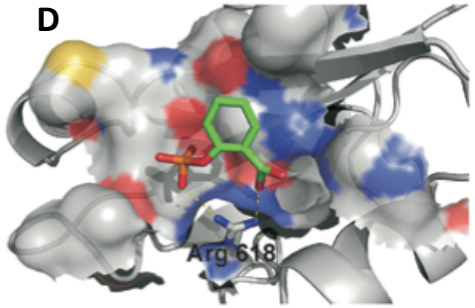
1f: R^1 = H, R^2 = COOH
1g: R^1 = PO_3H_2, R^2 = H



(B) Activity and selectivity of fosfosal analyzed in fluorescence polarization assays.



(C) Structure activity relationships for fosfosal analyzed in fluorescence polarization assays.



(D) Binding model of fosfosal (**1a**) suggested by molecular modeling

Reported STAT5 SH2 domain inhibitors

III. Salicylic acid-based inhibitors (nonphosphorylated small molecule inhibitors of STAT5)

Gunning *et al. J. Med. Chem.* **2012**, *55*, 1047.
Gunning *et al. ACS Med. Chem. Lett.* **2014**, *5*, 1202.

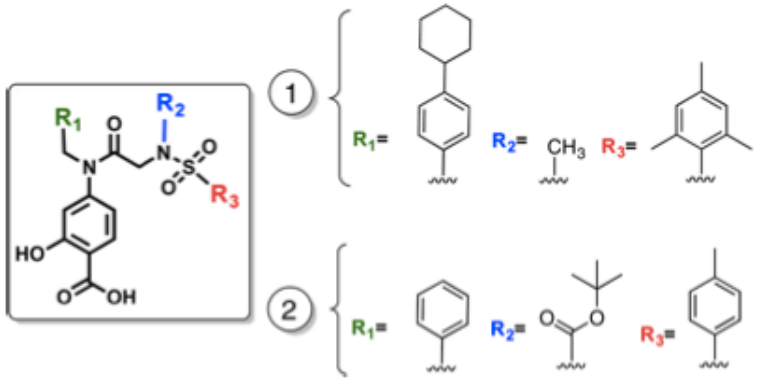
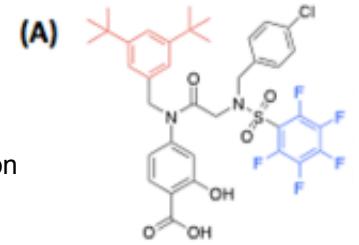


Figure 1. STAT5 inhibitors BP-1-108 (**1**) and SF-1-088 (**2**).

BP-1-108
STAT5, K_i = 2.8 μM

Structural optimization

SF-1-088
STAT5, K_i = 8.3 μM



13a

STAT5b, K_i = 145 nM
(STAT3, K_i = 143 μM)

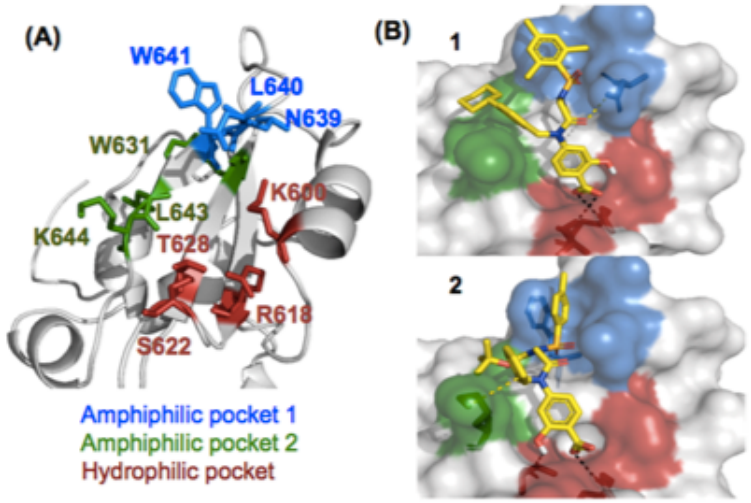
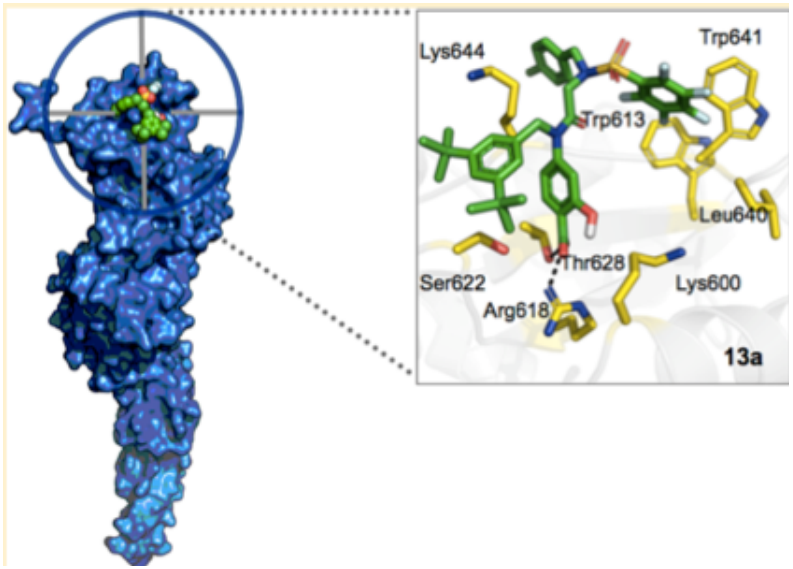


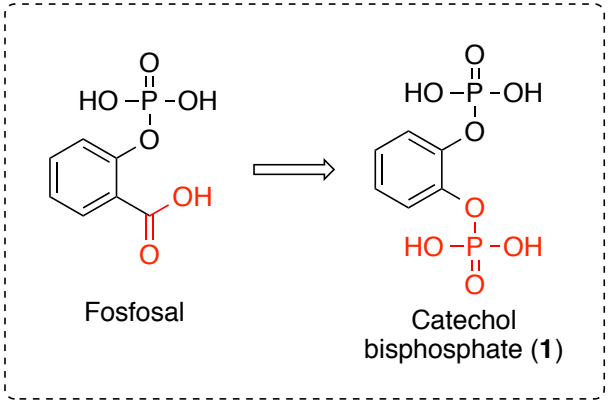
Figure 2. (A) STAT5a's SH2 domain with three binding pockets: hydrophilic, red; amphiphilic, green; amphiphilic, blue; (B) *in silico* docking of **1** interacting with R618, S622, and N639; **2** interacting with R618 and S622, as well as a cationic–π interaction of the R_1 benzyl with K644.



