DOI: 10.1002/cbic.200700701

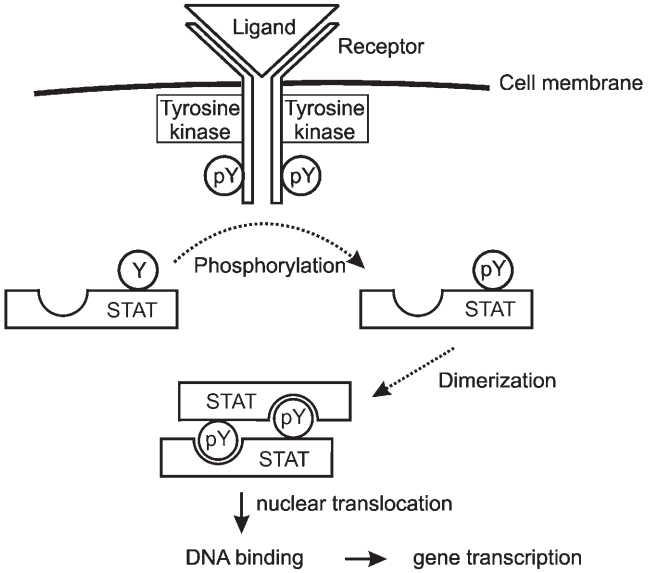
Discovery of Chromone-Based Inhibitors of the Transcription Factor STAT5

Judith Müller, Bianca Sperl, Wolfgang Reindl, Anke Kiessling, and Thorsten Berg*[a]

Molecular signals originating at the cell surface are conveyed by a complex system of interconnected signaling pathways to the nucleus. They converge at transcription factors, which in turn regulate the transcription of sets of genes which ultimately determine the cellular phenotype. Whereas enzymes involved in signaling pathways, that is, intracellular kinases and phosphatases and receptor tyrosine kinases, have been recognized and exploited as intervention points for modulating cellular properties with small organic molecules,[1] transcription factors are often considered "nondruggable" because of their lack of enzymatic activities. However, as many transcription factors require interactions with themselves or other proteins, cell-permeable inhibitors of protein–protein interactions could provide an approach towards the inhibition of this important class of proteins, and would thereby allow for the analysis of transcription factor functions and for therapeutic intervention of diseased states.[2,3] Initially regarded as unfeasible, a growing body of evidence indicates that the inhibition of protein–protein interactions can be potently and selectively achieved by drug-like molecules,[4–11] some of which are even undergoing clinical trials.[12,13]

STATs (signal transducers and activators and transcription) are a family of transcription factors, which require their Src-homology 2 (SH2) domain at two steps of the signaling process to be active. Firstly, STATs need to bind via their SH2 domain to activated receptors and nonreceptor tyrosine kinases (NRTKs), and can subsequently be phosphorylated at a conserved tyrosine residue C-terminal of their SH2 domain (Scheme 1). Secondly, upon tyrosine phosphorylation, STATs dissociate from the respective receptor or NRTK, and form dimers via reciprocal interactions between their SH2 domains and the sequences surrounding the phosphorylated tyrosine residue. Therefore, a small molecule which inhibits the protein–protein interactions mediated by the SH2 domain of STATs could inhibit STAT functions efficiently (Scheme 1).[14] Direct inhibition of STATs is less likely to result in unintentional inhibition of additional signaling pathways than the targeting of upstream kinases.

Two STAT family members, STAT3 and STAT5, have been recognized as therapeutic targets for many human tumors.[15,16] We have recently identified a small-molecule inhibitor of STAT3, which acts by selectively inhibiting the function of the STAT3 SH2 domain, thus validating the outlined approach



Scheme 1. Simplified model of STAT signaling induced by activated cytokine receptors. The signaling steps indicated by the dashed arrows (phosphorylation and dimerization) could be inhibited by an inhibitor of the SH2 domain of STAT family members.

