Small Molecule Inhibitors of Signal Transducer and Activator of Transcription 3 (Stat3) Protein

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1. INTRODUCTION

Signal transducers and activators of transcription (STATs) are 79−113 kDa proteins playing dual roles as signal transducers and transcription factors. At least seven members have been identified in this family, including Stat1, Stat2, Stat3, Stat4, Stat5A, Stat5B, and Stat6. Although the Stat proteins are structurally related, they participate in different cellular processes.[1,2] Among the members in the STAT family, Stat3 has received particular attention and is the most studied member primarily because of its role in cancer progression, inflammation, cardiomyogenesis, ischemia/reperfusion injury, and stem cell self-renewal.

Stat3 was independently discovered and studied by two research groups and described in 1994. Akira et al. purified and cloned Stat3 from mouse liver nuclear extracts, named it as acute-phase response factor (APRF), and also identified Stat3 as a DNA-binding factor that selectively binds to the IL-6-responsive element within the acute-phase gene promoter.[3] Zhong et al. discovered Stat3 as a DNA-binding protein in response to epidermal growth factor.[4] Since then, multiple Stat3 isoforms have been identified, including the long form Stat3α, the truncated forms Stat3β and Stat3γ, and a putative novel form Stat3δ,[5] all derived from a single gene located within chromosome 17q21 via alternative splicing of the transcript's 3′ end.[5] Stat3α (p92), a 770 amino acid protein, is the predominantly expressed form of Stat3 in most cell types.[6] Stat3β (p83) is an alternatively spliced RNA form of Stat3α, in which the 55 C-terminal amino acids of the transactivation domain are replaced by seven distinct amino acids. Stat3β was generally regarded as a dominant negative Stat3 isoform[7] until recent in vivo experimental evidence showed that Stat3β rescued the embryonic lethality of a Stat3-null mutation and was capable by itself of activating the expression of Stat3 target genes.[8] Compared with Stat3α and Stat3β, the physiologic roles of Stat3γ and Stat3δ are less clear. Stat3γ (p72) is another C-terminal truncated form of Stat3α derived post-translationally through limited proteolysis. Stat3γ is primarily activated in terminally differentiated neutrophils.[9] Stat3δ exists at low levels and decreases with cell differentiation.[5]

In this review, we summarize the signaling pathways of Stat3, its role in different diseases as well as in stem cell maintenance, and the progress in the design, discovery, and development of Stat3 inhibitors since 2006.

2. STAT3 PROTEIN STRUCTURE

The Stat3β structure consists of a coiled coil, a DNA binding, a linker, as well as an SH2 domain and lacks the N-terminal cooperative and C-terminal transactivation domains (Figure 1a). Currently, two crystal structures of mouse Stat3β (1BG1 and 3CWG) are available in the Protein Data Bank.[10,11] 1BG1 consists of a Stat3β homodimer bound to its DNA target sequence with a 2.25 Å resolution (Figure 1b).[10] The phosphorylated Tyr705 along with neighboring residues (702−709) in each monomer (amino acid residues 136−716) is bound to the Src homology 2 (SH2) domain in the other monomer. The SH2 domain comprises three subpockets that can be targeted by small-molecule inhibitors. Residues Lys591, Arg609, Ser611, and Ser613 from the SH2 domain are involved in polar interactions with phospho-Tyr705 (Figure 1c). Leu706 of the phosphopeptide is bound to a hydrophobic pocket of the SH2 domain. Four loops, three from the DNA binding domain and one from the linker domain, form interactions with both DNA strands.[10] Figure 1d shows that the unphosphorylated Stat3 core fragment comprising amino acid residues 136−688 is loosely bound through the SH2 domain (PDB code 3CWG).[11] In the 3CWG crystal structure, Stat3 does not bind DNA because the DNA binding domains are away from each other and are in reverse orientation compared to the 1BG1 structure. The root-mean-square deviation (rmsd) value for C_α atoms of these two crystal structures (core fragment of monomers) is only 0.9 Å, suggesting minor conformational changes between the phosphorylated and unphosphorylated forms.[11]

3. STAT3 SIGNALING PATHWAY

As part of the Janus kinase (JAK) Stat pathway, Stat3 signaling can be activated by both receptor and nonreceptor tyrosine kinases via the tyrosine phosphorylation cascade (Figure 2). The growth factor receptors that are known to cause the activation of Stat3 include epidermal growth factor receptors (EGFRs), human epidermal growth factor receptor (HER2, also known as NEU), fibroblast growth factor receptors (FGFRs), insulin-like growth factor receptors (IGFRs), hepatocyte growth factor receptors (HGFRs, also known as MET), platelet-derived growth factor receptors (PDGFRs), and vascular endothelial growth factor receptors (VEGFRs). After autocrine or paracrine cytokines/growth factors binding to their respective receptors, they undergo homo- or heterodimerization leading to the subsequent activation of intrinsic receptor tyrosine kinases that culminate in the phosphorylation of Stat3 at Tyr705. For receptors lacking intrinsic tyrosine-kinase activity, ligand engagement induces recruitment and activation of receptor-associated tyrosine kinases, such as JAK and SRC. Subsequently, JAK and SRC proteins phosphorylate certain tyrosine residues in the intracellular domain of the receptor, creating docking sites for cytosolic Stat3 via its SH2 domain.
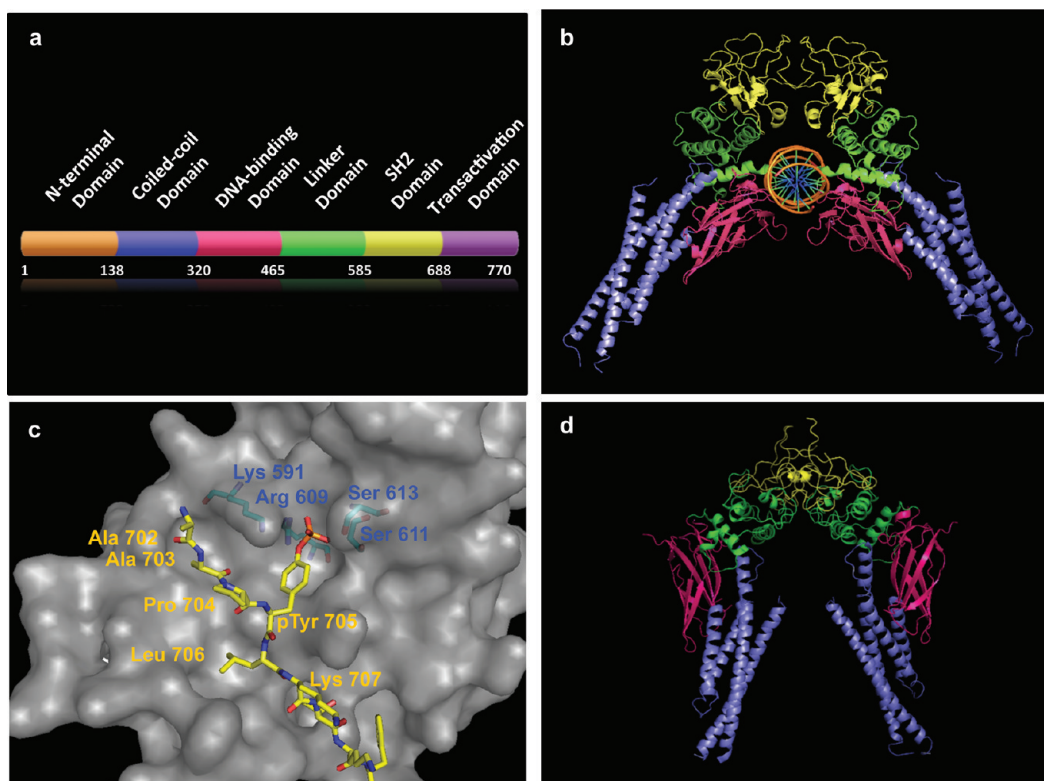
Figure 1. Structural organization of Stat3: (a) structural domains of Stat3; (b) structure of Stat3-β homodimer bound to its DNA recognition site (PDB code 1BG1); (c) Stat3 phosphopeptide (yellow) bound in the Stat3 SH2; (d) structure of Stat3 core fragment homodimer (PDB code 3CWG).

Once recruited, Stat3 becomes phosphorylated at Tyr705 by JAK or SRC. In addition to JAK and SRC proteins, other classes of nonreceptor protein tyrosine kinases have also been reported to stimulate Stat3 activation. One example is Fes that activates Stat3 in human cells and Sf-9 insect cells, suggesting Stat3 may be a direct substrate for Fes.[12] In transformed cells, Stat3 can also be directly activated by constitutively active nonreceptor tyrosine kinases, such as SRC and ABL.[13]

Tyr705 phosphorylation converts Stat3 from an inactive form to an active form. Following phosphorylation and activation, Stat3 proteins undergo dimerization via the interaction between the phospho-Tyr705 within one monomer and the SH2 domain within the other. Stat3 dimers then disassociate from the receptor and translocate into the nucleus, where they bind to specific DNA sequences such as the cis element IFN-stimulated response element (ISRE) and regulate the transcription of target genes. In addition, Ser727 located in the transactivation domain of Stat3 is further activated by the MAPK or mTOR pathways regulating Stat3 transcriptional activity[14] and is suggested to be necessary for its full activation.[15] Besides phosphorylation at Tyr705 and Ser727, Stat3 activity is also regulated by acetylation at Lys685 that stabilizes Stat3 dimers.[16] Stat3 transcriptional activity requires the association with coactivators such as CREB-binding protein (CBP)/p300, APE1/Ref-1, and NCOA/SRC1a.[17,18] The interaction of Stat3 with c-Jun has also been reported to yield maximal enhancer function for a number of genes, including the gene encoding the interleukin-6 (IL-6) induced acute-phase response protein α2-macroglobulin.[19] Additionally, Stat3 activates the transcription of several genes involved in cell cycle progression such as Fos, p21$^{WAF1/CIP2}$, cyclins D1, D2, D3, CDC25A, APE1/Ref-1, c-Myc, and Pim1, genes involved in

angiogenesis such as VEGF, and antiapoptotic genes such as survivin, Bcl-2, and Bcl-xL. Indeed, most Stat3 target genes are key components in the regulation of cell growth, transformation, cell cycle progression, survival, metastasis, and invasion. Apart from gene transcriptional promotion, Stat3 also plays a role in transcriptional suppression. Through its cooperation with c-Jun, active Stat3 down-regulates Fas/CD95 transcription as well as Fas surface expression, which may be mediated by PI3K-AKT signaling.[20] Recently, in vitro and in vivo data further showed that knockdown of Stat3 using Stat3-siRNA in breast cancer resulted in up-regulation of Fas and FADD expression.[21] These findings suggest that Stat3 activities can also be mediated by suppressing the expression of some proapoptotic proteins. It is important to mention that Stat3 not only regulates gene transcription but also participates in some transcription-independent cellular processes.[22] In addition to the well-known localization of Stat3 in the cytosol, associated with receptors at the plasma membrane and in the nucleus, Stat3 was also discovered to be present in isolated mitochondria, suggesting that Stat3 exerts a direct impact on mitochondrial function.[2]

In normal cells, and under physiological conditions, Stat3 activation is a tightly controlled transient process, lasting from 30 min to several hours. Upon inactivation via tyrosine dephosphorylation by nuclear protein-tyrosine phosphatases such as TC-PTP and TC45,[23,24] nuclear Stat3 shuttles back to the cytoplasm through nuclear pores. Apart from the nuclear protein-tyrosine phosphatases, several other negative regulatory mechanisms normally serve to suppress Stat3 activation at different levels of the signal transduction pathway. Upon binding of the correspondent ligands, cytokine or growth factor receptors are rapidly internalized and subsequently recycled or
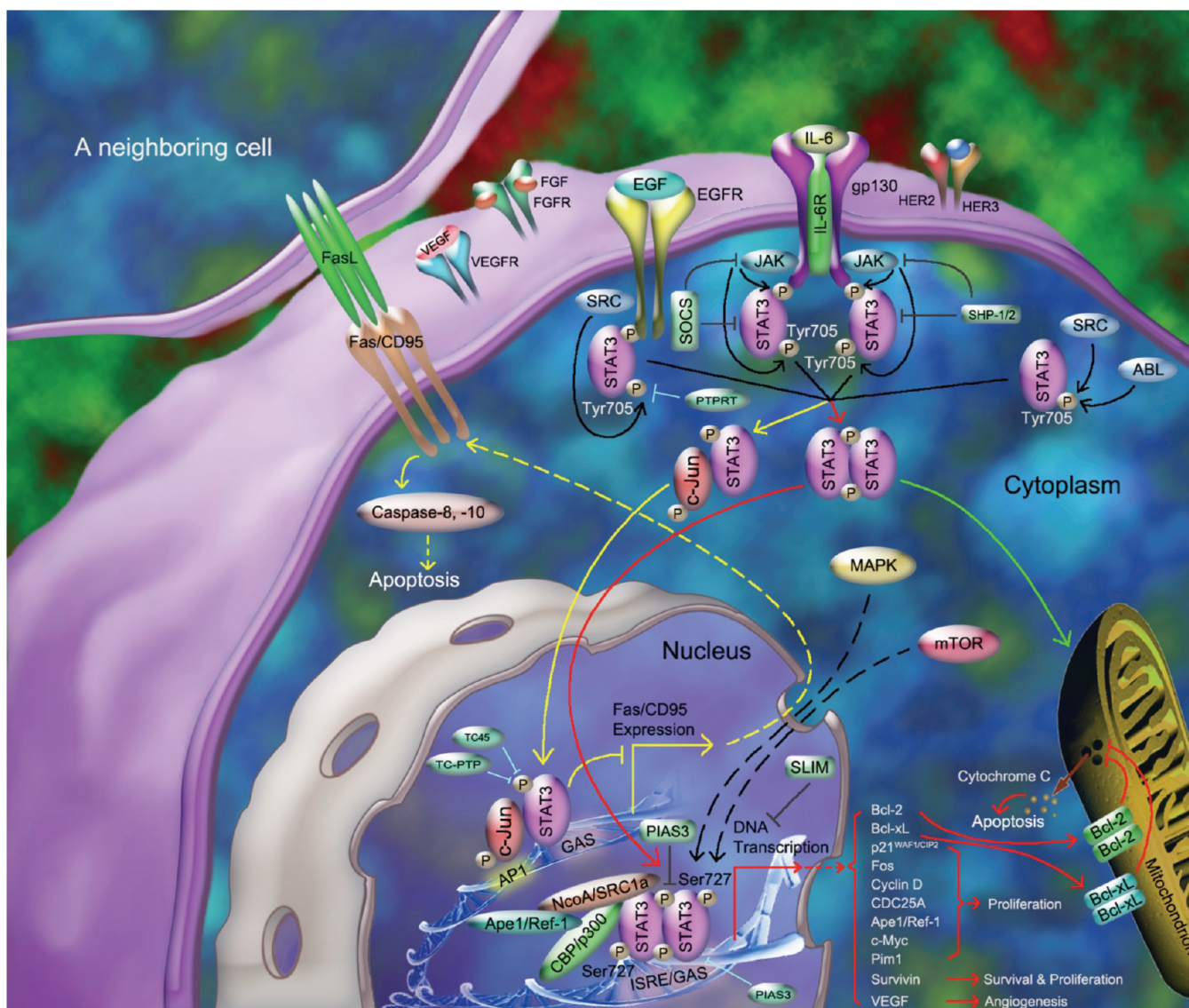
Figure 2. Stat3 signaling pathway. Upon cytokines binding to and activating respective receptors, Stat3 is recruited to the cytoplasmic domain of the receptors, where it is phosphorylated and activated. Phosphorylated Stat3 detaches from the cell surface and forms homo- or heterodimers, which translocate to the nucleus and regulate gene expression. Stat3 activation is negatively regulated by SOCS, PIAS, and SHP-1/2.

degraded by the ubiquitin proteasome pathway.[25] The Src homology domain-containing tyrosine phosphatases 1/2 (SHP-1/2) interact with the intracellular domain of cytokine receptors and dephosphorylate and inactivate JAK, Stat3, and the receptor.[26,27] In addition, active Stat3 binds to the promoter regions of suppressor of cytokine signaling (SOCS) genes.[28] Similar to SHP-1/2, SOCS-1 and SOCS-3 interact with the kinase domain of various JAK proteins or the phosphotyrosine residues in the intracellular portion of the respective receptor, leading to the reduction of Stat3 activation. This is another negative feedback mechanism of the Stat3 signaling pathway and its upstream kinases. However, SOCS-3 does not affect SRC-induced Stat3 activation.[29] Besides SHPs and SOCSs, protein inhibitor of activated Stat3 (PIAS3) specifically inhibits the binding of dimerized phosphorylated Stat3 to DNA, thus abolishing the transcription of Stat3 target genes.[30] Recently, the protein tyrosine phosphatase receptor T (PTPRT) has been shown to specifically dephosphorylate Stat3 at Tyr705 and thereby regulates its cellular localization as well as Stat3 target gene expression.[31]