In this study

- SARs of fosfosal: Deleting the carboxylic acid retained partial activity, deleting the phosphate led to a complete loss of activity against STAT5b.

- Catechol bisphosphate (**1**) is a selective inhibitor of the STAT5b SH2 domain.



- Structure optimization: inspiration from natural product & docking simulation

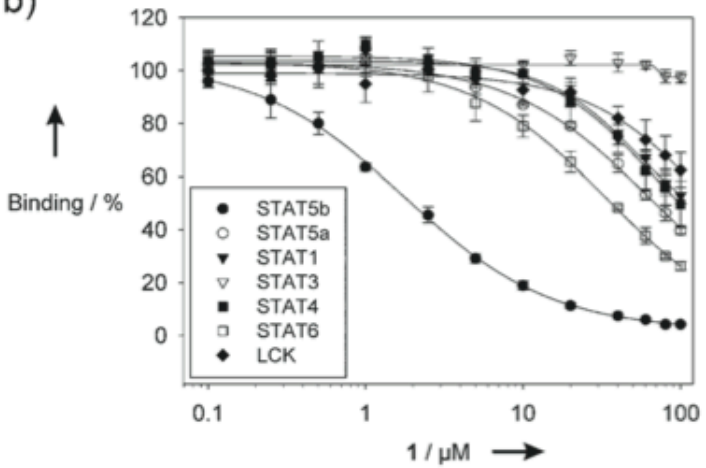
- Molecular docking studies using the homology model of the STAT5b SH2 domain based on the crystal structure of STAT5a.

- Binding site was validated by functional analysis of point mutants.

→ Low-nanomolar inhibitor, ***Stafib-1*** (compound 13), has developed.

Structure modification I.

b)

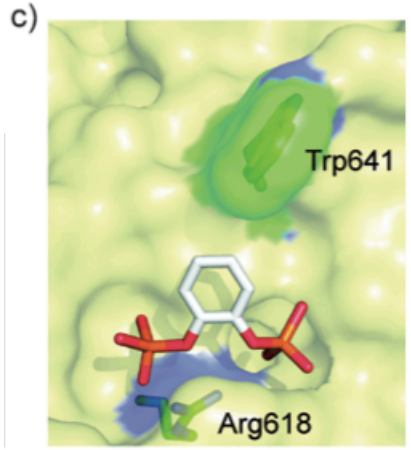


Protein	IC_{50} (µM) or maximum inhibition (%)	K_i (µM)
STAT5b	1.95 ± 0.15	0.93 ± 0.07
STAT5a	69 ± 5	34 ± 3
STAT1	90 ± 3	44 ± 1
STAT3	3 ± 2% inhibition at 100 µM	n/a
STAT4	68 ± 6	34 ± 3
STAT6	36 ± 2	18 ± 1
Lck SH2	38 ± 7 % inhibition at 100 µM	n/a

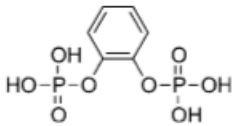
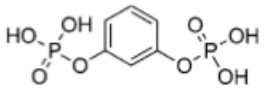
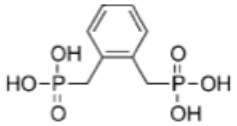
n/a: not applicable

b) Activities of **1** against the SH2 domains of several classes

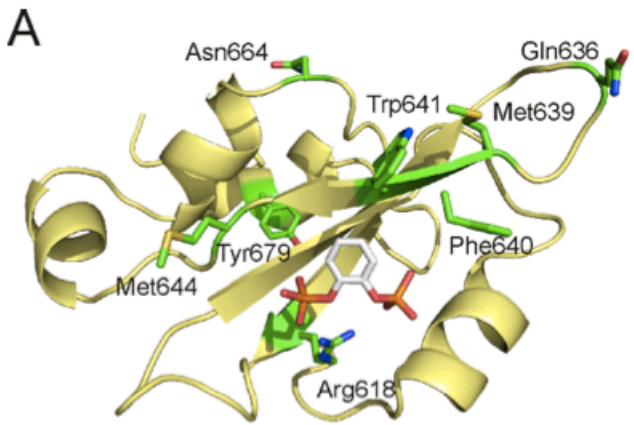
c)



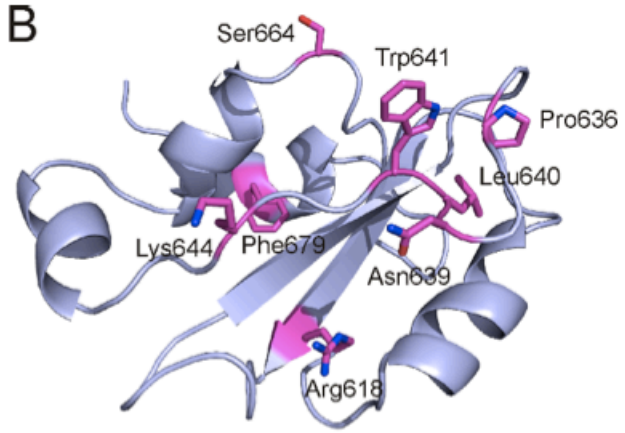
c) Binding of **1** to the STAT5b SH2 domain as predicted by docking with AutoDock Vina.

Compounds		K_i [µM] (STAT5b)	K_i [µM] (STAT5a)	Selectivity factor
1		0.93 ± 0.07	34 ± 3	37
2		27 ± 4	24 ± 3	0.9
3		n.a.	n.a.	n.a.