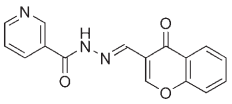
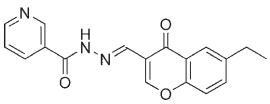
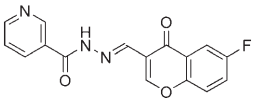
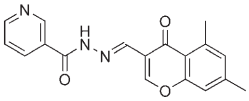
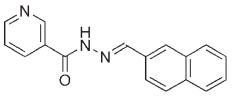
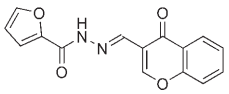
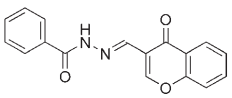
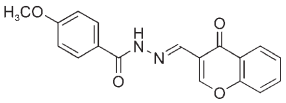
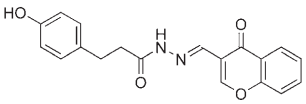
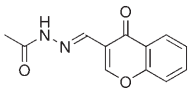
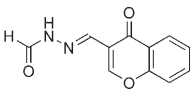
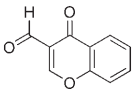
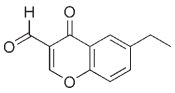
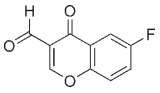
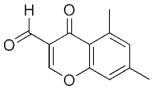
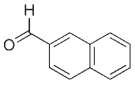
toward STAT inhibition.[17] Two isoforms of STAT5 exist, dubbed STAT5a and STAT5b, which are 93% identical at the amino acid level. STAT5 is overactive in several kinds of leukemias, and also in breast cancer, uterine cancer, prostate cancer, and squamous cell carcinoma of the head and neck (SCCHN).[18] As the inhibition of signaling by STAT5 has been shown to inhibit tumor growth and to induce apoptosis of tumor cells,[19–21] direct inhibition of the STAT5 protein would be desirable to help dissect and counteract the role of STAT5 in cancer. Small-molecule inhibitors of STAT5 could furthermore be useful tools to clarify the relevance of STAT5 for various cellular processes in genetically unmodified systems.[22] Despite the significant interest in small-molecule inhibitors of STAT5, to the best of our knowledge, nonpeptidic molecules which inhibit the function of the STAT5 SH2 domain have not been published to date.

To identify organic molecules which can inhibit the function of the SH2 domain of STAT5, we used a homogeneous assay based on fluorescence polarization which monitors binding of the peptide 5-carboxyfluorescein-GY(PO_3H_2)LVLDKW, which is derived from the erythropoietin (EPO) receptor,[23,24] to the SH2 domain of STAT5b.[17,25] Screening of diverse chemical libraries consisting of a total of 17298 molecules for compounds which disrupt the interaction between STAT5b and its binding peptide led to the identification of the chromone-derived acyl hydrazone **1** (Table 1, apparent $IC_{50} = 47 \pm 17$ μM). The functions of the SH2 domains of STAT3, STAT1, and of the tyrosine kinase Lck were inhibited to a lesser extent (Table 1, Figure 1).

[a] *J. Müller, B. Sperl, W. Reindl, Dr. A. Kiessling, Dr. T. Berg*
Max Planck Institute of Biochemistry, Department of Molecular Biology
Am Klopferspitz 18, 82152 Martinsried (Germany)
and Munich Center for Integrated Protein Science (CiPSM)
Fax: (+49) 89-8578-2454
E-mail: berg@biochem.mpg.de

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

Table 1. Activities of compounds **1**–**16** against the SH2 domains of STAT5b, STAT3, STAT1, and Lck in the fluorescence polarization assay.

