Chemistry & Biology *13*, November 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.chembiol.2006.11.001

A New Small-Molecule Stat3 Inhibitor

In this issue of *Chemistry & Biology*, Schust et al. [1] report the discovery of a small molecule (Stattic) that inhibits the binding of a high affinity phosphopeptide for the SH2 domain of Stat3. Stattic is a new tool for studying Stat3 signaling and demonstrates that the SH2 domain is not a dead target.

Signal transducer and activator of transcription 3 (Stat3) has received a lot of attention in the past decade because its constitutive activation leads to aberrant growth and survival of human tumors [2]. Stat3 transduces signals from IL-6 family cytokines, epidermal growth factor, platelet-derived growth factor, and others. When the cytokine or growth factor binds to its receptor on the cell surface, the receptors become phosphorylated on tyrosine residues and Stat3 is recruited through its SH2 domain. Tyr705 of Stat3 then becomes phosphorylated by JAK kinases, Src-family kinases, or the kinase activity of the receptor. On dissociating from the receptor, two Stat3 molecules dimerize via reciprocal pTyr705-SH2 domain interactions and the dimer translocates to the nucleus where it initiates the transcription of various genes. Relevant to cancer are the Bcl-2-family of antiapoptotic proteins, cyclin D1, c-*myc*, and others related to survival and cell cycling. Stat3 is a recognized target for anticancer drug development [3], and to inhibit its activity one could potentially target the SH2 domain, the DNA binding domain, or the transactivation domain. Targeting the SH2 domain would prevent recruitment to growth factor or cytokine receptors, impede dimer formation, and block transcriptional activity.

SH2 domains, found in a variety of proteins, are approximately 100-residue cassettes that recognize phosphotyrosine residues and enable recruitment of proteins to cell surface receptors or focal adhesions to form signaling complexes [4]. Amino acids that determine the binding specificity for individual SH2 domains are located 2-5 positions C-terminal to the pTyr [5]. Phosphopeptide complexes with the SH2 domains of important targets such as Src, Lck, and Grb2 were easily studied by NMR and X-ray crystallography, which led to very successful structure-based peptidomimetic inhibitor development programs in industry and academia [6].

The consensus sequence for Stat3 SH2 domain recognition was found to be pYXXQ [7, 8], which was confirmed by a combinatorial phosphopeptide library [9] and a survey of Stat3 docking sites [10]. Unfortunately, NMR or crystal structures of pYXXQ peptide bound to Stat3 have not been published to date, so SH2 domain inhibitor development has been done with targeted libraries based on phosphopeptides [10–14]. One structure-affinity study resulted in a high affinity modified peptide (**2**) which showed an IC_{50} of 125 nM in a fluorescence polarization assay [14] (Figure 1).

The pharmaceutical industry has largely abandoned the SH2 domain as a target because of its requirement of a negatively charged phosphotyrosine or pTyr mimic, which is difficult to deliver to intracellular targets. To date, no high throughput screens targeting SH2 domains have appeared in the literature.

Schust et al. [1] have stepped in and screened over 17,000 small molecules for their ability to compete with a high-affinity phosphopeptide targeted to the SH2 domain of Stat3. Interestingly, 144 compounds inhibited phosphopeptide-protein interactions by >60%, the activity cut-off in their screen. Secondary screens of inhibition of IL-6 driven Stat3 nuclear localization and inhibition of phopsho-Stat3-DNA binding identified 6-nitrobenzo[b]thiophene-1,1-dioxide as a top candidate (**1**, Figure 1). This compound, named Stattic (*Stat*3 *t*hree *i*nhibitory *c*ompound), is selective for Stat3 over Stat1, Stat5, and Lck. Stattic appears to be susceptible to Michael addition because it is only inhibitory in the absence of dithiothreitol in a fluorescence polarization assay. Inhibition increases with time and temperature which suggests alkylation of Stat3. Cys687, on the opposite side of the protein from the phosphopeptide binding face, may be the modified residue. If so, Stattic may not be a direct competitor of phosphopeptide binding, but it may instead alter the conformation of the SH2 domain. The corresponding residue in Stat1 and Stat5 is tyrosine, and Cys687 does not appear to have a corresponding amino acid in Lck, which might account for selective inhibition of Stat3. Unfortunately, attempts to localize the interaction using mass spectrometry have not been conclusive to date. We await the successful completion of these experiments.

Other approaches to inhibitor discovery for SH2 domains have been tried. Virtual screening exercises have resulted in compounds with much lower affinity than phosphopeptides for Lck [15] and Src [16]. Song et al. [17] performed a virtual screen against the SH2 domain of Stat3 and discovered STA-21 (**3**, Figure 1). Although this compound inhibited Stat3 activity in cells as well as binding of activated protein to DNA, no evidence was offered that it actually inhibited phosphopeptide binding to the protein. Could STA-21 bind to the DNA binding domain?