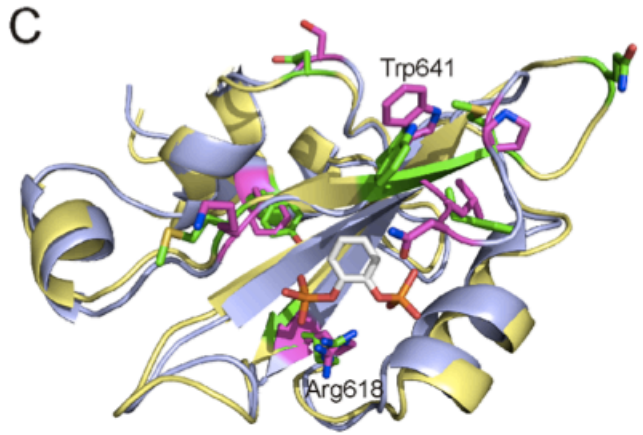
Molecular docking study: Putative binding of 1

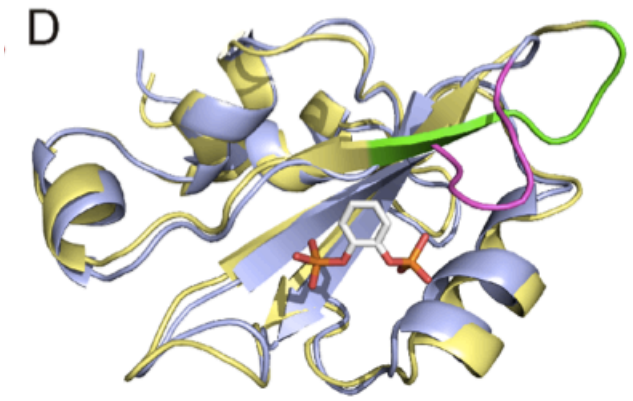


A) Model of **1** bound to the STAT5b SH2 domain (Homology model based on the STAT5a SH2 domain)



B) X-ray crystal structure of STAT5a SH2 domain (PDB: 1Y1U)

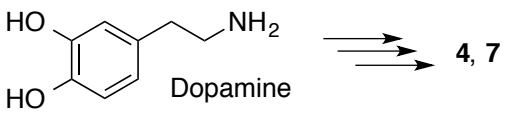




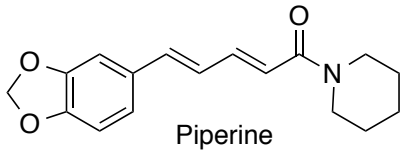
C) and D) Overlay of A (STAT5b, yellow) and B (STAT5a, lightblue)

→ Small differences in the primary structure of STAT5a and STAT5b might cause a partial change in secondary structure. But, detailed comparative analysis needed (Crystallographic or NMR methods)

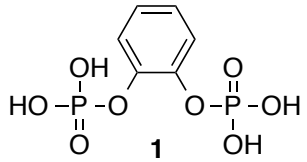
Structure modification II: Inspiration from natural product



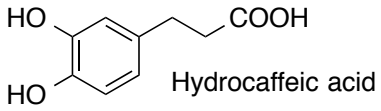
4, 7



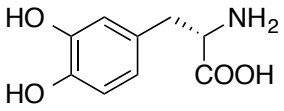
6



STAT5b, K_i = 0.93 μM
STAT5a, K_i = 34 μM
(Selectivity factor: 37)

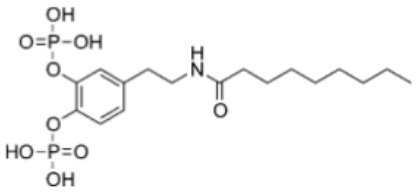
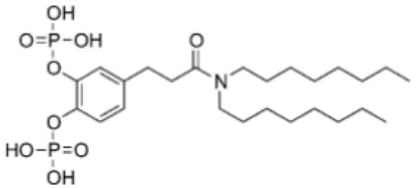
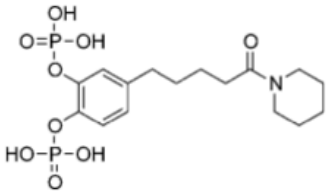


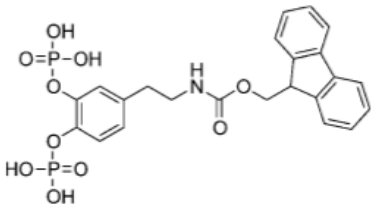
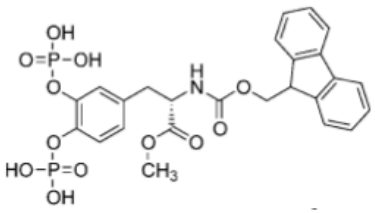
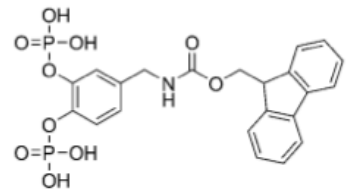
5



L-DOPA

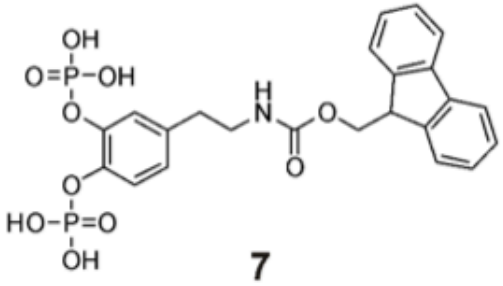
8

Compounds	K_i [μM] (STAT5b)	K_i [μM] (STAT5a)	Selectivity factor
4 	0.69 ± 0.04	19.2 ± 2.7	28
5 	0.82 ± 0.05	2.5 ± 0.2	3
6 	0.73 ± 0.07	21 ± 5	30

Compounds	K_i [μM] (STAT5b)	K_i [μM] (STAT5a)	Selectivity factor
7 	0.45 ± 0.04	16.0 ± 0.8	35
8 	0.46 ± 0.08	7.8 ± 1.0	17
9 	0.21 ± 0.04	11 ± 2	52

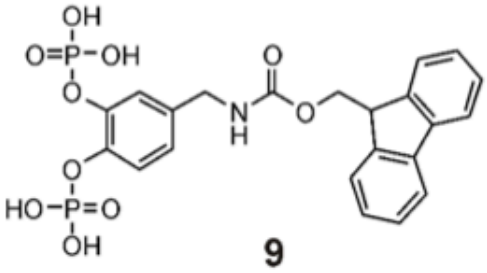
Molecular docking study

STAT5b, K_i = 0.45 µM
STAT5a, K_i = 16 µM
(Selectivity factor: 35)

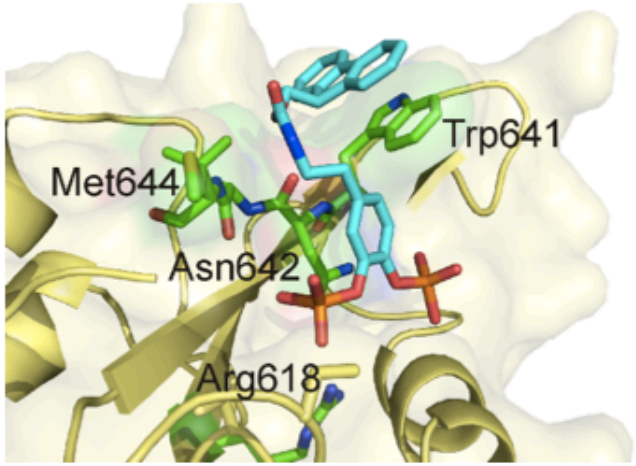


7

STAT5b, K_i = 0.21 µM
STAT5a, K_i = 11 µM
(Selectivity factor: 52)