No	Structure	STAT5b apparent IC_{50} [μM] or inhibition [%][a]	STAT3 apparent IC_{50} [μM] or inhibition [%][a,b]	STAT1 apparent IC_{50} [μM] or inhibition [%][a,b]	Lck apparent IC_{50} [μM] or inhibition [%][a,b]
1		47 ± 17 μM	36 ± 4% inhibition at 500 μM	26 ± 3% inhibition at 500 μM	7 ± 7% inhibition at 500 μM
2		53 ± 32 μM	54 ± 8 μM	52 ± 1 μM	34 ± 4% inhibition at 500 μM
3		79 ± 20 μM	159 ± 19 μM	396 ± 84 μM	12 ± 1% inhibition at 500 μM
4		217 ± 45 μM	107 ± 9 μM	162 ± 36 μM	340 ± 2 μM
5		311 ± 61 μM	n.d.	n.d.	n.d.
6		56 ± 10 μM	50 ± 3% inhibition at 500 μM	43 ± 3% inhibition at 500 μM	17 ± 8% inhibition at 500 μM
7		53 ± 24 μM	241 ± 55 μM	38 ± 2% inhibition at 500 μM	23 ± 8% inhibition at 500 μM
8		64 ± 26 μM	176 ± 22 μM	351 ± 23 μM	38 ± 3% inhibition at 500 μM
9		90 ± 30 μM	47 ± 3% inhibition at 500 μM	42 ± 8% inhibition at 500 μM	18 ± 6% inhibition at 500 μM
10		86 ± 27 μM	242 ± 43 μM	46 ± 1% inhibition at 500 μM	7 ± 6% inhibition at 500 μM
11		92 ± 13 μM	343 ± 14 μM	36 ± 4% inhibition at 500 μM	6 ± 4% inhibition at 500 μM
12		15 ± 1 μM	54 ± 7 μM	69 ± 5 μM	381 ± 48 μM
13		11 ± 2 μM	20 ± 2 μM	34 ± 5 μM	111 ± 5 μM
14		22 ± 4 μM	41 ± 11 μM	64 ± 8 μM	287 ± 57 μM
15		51 ± 10 μM	n.d.	n.d	n.d
16		0% inhibition at 500 μM	n.d	n.d	n.d

[a] Proteins were incubated with compounds for 1 h at 22 °C before addition of fluorophore-labeled peptide. See experimental procedures for details. [b] n.d.: not determined.

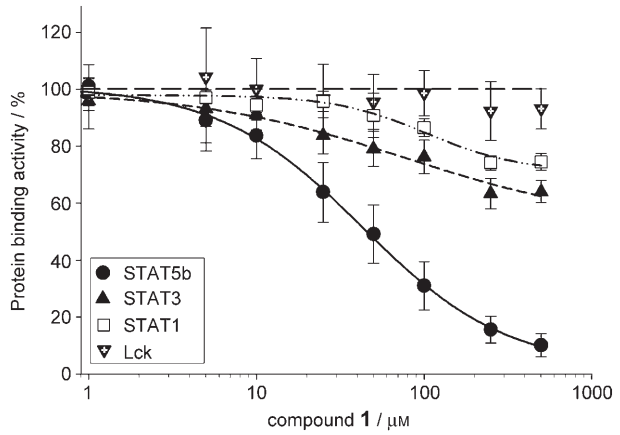


Figure 1. Activity profile of compound **1** against the SH2 domains of STAT5b, STAT3, STAT1, and Lck in the fluorescence polarization assay at 22 °C.

The chemical structure of **1** was confirmed by resynthesis (see the Supporting Information for synthesis of **1** and selected derivatives). Substitution of the hydrogen at C6 of the chromone ring by an ethyl group (compound **2**) did not affect activity of **1** against STAT5b, but led to complete loss of selectivity against other STAT family members (Table 1). A fluorine atom at C6 (compound **3**) decreased activity against STAT5 slightly and also negatively affected the activity profile. Simultaneous introduction of two methyl groups in positions 5 and 7 of the chromone ring decreased the activity against STAT5 by more than fourfold (compound **4**), and even caused preferential inhibition of STAT3 over STAT5b. Substitution of the chromone group for a naphthyl ring (compound **5**) reduced activity against STAT5b by more than sixfold. These data indicate recognition of the chromone part of **1**–**3** by STAT family members.