4. STAT3 AND CANCER

Accumulating evidence in the past few years strongly implicates the critical role of aberrant Stat3 activation in malignant transformation and tumorigenesis. In contrast to the transient nature of Stat3 activation in normal cells, overexpression and/or persistent activation of Stat3 has been reported in most human solid and hematological tumors,[32] including ovarian, endometrial, cervical, breast, colon, pancreatic, lung, brain, renal, and prostate cancers, head and neck squamous cell carcinoma (HNSCC), glioma, melanoma, lymphomas, and leukemias. The expression and activation levels of Stat3 in select cancer and normal tissues as well as cell lines are shown in Figure 3. Stat3 is significantly expressed in some normal tissues including pericardium, neutrophils of bone marrow, leukocytes of peripheral blood, peritoneum, and mammary gland (Figure 3a) and in systems including digestive tract, kidney, bladder, hematopoietic, lung, and peripheral nervous system (PNS) (Figure 3b), indicating that Stat3 plays an important role in the physiological processes in these tissues
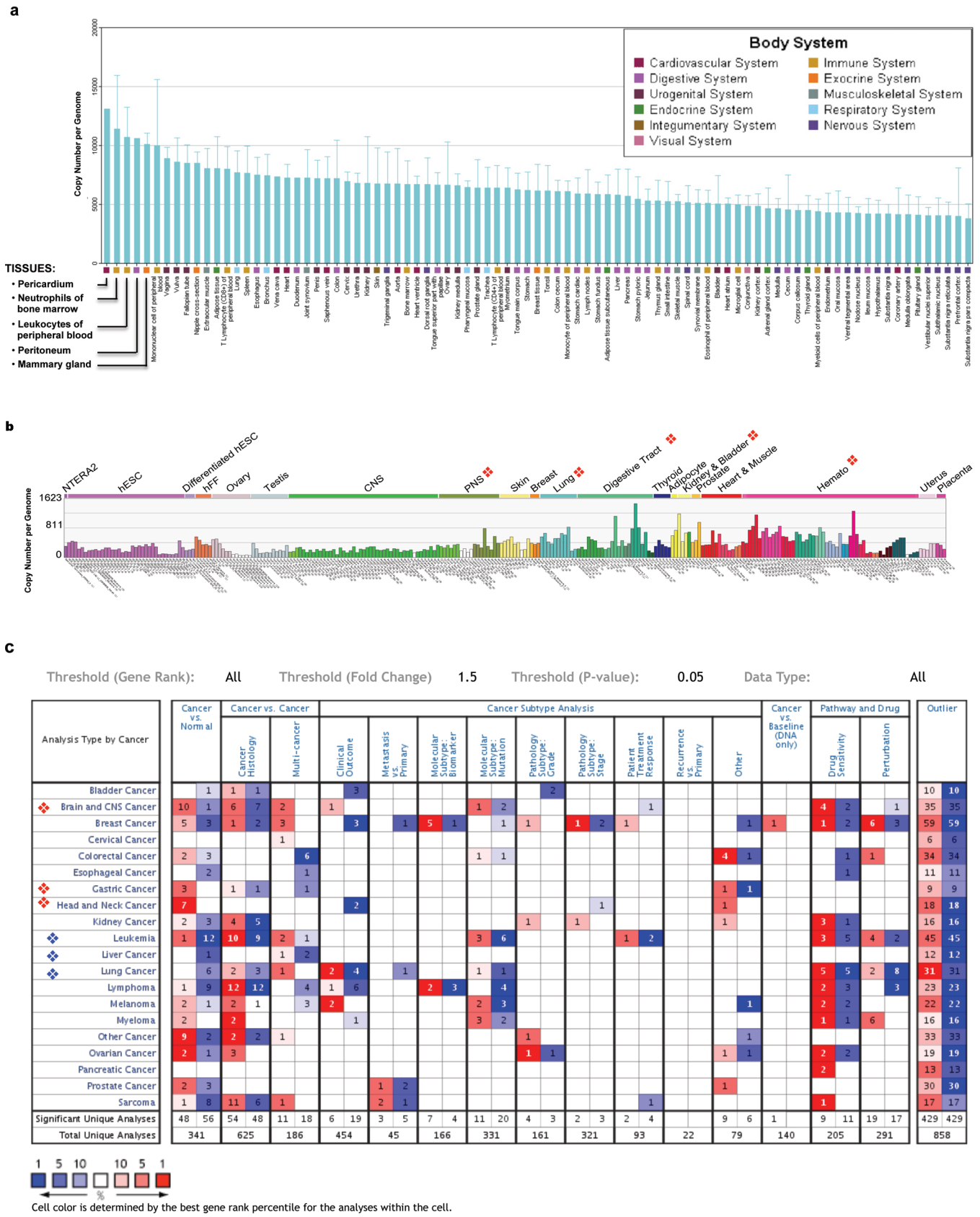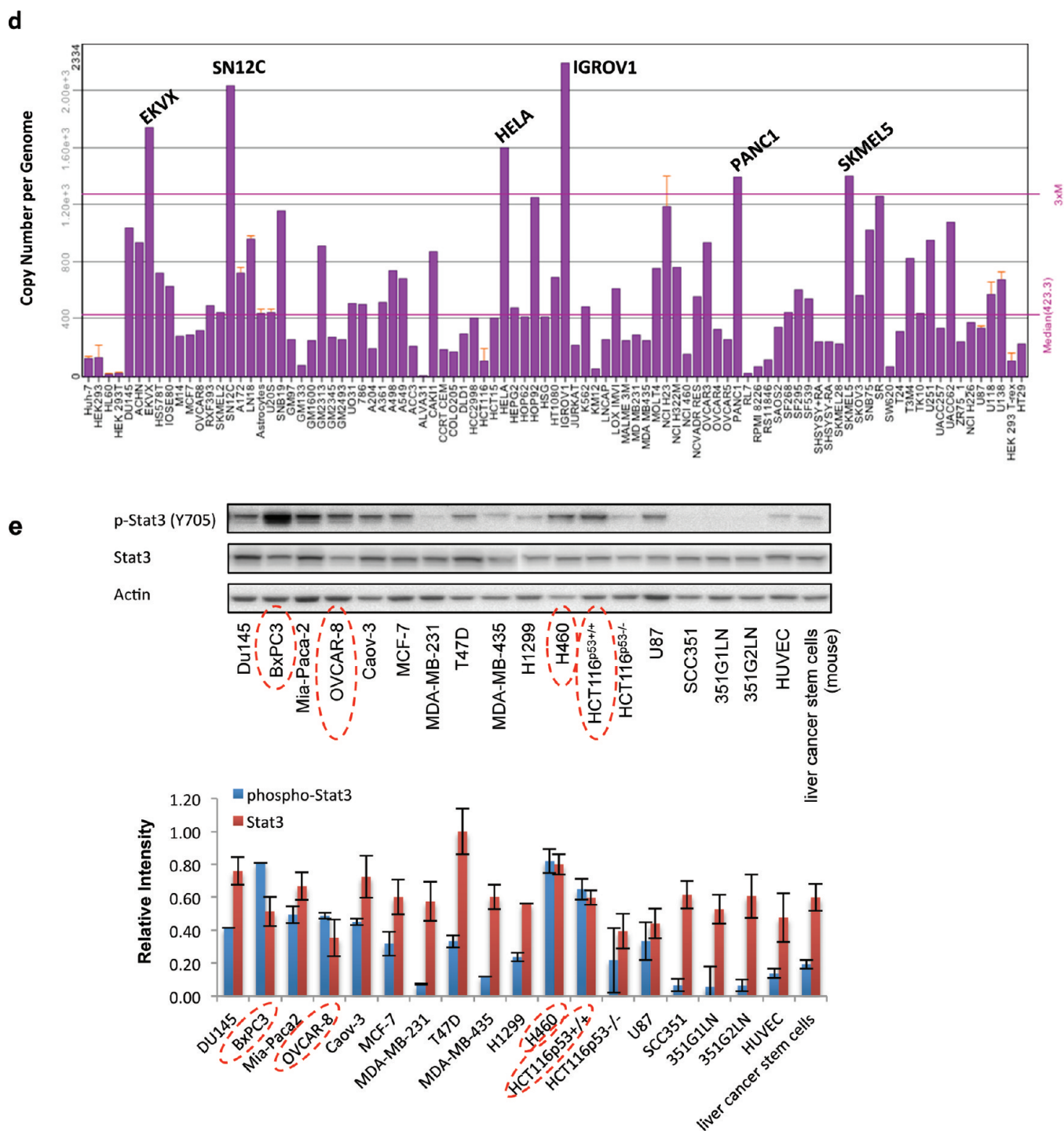
Figure 3. continued

Figure 3. Stat3 expression and activation in human normal and cancer tissues and cell lines. Stat3 levels in various body tissues are displayed (a) from highest to lowest (image generated using NEXTBIO, http://www.nextbio.com[170]) and (b) in groups of tissues (image generated using Amazonia!, http://amazonia.transcriptome.eu[171]). (c) Comparison of the expression levels of Stat3 in selected cancers and respective normal tissues. Image is generated using Oncomine (Compendia Bioscience, Ann Arbor, MI) analysis and visualization. (d) Comparison of the expression levels of Stat3 in NCI-60 human cancer cell lines. Image is generated using BioGPS, http://biogps.org/#goto=welcome.[172] (e) Stat3 expression and phosphorylation at Tyr705 in indicated cell lines analyzed by immunoblotting. Bar indicates SEM.

and organs. Compared with normal tissues, Stat3 is overexpressed in brain and central nervous systems (CNS), head and neck, and gastric cancers but down-regulated in leukemia, liver and lung cancers, lymphomas, and sarcomas (Figure 3c). Among the NCI-60 cell lines, IGROV1, SN12C, EKVX, HELA, PANC1, and SKMEL5 display the highest mRNA levels of Stat3 (Figure 3d). Compared with total Stat3 expression,

phospho-Stat3 (Y705) levels are more functionally related to tumorigenesis. Unlike the transient activation of Stat3 in normal cells, constitutively active Stat3 is present in most human cancer cell lines, especially H460, OVCAR-8, BxPC3, and HCT116$^{p53+/+}$ (Figure 3e). Studies have demonstrated that expression of mutant Stat3 is able to induce oncogenesis in cultured cells and nude mice.[33] While the mechanisms of

constitutively active Stat3 in tumor formation continue to be elucidated, studies on different cancers implicate dysregulation of Stat3 as a key participant in cancer cell growth, proliferation, survival, angiogenesis, metastasis, and invasion and are often correlated with a more malignant tumor phenotype. Additionally, the maintenance of multipotency in glioblastoma stem cells requires receptor signaling through Stat3, the inhibition of which results in suppression of cell growth and induction of differentiation as well as apoptosis,[34] suggesting that Stat3 is also essential for cancer stem cell (CSC) survival. Moreover, active Stat3 plays a role in suppressing the host's immune surveillance of cancer, which also contributes to tumor progression.[35,36] On the basis of this evidence, Stat3 has emerged as a promising drug target for cancer treatment.

5. STAT3 AND OTHER DISEASES

While Stat3's role in cancer remains a major topic in biomedical research, compelling evidence has shown that activation or suppression of Stat3 signaling has a significant contribution in the progression or remission of a series of other human diseases, including fibrosis in kidney, lung, and liver, cardiovascular diseases, rheumatoid arthritis (RA), Alzheimer's disease (AD), psoriasis, and inflammatory bowel disease (IBD) (Table 1).

Table 1. Levels of Stat3 in Pathological Phenotypes

disease condition	stat3 level	ref
cardiomyopathy	↓ pStat3	38
myocardial ischemia/reperfusion injury	↑ pStat3	2
rheumatoid arthritis	↑ pStat3	39
cancer	↑ pStat3	32, 35, 36, 139, 147−165
Alzheimer's disease	??	42
renal fibrosis	↑ pStat3	45
liver fibrosis	↓ pStat3	46, 47
inflammatory lung diseases/pulmonary fibrosis	↑ pStat3	48, 49
psoriasis	↑ pStat3	52, 166, 167
inflammatory bowel disease	↑ pStat3	56
obesity and diabetes type 2	↓ pStat3	57, 58
atherosclerosis	↑ pStat3	60

5.1. Stat3 and Cardiovascular Diseases. Stat3 plays an important role in cardioprotection. Increased Stat3 expression was observed in rat myocardium subjected to ischemia compared with sham operated rats. Cardiospecific overexpression of constitutively active Stat3 in transgenic mice provides protection from myocardial ischemia/reperfusion injury,[2] and cardiospecific activation of Stat3 by IL-11 attenuated cardiac fibrosis after myocardial infarction.[37] In contrast, cardiomyocyte-specific deletion of *Stat3* gene causes postpartum cardiomyopathy (PPCM) in female mice, and decreased levels of Stat3 in myocardium are associated with PPCM patients.[38]

5.2. Stat3 and Rheumatoid Arthritis. Phosphospecific activation of Stat3 is significantly increased in human peripheral blood fibrocytes derived from RA patients. Survival and abnormal growth of RA synoviocytes are associated with Stat3 activity. Stat3-YF (a dominant negative mutant of Stat3) transduced RA synoviocytes failed to grow and survive with markedly diminished [³H]thymidine incorporation.[39] Hyperactivation of Stat3 was observed in RA patients and an RA

mouse model.[40] Mice bearing a single amino acid substitution in gp130 (Y759F) have been reported to exhibit enhanced Stat3 activation that promotes RA development associated with IL-6 stimulation and loss of SOCS3-mediated negative feedback.[41]

5.3. Stat3 and Alzheimer's Disease. Tyk2/Stat3 signaling is involved in β-amyloid-induced neuronal cell death in AD. However, whether Stat3 level and activity is up- or down-regulated in AD is still controversial. Elevated levels of phospho-Stat3 (Tyr705) were observed in the cortex and hippocampus of APP/PS1 transgenic mice and in post-mortem brains of AD patients.[42] Overexpression and activation of Stat3 induce Aβ-mediated neuronal apoptosis and thus cause neuronal cell death.[42] On the other hand, polypeptide humanin and colivelin attenuate AD-related neuronal death by activating Stat3 in vitro. Colivelin ameliorates AD-related memory impairment in vivo by activating Stat3 in the septohippocampal region.[43]

5.4. Stat3 and Renal Fibrosis. Activated Stat3 is preferentially overexpressed in rat tubular epithelial cells and myofibroblasts after unilateral ureteral obstruction. Immuno-histochemical staining in rat kidney tissues on day 7 showed a 6-fold elevation of Stat3 phosphorylation in tubular epithelial cells and a 2500-fold increase in interstitial cells.[44] Inhibition of Stat3 signaling by a Stat3 inhibitor (blocks Stat3 phosphorylation and dimerization) induced apoptosis in renal fibroblasts of obstructed kidney.[45]

5.5. Stat3 and Liver Fibrosis. Stat3 plays a protective role in liver injury and fibrosis in sclerosing cholangitis. Stat3 phosphorylation is significantly down-regulated in cholestatic cirrhosis compared with noncholestatic cirrhosis in adults.[46] Conditional inactivation of Stat3 in hepatic cells was reported to strongly aggravate bile acid induced hepatic injury and fibrosis in mice lacking the multidrug resistance gene 2 (mdr2−/−), whereas Stat3-deficient hepatocytes exhibited up-regulation of bile acid biosynthesis and down-regulation of hepatoprotective growth factors.[47]

5.6. Stat3 and Inflammatory Lung Diseases/Pulmonary Fibrosis. Several studies have investigated Stat3 in inflammatory lung diseases/pulmonary fibrosis.[48,49] Stat3 was overexpressed in different airway tissues of silica-exposed mice[48] and in lung tissues of chronic obstructive pulmonary disease (COPD) patients.[50]

5.7. Stat3 and Psoriasis. Stat3 is involved in epidermal regenerative or stress responses like wound healing and psoriasis.[51,52] During wound healing Stat3 becomes activated, whereas disrupted Stat3 signaling delays healing in mice.[51] On the other hand, Stat3 becomes phosphorylated in lesional keratinocytes in psoriatic patients. Transgenic mice with constitutively active Stat3 in keratinocytes spontaneously developed psoriatic-like skin lesions accompanied by T-cell activation,[52] whereas disruption of Stat3 activity reversed established psoriasis in these transgenic mice.[52] In addition, inhibition of Stat3 by the JAK2 inhibitor WP1066 suppressed psoriasis development in PPARβ/δ transgenic mice.[53]

5.8. Stat3 and Inflammatory Bowel Disease. Stat3 regulates intestinal homeostasis. Abnormal Stat3 expression and/or phosphorylation is present in IBD. Stat3 activation is elevated in intestinal epithelial cells from patients suffering from IBD, whereas inhibition of Stat3 delays and suppresses wound healing of intestinal mucosa.[54] There was an over 2-fold increase in the number of phospho-Stat3 positive peripheral blood granulocytes from patients with clinically active IBD

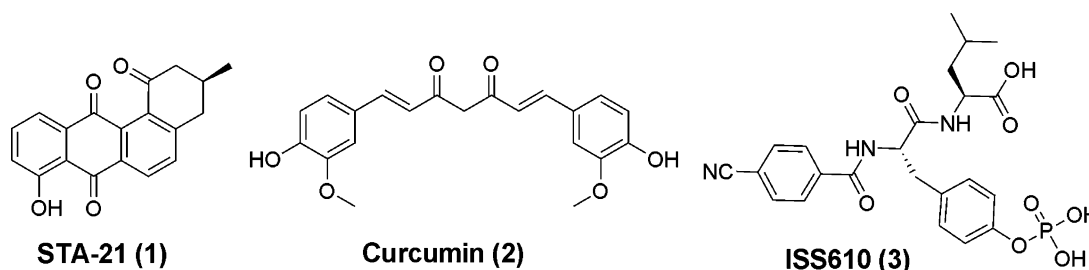**Figure 4.** Stat3 inhibitors prior to the year 2006.

compared to those in remission.[55] Elevated expression as well as nuclear translocation of Stat3 is found in Crohn's disease, a type of IBD. Mice treated with Stat3 antisense oligonucleotide showed less colonic tissue damage with reduced proinflammatory cytokines such as TNF-α and INF-γ in mucosa in TNBS-induced colitis model.[56]

5.9. Stat3 and Obesity/Diabetes. The JAK2/Stat3 pathway in the brain is required for energy homeostasis actions of leptin. Leptin stimulates phosphorylation of Stat3 in hypothalamus. In response to leptin, Stat3 binds the leptin receptor LEPRb and thus is activated to regulate energy homeostasis.[57] Disruption of the Stat3 binding to LEPRb, or deletion of neuronal Stat3, resulted in severe hyperphagia and morbid obesity, as well as hyperglycemia and hyperinsulinemia.[58] Leptin down-regulated fat-mass- and obesity-associated gene in in vitro arcuate nucleus of hypothalamus cultures and in mice through activation of the Stat3 signaling pathway.[59]

5.10. Stat3 and Atherosclerosis. Elevated phospho-Stat3 level in blood vessels is associated with atherosclerotic lesions. Significantly higher levels of Stat3 activation were observed in endothelium and monocytes in human atherosclerotic lesions.[60] The endothelium-specific Stat3 knockout mice with atherogenic diets exhibited reduced fatty streak formation compared to wild type.[60] Delivery of a human Stat3 gene inhibited aortic structural changes associated with atherosclerosis and down-regulated inflammation/monocyte/macrophage (Mo/MΦ) burden during atherosclerosis, resulting in a significant reduction in atherosclerotic lesions.[61] In addition, there was a significant correlation between pravastatin (a HMG-CoA inhibitor) caused atherosclerotic lesion reduction and decreased Stat3 phosphorylation.[62]

6. STAT3 AND STEM CELLS

Stat3 mediates cytokine leukemia inhibitory factor (LIF) induced self-renewal of mouse embryonic stem cells (ESC) in the presence of serum. In the absence of LIF, artificial activation of Stat3 is sufficient to maintain mouse ESC self-renewal.[63] Binding of LIF to its receptor LIFR causes heterodimerization of LIFR and glycoprotein-130 (gp130), resulting in JAK/TIK-mediated Stat3 activation and subsequently the expression of Stat3 target genes essential for maintaining the undifferentiated state of mouse ESCs.[64] The interactions between Stat3 and other proteins, such as the transcription factor Nanog[65] and the molecular chaperone heat shock protein 90 (Hsp90),[66] suggest that Stat3 functions in protein complexes to maintain ESC pluripotency. Bone morphogenetic protein (BMP) induction of Id proteins was also documented to collaborate with Stat3 in sustaining ESC self-renewal.[67] Loss of Stat3 function results in ESC differentiation in vitro and mouse embryonic lethality (E6.5-7.5) in vivo.[68] Recently, Stat3 activation has been demonstrated to be limiting for reprogramming to ground

state pluripotency in both EpiSCs (heterogeneous cell lines established by culture of postimplantation epiblast) and somatic cells.[69] In contrast to the essential role of LIF/Stat3 signaling in mouse ESCs, this pathway fails to maintain self-renewal of human ESC (hESCs) in spite of the presence of LIF and LIFR.[70,71] Besides ESCs, Stat3 has also been documented to regulate hematopoietic stem cell (HSC) regeneration in the early phase,[72] granulocytic differentiation,[5] dendritic cell (DC) development and function,[73] B-cell development,[74] mouse spermatogonial stem cell (SSC) differentiation,[75] and muscle stem cell differentiation.[76]

7. STAT3 INHIBITION

As Stat3 plays important pathophysiological roles in various diseases, Stat3 inhibitors are currently under intense development preclinically and clinically in many therapeutic areas. There are two strategies to inhibit the Stat3 signaling pathway: indirectly block the upstream molecules of Stat3 signaling pathway and directly target Stat3 protein. Direct inhibition of Stat3 can be achieved via targeting one of three structural domains of Stat3, (a) SH2 domain, (b) DNA binding domain, and (c) N-terminal domain, which therefore suppresses processes related to Stat3 signaling, such as Stat3 phosphorylation, Stat3 dimerization, nuclear translocation, Stat3-DNA binding, and expression of Stat3 target genes. Ideally, direct Stat3 inhibitors should target Stat3 protein without blocking upstream molecules and other Stat members. Stat3 inhibitors can be broadly classified in two categories: (i) peptide and peptidomimetics; (ii) small molecular nonpeptidic inhibitors.