9



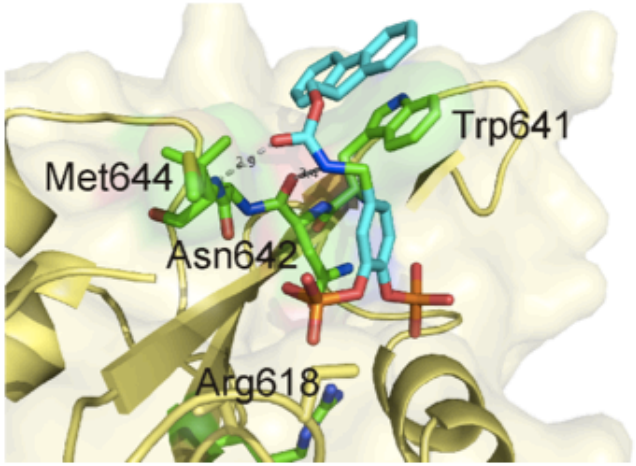
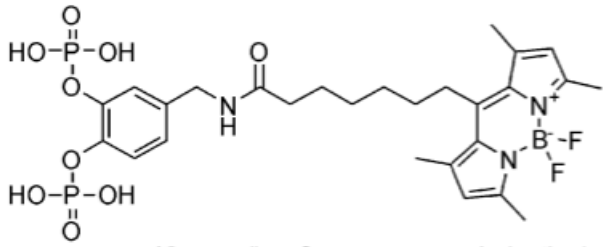


Figure S4. Docking of compounds **7** and **9** into the STAT5b SH2 domain. The amide bond of compound **9** is directed towards the protein, suggesting the possibility of hydrogen bonding with the protein backbone at positions 642 and 644. In contrast, the amide bond of **7** is orientated parallel to the protein backbone, which make hydrogen bonds less likely.

Binding site verification

a)

Tracer molecule for direct binding assay

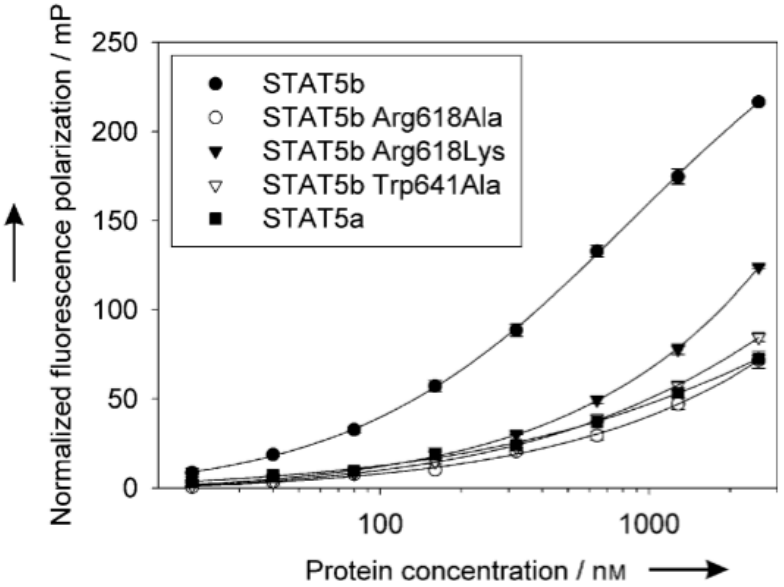


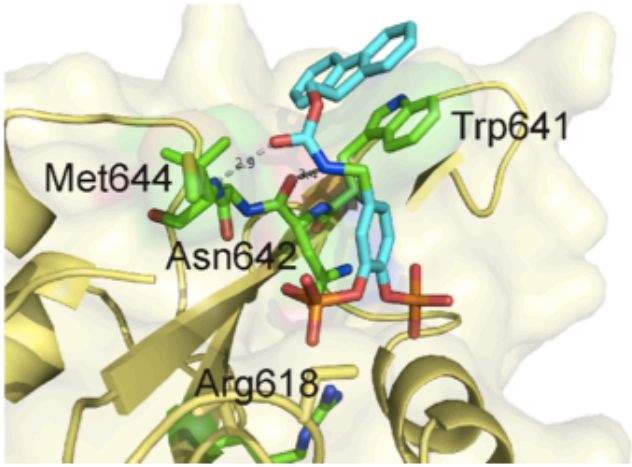
10 (low fluorescence polarization)

Protein

[Protein-**10**] (high fluorescence polarization)

b)

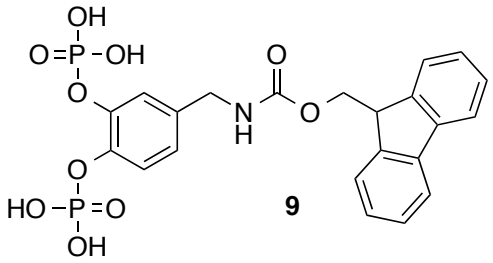




*Binding of point mutant,
Arg618Ala: Markedly reduced
Arg618Lys: Partially reduced
Trp641Ala: Significantly reduced

Figure 2. Validation of the binding mode and specificity of catechol bisphosphate derivatives. Binding was detected by an increased in fluorescence polarization.