Analysis of the requirements for the acyl hydrazone parts of the molecules revealed a surprisingly large tolerance for various groups. Substitution of the nicotinoyl residue for furan-2-carbonyl (compound **6**) or benzoyl (compound **7**) resulted in similar potency against STAT5b as that observed for **1** (Table 1). The 4-methoxybenzoyl group also conferred activity, albeit with decreased selectivity (compound **8**). Insertion of a C_2 spacer between the aromatic ring and the acyl carbon (compound **9**) was tolerated with a slight decrease in activity. Stimulated by the wide range of tolerated acyl hydrazone substituents, we investigated the activity of acetyl hydrazone **10** and formyl hydrazone **11**. Both compounds turned out to be only about twofold less active than **1**, which led us to investigate whether chromone aldehydes formed by cleavage of the corresponding acyl hydrazones in aqueous media might be involved in STAT inhibition. In fact, formyl chromone **12** was threefold more active than acyl hydrazone **1** (Table 1). Analogues to the structure–activity relationships observed for hydrazones **2** and **3**, introduction of an ethyl group (compound **13**) or a fluorine (compound **14**) in the 6-position of the chromone moiety changed activity against STAT5b only slightly. As had been observed for the nicotinoyl hydrazones **4** and **5**, the presence of methyl groups in positions 5 and 7 of the chromone ring (compound **15**) or substitution of the chromone ring for a naphthyl group in (compound **16**) led to significant or even complete loss of activity, arguing for recognition of the chromone moiety by STAT5.

The discovery of chromone aldehydes as inhibitors of STAT family proteins might suggest a model by which, under the assay conditions, acyl hydrazones **1**–**11** are cleaved to the respective aldehydes which represent the active species. However, the following three observations argue for a more complex inhibition mode, as they suggest a contribution of the acyl hydrazone parts towards protein recognition. Firstly, the activity profile of aldehydes **12** and **13** against STAT family members differed from the activity profile of the corresponding nicotinoyl hydrazones **1** and **2**. In the case of the unsubstituted chromone core, nicotinoyl hydrazone **1** showed more selective activity than aldehyde **12**, whereas in the context of 6-ethyl chromone, the free aldehyde **13** was more selective than the corresponding nicotinoyl hydrazone **2** (Table 1). Secondly, the specificities of hydrazones bearing the same chromone group differ (compare the activity profile of **1** for STAT5b over STAT3 to the corresponding profiles of **8**, **10**, or **11**). Thirdly, the activities of compounds **1** and **6**–**11** containing the unsubstituted chromone moiety against STAT5b differed from each other, even if only by a factor of two (compare the activities of **1** and **6** to the activities of **9**–**11** in Table 1). Differences in activities and specificities within the group of acyl hydrazones carrying the unsubstituted chromone core were also observed in the assays described in the following paragraphs.

In principle, inhibitors of STAT SH2 domains can be expected to interfere with DNA binding of STATs, as DNA binding requires dimerization of phosphorylated STAT subunits. However, dissociation of preformed, phosphorylated STAT5 dimers, which interact via two reciprocal SH2 domain–pTyr interactions, is a significantly harder task than the inhibition of association between a single SH2 domain and its pTyr-containing ligand. Using the high-affinity peptide QDTpYLVLDKWL known to bind to the STAT5 SH2 domain, we found that dissociation of preformed STAT5 dimers[23] requires approximately 100-fold higher concentrations of this SH2-domain binding peptide ($IC_{50} \approx 50$ μM, Figure S1 in the Supporting Information) than inhibition of binding between a single SH2 domain and its binding motif in the fluorescence polarization assay ($IC_{50} \approx 0.5$ μM).[25] Nevertheless, the most active and selective acyl hydrazones from the fluorescence polarization assay, **1** and **6**, strongly inhibited STAT5 DNA binding at concentrations of 400 μM and higher (Figure 2A). DNA binding of the structurally unrelated transcription factor dimers consisting of Jun und Fos, which need to bind to each other to stably bind to DNA, was not significantly affected by **1** and **6** at 400 μM (Figure 2B). Derivative **9**, and especially the less selective derivative **8** showed only partial, if any, selectivity in this assay (Figure S2 in the Supporting Information).