In our previous review we reported inhibitors of the Stat3 signaling pathway up to the year 2006.[77] In this article we present Stat3 inhibitors not covered in our previous article[77] and new inhibitors reported since 2006. As in our previous review, we have attempted to be inclusive and cite all active inhibitors. Where possible we included pertinent analogues to shed light on potential trends and structure–activity relationships. However, it is important to note that most of the inhibitors need further characterization to be considered true Stat3 inhibitors. An unsettling point is the fact that many of the alleged inhibitors are nonspecific, contain reactive groups, or are not potent in cell-based assays. Future studies will winnow out uninteresting compounds, and a few bona fide inhibitors could be further optimized for clinical use.

7.1. Small-Molecule Inhibitors. Small molecules constitute the largest class of Stat3 inhibitors for cancer prevention and treatment.[78−81] To identify novel small molecule inhibitors of Stat3 multiple approaches, such as high-throughput screening of large chemical libraries,[82] virtual screening,[83,84] rational design based on peptides and peptidomimetic inhibitors,[85,86] and fragment-based drug design and drug repositioning using multiple ligand simultaneous docking (MLSD),[87] were used to

disrupt Stat3 phosphorylation, dimerization, nuclear translocation, and/or DNA binding. Several classes of such small molecule inhibitors are discussed below. Unfortunately, there is no uniformity among different publications reporting Stat3 inhibitors in terms of assay systems used to evaluate potency, making comparisons across different classes of inhibitors difficult.

Our previous review covered inhibitors of Stat3 signaling pathway reported prior to 2006.[77] Most of those inhibitors were identified by screening natural product databases, organometallics, peptides, and peptidomimetics. Many of those compounds are not specific inhibitors of Stat3.[77] Figure 4 lists a few selected compounds such as STA-21 (**1**), curcumin (**2**), and ISS610 (**3**)[77] that were further characterized in subsequent years for their Stat3 inhibitory effects or used for lead optimization.

7.1.1. Compound 1 and Its Analogues. The first non-peptide small molecule Stat3 inhibitor, compound **1**, was discovered through virtual screening.[83] It is a natural product and deoxy analogue of tetrangomycin (**4**) (Figure 5) that was
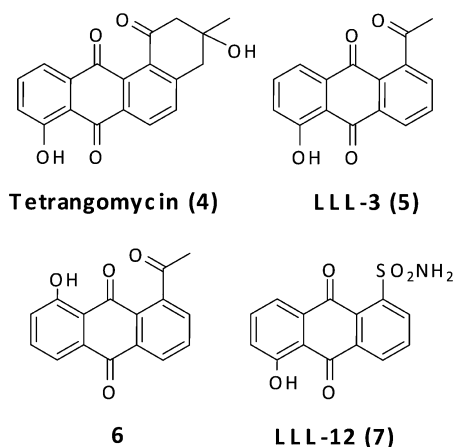

Figure 5. Analogues of compound **1**.

originally discovered as an antibiotic isolated from cultures of a variant strain of *Streptomyces rimosus*.[88] Compound **1** inhibited Stat3-dependent luciferase activity in breast and ovarian cancer cell lines. In the MDA-MB-435 cells stably transfected with a Stat3-dependent luciferase reporter pLucTKS3, compound **1** at 20 μM inhibited luciferase activity by 5-fold compared to untreated control.[83] It also inhibited Stat3 dimerization, nuclear translocation, Stat3-DNA binding, as well as the expression of Stat3-regulated genes in cancer cells with constitutively active Stat3.[83] Compound **1** at 30 μM inhibited Stat3-DNA binding activity in MDA-MB-435s cells.[83] Several analogues (**5**–**7**) of compound **1** were also reported to target Stat3 SH2 domain (Figure 5).[89−91] Among these, LLL-3 (**5**) and **6** along with compound **1** showed antiproliferative activity in cancer cells with persistently active Stat3 (Table 2). The cytotoxic activities of these compounds were directly proportional to Stat3 expression levels from highest in DU145 to lowest or no expression in MCF-7.[89] Acetyl and hydroxyl groups in trans positions favored cytotoxicity. Compound **5** also induced apoptosis in glioblastoma cells by inhibiting Stat3 activities and suppressed tumor growth and metastasis in a glioblastoma xenograft mouse model, resulting in prolonged survival.[92] In addition, compound **5** inhibited cell growth, activated caspases-3/7, and induced apoptosis in K562 cells. It also enhanced the

Table 2. Cytotoxic Activities of Compound 1 and Its Analogues in a Panel of Cancer Cell Lines

compd	IC$_{50}$ (μM)				ref
	DU145	PC3	LNCaP	MCF-7	
1	12.2	18.7	not tested	>100	89
5	16.2	13.4	34.1	88.5	89
6	31.5	32.4	31.5	not tested	89

effect of imatinib on BCR-ABL positive cells.[93] Another analogue, LLL-12 (**7**), selectively inhibited Stat3 phosphorylation in glioblastoma, breast, and pancreatic cancer cells expressing elevated levels of Stat3, without significant effect on kinases such as ERK1/2, mTOR, and Src.[90] Compound **7** also selectively inhibited Stat3-DNA binding and Stat3-dependent transcriptional activities, reduced the secretion of IL-6 and LIF in medulloblastoma and glioblastoma cell lines,[94] and suppressed tumor growth in mouse xenograft models of breast cancer and glioblastoma.[90] In a panel of cancer cell lines, compound **7** at 10 μM significantly inhibited Stat3-DNA binding.[90] Compound **7** is more potent than the original lead, compound **1**, and they represent a class of selective small-molecule Stat3 inhibitors with druglike properties.

7.1.2. Stattic (8)[95] and Its Analogues. Another small molecule, compound **8** (Figure 6a), was discovered as a Stat3-SH2 domain inhibitor via high-throughput screening of a diverse chemical library.[95] Compound **8** exhibited an IC$_{50}$ of 5.1 μM in a fluorescence polarization (FP) assay and showed selectivity over other proteins containing SH2 domain, including Lck kinase, Stat1, and Stat5. It also inhibited prephosphorylated Stat3 homodimers from binding DNA in electrophoretic mobility shift assays (EMSA) at 10 μM. However, no inhibitory effects were observed on prephosphorylated Stat1 homodimers even at higher concentrations. Compound **8** selectively down-regulated phospho-Stat3 in liver carcinoma HepG2 cells and breast cancer MDA-MB-231 and MDAMB-435S cells.[95] By inhibiting Stat3, compound **8** impaired proliferation and neurosphere initiation in tumor-initiating stem cells (TISC) from glioblastoma and sensitized TISCs to temozolomide.[96] However, compound **8** may lack target selectivity because of its simple chemical structure. For example, we demonstrated that compound **8** equally inhibited the phosphorylation of Stat3 and Stat1 induced by IL-6 or LIF in human ovarian cancer OVCAR-8 cells (Figure 6b). Several close analogues of compound **8** were reported in an attempt to optimize the lead (compounds **9**−**11**, Figure 6a). Structure−activity relationship (SAR) analysis of compound **8** and its analogues suggests that replacement of the NO$_2$ group by NH$_2$ or H results in loss of Stat3 inhibitory activity in both FP and EMSA assays. Furthermore, saturation of the five-membered ring led to complete loss of activity.[95] The SAR results suggest that the NO$_2$ group and an unsaturated five-membered ring are important for the Stat3 inhibitory activity of this class of compounds.

7.1.3. Curcumin and Its Analogues. Curcumin, the principal curcuminoid of the Indian spice turmeric, inhibits IL-6-induced Stat3 phosphorylation and nuclear translocation. Several compounds were designed as Stat3 inhibitors based on curcumin (Figure 7).[97−99] FLLL11 (**12**) and FLLL12 (**13**) inhibited Stat3 phosphorylation and induced apoptosis in pancreatic cancer cell lines in a dose-dependent manner.[97] Although both compounds inhibited Stat3 phosphorylation, Stat3-DNA binding, and transcriptional activity at 10 μM in
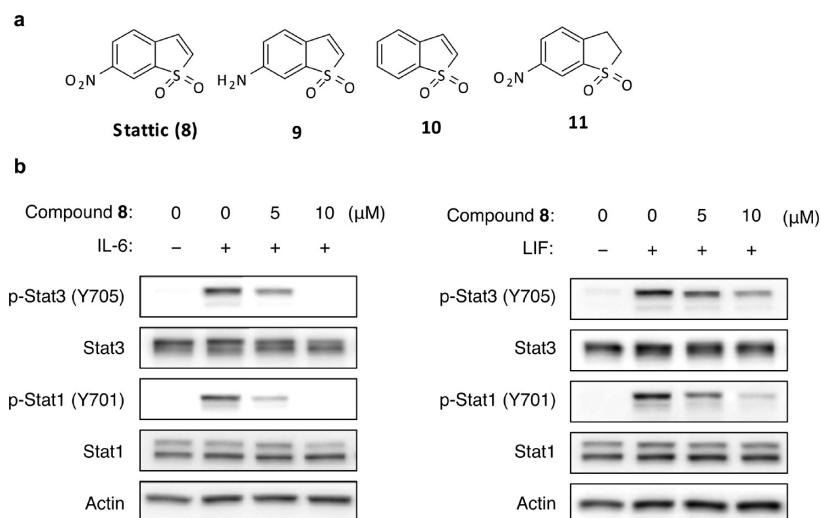
Figure 6. (a) Compound **8** and its analogues. (b) Compound **8** inhibits Stat3 and Stat1 phosphorylation. Human ovarian cancer OVCAR-8 cells were serum-starved overnight. Cells were then treated with compound **8** at indicated concentrations for 4 h before IL-6 (50 ng/mL) or LIF (50 ng/mL) stimulation for 20 min. Whole cell lysates were prepared for Western blotting analysis using specific antibodies (Cell Signaling Inc.) for indicated proteins.
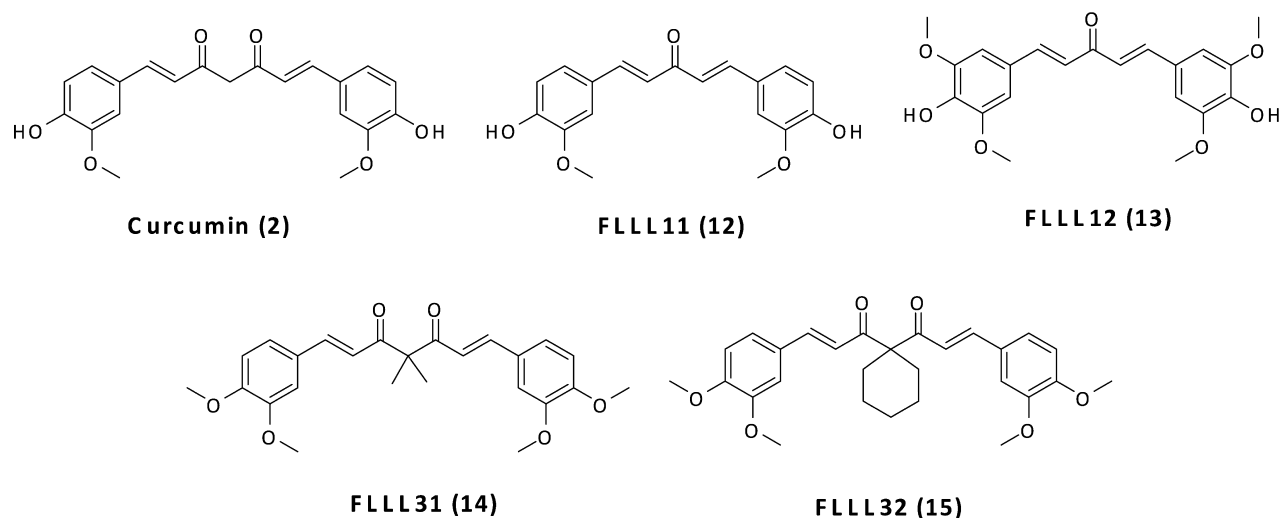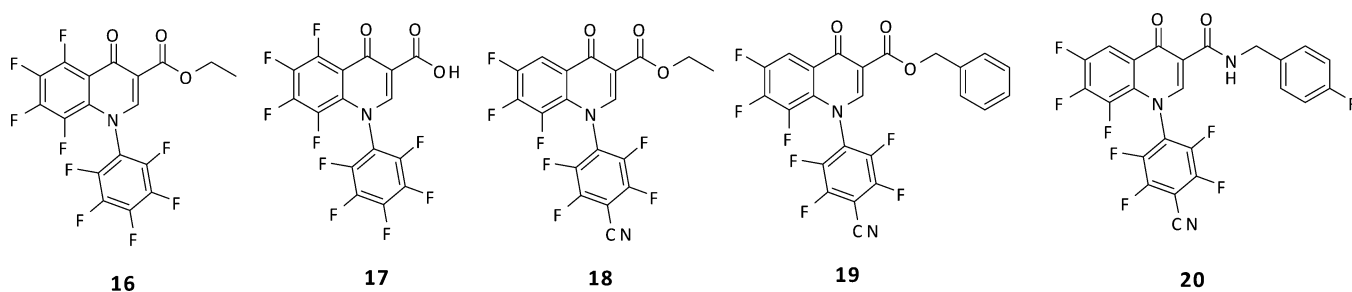

Figure 7. Analogues of curcumin.

Figure 8. Analogues of quinolinone.

breast and prostate cancer cells, **13** was more potent than **12** and curcumin.[98] FLLL31 (**14**) and FLLL32 (**15**) were also cytotoxic in several cancer cell lines.[100] Both compounds at 10 μM down-regulated Stat3 phosphorylation, DNA binding, and the expression of Stat3 target genes in MDA-MB-231 and PANC1 cells. Compound **15** showed little inhibitory effect on tyrosine kinases and other non-tyrosine kinases, such as AKT2, CDK-2, EGFR, HER-2, and Met. Both **14** and **15**

demonstrated JAK2 kinase inhibitory activity at 5 μM (approximately 60% and 75% reduction, respectively).[99] Compound **15** specifically inhibited Stat3 phosphorylation at Tyr705 and induced caspase-dependent apoptosis in phospho-Stat3-positive human melanoma cell lines in the low micromolar range.[101]

7.1.4. Quinolinone Analogues. Several quinolinones were reported as JAK-Stat3 pathway inhibitors.[102] These compounds

(Figure 8) inhibited Stat3 phosphorylation at Tyr705 (Table 3). SAR analysis revealed that the ester group is important for

Table 3. Inhibition of Stat3 Phosphorylation by Quinolinone Analogues

compd	inhibition of Stat3 phosphorylation, EC_{50} (μM)	ref
16	4.6	102
17	46	102
18	0.17	102
19	0.13	102
20	0.20	102

inhibitory activity, as hydrolysis of the ester bond leads to a 10-fold loss of activity (**16** vs **17**). Introduction of a cyano group (compound **18**) leads to a 27-fold increase in potency. Replacement of ethyl ester with a benzyl ester (**19**) or benzylamino (**20**) group did not affect activity. Compound **18** inhibited activation of JAKs but did not directly affect the enzymatic activity of JAK1, JAK2, and JAK3.[102] Further investigation is underway to elucidate the actual molecular targets of this class of compounds.

7.1.5. S3I-201 (21)[84] and Its Analogues. Structure-based virtual screening of chemical libraries from the National Cancer

Institute led to the identification of a small molecule **21** (Figure 9) as a Stat3 inhibitor.[84] Compound **21** preferentially inhibits the DNA binding ability of Stat3/Stat3 dimer (IC_{50} = 86 μM), compared to Stat1/Stat3 dimers (IC_{50} = 160 μM) and Stat1/Stat1 dimers (IC_{50} > 300 μM).[84]

Compound **21** inhibited proliferation of hepatocellular carcinoma cells with decreased phospho-Stat3(S727) and aberrant TGF-β pathway. At 250 μM, it caused 80% inhibition of cell growth in cells with low phospho-Stat3 (S727) and disrupted TGF-β signaling, while only 20% inhibition was observed in cells with an intact TGF-β pathway. It suppressed tumor growth in mouse xenograft models of hepatocellular carcinoma and breast cancer.[84,103]

In an attempt to optimize compound **21**, structure-based modeling resulted in compounds **22**−**27** (Figure 9) with improved potency.[104,105] Compounds **22**−**27** inhibited Stat3-DNA binding in a dose dependent manner with IC_{50} values in the range 19.7−50 μM in EMSA and disrupted Stat3−phosphopeptide binding in FP assays (Table 4).[104−106] Incorporation of hydrophobic substitutions at the amide nitrogen improved Stat3-DNA binding inhibition by 2- to 3-fold over compound **21**. Compound **23** at 50 μM inhibited constitutive Stat3 activation in NIH3T3/v-Src, Panc-1, and MDA-MB-231 cells. Compound **23** bound to the SH2 domain
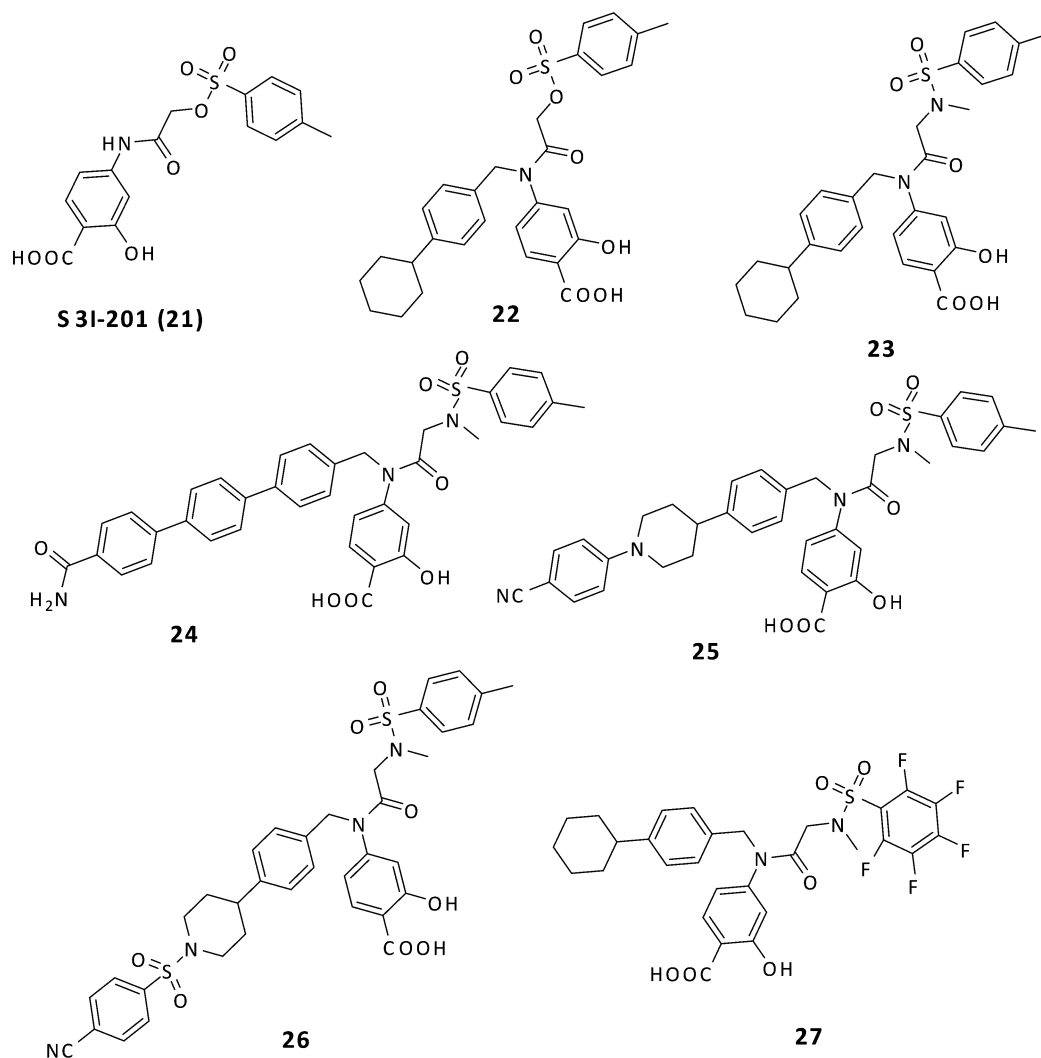

Figure 9. Analogues of compound **21**.