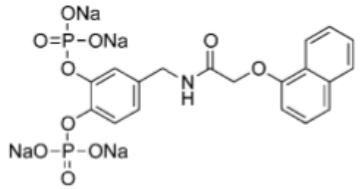
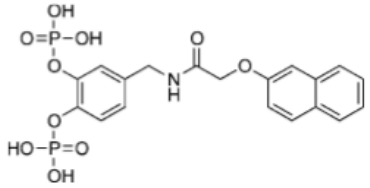
Structure modification III



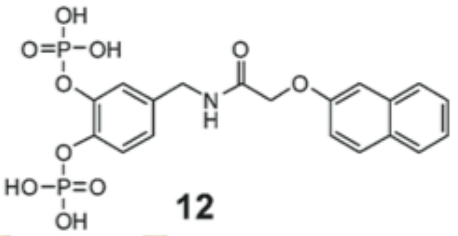
9

STAT5b, K_i = 0.21 μM
STAT5a, K_i = 11 μM
(Selectivity factor: 52)

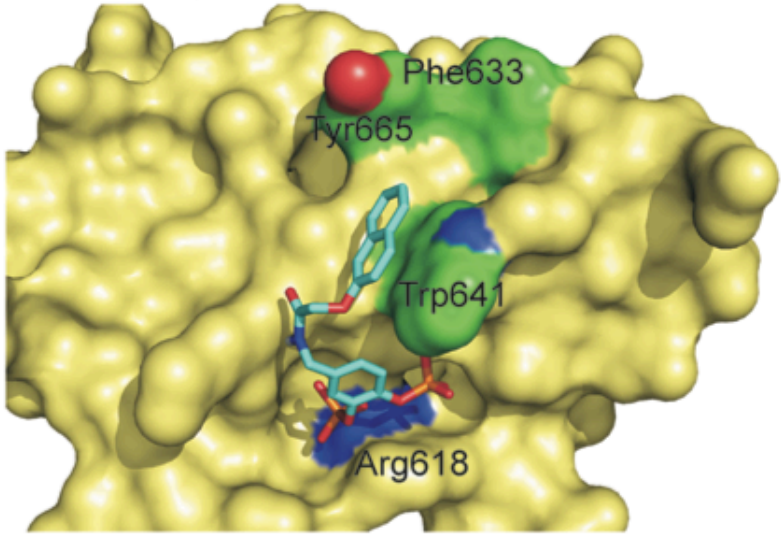
Fmoc to naphthyl group

Compounds	K_i [μM] (STAT5b)	K_i [μM] (STAT5a)	Selectivity factor
11 	0.24 ± 0.01	16.1 ± 1.1	67
12 	0.28 ± 0.02	9.3 ± 0.3	33

a)