To validate the results of the fluorescence polarization assay in a more relevant cellular setting, we analyzed the effects of the test compounds on STAT activation, that is, phosphorylation of STATs at the conserved tyrosine residue adjacent to

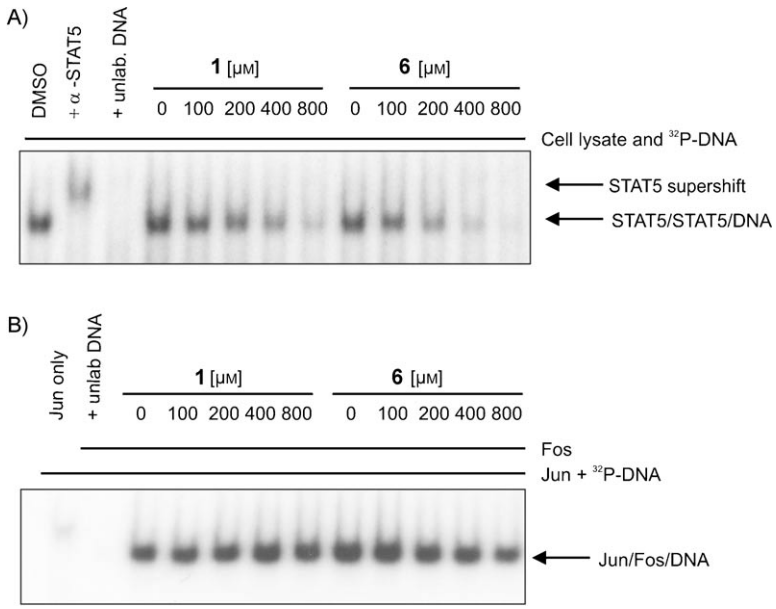


Figure 2. A) DNA binding of pre-phosphorylated STAT5 is inhibited by acyl hydrazones **1** and **6** as analyzed by electrophoretic mobility shift assay (EMSA). All lanes contain ^{32}P-labeled DNA comprising the STAT5 binding site and lysates from K562 cells, which contain activated, that is, tyrosine phosphorylated, STAT5. An antibody against STAT5 in the second lane increases the molecular weight of the STAT5/STAT5/DNA complex and thus causes a reduced mobility of the complex, thereby confirming the identity of the STAT5/STAT5/DNA band. An excess of unlabeled DNA in the third lane competes with the ^{32}P-labeled DNA for the proteins, and thereby abolishes the signal. Experimental conditions used for lanes 1 and 4 are identical. Both compounds **1** and **6** decrease the concentration of the STAT/STAT5/DNA complex in a dose-dependent manner. B) DNA binding of Jun/Fos is not significantly inhibited by **1** and **6** in EMSA. All lanes contain ^{32}P-labeled DNA comprising the Jun/Fos binding site, Jun, and Fos, except for the first lane, which lacks Fos and thereby excludes the possibility that Jun/Jun homodimers are bound to DNA. Fos cannot bind DNA on its own. The second lane contains an excess of unlabeled competitor DNA to verify the identity of the band.