Table 4. Inhibition of Stat3-DNA Binding in EMSA Assay and Stat3−Phosphopeptide Binding in FP Assay by Compound 21 and Its Analogues

compd	EMSA, IC$_{50}$ (μM)	FP, K_i (μM)	ref
21	86	80	104, 168
22	43	50	104
23	35	20, 15	104, 168, 105, 85
24	43	16.7	105
25	45	18.7	105
26	50	21.5	105
27	19.7	12.8	106

with a K_d of 2.74 μM and inhibited Stat3-DNA binding with an IC$_{50}$ of 35 μM.[86] The SH2 domain comprises three subpockets. Compound **21** only interacts with two subpockets. Additional N-substitution in compound **23** allowed hydrophobic interactions with the third pocket.[86,167] Compounds **22** and **23**, in a concentration-dependent manner, disrupted Stat3 interaction with phosphopeptide in a FP assay with IC$_{50}$ of 50 and 20 μM, respectively.[104] Compound **23** also significantly inhibited proliferation of malignant cells with aberrant Stat3, such as NIH3T3/v-Src, Panc-1, and MDA-MB-231 cells, compared to cells that do not harbor aberrant Stat3, such as NIH3T3, normal human pancreatic duct epithelial cells (HPDEC), and mouse thymus epithelial stromal cells (TE-71). The IC$_{50}$ values in NIH3T3/v-Src, Panc-1, and MDA-MB-231 cells were 35, 48, and 37 μM, respectively, while the IC$_{50}$ values were over 200 μM in NIH3T3, HPDEC, TE-71 cells. It also repressed the expression of Stat3 target genes and the growth of human breast tumors in xenograft models.[86] Compound **23** exhibited selectivity toward Stat3 over Stat1 in a FP assay ($K_{i(Stat3)}$ = 15 μM vs $K_{i(Stat1)} \geq$ 50 μM) and inhibited growth of a panel of leukemia, breast, and prostate cancer cell lines.[105] Compound **27** is a more potent analogue of **23**, inhibits Stat3-DNA binding with an IC$_{50}$ of 19.7 μM, and binds the Stat3-SH2 domain with a K_i of 12.8 μM in a FP assay.[106]

7.1.6. Small-Molecule Stat3 Inhibitors Identified from MLSD, High-Throughput Screening, and Virtual Screening. Several drugs and bioactive compounds were identified as Stat3 inhibitors using high-throughput screenings (Figure 10). The antimalarial drug pyrimethamine (**28**),[107] the antidiarrheal agent nifuroxazide (**29**),[108] and 5,15-diphenylporphyrin (5,15-DPP, **30**)[82] showed inhibitory activity against Stat3. Nifuroxazide inhibited Stat3 in a Stat3-dependent luciferase assay using U3A cells (EC$_{50}$ = 3 μM) and down-regulated Stat3 phosphorylation in a dose dependent manner.[108] Porphyrin analogue **30**, identified as Stat3-SH2 domain antagonist in a high-throughput α screening assay (inhibition of Stat3−phosphopeptide interaction), selectively inhibited Stat3 (IC$_{50}$ = 0.28 μM) over Stat1 (IC$_{50}$ = 10 μM).[82]

The COX2 inhibitor celecoxib (**31**) and other two compounds (**32** and **33**) were identified as Stat3 inhibitors through fragment-based drug design followed by MLSD and drug repositioning.[87] They inhibited Stat3 phosphorylation in both dose- and time-dependent manners. Phosphorylation of Stat3 was inhibited by compounds **32** and **33** at 10−30 μM and by celecoxib at 25−50 μM.[87]

Various Stat3 inhibitors (**34−39**, Table 5) targeting the SH2 domain were identified from virtual screening of one million druglike compounds and subsequent in vitro testing.[109] Interestingly, all of these compounds have either free carboxylic acid or carboxylic acid ester. Further optimization through scaffold hopping led to the identification of a small molecule (C188-9, structure not disclosed) with potent activity against Stat3 in acute myeloid leukemia cells.[110]

All these compounds identified from virtual or high-throughput screening need to be further characterized to elucidate their actual molecular targets. A troubling trend is the fact that many of these compounds have α,β unsaturated ketone (Michael acceptor) that could be responsible for their nonspecific interaction with Stat3 as well as with a myriad of other cellular targets. Therefore, at this stage it is too early to classify such compounds as bona fide Stat3 inhibitors. It is not
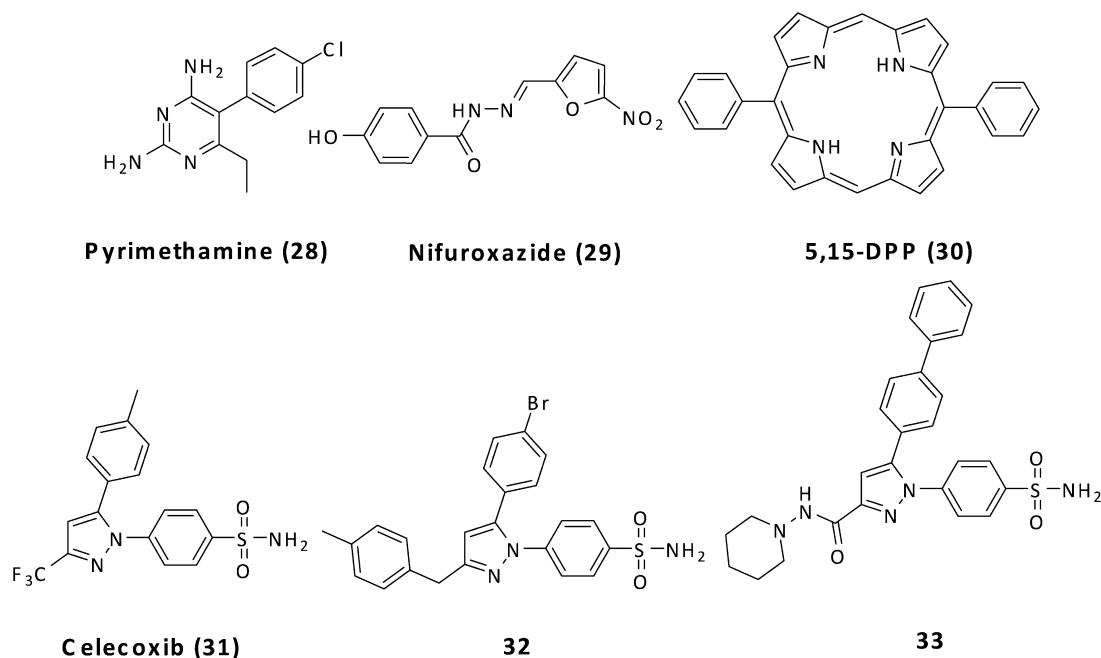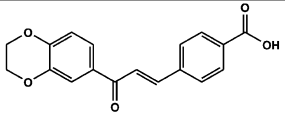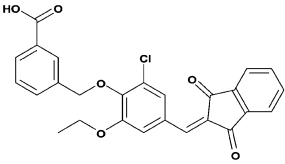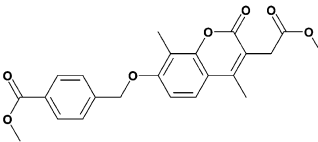

Pyrimethamine (28)   **Nifuroxazide (29)**   **5,15-DPP (30)**

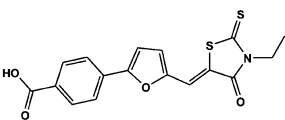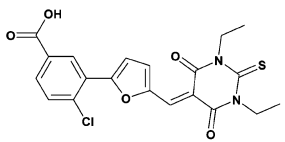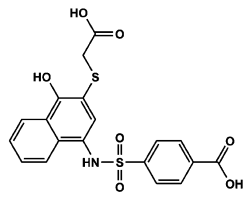**Celecoxib (31)**   **32**   **33**

Figure 10. Stat3 inhibitors identified from high-throughput and virtual screening.

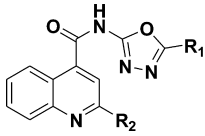**Table 5. Selected Examples of Stat3 Inhibitors Discovered by Virtual Screening**

Compound	Structure	Stat3/pY-peptide binding assay (IC$_{50}$, µM)	IL-6-mediated phosphorylation of Stat3 (Immunoblot assay) (IC$_{50}$, µM)	References
34		447	91	[109]
35		256	144	[109]
36		137	63	[109]
37		30	18	[109]
38		114	60	[109]
39		20	73	[109]

our intention to critically appraise the merit of each compound as a selective Stat3 inhibitor but rather to make the reader aware of the fact that most of these so-called Stat3 inhibitors will eventually fail upon further scrutiny.

Recently, a quinoline analogue STX-0119 (**40**) was identified as a Stat3 dimerization inhibitor through virtual screening.[111] Table 6 shows several analogues (**41−46**) of compound **40**, highlighting the structural features required for Stat3 inhibition. Compound **40** inhibited Stat3 dimerization as well as transcription but failed to hinder Stat3 phosphorylation. These observations suggest that it inhibits Stat3 through direct binding instead of targeting upstream JAK kinases controlling phosphorylation of Stat3.[111]

7.1.7. Other Small-Molecule Inhibitors. Additional compounds have been identified as Stat3 inhibitors that directly or indirectly target Stat3. Figure 11 lists Stat3 inhibitors from different sources. A natural product, cryptotanshinone (**47**), from *Salvia miltiorrhiza* Bunge (Danshen root) was identified as a Stat3 inhibitor.[112] It inhibits Stat3-dependent luciferase activity in colon cancer cells in a dose-dependent manner with an IC$_{50}$ of 4.6 µM and also selectively blocked Y705 phosphorylation of Stat3 in DU145 cells at 7 µM. Cryptotanshinone does not show equal inhibitory activity against upstream tyrosine kinases, such as JAKs, c-Src, and EGFR. However, it inhibits JAK2 kinase at 5 µM after 4 h of treatment, while the inhibition of Stat3 phosphorylation occurs within 30 min. Moreover, compound **47** inhibited Stat3

Table 6. Compound 40 and Its Analogues with Inhibitory Activities on Stat3 Transcription and Dimerization

Compound	R_1	R_2	Stat3 transcription IC$_{50}$ (μM)	Stat3 dimerization 50 μM (%)	10 μM (%)	References
40	2-furyl	phenyl	74	62	9	111
41	2-furyl	naphthyl	55	70	14	111
42	2-furyl	thienyl	63	56	18	111
43	phenyl	phenyl	66	60	43	111
44	benzyl	phenyl	75	61	25	111
45	3-furyl	phenyl	72	55	8	111
46	2-thienyl	phenyl	75	56	36	111

dimerization in EMSA assays by binding to the SH2 domain of the Stat3 monomer.[112]

An antirheumatic sulfur-containing organogold compound, auranofin (48), was reported to inhibit IL6-induced Stat3 phosphorylation in HepG2 cells, fibroblast-like synoviocytes from rheumatoid arthritis patients, human umbilical vein endothelial cells (HUVECs), and rat astrocytes, but the exact molecular mechanism of this compound is unclear.[113] Dobesilate (49), a drug commonly used in the treatment of diabetic retinopathy and chronic venous insufficiency, inhibited Stat3 activation and downstream gene expression in glioma cells.[114] Nitric oxide-releasing aspirin derivative NCX-4016 (50) down-regulated Stat3 protein levels and inhibited phosphorylation of Stat3 at Tyr705 and Ser727 in ovarian cancer cells.[115] An orally bioavailable farnesyl protein transferase inhibitor, SCH 66336 (51), inhibited Stat3 phosphorylation at 4 μM in non-small-cell lung carcinoma (NSCLC) cell lines.[116] A synthetic triterpenoid, 2-cyano-3,12-dioxoolen-1,9-dien-28-oic acid imidazolide (CDDO-Im, 52) inhibited both constitutive and inducible Stat3 phosphorylation in human cancer cells in a dose- and time-dependent manner and down-regulated Stat3 target genes. It also inhibited Stat5 phosphorylation.[117] Methyl ester of 2-cyano-3,12-dioxoolen-1,9-dien-28-oic acid (CDDO-Me, RTA 402, 53) inhibited Stat3 activity at 500 nM in metastatic murine breast cancer cells. It also inactivated Akt and Src kinases and down-regulated c-Myc by 4-fold.[118] In an in vivo murine breast tumor model, compound 53 inhibited tumor cell growth but did not induce apoptosis.[118] Actual mechanisms behind Stat3 inhibition of compound 53 are unclear.

Chalcone (54) and a chalcone derivative (2′-hydroxychalcone, 55) were reported to inhibit IL-6-induced Stat3 phosphorylation in endothelial cells in a dose-dependent

manner. The α,β-unsaturated carbonyl moiety was important for the inhibition of Stat3 phosphorylation, as other flavonoids without this moiety failed to show any inhibitory activity.[119] Another chalcone derivative, butein (3,4,2′,4′-tetrahydroxychalcone, 56), inhibited tumor cell proliferation through Stat3 suppression. Compound 56 inhibited both constitutive and IL-6-induced Stat3 activation in multiple myeloma cells through the induction of SHP-1 and also suppressed constitutive activation of JAK1 and c-Src.[120]

Silibinin (57), a flavanone from *Silybum marianum*, inhibited constitutively active Stat3 phosphorylation, activated caspases, and induced apoptosis in DU145 prostate cancer cells in a dose dependent manner.[121] It also suppressed the expression of Stat3 target genes.[121] Capsaicin (58), a vanilloid constituent of green and red peppers, preferentially inhibited constitutive Stat3 phosphorylation in multiple myeloma cells in a dose- and time-dependent manner through the inhibition of JAK1 and c-Src. It down-regulated the expression of Stat3 target genes and inhibited growth of human multiple myeloma cells in vitro and in vivo.[122] Ursolic acid (59), a natural triterpenoid isolated from different fruits and vegetables, inhibited both constitutive and IL-6-induced Stat3 activation in multiple myeloma cells through the blockade of JAK1, JAK2, and c-Src.[123] It inhibited Stat3 phosphorylation, DNA binding, and the expression of Stat3 target genes. It also induced apoptosis in multiple myeloma cells in a time-dependent manner.[123] Betulinic acid (60), a pentacyclic triterpenoid from the stem bark of the plant *Zizyphus mauritiana*, blocked Stat3 activation in human multiple myeloma cells through the induction of SHP-1.[124] It down-regulated the expression of Bcl-2, Bcl-xL, cyclin D1, and survivin and enhanced apoptosis induced by thalidomide and bortezomib.[124] Farnesoid X receptor antagonist guggulsterone (61) inhibited constitutive as well as IL-6-induced Stat3
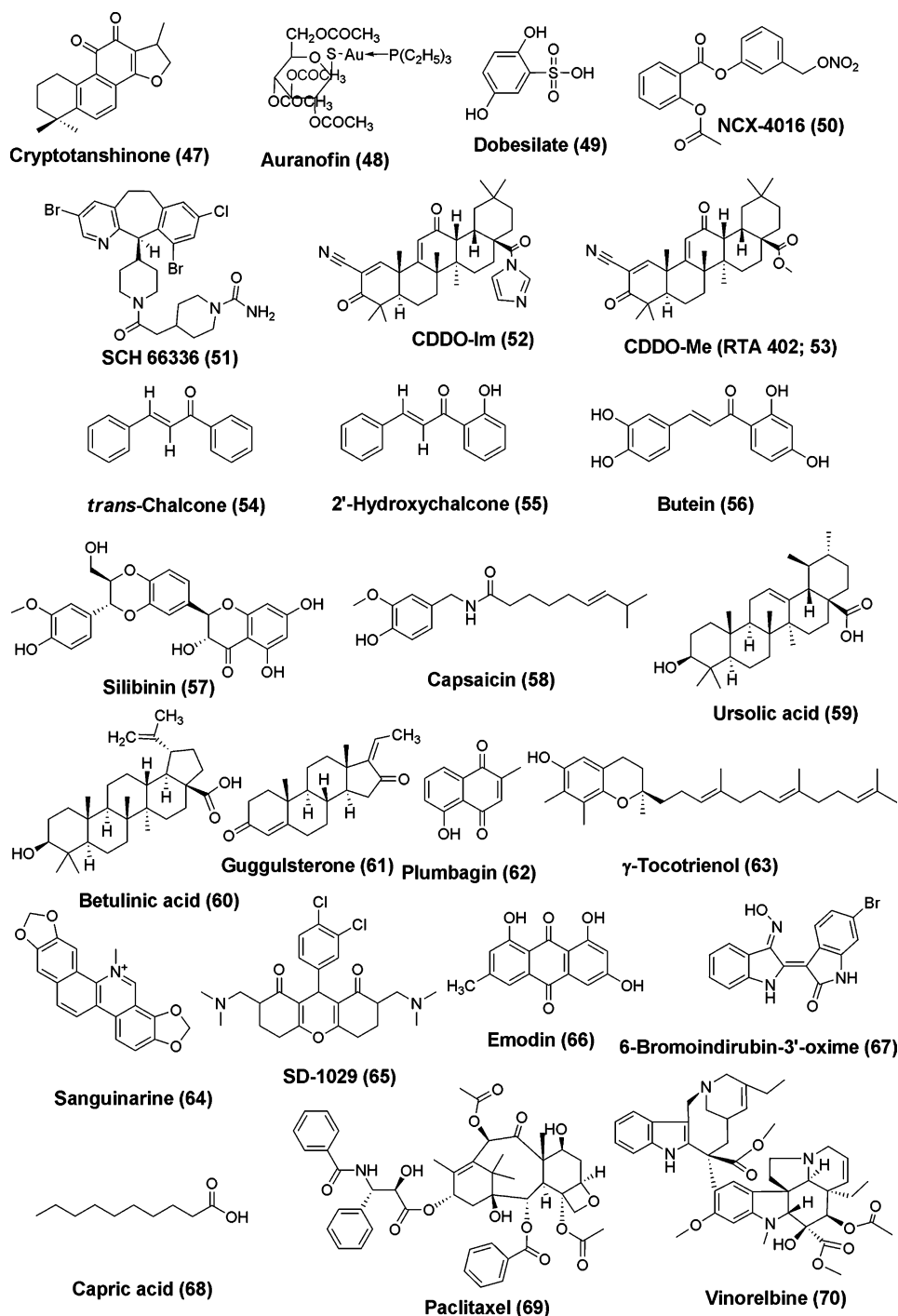
Figure 11. Other small molecule Stat3 inhibitors.