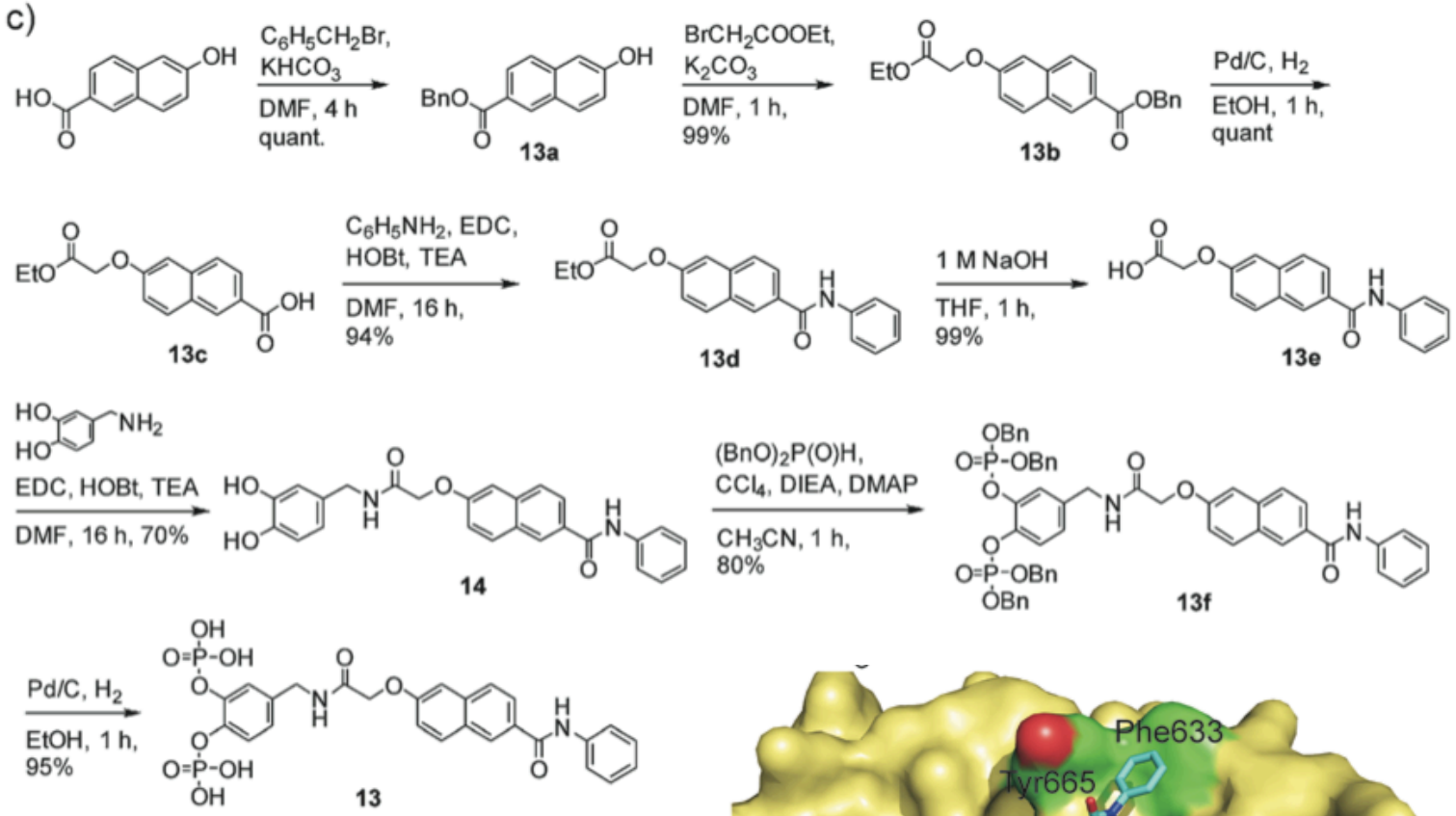


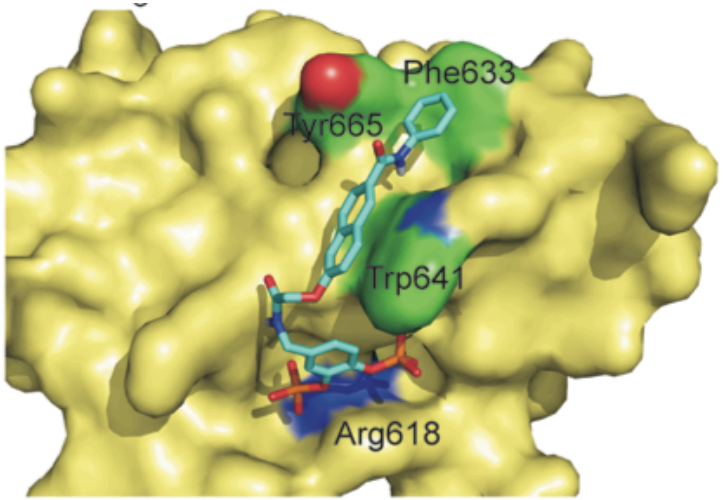
12



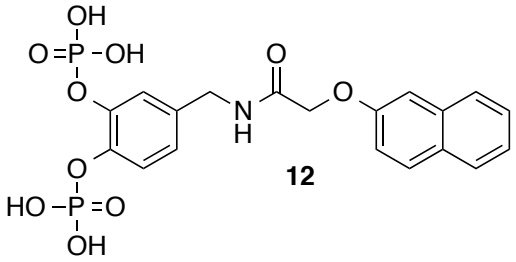
→ Further modification to target hydrophobic pocket created by Phe633 and Tyr665

Structure Modification III



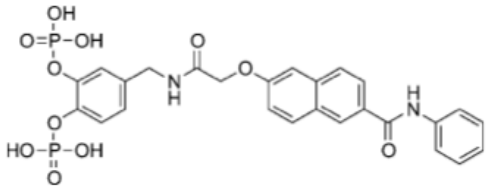
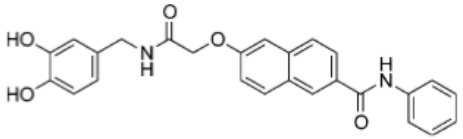
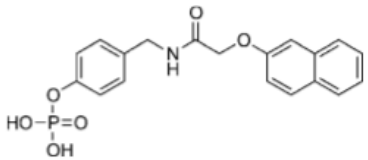


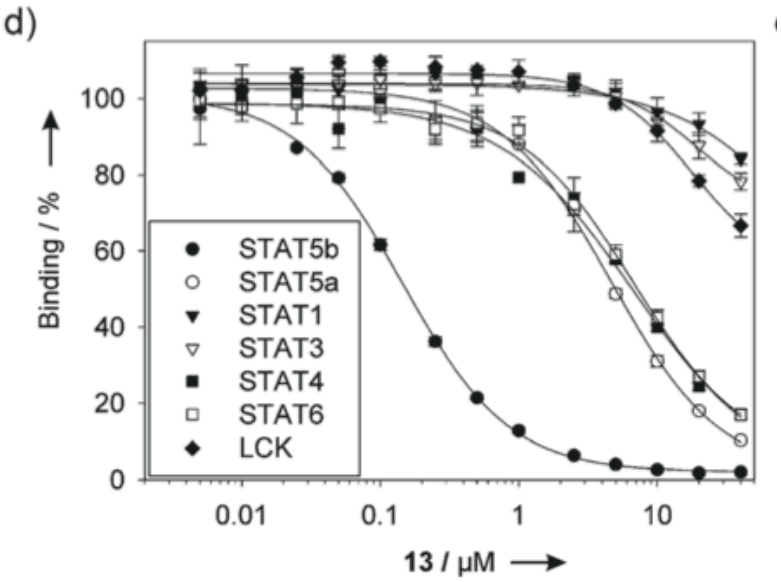
Activities of compounds 13-16

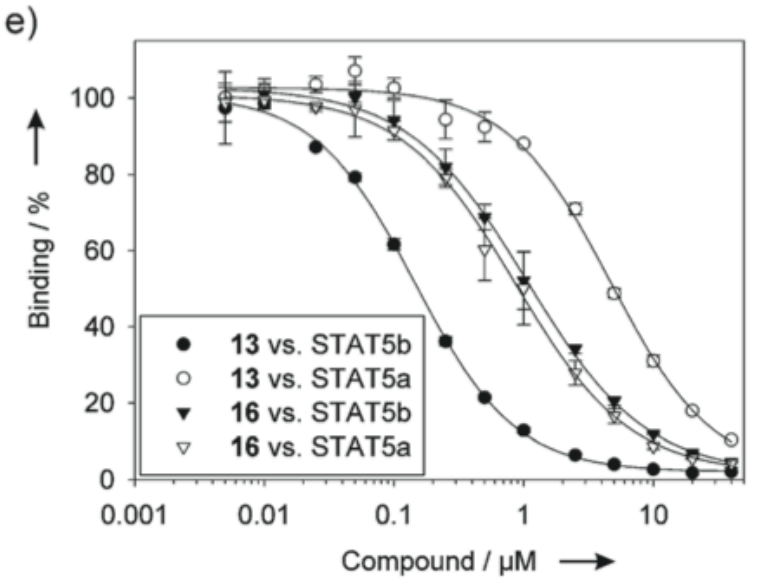


12

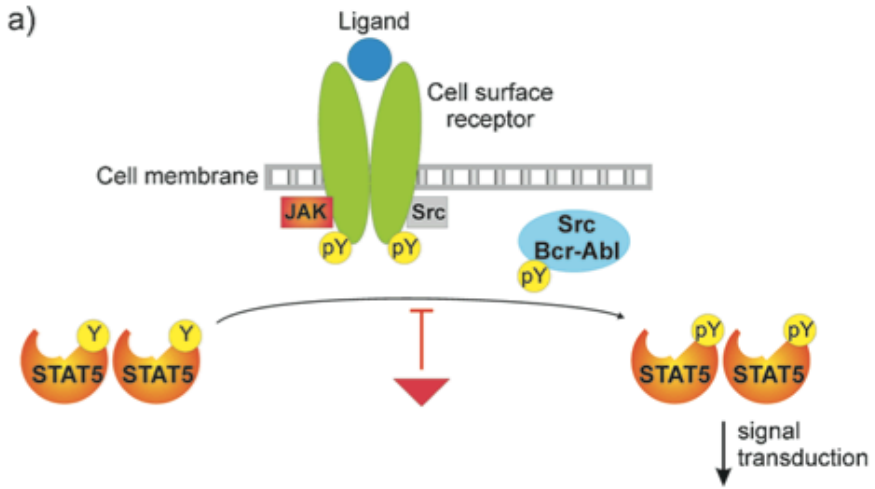
STAT5b, K_i = 0.28 μM
STAT5a, K_i = 9.3 μM
(Selectivity factor: 33)