A distinction should be made between a Stat3 inhibitor and a Stat3 pathway inhibitor. The former inhibits the protein by direct binding, as in the case of Stattic and the phosphopeptides, whereas the latter refers to compounds that result in reduced activation of Stat3 by indirect means. In recent years, several compounds have been reported to reduce Stat3 phosphorylation and/or expression, but they do not bind to Stat3. For example, in a screening program to discover Stat3 inhibitors, Blaskovitch et al. [18] discovered that cucurbitacin (**4**), a steroidal natural product, reduces Stat3 phosphorylation, but does not directly bind to Stat3. The analog

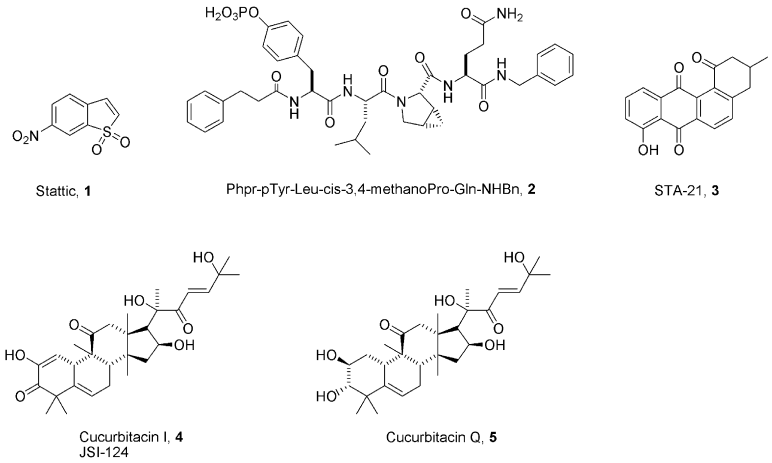

Figure 1. Structures of Compounds Discovered in the Search for Stat3 Inhibitors

cucurbitacin Q (5) is also a potent Stat3 pathway inhibitor [19]. Given the interest in this pathway, other small molecules will undoubtedly continue to appear in the literature.

This discovery of a small molecule that can inhibit phosphopeptide binding to an SH2 domain is a great accomplishment which shows that these domains are still potential targets for drug delivery. In a broader sense, Stattic is an example of a small molecule inhibitor of protein-protein interactions, which are very important for signal transduction in the cell. The big advantage that Stattic holds over phosphopeptides and their derivatives is its ready transportation across cell membranes. When interpreting the effects on cells, however, caution must be exercised. Stattic with its Michael acceptor potential may be subject to redox reactions and several biochemical reactions in the cell that may affect pathways other than Stat3.

John S. McMurray[1]
[1] Department of Experimental Therapeutics
The University of Texas M.D. Anderson Cancer Center
1515 Holcombe Boulevard, Unit 36
Houston, Texas 77030

Selected Reading

1. Schust, J., Sperl, B., Hollis, A., Mayer, T.U., and Berg, T. (2006). Chem. Biol. *13*, this issue, 1235–1242.
2. Levy, D.E., and Darnell, J.E., Jr. (2002). Nat. Rev. Mol. Cell Biol. *3*, 651–662.
3. Yu, H., and Jove, R. (2004). Nat. Rev. Cancer *4*, 97–105.
4. Pawson, T. (1995). Nature *373*, 573–580.
5. Songyang, Z., and Cantley, L.C. (1995). Trends Biochem. Sci. *20*, 470–475.
6. Metcalf, C.A., III, and Sawyer, T. (2004). In Drug Discovery Strategies and Methods, A. Makriyannis and D. Biegel, eds. (New York: Marcel Dekker, Inc.), pp. 23–59.
7. Stahl, N., Farruggella, T.J., Boulton, T.G., Zhong, Z., Darnell, J.E., and Yancopoulos, G.D. (1995). Science *267*, 1349–1353.
8. Gerhartz, C., Heesel, B., Sasse, J., Hemmann, U., Landgraf, C., Schneider-Mergener, J., Horn, F., Heinrich, P.C., and Graeve, L. (1996). J. Biol. Chem. *271*, 12991–12998.
9. Wiederkehr-Adam, M., Ernst, P., Muller, K., Bieck, E., Gombert, F.O., Ottl, J., Graff, P., Grossmuller, F., and Heim, M.H. (2003). J. Biol. Chem. *278*, 16117–16128.
10. Ren, Z., Cabell, L.A., Schaefer, T.S., and McMurray, J.S. (2003). Bioorg. Med. Chem. Lett. *13*, 633–636.
11. Turkson, J., Kim, J.S., Zhang, S., Yuan, J., Huang, M., Glenn, M., Haura, E., Sebti, S., Hamilton, A.D., and Jove, R. (2004). Mol. Cancer Ther. *3*, 261–269.
12. Turkson, J., Ryan, D., Kim, J.S., Zhang, Y., Chen, Z., Haura, E., Laudano, A., Sebti, S., Hamilton, A.D., and Jove, R. (2001). J. Biol. Chem. *276*, 45443–45455.
13. Shao, H., Cheng, H.Y., Cook, R.G., and Tweardy, D.J. (2003). Cancer Res. *63*, 3923–3930.
14. Coleman, D.R., IV, Ren, R., Mandal, P.K., Cameron, A.G., Dyer, G.A., Muranjan, S., Chen, X., and McMurray, J.S. (2005). J. Med. Chem. *48*, 6661–6670.
15. Huang, N., Nagarsekar, A., Xia, G., Hayashi, J., and MacKerell, A.D., Jr. (2004). J. Med. Chem. *47*, 3502–3511.
16. Lange, G., Lesuisse, D., Deprez, P., Schoot, B., Loenze, P., Benard, D., Marquette, J.-P., Broto, P., Sarubbi, E., and Mandine, E. (2003). J. Med. Chem. *46*, 5184–5195.
17. Song, H., Wang, R., Wang, S., and Lin, J. (2005). Proc. Natl. Acad. Sci. USA *102*, 4700–4705.
18. Blaskovich, M.A., Sun, J., Cantor, A., Turkson, J., Jove, R., and Sebti, S.M. (2003). Cancer Res. *63*, 1270–1279.
19. Sun, J., Blaskovich, M.A., Jove, R., Livingston, S.K., Coppola, D., and Sebti, S.M. (2005). Oncogene *24*, 3236–3245.