phosphorylation.[125] Additionally, it suppressed the phosphorylation of JAK2 and c-Src kinases.[125] Inhibition of Stat3 phosphorylation by compound **61** was due to the induction of SHP-1.[125] Moreover, compound **61** down-regulated the expression of Stat3 target genes and inhibited cell proliferation, arrested cells in the sub-G1 phase of cell cycle, and induced apoptosis in multiple myeloma cells.[125]

Plumbagin (**62**), a vitamin K3 analogue, inhibited Stat3 activation also through the induction of SHP-1. It inhibited Stat3 phosphorylation, nuclear translocation, DNA binding, and the expression of Stat3 target genes in human multiple myeloma cells. Plumbagin also inhibited the activation of JAK1 and JAK2 and induced apoptosis in tumor cells.[126]

γ-Tocotrienol (**63**), a vitamin E isolated from palm and rice bran oil, showed cytotoxicity through induction of SHP-1, causing inhibition of the Stat3 signaling pathway.[127] It also hindered Stat3-DNA binding as well as the activation of JAK1, JAK2, and c-Src kinases and sensitized tumor cells to anticancer agents.[127]

Sanguinarine (**64**), a benzylisoquinoline alkaloid extracted primarily from the bloodroot plant, inhibited both constitutive and IL-6 induced Stat3 phosphorylation in prostate cancer cells.[128] It also down-regulated the expression of Stat3 target
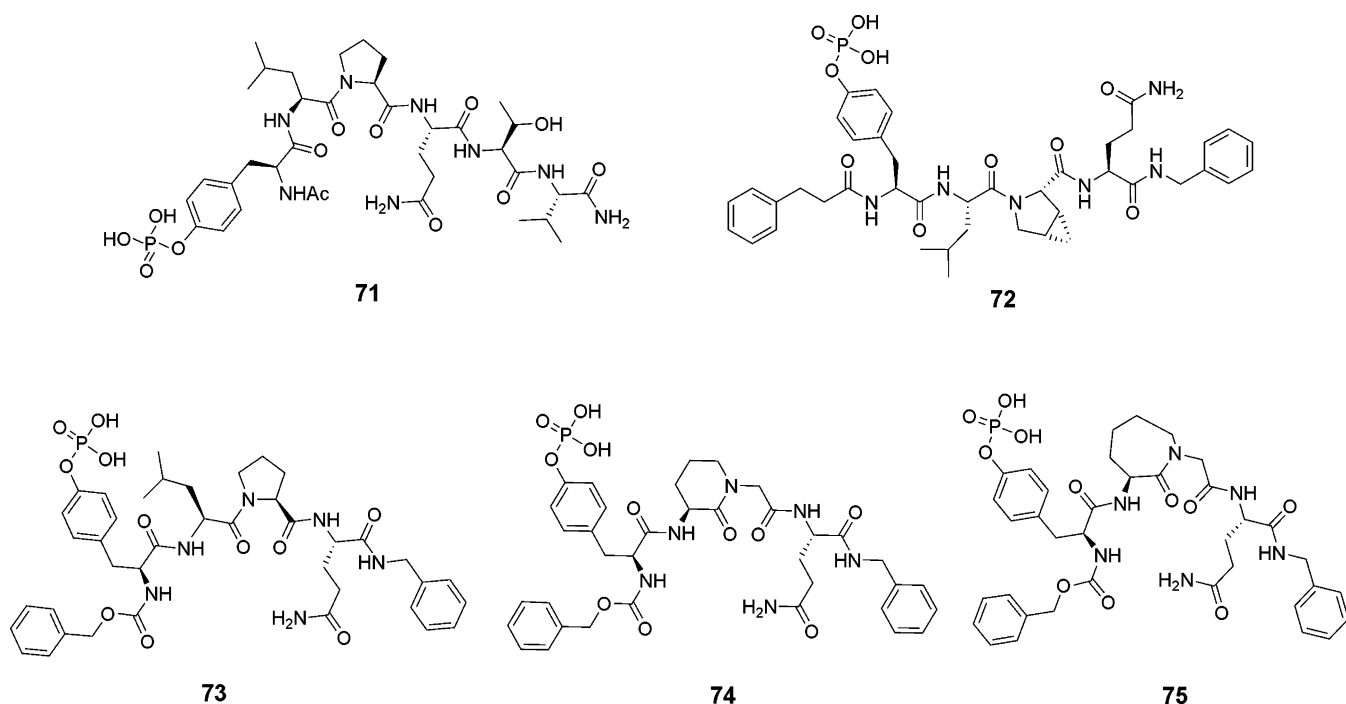
Figure 12. Peptide and peptidomimetic inhibitors.
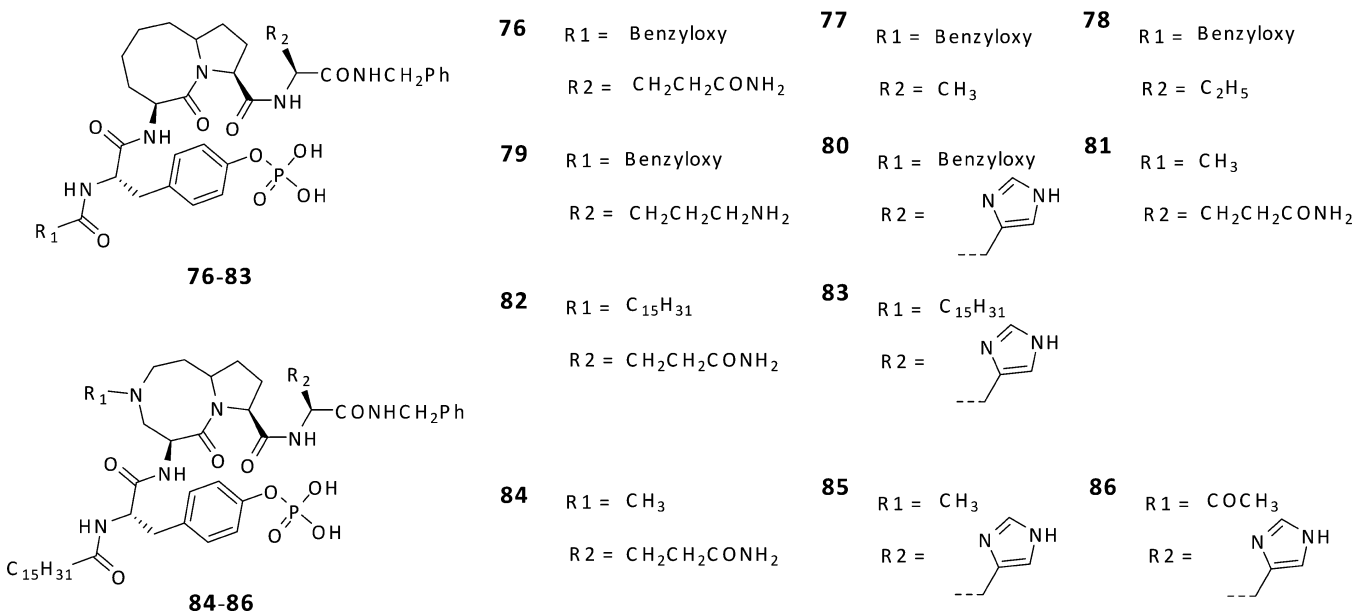
Figure 13. Peptidomimetic Stat3 inhibitors with bicyclic lactam.

genes, such as c-myc and survivin.[128] The NCI bioactive compound SD-1029 (**65**) was identified as an inhibitor of IL-6 or oncostatin-induced Stat3 nuclear translocation in the micromolar range.[129] Compound **65** inhibited phosphorylation of JAK2, down-regulated Stat3 signaling pathway downstream genes, and induced apoptosis in a panel of breast and ovarian cancer cells.[129]

Emodin (**66**), a purgative resin isolated from the root and rhizomes of *Rheum palmatum* L., inhibited Stat3 phosphorylation by targeting JAK2.[130] It inhibited cell growth and induced apoptosis in multiple myeloma cells.[130] Another small molecule, 6-bromoindirubin-3′-oxime (**67**), inhibited Stat3 phosphorylation by blocking JAK/Stat3 signaling and induced

apoptosis in human melanoma cells in a dose- and time-dependent manner.[131] Recently, it was reported that capric acid (**68**), a saturated fatty acid from coconut oil, palm kernel oil, and milk of various mammals, inhibited nitric oxide production during LPS induced osteoclastogenesis via Stat3 phosphorylation at Ser727 but had no effects on NF-κB, JNK, ERK1/2, and Stat1 pathways.[132]

Microtubule-targeted drugs such as paclitaxel (**69**) and vinorelbine (**70**) inhibited Stat3 phosphorylation in cancer cells with constitutively active Stat3 and down-regulated the expression of Stat3 dependent genes.[133] Cytotoxic activity of paclitaxel in breast cancer cells correlates with its Stat3 inhibition.[133]

Purported mechanisms behind these compounds need to be further validated in order to conclude whether these are direct inhibitors of Stat3 or whether they affect pathways leading to Stat3 phosphorylation.

7.2. Peptide and Peptidomimetic Inhibitors. Several peptides and peptidomimetics have been reported to inhibit Stat3 (Figure 12). On the basis of truncated gp130 sequence, Ren et al. discovered the phosphopeptide Ac-pTyr-Leu-Pro-Gln-Thr-Val-NH$_2$ (**71**) as a Stat3 inhibitor. This peptide inhibited Stat3-DNA binding with an IC$_{50}$ of 150 nM in EMSA.[134] Another peptidic inhibitor, hydrocinnamoyl-Tyr-(PO3H2)-Leu-*cis*-3,4-methanoPro-Gln-NHBn (**72**), binds to the Stat3-SH2 domain and is over 2-fold more potent than peptide **71**.[135] In a FP assay, the IC$_{50}$ of peptide **72** was 125 nM while that of peptide **71** was 290 nM.[135] Although peptides face several challenges in terms of membrane permeability and stability, they are valuable starting leads for the rational design of peptidomimetic and small-molecule Stat3 inhibitors.

On the basis of the peptide **71**, Gomez et al. designed peptidomimetics incorporating Freidinger lactams as Stat3 inhibitors (Figure 12).[136] A shortened version of the gp130 peptide, compound **73**, bound to Stat3 with a K_i of 350 nM in a FP assay. Compounds **74** and **75** were designed based on compound **73**'s chemical structure by incorporating six- and seven-membered Freidinger lactams, respectively.[136] The six-membered lactam (**74**, K_i = 3.13 μM) lost 9-fold potency, while the seven-membered lactam (**75**, K_i = 190 nM) gained 2-fold potency over compound **73**.[136]

Cyclization of isobutyl group of leucine with a five-membered ring of proline led to bicyclic lactam derivatives (**76−86**) of compound **73** (Figure 13).[137] Table 7 lists binding affinities and cytotoxic activities of these compounds. A conformationally constrained derivative, compound **76**, was 20 times more potent than compound **73**. Various structural changes in the glutamine residue resulted in derivatives (**77−80**) less potent than compound **76**. This suggests that the

glutamine residue is structurally important for the binding affinity. Replacement of carbobenzyloxy (Cbz) with an acetyl group resulted in compound **81** without any loss of activity. Compounds **76−81** showed moderate to high activity in FP-based binding assays but failed to show cellular activity up to 100 μM probably because of their poor cellular uptake. Substituting the acetyl group of compound **81** with a lipid chain led to a cell-permeable derivative, compound **82**, which exhibited a K_i of 10 nM in a binding assay and inhibited the growth of breast cancer cells in a dose-dependent manner. In an effort to improve cell permeability, the glutamine was replaced with a histidine (compound **83**), resulting in a loss of binding affinity but an increase in cellular activity. Introduction of a positively charged nitrogen atom in the seven-membered ring to neutralize the negatively charged phosphotyrosine group resulted in compounds **84** and **85** retaining binding activity. Compound **85** showed high potency in cell growth inhibition with IC$_{50}$ of 11.2 and 3.6 μM in MDA-MB-231 and MDA-MD-468 cells, respectively.[137]

Peptidomimetics with glutamine mimics incorporated into the phosphopeptide were reported as Stat3 inhibitors targeting the SH2 domain (**87−89**) with nanomolar potency in FP assays (Figure 14).[138] A series of small-molecule analogues (compounds **90−94**, Figure 15) were designed based on the peptidomimetic Stat3 inhibitor **3**,[85] which were capable of inhibiting Stat3-DNA binding with IC$_{50}$ < 100 μM (Table 8). SAR analysis of these analogues revealed that a thiazole moiety is more preferable than an oxazole moiety for the inhibition of Stat3-DNA binding, as thiazole analogues (**92** and **94**) were at least 2- to 3-fold more potent than their oxazole analogues (**91** and S3I-M2001 (**93**)). Compound **93** preferentially inhibited Stat3-DNA binding over Stat1 in EMSA assays with nuclear extracts or lysates from Sf-9 cells and also inhibited Stat3 phosphorylation in MDA-MB-231 and MDA-MB-435 cells.[139,140] Moreover, **93** inhibited the migration of malignant tumor cells and repressed the growth of human breast cancers in xenograft models.[139,140]

A novel class of hybrid peptidomimetics was designed by conjugating compound **3** with high-affinity peptides from gp130 (Figure 16). Compound **95** strongly interacted with Stat3 (K_d = 205 nM) and disrupted Stat3 interaction with phosphopeptide (K_i = 9 μM) in a FP assay. It also exhibited time-dependent inhibition of constitutively active Stat3 in NIH3T3/v-Src cells. A nonphosphorylated analogue (compound **96**) binds (K_d = 17 μM) Stat3 but fails to disrupt Stat3−phosphopeptide interaction.[141] This observation suggests that these compounds act through different mechanisms: compound **95** binds to the SH2 domain, while compound **96** binds a different site.

8. STAT3 INHIBITORS IN CLINICAL TRIALS

Several Stat3 inhibitors are in early phase clinical trials (Table 9). Compound **1** has completed phase I/II clinical trials for the treatment of psoriasis.[142] However, no results are publicly available at this time. Pyrimethamine is in phase I/II trials for the treatment of chronic lymphocytic leukemia and small lymphocytic leukemia.[143] Another Stat3 inhibitor, OPB-31121 (structure not disclosed), is currently in phase I in patients with advanced solid tumors.[144] NF-κB/Stat3 inhibitor, compound **53**, is currently undergoing phase I/II clinical testing for pancreatic cancer.[145] A phase I trial with compound **53** has been completed in patients with solid tumors and lymphoid malignancies. It was well tolerated with a maximum tolerated

Table 7. Stat3 Binding Affinity and Cytotoxic Activities of Select Peptides and Peptidomimetics in Breast Cancer Cells

compd	K_i[a] (μM)	IC$_{50}$[b] (μM)		ref
		MDA-MB-231	MDA-MB-468	
71	0.29	NA	NA	135
72	0.125	NA	NA	135
73	0.35	>100	>100	136, 137
74	0.19	NA	NA	136
75	3.13	NA	NA	136
76	0.017	>100	>100	137
77	3.23	36	52.5	137
78	8.57	>100	>100	137
79	0.21	>100	>100	137
80	0.44	>100	>100	137
81	0.015	>100	>100	137
82	0.010	25.6	35	137
83	0.46	50.4	70	137
84	0.030	42.6	20.6	137
85	0.95	11.2	3.6	137
86	0.76	41.5	36.1	137
87	0.068	NA	NA	138
88	0.094	NA	NA	138
89	0.069	NA	NA	138

[a]FP-based binding assay. [b]NA: not available.
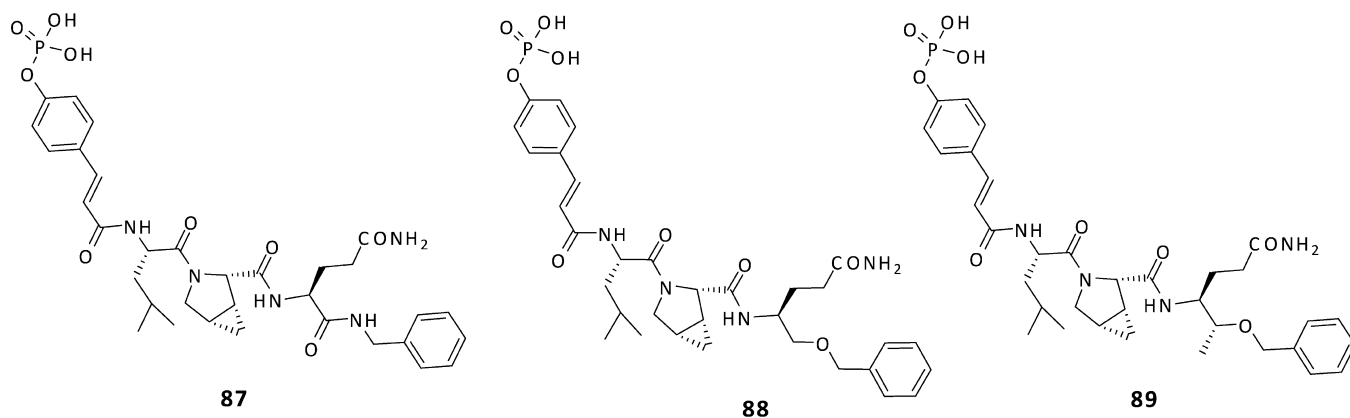
Figure 14. Peptidomimetic Stat3 inhibitors.
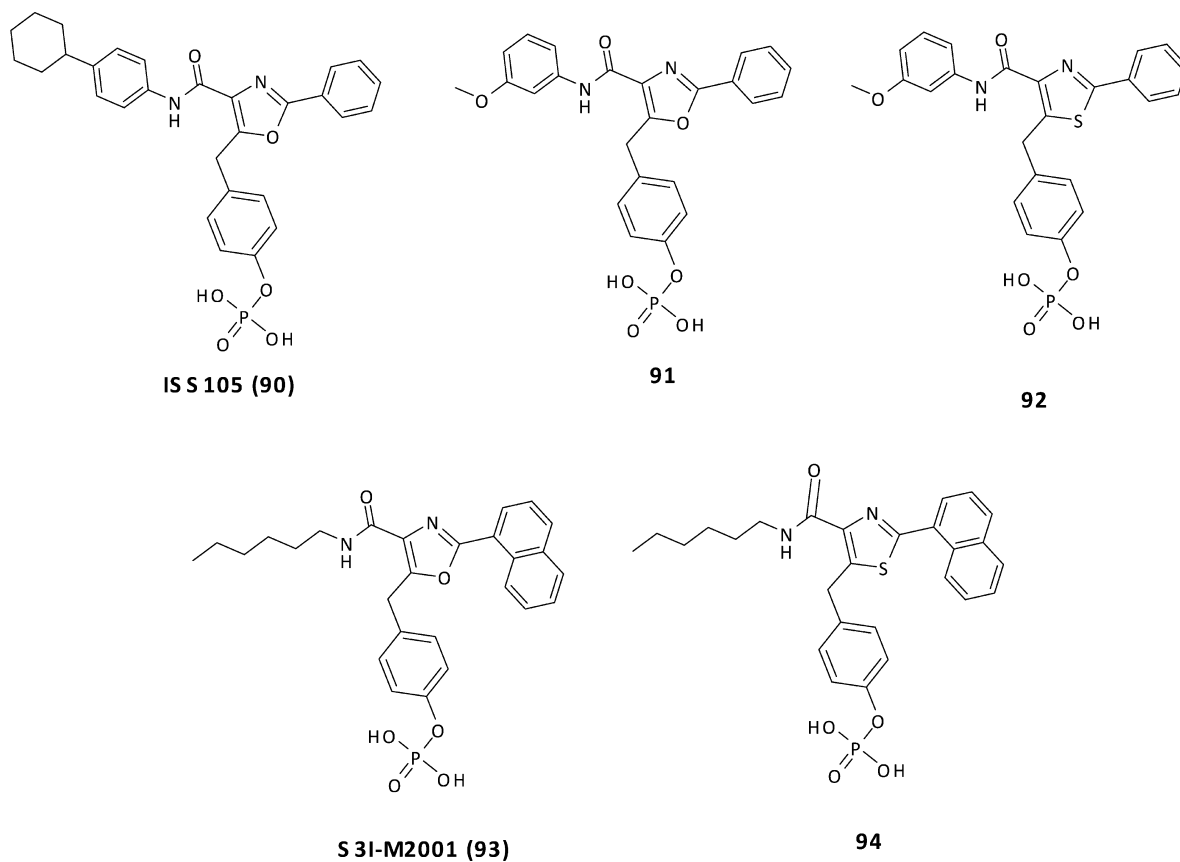
Figure 15. Analogues of compound 3.