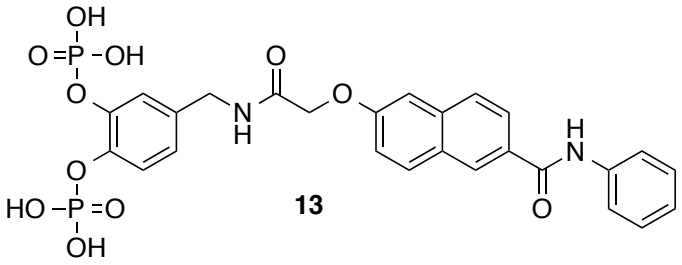
Compounds	K_i [μM] (STAT5b)	K_i [μM] (STAT5a)	Selectivity factor
13 	0.044 ± 0.001	2.42 ± 0.05	55
14 	n.a.	n.a.	n.a.
15 	50 ± 3	40 ± 2	0.80
16 QDTpYLVLDKWL (natural ligand, derived from the EPO receptor)	0.54 ± 0.08	0.41 ± 0.09	0.75





For cell-based assays





STAT5b, K_i = 0.044 μM
STAT5a, K_i = 2.42 μM
(Selectivity factor: 55)

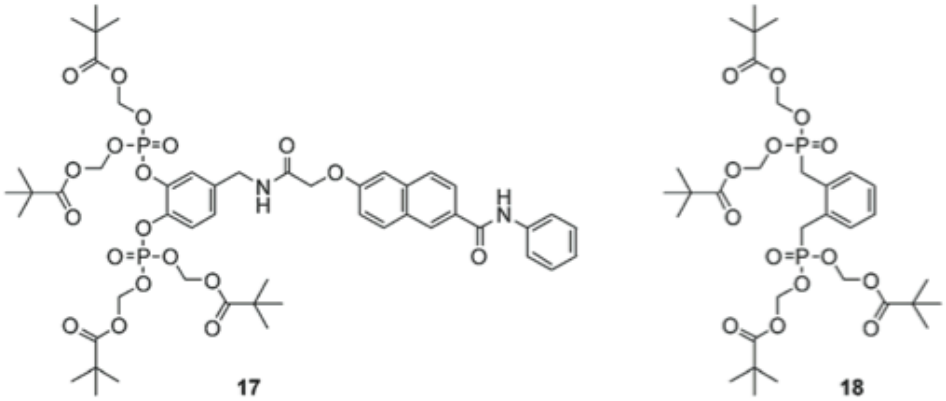
↓ Cell-permeable POM ester **17**

Lack of selective antibodies able to selectively recognize STAT5a/b

↓

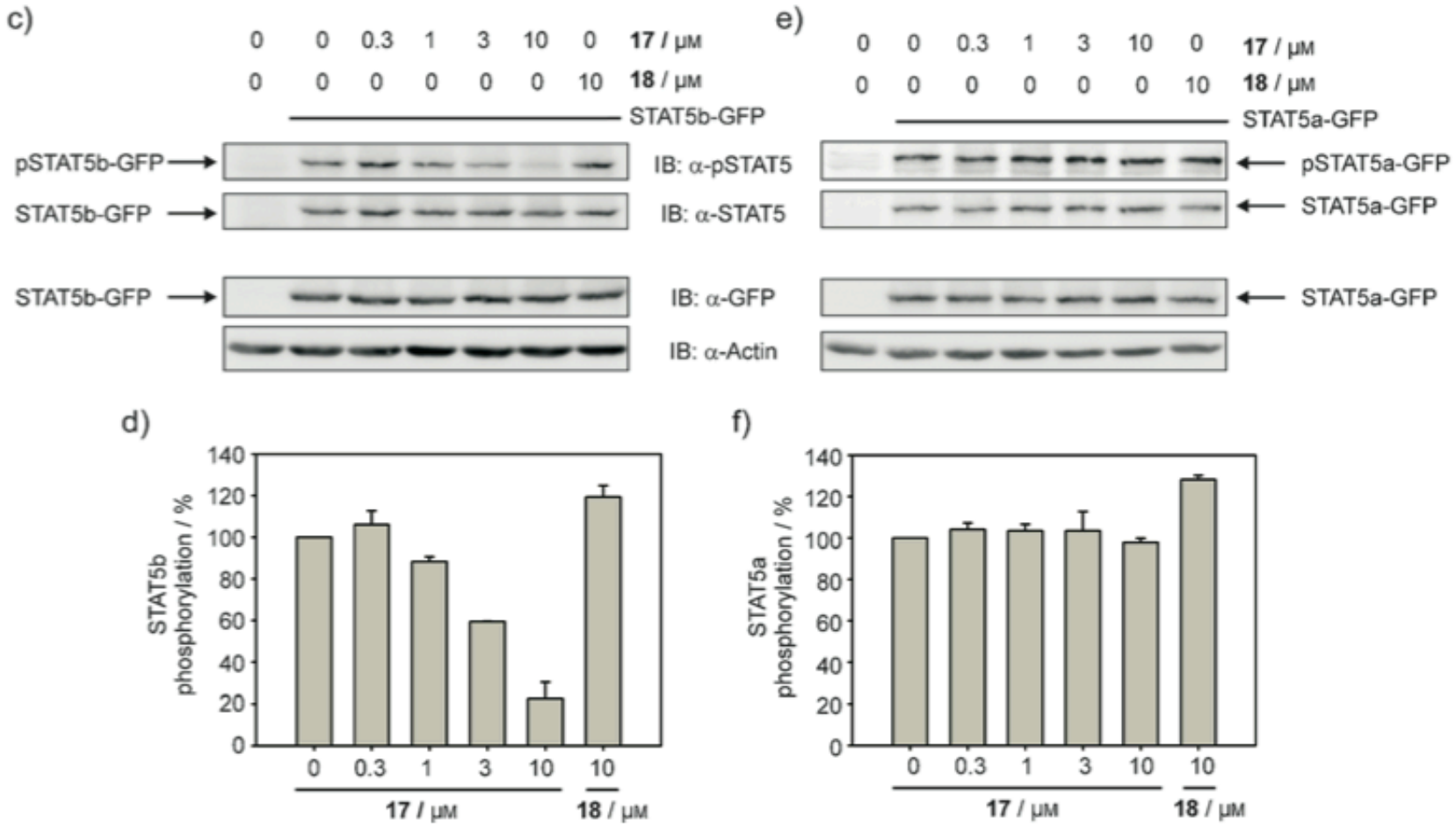
Transfect K562 cell with STAT5a-GFP or STAT5b-GFP

b)



a) STAT signaling is induced by activated cell surface receptors and non-receptor tyrosine kinases. A small-molecule inhibitor of a STAT SH2 domain (the red triangle) inhibits STAT signaling by inhibiting STAT phosphorylation at the conserved tyrosine residue.
b) Structure of **17** and the negative control compound **18**. Neither **17** nor **18** displayed activity against STAT5b in vitro.