their SH2 domains. The peptide motifs used in the fluorescence polarization assays are derived from the receptors to which STATs bind, and receptor binding is a prerequisite to STAT phosphorylation. Therefore, inhibition of a STAT SH2 domain should be reflected by decreased tyrosine phosphorylation of the respective STAT (Scheme 1). Stimulation of lymphoma cells (Daudi) with IFN-α leads to simultaneous tyrosine phosphorylation of STAT5, STAT1, and STAT3,[26] which is monitored by Western blot analysis using antibodies that recognize STAT proteins only when phosphorylated at their conserved tyrosine residue. Preincubation of the cells with the two most active and selective acyl hydrazones, compounds **1** and **6**, before IFN-α treatment inhibited IFN-α-induced STAT5 tyrosine phosphorylation, but not tyrosine phosphorylation of STAT3 and STAT1 (Figure 3 A). In addition, we analyzed the activity of derivatives **8** and **9**, which had proven less selective (**8**) or slightly less potent (**9**) in the fluorescence polarization assays (Table 1). Cellular activity profiles of **8** and **9** matched their in vitro activity profiles (Figure S3 in the Supporting Information). Compound **4**, which was significantly less active than **1** and **6** in vitro (Table 1), displayed only weak activity in the cellular assay (Figure 3 B). The good correlation between in vitro and cellular activities argues against the notion that the in vitro effects of the acyl hydrazones, albeit observed at relatively high concentrations, could have been influenced by effects not relevant under cellular conditions. As activation of STATs by tyrosine phosphorylation is indispensable for STAT dimerization via reciprocal SH2–phosphotyrosine interactions, which in turn is required for nuclear translocation, DNA binding, and target gene activation, the selective inhibition of STAT5 activation by **1** and **6** inhibits STAT5 signaling at a very early stage. As a result of the high concentrations of compounds needed in the gel shift analysis (see Figure 2), we assume that inhibition of STAT5 phosphorylation is the more relevant cellular mechanism of action than inhibition of dimerization or DNA binding. We did not observe any obvious morphological changes of the Daudi cells upon exposure to the test compounds in these experiments.

As aldehydes **12** and **13** were the most potent agents against STAT5b in the fluorescence polarization assay (Table 1), we also tested their effects in the cellular STAT activation assay. Despite their good potencies in vitro, their ability to inhibit tyrosine phosphorylation of STAT5 was not superior to the corresponding nicotinoyl hydrazone **1** (Figure S4 in the Supporting Information). The low cellular activities of aldehydes **12** and **13** could be caused by unspecific interactions with components of the tissue culture medium or the cell membrane, which would lead to their deactivation before reaching their intracellular targets. The aldehydes' cellular selectivity profiles resembled the selectivity profiles observed in vitro, in that **12** was more selective for STAT5 than **13** (Table 1).

To the best of our knowledge, this manuscript is the first report of nonpeptidic small molecules which inhibit activation of the cancer target STAT5 directly by targeting the function of its SH2 domain. In particular, chromone-based nicotinoyl hydrazone **1** selectively inhibited the function of the STAT5b domain and STAT5 DNA binding in vitro, and selectively inhibited activation of STAT5 in a cancer cell line. Our data provide further evidence for the feasibility of targeting the function of the SH2 domain of STATs with small organic molecules,[17,27,28] and add to the growing body of evidence that transcription factors are in fact amenable to inhibition by low-molecular weight compounds.[3,29,30] Future research will be aimed at understanding the binding mode of the inhibitors, and will assess the suitability of the compounds for the inhibition of STAT5 in a wider range of cell types.

Acknowledgements

This work was supported by the Bundesministerium für Forschung und Technik (NGFN-2, Grant 01GS0451 to T.B.), and the Max Planck Institute of Biochemistry, Department of Molecular Biology (Director: Axel Ullrich). We would like to thank T.U. Mayer (University of Konstanz) for providing access to his chemical