Table 8. Inhibition of Stat3-DNA Binding in EMSA Assay by Compound 3 and Its Analogues

compd	EMSA, IC$_{50}$ (μM)	ref
3	42	139, 140
90	33	85, 169
91	90	85, 169
92	28	85, 169
93	58, 79	85, 139, 140, 169
94	25	85, 169
95	5	141

dose of 900 mg/day with prolonged exposure up to 12 months. Partial clinical efficacy was observed in a patient with thyroid cancer. Furthermore, a phase II trial with compound **53** is recruiting patients with solid tumors and lymphoid malignancies.[146]

9. CONCLUSION

Our understanding of the involvement of Stat3 in numerous signaling pathways has grown exponentially in the past decade, reinforcing Stat3 as an important and a valid therapeutic target for many diseases. There is a great potential for the development of Stat3 inhibitors as therapy for cancer and other diseases, such as cardiovascular, rheumatoid arthritis, psoriasis, renal and pulmonary fibrosis, and Alzheimer's disease. After a decade of experience with preclinical evaluations of Stat3 inhibitors, limited translational studies are currently in progress.

95

96

Figure 16. Hybrid analogues of compound **3**.

Table 9. Stat3 Inhibitors in Clinical Trials

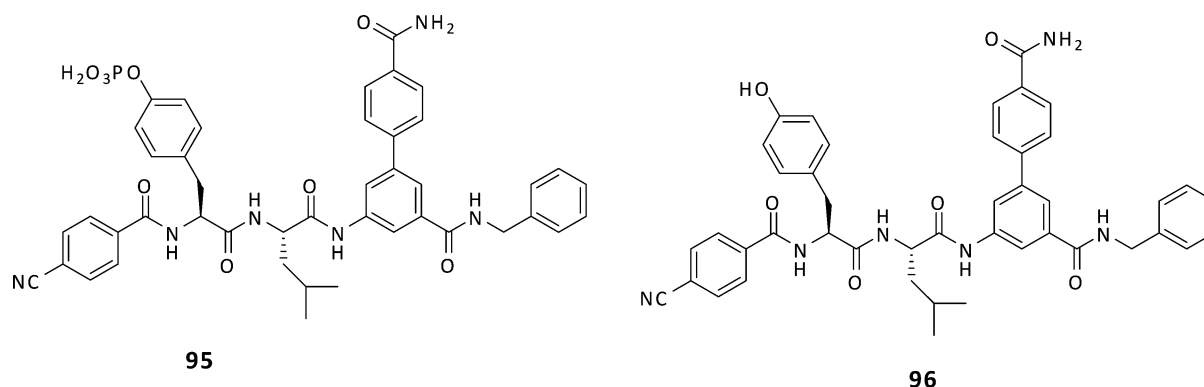| Agent	Structure	Trial phase	Indication	References
1		Phase I/II	Psoriasis	[142]
Pyrimethamine		Phase I/II	Chronic lymphocytic leukemia / Small lymphocytic lymphoma	[143]
OPB-31121	Structure not disclosed	Phase I	Advanced solid tumor	[144]
53		Phase I/II	Pancreatic cancer	[145]
		Phase II	Solid tumors and lymphoid malignancies	[146]

Virtual and high-throughput screenings as well as structure- and ligand-based molecular modeling yielded little success in identifying highly selective Stat3 inhibitors compared to other upstream or downstream targets in the Stat3-signaling pathway. The cellular targets of the putative Stat3 inhibitors identified from different virtual and high-throughput screening are unclear and require further validation and triaging. Moreover, many purported inhibitors possess biologically labile groups that may react with multiple targets, emphasizing the need to develop more selective inhibitors. Nevertheless, the diverse set of compounds reported in this article can be used for the optimization of leads via ligand-based and structure-based modeling and scaffold hopping approaches. For example, peptides and peptidomimetics should be further explored to develop physiologically stable small molecule Stat3 inhibitors. Several interesting inhibitors, such as compounds **7**, **8**, and **27**, could be utilized for lead optimization and subsequent clinical development. Although there has been some progress in the development of Stat3 SH2 domain binding inhibitors, almost no progress has been made in the development of selective DNA-binding and N-terminal domain inhibitors. Medicinal chemistry efforts are warranted to develop inhibitors for these two domains.

The design and discovery of truly specific and potent Stat3 inhibitors have been very challenging. Therefore, there is a need to reassess the ongoing strategies to develop clinically useful drugs. Robust high-throughput screens using reliable assays and rational computational approaches are being considered to improve upon existing inhibitors. In the near future it will become clear that transcription factors like Stat3 are indeed druggable, opening a whole new field for medicinal chemists to affect patient care.

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■ ABBREVIATIONS USED

APRF, acute-phase response factor; CBP, CREB-binding protein; CDDO-Im, 2-cyano-3,12-dioxoolen-1,9-dien-28-oic acid imidazolide; CDDO-Me, methyl ester of 2-cyano-3,12-dioxoolen-1,9-dien-28-oic acid; EGFR, epidermal growth factor receptor; EMSA, electrophoretic mobility shift assay; EpiSC, epiblast stem cell; FGFR, fibroblast growth factor receptor; FP, fluorescence polarization; HER2, human epidermal growth factor receptor 2; HGFR, hepatocyte growth factor receptor; Hsp90, heat shock protein 90; HUVEC, human umbilical vein endothelial cell; IGFR, insulin-like growth factor receptor; ISRE, IFN-stimulated response element; LIF, leukemia inhibitory factor; MLSD, multiple ligand simultaneous docking; NSCLC, non-small-cell lung carcinoma; PDGFR, platelet-derived growth factor receptor; SAR, structure−activity relationship; SH2, Src homology 2; SHP-1/2, Src homology domain-containing tyrosine phosphatases 1/2; SOCS, suppressor of cytokine signaling; Stat3, signal transducer and activator of transcription 3; VEGFR, vascular endothelial growth factor receptor

■ REFERENCES

(1) Yu, H.; Pardoll, D.; Jove, R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat. Rev. Cancer* **2009**, *9*, 798−809.

(2) Boengler, K.; Hilfiker-Kleiner, D.; Drexler, H.; Heusch, G.; Schulz, R. The myocardial JAK/STAT pathway: from protection to failure. *Pharmacol. Ther.* **2008**, *120*, 172−185.

(3) Akira, S.; Nishio, Y.; Inoue, M.; Wang, X. J.; Wei, S.; Matsusaka, T.; Yoshida, K.; Sudo, T.; Naruto, M.; Kishimoto, T. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell* **1994**, *77*, 63−71.

(4) Zhong, Z.; Wen, Z.; Darnell, J. E., Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science* **1994**, *264*, 95−98.

(5) Hevehan, D. L.; Miller, W. M.; Papoutsakis, E. T. Differential expression and phosphorylation of distinct STAT3 proteins during granulocytic differentiation. *Blood* **2002**, *99*, 1627−1637.

(6) Chakraborty, A.; Tweardy, D. J. Stat3 and G-CSF-induced myeloid differentiation. *Leuk. Lymphoma* **1998**, *30*, 433−442.

(7) Caldenhoven, E.; van Dijk, T. B.; Solari, R.; Armstrong, J.; Raaijmakers, J. A.; Lammers, J. W.; Koenderman, L.; de Groot, R. P. STAT3beta, a splice variant of transcription factor STAT3, is a dominant negative regulator of transcription. *J. Biol. Chem.* **1996**, *271*, 13221−13227.

(8) Maritano, D.; Sugrue, M. L.; Tininini, S.; Dewilde, S.; Strobl, B.; Fu, X.; Murray-Tait, V.; Chiarle, R.; Poli, V. The STAT3 isoforms alpha and beta have unique and specific functions. *Nat. Immunol.* **2004**, *5*, 401−409.

(9) Chakraborty, A.; Tweardy, D. J. Granulocyte colony-stimulating factor activates a 72-kDa isoform of STAT3 in human neutrophils. *J. Leukocyte Biol.* **1998**, *64*, 675−680.

(10) Becker, S.; Groner, B.; Muller, C. W. Three-dimensional structure of the Stat3beta homodimer bound to DNA. *Nature* **1998**, *394*, 145−151.

(11) Ren, Z.; Mao, X.; Mertens, C.; Krishnaraj, R.; Qin, J.; Mandal, P. K.; Romanowski, M. J.; McMurray, J. S.; Chen, X. Crystal structure of unphosphorylated STAT3 core fragment. *Biochem. Biophys. Res. Commun.* **2008**, *374*, 1−5.

(12) Nelson, K. L.; Rogers, J. A.; Bowman, T. L.; Jove, R.; Smithgall, T. E. Activation of STAT3 by the c-Fes protein-tyrosine kinase. *J. Biol. Chem.* **1998**, *273*, 7072−7077.

(13) Yu, H.; Kortylewski, M.; Pardoll, D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. *Nat. Rev. Immunol.* **2007**, *7*, 41−51.

(14) Yokogami, K.; Wakisaka, S.; Avruch, J.; Reeves, S. A. Serine phosphorylation and maximal activation of STAT3 during CNTF signaling is mediated by the rapamycin target mTOR. *Curr. Biol.* **2000**, *10*, 47−50.

(15) Aznar, S.; Valeron, P. F.; del Rincon, S. V.; Perez, L. F.; Perona, R.; Lacal, J. C. Simultaneous tyrosine and serine phosphorylation of STAT3 transcription factor is involved in Rho A GTPase oncogenic transformation. *Mol. Biol. Cell* **2001**, *12*, 3282−3294.

(16) Yuan, Z. L.; Guan, Y. J.; Chatterjee, D.; Chin, Y. E. Stat3 dimerization regulated by reversible acetylation of a single lysine residue. *Science* **2005**, *307*, 269−273.

(17) Giraud, S.; Bienvenu, F.; Avril, S.; Gascan, H.; Heery, D. M.; Coqueret, O. Functional interaction of STAT3 transcription factor with the coactivator NcoA/SRC1a. *J. Biol. Chem.* **2002**, *277*, 8004−8011.

(18) Gray, M. J.; Zhang, J.; Ellis, L. M.; Semenza, G. L.; Evans, D. B.; Watowich, S. S.; Gallick, G. E. HIF-1alpha, STAT3, CBP/p300 and Ref-1/APE are components of a transcriptional complex that regulates Src-dependent hypoxia-induced expression of VEGF in pancreatic and prostate carcinomas. *Oncogene* **2005**, *24*, 3110−3120.

(19) Zhang, X.; Wrzeszczynska, M. H.; Horvath, C. M.; Darnell, J. E., Jr. Interacting regions in Stat3 and c-Jun that participate in cooperative transcriptional activation. *Mol. Cell. Biol.* **1999**, *19*, 7138−7146.

(20) Ivanov, V. N.; Krasilnikov, M.; Ronai, Z. Regulation of Fas expression by STAT3 and c-Jun is mediated by phosphatidylinositol 3-kinase-AKT signaling. *J. Biol. Chem.* **2002**, *277*, 4932−4944.

(21) Kunigal, S.; Lakka, S. S.; Sodadasu, P. K.; Estes, N.; Rao, J. S. Stat3-siRNA induces Fas-mediated apoptosis in vitro and in vivo in breast cancer. *Int. J. Oncol.* **2009**, *34*, 1209−1220.

(22) Fischer, P.; Hilfiker-Kleiner, D. Survival pathways in hypertrophy and heart failure: the gp130-STAT axis. *Basic Res. Cardiol.* **2007**, *102*, 393−411.

(23) Yamamoto, T.; Sekine, Y.; Kashima, K.; Kubota, A.; Sato, N.; Aoki, N.; Matsuda, T. The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathway through STAT3 dephosphorylation. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 811−817.

(24) Muromoto, R.; Sekine, Y.; Imoto, S.; Ikeda, O.; Okayama, T.; Sato, N.; Matsuda, T. BART is essential for nuclear retention of STAT3. *Int. Immunol.* **2008**, *20*, 395−403.

(25) Hirano, T.; Nakajima, K.; Hibi, M. Signaling mechanisms through gp130: a model of the cytokine system. *Cytokine Growth Factor Rev.* **1997**, *8*, 241−252.

(26) Bousquet, C.; Susini, C.; Melmed, S. Inhibitory roles for SHP-1 and SOCS-3 following pituitary proopiomelanocortin induction by leukemia inhibitory factor. *J. Clin. Invest.* **1999**, *104*, 1277−1285.

(27) Kim, H.; Baumann, H. Dual signaling role of the protein tyrosine phosphatase SHP-2 in regulating expression of acute-phase plasma proteins by interleukin-6 cytokine receptors in hepatic cells. *Mol. Cell. Biol.* **1999**, *19*, 5326−5338.

(28) Naka, T.; Narazaki, M.; Hirata, M.; Matsumoto, T.; Minamoto, S.; Aono, A.; Nishimoto, N.; Kajita, T.; Taga, T.; Yoshizaki, K.; Akira, S.; Kishimoto, T. Structure and function of a new STAT-induced STAT inhibitor. *Nature* **1997**, *387*, 924−929.

(29) Herrmann, A.; Vogt, M.; Monnigmann, M.; Clahsen, T.; Sommer, U.; Haan, S.; Poli, V.; Heinrich, P. C.; Muller-Newen, G. Nucleocytoplasmic shuttling of persistently activated STAT3. *J. Cell Sci.* **2007**, *120*, 3249−3261.

(30) Junicho, A.; Matsuda, T.; Yamamoto, T.; Kishi, H.; Korkmaz, K.; Saatcioglu, F.; Fuse, H.; Muraguchi, A. Protein inhibitor of activated STAT3 regulates androgen receptor signaling in prostate carcinoma cells. *Biochem. Biophys. Res. Commun.* **2000**, *278*, 9−13.

(31) Zhang, X.; Guo, A.; Yu, J.; Possemato, A.; Chen, Y.; Zheng, W.; Polakiewicz, R. D.; Kinzler, K. W.; Vogelstein, B.; Velculescu, V. E.; Wang, Z. J. Identification of STAT3 as a substrate of receptor protein tyrosine phosphatase T. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 4060−4064.

(32) Yu, H.; Jove, R. The STATs of cancer—new molecular targets come of age. *Nat. Rev. Cancer* **2004**, *4*, 97−105.

(33) Bromberg, J. F.; Wrzeszczynska, M. H.; Devgan, G.; Zhao, Y.; Pestell, R. G.; Albanese, C.; Darnell, J. E., Jr. Stat3 as an oncogene. *Cell* **1999**, *98*, 295−303.

(34) Li, G. H.; Wei, H.; Lv, S. Q.; Ji, H.; Wang, D. L. Knockdown of STAT3 expression by RNAi suppresses growth and induces apoptosis and differentiation in glioblastoma stem cells. *Int. J. Oncol.* **2010**, *37*, 103−110.

(35) Kortylewski, M.; Yu, H. Stat3 as a potential target for cancer immunotherapy. *J. Immunother.* **2007**, *30*, 131−139.

(36) Wang, T.; Niu, G.; Kortylewski, M.; Burdelya, L.; Shain, K.; Zhang, S.; Bhattacharya, R.; Gabrilovich, D.; Heller, R.; Coppola, D.; Dalton, W.; Jove, R.; Pardoll, D.; Yu, H. Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. *Nat. Med.* **2004**, *10*, 48−54.

(37) Obana, M.; Maeda, M.; Takeda, K.; Hayama, A.; Mohri, T.; Yamashita, T.; Nakaoka, Y.; Komuro, I.; Matsumiya, G.; Azuma, J.; Fujio, Y. Therapeutic activation of signal transducer and activator of transcription 3 by interleukin-11 ameliorates cardiac fibrosis after myocardial infarction. *Circulation* **2010**, *121*, 684−691.

(38) Hilfiker-Kleiner, D.; Kaminski, K.; Podewski, E.; Bonda, T.; Schaefer, A.; Sliwa, K.; Forster, O.; Quint, A.; Landmesser, U.; Doerries, C.; Luchtefeld, M.; Poli, V.; Schneider, M. D.; Balligand, J. L.; Desjardins, F.; Ansari, A.; Struman, I.; Nguyen, N. Q.; Zschemisch, N. H.; Klein, G.; Heusch, G.; Schulz, R.; Hilfiker, A.; Drexler, H. A cathepsin D-cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy. *Cell* **2007**, *128*, 589−600.

(39) Krause, A.; Scaletta, N.; Ji, J. D.; Ivashkiv, L. B. Rheumatoid arthritis synoviocyte survival is dependent on Stat3. *J. Immunol.* **2002**, *169*, 6610−6616.

(40) Shouda, T.; Yoshida, T.; Hanada, T.; Wakioka, T.; Oishi, M.; Miyoshi, K.; Komiya, S.; Kosai, K.; Hanakawa, Y.; Hashimoto, K.; Nagata, K.; Yoshimura, A. Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. *J. Clin. Invest.* **2001**, *108*, 1781−1788.

(41) Hirano, T. Interleukin 6 in autoimmune and inflammatory diseases: a personal memoir. *Proc. Jpn. Acad., Ser. B: Phys. Biol. Sci.* **2010**, *86*, 717−730.

(42) Wan, J.; Fu, A. K.; Ip, F. C.; Ng, H. K.; Hugon, J.; Page, G.; Wang, J. H.; Lai, K. O.; Wu, Z.; Ip, N. Y. Tyk2/STAT3 signaling mediates beta-amyloid-induced neuronal cell death: implications in Alzheimer's disease. *J. Neurosci.* **2010**, *30*, 6873−6881.

(43) Yamada, M.; Chiba, T.; Sasabe, J.; Terashita, K.; Aiso, S.; Matsuoka, M. Nasal colivelin treatment ameliorates memory impairment related to Alzheimer's disease. *Neuropsychopharmacology* **2008**, *33*, 2020−2032.

(44) Kuratsune, M.; Masaki, T.; Hirai, T.; Kiribayashi, K.; Yokoyama, Y.; Arakawa, T.; Yorioka, N.; Kohno, N. Signal transducer and activator of transcription 3 involvement in the development of renal interstitial fibrosis after unilateral ureteral obstruction. *Nephrology* **2007**, *12*, 565−571.

(45) Pang, M.; Ma, L.; Gong, R.; Tolbert, E.; Mao, H.; Ponnusamy, M.; Chin, Y. E.; Yan, H.; Dworkin, L. D.; Zhuang, S. A novel STAT3 inhibitor, S3I-201, attenuates renal interstitial fibroblast activation and interstitial fibrosis in obstructive nephropathy. *Kidney Int.* **2010**, *78*, 257−268.