Selective inhibition of STAT5b tyrosine phosphorylation by 17

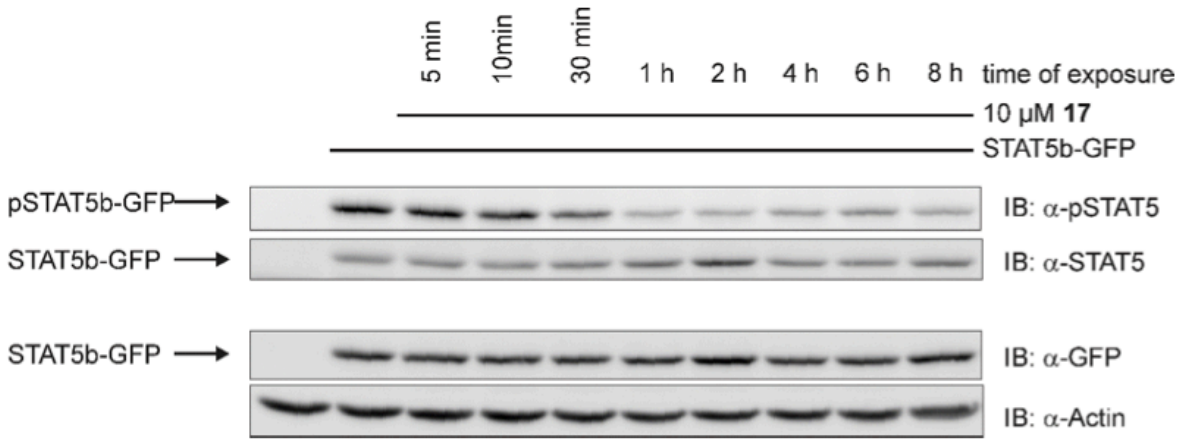


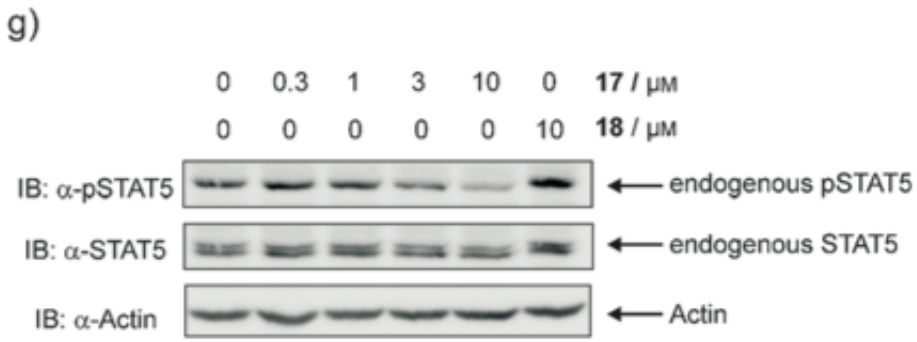
c) Inhibition of STAT5b tyrosine phosphorylation by **17** in K562 cells transfected with STAT5b-GFP. A separate gel was run to confirm even transfection via the GFP tag.
d) Quantification of the pSTAT5b-GFP bands, normalized against total STAT5b- GFP. Error bars represent the standard deviations from two independent experiments.

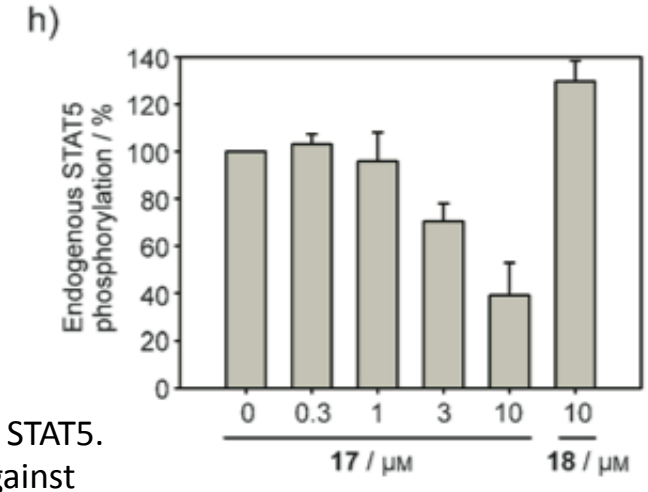
e) Tyrosine phosphorylation of STAT5a in K562 cells transfected with STAT5a-GFP is not inhibited by **17**.
f) Quantification of the pSTAT5a-GFP bands, normalized against total STAT5a-GFP.

Selective inhibition of STAT5b tyrosine phosphorylation by 17

Figure S5 Time course of STAT5b inhibition in K562 cells.
Inhibition was still observed after 8 hours.

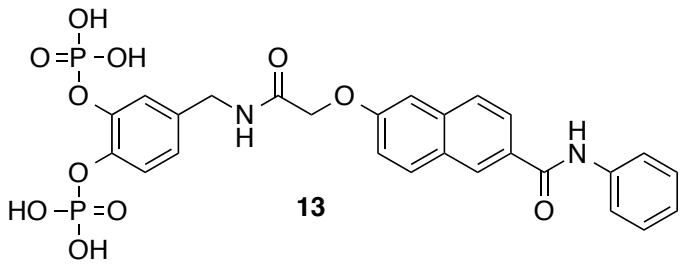






g) Effect of **17** and **18** on tyrosine phosphorylation of endogenous STAT5.
h) Quantification of the endogenous pSTAT5 bands, normalized against total endogenous STAT5.

Summary



13

STAT5b, K_i = 0.044 μM
STAT5a, K_i = 2.42 μM
(Selectivity factor: 55)

- Compound **13** (Stafib-1) selectively inhibits STAT5b

: the first small molecule that can differentiate between the two highly homologous STAT5 proteins

- The first report describing strongly divergent affinities of the SH2 domains of STAT5a/b for a chemical entity, both in vitro and in cultured human cells.

- An example of potent and selective small molecule inhibitors of highly similar protein-protein interaction domains.

- Detailed comparative structural analysis needed: STAT5b vs STAT5a

- Activity against other types of SH2 domain containing proteins?