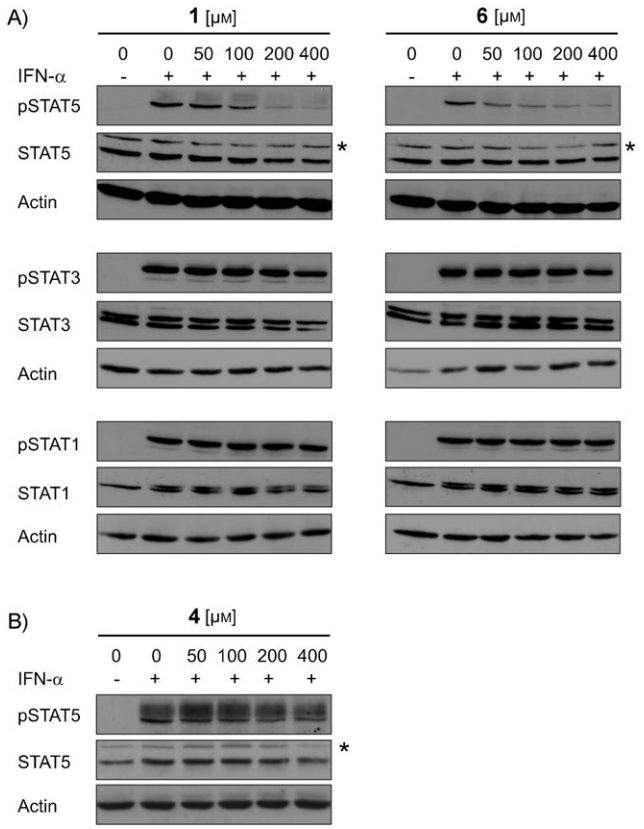


Figure 3. A) Inhibition of STAT5 tyrosine phosphorylation, but not of STAT3 and STAT1 tyrosine phosphorylation, by inhibitors **1** and **6**. Lysates of unstimulated Daudi cells are shown in the first lane. In all other lanes, cells were treated with IFN-α to induce phosphorylation of STAT5, STAT3, and STAT1. Cells were incubated with the indicated concentrations of compounds for 1 h before IFN-α stimulation. STAT phosphorylation was monitored using phosphospecific antibodies which only recognize the respective STAT proteins when the conserved tyrosine residue at the C terminus of their SH2 domain is phosphorylated. Reblots using antibodies which recognize STATs regardless of their phosphorylation state, and antibodies against actin were performed to control for equal loading of the lanes. The STAT5 antibodies do not discriminate between STAT5 isoforms. B) Compound **4** had only a weak effect on STAT5 tyrosine phosphorylation. * denotes an additional protein recognized by the STAT5 antibody.

library, Lothar Hennighausen (NIH, Bethesda, USA) for initial discussions on STATs, and Angela Hollis and Jochen Schust for preliminary studies.

Keywords: inhibitors · protein–protein interactions · screening · signal transduction · transcription factor