(46) Huang, Y. H.; Chuang, J. H.; Yang, Y. L.; Huang, C. C.; Wu, C. L.; Chen, C. L. Cholestasis downregulate hepcidin expression through inhibiting IL-6-induced phosphorylation of signal transducer and activator of transcription 3 signaling. *Lab. Invest.* **2009**, *89*, 1128−1139.

(47) Mair, M.; Zollner, G.; Schneller, D.; Musteanu, M.; Fickert, P.; Gumhold, J.; Schuster, C.; Fuchsbichler, A.; Bilban, M.; Tauber, S.; Esterbauer, H.; Kenner, L.; Poli, V.; Blaas, L.; Kornfeld, J. W.; Casanova, E.; Mikulits, W.; Trauner, M.; Eferl, R. Signal transducer and activator of transcription 3 protects from liver injury and fibrosis in a mouse model of sclerosing cholangitis. *Gastroenterology* **2010**, *138*, 2499−2508.

(48) Tripathi, S. S.; Mishra, V.; Shukla, M.; Verma, M.; Chaudhury, B. P.; Kumar, P.; Chhabra, J. K.; Pandey, H. P.; Paul, B. IL-6 receptor-mediated lung Th2 cytokine networking in silica-induced pulmonary fibrosis. *Arch. Toxicol.* **2010**, *84*, 947−955.

(49) Gao, H.; Ward, P. A. STAT3 and suppressor of cytokine signaling 3: potential targets in lung inflammatory responses. *Expert Opin. Ther. Targets* **2007**, *11*, 869−880.

(50) Qu, P.; Roberts, J.; Li, Y.; Albrecht, M.; Cummings, O. W.; Eble, J. N.; Du, H.; Yan, C. Stat3 downstream genes serve as biomarkers in human lung carcinomas and chronic obstructive pulmonary disease. *Lung Cancer* **2009**, *63*, 341−347.

(51) Dauer, D. J.; Ferraro, B.; Song, L.; Yu, B.; Mora, L.; Buettner, R.; Enkemann, S.; Jove, R.; Haura, E. B. Stat3 regulates genes common to both wound healing and cancer. *Oncogene* **2005**, *24*, 3397−3408.

(52) Sano, S.; Chan, K. S.; Carbajal, S.; Clifford, J.; Peavey, M.; Kiguchi, K.; Itami, S.; Nickoloff, B. J.; DiGiovanni, J. Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat. Med.* **2005**, *11*, 43−49.

(53) Romanowska, M.; Reilly, L.; Palmer, C. N.; Gustafsson, M. C.; Foerster, J. Activation of PPARbeta/delta causes a psoriasis-like skin disease in vivo. *PLoS One* **2010**, *5*, e9701.

(54) Neufert, C.; Pickert, G.; Zheng, Y.; Wittkopf, N.; Warntjen, M.; Nikolaev, A.; Ouyang, W.; Neurath, M. F.; Becker, C. Activation of epithelial STAT3 regulates intestinal homeostasis. *Cell Cycle* **2010**, *9*, 652−655.

(55) Carey, R.; Jurickova, I.; Ballard, E.; Bonkowski, E.; Han, X.; Xu, H.; Denson, L. A. Activation of an IL-6:STAT3-dependent transcriptome in pediatric-onset inflammatory bowel disease. *Inflamm. Bowel Dis.* **2008**, *14*, 446−457.

(56) Bai, A.; Hu, P.; Chen, J.; Song, X.; Chen, W.; Peng, W.; Zeng, Z.; Gao, X. Blockade of STAT3 by antisense oligonucleotide in TNBS-induced murine colitis. *Int. J. Colorectal Dis.* **2007**, *22*, 625−635.

(57) Bates, S. H.; Stearns, W. H.; Dundon, T. A.; Schubert, M.; Tso, A. W.; Wang, Y.; Banks, A. S.; Lavery, H. J.; Haq, A. K.; Maratos-Flier, E.; Neel, B. G.; Schwartz, M. W.; Myers, M. G., Jr. STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* **2003**, *421*, 856−859.

(58) Puglianiello, A.; Germani, D.; Cianfarani, S. Exposure to uteroplacental insufficiency reduces the expression of signal transducer

and activator of transcription 3 and proopiomelanocortin in the hypothalamus of newborn rats. *Pediatr. Res.* **2009**, *66*, 208−211.

(59) Wang, P.; Yang, F. J.; Du, H.; Guan, Y. F.; Xu, T. Y.; Xu, X. W.; Su, D. F.; Miao, C. Y. Involvement of leptin receptor (LepRb)-STAT3 signaling pathway in brain FTO downregulation during energy restriction. *Mol. Med.* **2011**, *17*, 523−532.

(60) Gharavi, N. M.; Alva, J. A.; Mouillesseaux, K. P.; Lai, C.; Yeh, M.; Yeung, W.; Johnson, J.; Szeto, W. L.; Hong, L.; Fishbein, M.; Wei, L.; Pfeffer, L. M.; Berliner, J. A. Role of the Jak/STAT pathway in the regulation of interleukin-8 transcription by oxidized phospholipids in vitro and in atherosclerosis in vivo. *J. Biol. Chem.* **2007**, *282*, 31460−31468.

(61) Khan, J. A.; Cao, M.; Kang, B. Y.; Liu, Y.; Mehta, J. L.; Hermonat, P. L. AAV/hSTAT3-gene delivery lowers aortic inflammatory cell infiltration in LDLR KO mice on high cholesterol. *Atherosclerosis* **2010**, *213*, 59−66.

(62) Zhou, X.; Li, D.; Yan, W.; Li, W. Pravastatin prevents aortic atherosclerosis via modulation of signal transduction and activation of transcription 3 (STAT3) to attenuate interleukin-6 (IL-6) action in ApoE knockout mice. *Int. J. Mol. Sci.* **2008**, *9*, 2253−2264.

(63) Niwa, H.; Ogawa, K.; Shimosato, D.; Adachi, K. A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. *Nature* **2009**, *460*, 118−122.

(64) Boiani, M.; Scholer, H. R. Regulatory networks in embryo-derived pluripotent stem cells. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 872−884.

(65) Torres, J.; Watt, F. M. Nanog maintains pluripotency of mouse embryonic stem cells by inhibiting NFkappaB and cooperating with Stat3. *Nat. Cell Biol.* **2008**, *10*, 194−201.

(66) Setati, M. M.; Prinsloo, E.; Longshaw, V. M.; Murray, P. A.; Edgar, D. H.; Blatch, G. L. Leukemia inhibitory factor promotes Hsp90 association with STAT3 in mouse embryonic stem cells. *IUBMB Life* **2010**, *62*, 61−66.

(67) Ying, Q. L.; Nichols, J.; Chambers, I.; Smith, A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* **2003**, *115*, 281−292.

(68) Boyer, L. A.; Mathur, D.; Jaenisch, R. Molecular control of pluripotency. *Curr. Opin. Genet. Dev.* **2006**, *16*, 455−462.

(69) Yang, J.; van Oosten, A. L.; Theunissen, T. W.; Guo, G.; Silva, J. C.; Smith, A. Stat3 activation is limiting for reprogramming to ground state pluripotency. *Cell Stem Cell* **2010**, *7*, 319−328.

(70) Humphrey, R. K.; Beattie, G. M.; Lopez, A. D.; Bucay, N.; King, C. C.; Firpo, M. T.; Rose-John, S.; Hayek, A. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. *Stem Cells* **2004**, *22*, 522−530.

(71) Daheron, L.; Opitz, S. L.; Zaehres, H.; Lensch, M. W.; Andrews, P. W.; Itskovitz-Eldor, J.; Daley, G. Q. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. *Stem Cells* **2004**, *22*, 770−778.

(72) Chung, Y. J.; Park, B. B.; Kang, Y. J.; Kim, T. M.; Eaves, C. J.; Oh, I. H. Unique effects of Stat3 on the early phase of hematopoietic stem cell regeneration. *Blood* **2006**, *108*, 1208−1215.

(73) Onai, N.; Manz, M. G. The STATs on dendritic cell development. *Immunity* **2008**, *28*, 490−492.

(74) Chou, W. C.; Levy, D. E.; Lee, C. K. STAT3 positively regulates an early step in B-cell development. *Blood* **2006**, *108*, 3005−3011.

(75) Oatley, J. M.; Kaucher, A. V.; Avarbock, M. R.; Brinster, R. L. Regulation of mouse spermatogonial stem cell differentiation by STAT3 signaling. *Biol. Reprod.* **2010**, *83*, 427−433.

(76) Yang, Y.; Xu, Y.; Li, W.; Wang, G.; Song, Y.; Yang, G.; Han, X.; Du, Z.; Sun, L.; Ma, K. STAT3 induces muscle stem cell differentiation by interaction with myoD. *Cytokine* **2009**, *46*, 137−141.

(77) Deng, J.; Grande, F.; Neamati, N. Small molecule inhibitors of Stat3 signaling pathway. *Curr. Cancer Drug Targets* **2007**, *7*, 91−107.

(78) Zhao, M.; Jiang, B.; Gao, F. H. Small molecule inhibitors of STAT3 for cancer therapy. *Curr. Med. Chem.* **2011**, *18*, 4012−4018.

(79) Mankan, A. K.; Greten, F. R. Inhibiting signal transducer and activator of transcription 3: rationality and rationale design of inhibitors. *Expert Opin. Invest. Drugs* **2011**, *20*, 1263−1275.

(80) Lavecchia, A.; Di Giovanni, C.; Novellino, E. STAT-3 inhibitors: state of the art and new horizons for cancer treatment. *Curr. Med. Chem.* **2011**, *18*, 2359−2375.

(81) Page, B. D.; Ball, D. P.; Gunning, P. T. Signal transducer and activator of transcription 3 inhibitors: a patent review. *Expert Opin. Ther. Pat.* **2011**, *21*, 65−83.

(82) Uehara, Y.; Mochizuki, M.; Matsuno, K.; Haino, T.; Asai, A. Novel high-throughput screening system for identifying STAT3-SH2 antagonists. *Biochem. Biophys. Res. Commun.* **2009**, *380*, 627−631.

(83) Song, H.; Wang, R.; Wang, S.; Lin, J. A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4700−4705.

(84) Siddiquee, K.; Zhang, S.; Guida, W. C.; Blaskovich, M. A.; Greedy, B.; Lawrence, H. R.; Yip, M. L.; Jove, R.; McLaughlin, M. M.; Lawrence, N. J.; Sebti, S. M.; Turkson, J. Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7391−7396.

(85) Gunning, P. T.; Glenn, M. P.; Siddiquee, K. A.; Katt, W. P.; Masson, E.; Sebti, S. M.; Turkson, J.; Hamilton, A. D. Targeting protein−protein interactions: suppression of Stat3 dimerization with rationally designed small-molecule, nonpeptidic SH2 domain binders. *ChemBioChem* **2008**, *9*, 2800−2803.

(86) Zhang, X.; Yue, P.; Fletcher, S.; Zhao, W.; Gunning, P. T.; Turkson, J. A novel small-molecule disrupts Stat3 SH2 domain-phosphotyrosine interactions and Stat3-dependent tumor processes. *Biochem. Pharmacol.* **2010**, *79*, 1398−1409.

(87) Li, H.; Liu, A.; Zhao, Z.; Xu, Y.; Lin, J.; Jou, D.; Li, C. Fragment-based drug design and drug repositioning using multiple ligand simultaneous docking (MLSD): identifying celecoxib and template compounds as novel inhibitors of signal transducer and activator of transcription 3 (STAT3). *J. Med. Chem.* **2011**, *54*, 5592−5596.

(88) Dann, M.; Lefemine, D. V.; Barbatschi, F.; Shu, P.; Kunstmann, M. P.; Mitscher, L. A.; Bohonos, N. Tetrangomycin, a new quinone antibiotic. *Antimicrob. Agents Chemother.* **1965**, *5*, 832−835.

(89) Bhasin, D.; Cisek, K.; Pandharkar, T.; Regan, N.; Li, C.; Pandit, B.; Lin, J.; Li, P. K. Design, synthesis, and studies of small molecule STAT3 inhibitors. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 391−395.

(90) Lin, L.; Hutzen, B.; Li, P. K.; Ball, S.; Zuo, M.; DeAngelis, S.; Foust, E.; Sobo, M.; Friedman, L.; Bhasin, D.; Cen, L.; Li, C.; Lin, J. A novel small molecule, LLL12, inhibits STAT3 phosphorylation and activities and exhibits potent growth-suppressive activity in human cancer cells. *Neoplasia* **2010**, *12*, 39−50.

(91) Song, H.; Wang, R.; Wang, S.; Lin, J. Small Molecule Inhibitors of Stat3 and the Uses Thereof. WO 2006/091837 A2, 2006.

(92) Fuh, B.; Sobo, M.; Cen, L.; Josiah, D.; Hutzen, B.; Cisek, K.; Bhasin, D.; Regan, N.; Lin, L.; Chan, C.; Caldas, H.; DeAngelis, S.; Li, C.; Li, P. K.; Lin, J. LLL-3 inhibits STAT3 activity, suppresses glioblastoma cell growth and prolongs survival in a mouse glioblastoma model. *Br. J. Cancer* **2009**, *100*, 106−112.

(93) Mencalha, A. L.; Du Rocher, B.; Salles, D.; Binato, R.; Abdelhay, E. LLL-3, a STAT3 inhibitor, represses BCR-ABL-positive cell proliferation, activates apoptosis and improves the effects of imatinib mesylate. *Cancer Chemother. Pharmacol.* **2010**, *65*, 1039−1046.

(94) Ball, S.; Li, C.; Li, P. K.; Lin, J. The small molecule, LLL12, inhibits STAT3 phosphorylation and induces apoptosis in medulloblastoma and glioblastoma cells. *PLoS One* **2011**, *6*, e18820.

(95) Schust, J.; Sperl, B.; Hollis, A.; Mayer, T. U.; Berg, T. Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem. Biol.* **2006**, *13*, 1235−1242.

(96) Villalva, C.; Martin-Lanneree, S.; Cortes, U.; Dkhissi, F.; Wager, M.; Le Corf, A.; Tourani, J. M.; Dusanter-Fourt, I.; Turhan, A. G.; Karayan-Tapon, L. STAT3 is essential for the maintenance of neurosphere-initiating tumor cells in patients with glioblastomas: a potential for targeted therapy? *Int. J. Cancer* **2011**, *128*, 826−838.

(97) Friedman, L.; Lin, L.; Ball, S.; Bekaii-Saab, T.; Fuchs, J.; Li, P. K.; Li, C.; Lin, J. Curcumin analogues exhibit enhanced growth suppressive activity in human pancreatic cancer cells. *Anti-Cancer Drugs* **2009**, *20*, 444−449.

(98) Lin, L.; Hutzen, B.; Ball, S.; Foust, E.; Sobo, M.; Deangelis, S.; Pandit, B.; Friedman, L.; Li, C.; Li, P. K.; Fuchs, J.; Lin, J. New curcumin analogues exhibit enhanced growth-suppressive activity and inhibit AKT and signal transducer and activator of transcription 3 phosphorylation in breast and prostate cancer cells. *Cancer Sci.* **2009**, *100*, 1719−1727.

(99) Lin, L.; Hutzen, B.; Zuo, M.; Ball, S.; Deangelis, S.; Foust, E.; Pandit, B.; Ihnat, M. A.; Shenoy, S. S.; Kulp, S.; Li, P. K.; Li, C.; Fuchs, J.; Lin, J. Novel STAT3 phosphorylation inhibitors exhibit potent growth-suppressive activity in pancreatic and breast cancer cells. *Cancer Res.* **2010**, *70*, 2445−2454.

(100) Lin, L.; Deangelis, S.; Foust, E.; Fuchs, J.; Li, C.; Li, P. K.; Schwartz, E. B.; Lesinski, G. B.; Benson, D.; Lu, J.; Hoyt, D.; Lin, J. A novel small molecule inhibits STAT3 phosphorylation and DNA binding activity and exhibits potent growth suppressive activity in human cancer cells. *Mol. Cancer* **2010**, *9*, 217.

(101) Bill, M. A.; Fuchs, J. R.; Li, C.; Yui, J.; Bakan, C.; Benson, D. M., Jr.; Schwartz, E. B.; Abdelhamid, D.; Lin, J.; Hoyt, D. G.; Fossey, S. L.; Young, G. S.; Carson, W. E., 3rd; Li, P. K.; Lesinski, G. B. The small molecule curcumin analog FLLL32 induces apoptosis in melanoma cells via STAT3 inhibition and retains the cellular response to cytokines with anti-tumor activity. *Mol. Cancer* **2010**, *9*, 165.

(102) Xu, J.; Cole, D. C.; Chang, C. P.; Ayyad, R.; Asselin, M.; Hao, W.; Gibbons, J.; Jelinsky, S. A.; Saraf, K. A.; Park, K. Inhibition of the signal transducer and activator of transcription-3 (STAT3) signaling pathway by 4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxylic acid esters. *J. Med. Chem.* **2008**, *51*, 4115−4121.

(103) Lin, L.; Amin, R.; Gallicano, G. I.; Glasgow, E.; Jogunoori, W.; Jessup, J. M.; Zasloff, M.; Marshall, J. L.; Shetty, K.; Johnson, L.; Mishra, L.; He, A. R. The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with disrupted TGF-beta signaling. *Oncogene* **2009**, *28*, 961−972.

(104) Fletcher, S.; Singh, J.; Zhang, X.; Yue, P.; Page, B. D.; Sharmeen, S.; Shahani, V. M.; Zhao, W.; Schimmer, A. D.; Turkson, J.; Gunning, P. T. Disruption of transcriptionally active Stat3 dimers with non-phosphorylated, salicylic acid-based small molecules: potent in vitro and tumor cell activities. *ChemBioChem* **2009**, *10*, 1959−1964.

(105) Fletcher, S.; Page, B. D.; Zhang, X.; Yue, P.; Li, Z. H.; Sharmeen, S.; Singh, J.; Zhao, W.; Schimmer, A. D.; Trudel, S.; Turkson, J.; Gunning, P. T. Antagonism of the Stat3-Stat3 protein dimer with salicylic acid based small molecules. *ChemMedChem* **2011**, *6*, 1459−1470.

(106) Page, B. D.; Fletcher, S.; Yue, P.; Li, Z.; Zhang, X.; Sharmeen, S.; Datti, A.; Wrana, J. L.; Trudel, S.; Schimmer, A. D.; Turkson, J.; Gunning, P. T. Identification of a non-phosphorylated, cell permeable, small molecule ligand for the Stat3 SH2 domain. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5605−5609.

(107) Frank, D. A. STAT Modulators. WO 2008/156644 A2, 2008.

(108) Nelson, E. A.; Walker, S. R.; Kepich, A.; Gashin, L. B.; Hideshima, T.; Ikeda, H.; Chauhan, D.; Anderson, K. C.; Frank, D. A. Nifuroxazide inhibits survival of multiple myeloma cells by directly inhibiting STAT3. *Blood* **2008**, *112*, 5095−5102.

(109) Xu, X.; Kasembeli, M. M.; Jiang, X.; Tweardy, B. J.; Tweardy, D. J. Chemical probes that competitively and selectively inhibit Stat3 activation. *PLoS One* **2009**, *4*, e4783.

(110) Redell, M. S.; Ruiz, M. J.; Alonzo, T. A.; Gerbing, R. B.; Tweardy, D. J. Stat3 signaling in acute myeloid leukemia: ligand-dependent and -independent activation and induction of apoptosis by a novel small-molecule Stat3 inhibitor. *Blood* **2011**, *117*, 5701−5709.

(111) Matsuno, K.; Masuda, Y.; Uehara, Y.; Sato, H.; Muroya, A.; Takahashi, O.; Yokotagawa, T.; Furuya, T.; Okawara, T.; Otsuka, M.; Ogo, N.; Ashizawa, T.; Oshita, C.; Tai, S.; Ishii, H.; Akiyama, Y.; Asai, A. Identification of a New Series of STAT3 Inhibitors by Virtual Screening. *ACS Med. Chem. Lett.* **2010**, *1*, 371−375.

(112) Shin, D. S.; Kim, H. N.; Shin, K. D.; Yoon, Y. J.; Kim, S. J.; Han, D. C.; Kwon, B. M. Cryptotanshinone inhibits constitutive signal transducer and activator of transcription 3 function through blocking the dimerization in DU145 prostate cancer cells. *Cancer Res.* **2009**, *69*, 193−202.

(113) Kim, N. H.; Lee, M. Y.; Park, S. J.; Choi, J. S.; Oh, M. K.; Kim, I. S. Auranofin blocks interleukin-6 signalling by inhibiting phosphorylation of JAK1 and STAT3. *Immunology* **2007**, *122*, 607−614.

(114) Cuevas, P.; Diaz-Gonzalez, D.; Sanchez, I.; Lozano, R. M.; Gimenez-Gallego, G.; Dujovny, M. Dobesilate inhibits the activation of signal transducer and activator of transcription 3, and the expression of cyclin D1 and bcl-XL in glioma cells. *Neurol. Res.* **2006**, *28*, 127−130.

(115) Selvendiran, K.; Bratasz, A.; Tong, L.; Ignarro, L. J.; Kuppusamy, P. NCX-4016, a nitro-derivative of aspirin, inhibits EGFR and STAT3 signaling and modulates Bcl-2 proteins in cisplatin-resistant human ovarian cancer cells and xenografts. *Cell Cycle* **2008**, *7*, 81−88.

(116) Dowlati, A.; Kluge, A.; Nethery, D.; Halmos, B.; Kern, J. A. SCH66336, inhibitor of protein farnesylation, blocks signal transducer and activators of transcription 3 signaling in lung cancer and interacts with a small molecule inhibitor of epidermal growth factor receptor/human epidermal growth factor receptor 2. *Anti-Cancer Drugs* **2008**, *19*, 9−16.

(117) Liby, K.; Voong, N.; Williams, C. R.; Risingsong, R.; Royce, D. B.; Honda, T.; Gribble, G. W.; Sporn, M. B.; Letterio, J. J. The synthetic triterpenoid CDDO-imidazolide suppresses STAT phosphorylation and induces apoptosis in myeloma and lung cancer cells. *Clin. Cancer Res.* **2006**, *12*, 4288−4293.

(118) Ling, X.; Konopleva, M.; Zeng, Z.; Ruvolo, V.; Stephens, L. C.; Schober, W.; McQueen, T.; Dietrich, M.; Madden, T. L.; Andreeff, M. The novel triterpenoid C-28 methyl ester of 2-cyano-3,12-dioxoolen-1,9-dien-28-oic acid inhibits metastatic murine breast tumor growth through inactivation of STAT3 signaling. *Cancer Res.* **2007**, *67*, 4210−4218.

(119) Liu, Y. C.; Hsieh, C. W.; Wu, C. C.; Wung, B. S. Chalcone inhibits the activation of NF-kappaB and STAT3 in endothelial cells via endogenous electrophile. *Life Sci.* **2007**, *80*, 1420−1430.

(120) Pandey, M. K.; Sung, B.; Ahn, K. S.; Aggarwal, B. B. Butein suppresses constitutive and inducible signal transducer and activator of transcription (STAT) 3 activation and STAT3-regulated gene products through the induction of a protein tyrosine phosphatase SHP-1. *Mol. Pharmacol.* **2009**, *75*, 525−533.

(121) Agarwal, C.; Tyagi, A.; Kaur, M.; Agarwal, R. Silibinin inhibits constitutive activation of Stat3, and causes caspase activation and apoptotic death of human prostate carcinoma DU145 cells. *Carcinogenesis* **2007**, *28*, 1463−1470.

(122) Bhutani, M.; Pathak, A. K.; Nair, A. S.; Kunnumakkara, A. B.; Guha, S.; Sethi, G.; Aggarwal, B. B. Capsaicin is a novel blocker of constitutive and interleukin-6-inducible STAT3 activation. *Clin. Cancer Res.* **2007**, *13*, 3024−3032.

(123) Pathak, A. K.; Bhutani, M.; Nair, A. S.; Ahn, K. S.; Chakraborty, A.; Kadara, H.; Guha, S.; Sethi, G.; Aggarwal, B. B. Ursolic acid inhibits STAT3 activation pathway leading to suppression of proliferation and chemosensitization of human multiple myeloma cells. *Mol. Cancer Res.* **2007**, *5*, 943−955.

(124) Pandey, M. K.; Sung, B.; Aggarwal, B. B. Betulinic acid suppresses STAT3 activation pathway through induction of protein tyrosine phosphatase SHP-1 in human multiple myeloma cells. *Int. J. Cancer* **2010**, *127*, 282−292.

(125) Ahn, K. S.; Sethi, G.; Sung, B.; Goel, A.; Ralhan, R.; Aggarwal, B. B. Guggulsterone, a farnesoid X receptor antagonist, inhibits constitutive and inducible STAT3 activation through induction of a protein tyrosine phosphatase SHP-1. *Cancer Res.* **2008**, *68*, 4406−4415.

(126) Sandur, S. K.; Pandey, M. K.; Sung, B.; Aggarwal, B. B. 5-Hydroxy-2-methyl-1,4-naphthoquinone, a vitamin K3 analogue, suppresses STAT3 activation pathway through induction of protein

tyrosine phosphatase, SHP-1: potential role in chemosensitization. *Mol. Cancer Res.* **2010**, *8*, 107−118.

(127) Kannappan, R.; Yadav, V. R.; Aggarwal, B. B. γ-Tocotrienol but not γ-tocopherol blocks STAT3 cell signaling pathway through induction of protein tyrosine phosphatase SHP-1 and sensitizes tumor cells to chemotherapeutic agents. *J. Biol. Chem.* **2010**, *285*, 33520−33528.

(128) Sun, M.; Liu, C.; Nadiminty, N.; Lou, W.; Zhu, Y.; Yang, J.; Evans, C. P.; Zhou, Q.; Gao, A. C. Inhibition of Stat3 activation by sanguinarine suppresses prostate cancer cell growth and invasion. *Prostate* **2012**, *72*, 82−89.

(129) Duan, Z.; Bradner, J. E.; Greenberg, E.; Levine, R.; Foster, R.; Mahoney, J.; Seiden, M. V. SD-1029 inhibits signal transducer and activator of transcription 3 nuclear translocation. *Clin. Cancer Res.* **2006**, *12*, 6844−6852.

(130) Muto, A.; Hori, M.; Sasaki, Y.; Saitoh, A.; Yasuda, I.; Maekawa, T.; Uchida, T.; Asakura, K.; Nakazato, T.; Kaneda, T.; Kizaki, M.; Ikeda, Y.; Yoshida, T. Emodin has a cytotoxic activity against human multiple myeloma as a Janus-activated kinase 2 inhibitor. *Mol. Cancer Ther.* **2007**, *6*, 987−994.

(131) Liu, L.; Nam, S.; Tian, Y.; Yang, F.; Wu, J.; Wang, Y.; Scuto, A.; Polychronopoulos, P.; Magiatis, P.; Skaltsounis, L.; Jove, R. 6-Bromoindirubin-3′-oxime inhibits JAK/STAT3 signaling and induces apoptosis of human melanoma cells. *Cancer Res.* **2011**, *71*, 3972−3979.

(132) Park, E. J.; Kim, S. A.; Choi, Y. M.; Kwon, H. K.; Shim, W.; Lee, G.; Choi, S. Capric acid inhibits NO production and STAT3 activation during LPS-induced osteoclastogenesis. *PLoS One* **2011**, *6*, e27739.

(133) Walker, S. R.; Chaudhury, M.; Frank, D. A. STAT3 inhibition by microtubule-targeted drugs: dual molecular effects of chemotherapeutic agents. *Mol. Cell. Pharmacol.* **2011**, *3*, 13−19.

(134) Ren, Z.; Cabell, L. A.; Schaefer, T. S.; McMurray, J. S. Identification of a high-affinity phosphopeptide inhibitor of Stat3. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 633−636.

(135) Coleman, D. R. t.; Ren, Z.; Mandal, P. K.; Cameron, A. G.; Dyer, G. A.; Muranjan, S.; Campbell, M.; Chen, X.; McMurray, J. S. Investigation of the binding determinants of phosphopeptides targeted to the SRC homology 2 domain of the signal transducer and activator of transcription 3. Development of a high-affinity peptide inhibitor. *J. Med. Chem.* **2005**, *48*, 6661−6670.

(136) Gomez, C.; Bai, L.; Zhang, J.; Nikolovska-Coleska, Z.; Chen, J.; Yi, H.; Wang, S. Design, synthesis, and evaluation of peptidomimetics containing Freidinger lactams as STAT3 inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1733−1736.

(137) Chen, J.; Bai, L.; Bernard, D.; Nikolovska-Coleska, Z.; Gomez, C.; Zhang, J.; Yi, H.; Wang, S. Structure-based design of conformationally constrained, cell-permeable STAT3 inhibitors. *ACS Med. Chem. Lett.* **2010**, *1*, 85−89.

(138) Mandal, P. K.; Ren, Z.; Chen, X.; Xiong, C.; McMurray, J. S. Structure−affinity relationships of glutamine mimics incorporated into phosphopeptides targeted to the SH2 domain of signal transducer and activator of transcription 3. *J. Med. Chem.* **2009**, *52*, 6126−6141.

(139) Siddiquee, K. A.; Gunning, P. T.; Glenn, M.; Katt, W. P.; Zhang, S.; Schrock, C.; Sebti, S. M.; Jove, R.; Hamilton, A. D.; Turkson, J. An oxazole-based small-molecule Stat3 inhibitor modulates Stat3 stability and processing and induces antitumor cell effects. *ACS Chem. Biol.* **2007**, *2*, 787−798.

(140) Turkson, J.; Hamilton, A. D.; Jove, R.; Sebti, S. M. Stat3 Inhibitor Having Anti-Cancer Activity and Associated Methods. WO 2008/070697 A2, 2008.

(141) Shahani, V. M.; Yue, P.; Fletcher, S.; Sharmeen, S.; Sukhai, M. A.; Luu, D. P.; Zhang, X.; Sun, H.; Zhao, W.; Schimmer, A. D.; Turkson, J.; Gunning, P. T. Design, synthesis, and in vitro characterization of novel hybrid peptidomimetic inhibitors of STAT3 protein. *Bioorg. Med. Chem.* **2011**, *19*, 1823−1838.

(142) NCT01047943. Intrapatient Comparison Study of Efficacy of STA-21 Ointment on Psoriatic Skin Lesions. http://clinicaltrials.gov.

(143) NCT01066663. A Phase I/II Study of Pyrimethamine, a STAT3 Inhibitor, for the Treatment of Relapsed Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma. http://clinicaltrials.gov.

(144) NCT00955812. A Phase I, Open-label, Dose Escalation, Non-randomized Study To Assess the Pharmacokinetics, Dose Limiting Toxicity, and Maximum Tolerated Dose of OPB-31121 in Subjects with Advanced Solid Tumors. http://clinicaltrials.gov.

(145) NCT00529113. A Randomized Phase I/II Study with Gemcitabine and RTA 402 or Gemcitabine and Placebo for Patients with Unresectable Pancreatic Cancer. http://clinicaltrials.gov.

(146) Hong, D. S.; Kurzrock, R.; Supko, J. G.; Lawrence, D. P.; Wheler, J. J.; Meyer, C. J.; Mier, J. W.; Andreeff, M.; Shapiro, G. I.; Dezube, B. J. Phase I trial with a novel oral NF-κB/STAT3 inhibitor RTA 402 in patients with solid tumors and lymphoid malignancies. *J. Clin. Oncol.* **2008**, *26* (15S), 3517.

(147) Al Zaid Siddiquee, K.; Turkson, J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. *Cell Res.* **2008**, *18*, 254−267.

(148) Bowman, T.; Garcia, R.; Turkson, J.; Jove, R. STATs in oncogenesis. *Oncogene* **2000**, *19*, 2474−2488.

(149) Buettner, R.; Mora, L. B.; Jove, R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clin. Cancer Res.* **2002**, *8*, 945−954.

(150) Chen, C. L.; Hsieh, F. C.; Lieblein, J. C.; Brown, J.; Chan, C.; Wallace, J. A.; Cheng, G.; Hall, B. M.; Lin, J. Stat3 activation in human endometrial and cervical cancers. *Br. J. Cancer* **2007**, *96*, 591−599.

(151) Cheng, G. Z.; Zhang, W. Z.; Sun, M.; Wang, Q.; Coppola, D.; Mansour, M.; Xu, L. M.; Costanzo, C.; Cheng, J. Q.; Wang, L. H. Twist is transcriptionally induced by activation of STAT3 and mediates STAT3 oncogenic function. *J. Biol. Chem.* **2008**, *283*, 14665−14673.

(152) Duan, Z.; Foster, R.; Bell, D. A.; Mahoney, J.; Wolak, K.; Vaidya, A.; Hampel, C.; Lee, H.; Seiden, M. V. Signal transducers and activators of transcription 3 pathway activation in drug-resistant ovarian cancer. *Clin. Cancer Res.* **2006**, *12*, 5055−5063.

(153) Abdulghani, J.; Gu, L.; Dagvadorj, A.; Lutz, J.; Leiby, B.; Bonuccelli, G.; Lisanti, M. P.; Zellweger, T.; Alanen, K.; Mirtti, T.; Visakorpi, T.; Bubendorf, L.; Nevalainen, M. T. Stat3 promotes metastatic progression of prostate cancer. *Am. J. Pathol.* **2008**, *172*, 1717−1728.

(154) Corvinus, F. M.; Orth, C.; Moriggl, R.; Tsareva, S. A.; Wagner, S.; Pfitzner, E. B.; Baus, D.; Kaufmann, R.; Huber, L. A.; Zatloukal, K.; Beug, H.; Ohlschlager, P.; Schutz, A.; Halbhuber, K. J.; Friedrich, K. Persistent STAT3 activation in colon cancer is associated with enhanced cell proliferation and tumor growth. *Neoplasia* **2005**, *7*, 545−555.

(155) Dhir, R.; Ni, Z.; Lou, W.; DeMiguel, F.; Grandis, J. R.; Gao, A. C. Stat3 activation in prostatic carcinomas. *Prostate* **2002**, *51*, 241−246.

(156) Guo, C.; Yang, G.; Khun, K.; Kong, X.; Levy, D.; Lee, P.; Melamed, J. Activation of Stat3 in renal tumors. *Am. J. Transl. Res.* **2009**, *1*, 283−290.

(157) Schaefer, L. K.; Ren, Z.; Fuller, G. N.; Schaefer, T. S. Constitutive activation of Stat3alpha in brain tumors: localization to tumor endothelial cells and activation by the endothelial tyrosine kinase receptor (VEGFR-2). *Oncogene* **2002**, *21*, 2058−2065.

(158) Seki, Y.; Suzuki, N.; Imaizumi, M.; Iwamoto, T.; Usami, N.; Ueda, Y.; Hamaguchi, M. STAT3 and MAPK in human lung cancer tissues and suppression of oncogenic growth by JAB and dominant negative STAT3. *Int. J. Oncol.* **2004**, *24*, 931−934.

(159) Wei, D.; Le, X.; Zheng, L.; Wang, L.; Frey, J. A.; Gao, A. C.; Peng, Z.; Huang, S.; Xiong, H. Q.; Abbruzzese, J. L.; Xie, K. Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. *Oncogene* **2003**, *22*, 319−329.

(160) Abou-Ghazal, M.; Yang, D. S.; Qiao, W.; Reina-Ortiz, C.; Wei, J.; Kong, L. Y.; Fuller, G. N.; Hiraoka, N.; Priebe, W.; Sawaya, R.; Heimberger, A. B. The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. *Clin. Cancer Res.* **2008**, *14*, 8228−8235.

(161) Grandis, J. R.; Drenning, S. D.; Zeng, Q.; Watkins, S. C.; Melhem, M. F.; Endo, S.; Johnson, D. E.; Huang, L.; He, Y.; Kim, J. D. Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 4227−4232.

(162) Schuringa, J. J.; Wierenga, A. T.; Kruijer, W.; Vellenga, E. Constitutive Stat3, Tyr705, and Ser727 phosphorylation in acute myeloid leukemia cells caused by the autocrine secretion of interleukin-6. *Blood* **2000**, *95*, 3765−3770.

(163) Turkson, J. STAT proteins as novel targets for cancer drug discovery. *Expert Opin. Ther. Targets* **2004**, *8*, 409−422.

(164) Xie, T. X.; Huang, F. J.; Aldape, K. D.; Kang, S. H.; Liu, M.; Gershenwald, J. E.; Xie, K.; Sawaya, R.; Huang, S. Activation of stat3 in human melanoma promotes brain metastasis. *Cancer Res.* **2006**, *66*, 3188−3196.

(165) Zamo, A.; Chiarle, R.; Piva, R.; Howes, J.; Fan, Y.; Chilosi, M.; Levy, D. E.; Inghirami, G. Anaplastic lymphoma kinase (ALK) activates Stat3 and protects hematopoietic cells from cell death. *Oncogene* **2002**, *21*, 1038−1047.

(166) Sano, S.; Chan, K. S.; DiGiovanni, J. Impact of Stat3 activation upon skin biology: a dichotomy of its role between homeostasis and diseases. *J. Dermatol. Sci.* **2008**, *50*, 1−14.

(167) Liu, H.; Moroi, Y.; Yasumoto, S.; Kokuba, H.; Imafuku, S.; Nakahara, T.; Dainichi, T.; Uchi, H.; Tu, Y.; Furue, M.; Urabe, K. Immunohistochemical localization of activated Stat3 and hTERT protein in psoriasis vulgaris. *Eur. J. Dermatol.* **2006**, *16*, 205−207.

(168) Turkson, J.; Gunning, P. Compounds That Suppress Cancer Cells and Exhibit Antitumor Activity. WO2010117438 (A2), 2010.

(169) Turkson, J.; Hamilton, A. D. Inhibitors of STAT3. WO 2010/033685 A1, 2010.

(170) Kupershmidt, I.; Su, Q. J.; Grewal, A.; Sundaresh, S.; Halperin, I.; Flynn, J.; Shekar, M.; Wang, H.; Park, J.; Cui, W.; Wall, G. D.; Wisotzkey, R.; Alag, S.; Akhtari, S.; Ronaghi, M. Ontology-based meta-analysis of global collections of high-throughput public data. *PLoS One* **2010**, *5*, No. e13066.

(171) Tanguy Le Carrour, S. A.; Tondeur, S.; Lhermitte, L.; Lamb, N.; Reme, T.; Pantesco, V.; Hamamah, S.; Klein, B.; De Vos, J. Amazonia!: an online resource to google and visualize public human whole genome expression data. *Open Bioinf. J.* **2010**, *4*, 5−10.

(172) Wu, C.; Orozco, C.; Boyer, J.; Leglise, M.; Goodale, J.; Batalov, S.; Hodge, C. L.; Haase, J.; Janes, J.; Huss, J. W., 3rd; Su, A. I. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. *Genome Biol.* **2009**, *10*, R130.