[1] O. M. Fischer, S. Streit, S. Hart, A. Ullrich, *Curr. Opin. Chem. Biol.* **2003**, *7*, 490–495.
[2] J. E. Darnell, Jr., *Nat. Rev. Cancer* **2002**, *2*, 740–749.
[3] H. D. Arndt, *Angew. Chem.* **2006**, *118*, 4664–4673; *Angew. Chem. Int. Ed.* **2006**, *45*, 4552–4560.
[4] M. Arkin, *Curr. Opin. Chem. Biol.* **2005**, *9*, 317–324.
[5] T. Berg, *Angew. Chem.* **2003**, *115*, 2566–2586; *Angew. Chem. Int. Ed.* **2003**, *42*, 2462–2481.
[6] H. Yin, A. D. Hamilton, *Angew. Chem.* **2005**, *117*, 4200–4235; *Angew. Chem. Int. Ed.* **2005**, *44*, 4130–4163.
[7] M. R. Arkin, J. A. Wells, *Nat. Rev. Drug Discovery* **2004**, *3*, 301–317.
[8] D. C. Fry, L. T. Vassilev, *J. Mol. Med.* **2005**, *83*, 955–963.
[9] L. Zhao, J. Chmielewski, *Curr. Opin. Struct. Biol.* **2005**, *15*, 31–34.
[10] L. Pagliaro, J. Felding, K. Audouze, S. J. Nielsen, R. B. Terry, C. Krog-Jensen, S. Butcher, *Curr. Opin. Chem. Biol.* **2004**, *8*, 442–449.
[11] S. Fletcher, A. D. Hamilton, *Curr. Top. Med. Chem.* **2007**, *7*, 922–927.
[12] T. Oltersdorf, S. W. Elmore, A. R. Shoemaker, R. C. Armstrong, D. J. Augeri, B. A. Belli, M. Bruncko, T. L. Deckwerth, J. Dinges, P. J. Hajduk, M. K. Joseph, S. Kitada, S. J. Korsmeyer, A. R. Kunzer, A. Letai, C. Li, M. J. Mitten, D. G. Nettesheim, S. Ng, P. M. Nimmer, J. M. O'Connor, A. Oleksijew, A. M. Petros, J. C. Reed, W. Shen, S. K. Tahir, C. B. Thompson, K. J. Tomaselli, B. Wang, M. D. Wendt, H. Zhang, S. W. Fesik, S. H. Rosenberg, *Nature* **2005**, *435*, 677–681.
[13] M. A. Dechantsreiter, E. Planker, B. Matha, E. Lohof, G. Holzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem.* **1999**, *42*, 3033–3040.
[14] D. R. Coleman, Z. Ren, P. K. Mandal, A. G. Cameron, G. A. Dyer, S. Muranjan, M. Campbell, X. Chen, J. S. McMurray, *J. Med. Chem.* **2005**, *48*, 6661–6670.
[15] R. Buettner, L. B. Mora, R. Jove, *Clin. Cancer Res.* **2002**, *8*, 945–954.
[16] D. E. Levy, J. E. Darnell, Jr., *Nat. Rev. Mol. Cell. Biol.* **2002**, *3*, 651–662.
[17] J. Schust, B. Sperl, A. Hollis, T. U. Mayer, T. Berg, *Chem. Biol.* **2006**, *13*, 1235–1242.
[18] I. Wittig, B. Groner, *Curr. Drug Targets Immune Endocr. Metab. Disord.* **2005**, *5*, 449–463.
[19] S. Xi, Q. Zhang, W. E. Gooding, T. E. Smithgall, J. R. Grandis, *Cancer Res.* **2003**, *63*, 6763–6771.
[20] J. B. Demoulin, C. Uyttenhove, D. Lejeune, A. Mui, B. Groner, J. C. Renauld, *Cancer Res.* **2000**, *60*, 3971–3977.
[21] S. Mohapatra, B. Chu, S. Wei, J. Djeu, P. K. Epling-Burnette, T. Loughran, R. Jove, W. J. Pledger, *Cancer Res.* **2003**, *63*, 8523–8530.
[22] T. U. Mayer, *Trends Cell Biol.* **2003**, *13*, 270–277.
[23] F. W. Quelle, D. Wang, T. Nosaka, W. E. Thierfelder, D. Stravopodis, Y. Weinstein, J. N. Ihle, *Mol. Cell. Biol.* **1996**, *16*, 1622–1631.
[24] P. May, C. Gerhartz, B. Heesel, T. Welte, W. Doppler, L. Graeve, F. Horn, P. C. Heinrich, *FEBS Lett.* **1996**, *394*, 221–226.
[25] J. Müller, J. Schust, T. Berg, *Anal. Biochem.* **2008**; DOI: 10.1016/j.ab.2008.01.017.
[26] E. Fasler-Kan, A. Pansky, M. Wiederkehr, M. Battegay, M. H. Heim, *Eur. J. Biochem.* **1998**, *254*, 514–519.
[27] H. Song, R. Wang, S. Wang, J. Lin, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 4700–4705.
[28] K. Siddiquee, S. Zhang, W. C. Guida, M. A. Blaskovich, B. Greedy, H. R. Lawrence, M. L. Yip, R. Jove, M. M. McLaughlin, N. J. Lawrence, S. M. Sebti, J. Turkson, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 7391–7396.
[29] A. Kiessling, B. Sperl, A. Hollis, D. Eick, T. Berg, *Chem. Biol.* **2006**, *13*, 745–751.
[30] A. Kiessling, R. Wiesinger, B. Sperl, T. Berg, *ChemMedChem* **2007**, *2*, 627–630.

Received: November 16, 2007
Published online on February 5, 2008

