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Design, synthesis, and evaluation of peptidomimetics containing Freidinger lactams as STAT3 inhibitors

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ABSTRACT

The STAT3 oncogene is a promising molecular target for the design of a new class of anticancer drugs. In this letter, we describe the design, synthesis, and evaluation of peptidomimetics containing Freidinger lactams as novel STAT3 inhibitors. Compound **3** binds to STAT3 with a K_i value of 190 nM and is a promising lead compound for further design and optimization.

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Constitutive activation of the Signal Transducers and Activators of Transcription 3 (STAT3) is frequently detected in specimens from cancer patients with advanced disease and human cancer cell lines, but not in normal epithelial cells.[1–4] Persistent activation of STAT3 signaling has been demonstrated to contribute directly to oncogenesis by stimulating cell proliferation and preventing apoptosis in human cancer cells.[1–4] STAT3 activation not only provides a growth advantage to tumor cells, allowing their accumulation, but may also confer resistance to conventional therapies that rely on apoptotic machinery to eliminate tumor cells.[1–4] In cells, STAT3 is recruited from the cytosol by transmembrane cytokine and growth factor receptors and participates in specific interactions through its Src homology 2 (SH2) domain with the phosphotyrosine docking sites displayed by the receptors. It then is phosphorylated on tyrosine, Tyr705.[1–4] Tyrosine phosphorylation of STAT3 causes it to dimerize and translocate to the nucleus, where it binds to specific promoter sequences on its target genes.[1–4] Dimerization of STAT3 is a decisive event for its activation and its transcriptional activity.[1–5] Blocking STAT3 dimerization using a small-molecule inhibitor has been proposed as a potentially promising therapeutic approach to the development of molecularly targeted therapies for the treatment of human cancers with constitutively activated STAT3.[4–10]

Two approaches are currently being pursued in the design of small-molecule antagonists which inhibit STAT3 dimerization.[4–13] The first is the design of peptidomimetics[6–10] and the second is a search for non-peptidic small-molecules.[11–13] While a number of peptide-based ligands can achieve quite high binding affinities to STAT3,[6–10] they generally have no or very poor cellular activity due to their peptidic nature and/or the negatively charged phosphotyrosine amino acid residue. The major advantage of non-peptide small-molecule inhibitors is that they are cell permeable, but the inhibitors reported to date typically have weak binding affinities to STAT3 and may not be very specific against STAT3.[11–13] Therefore, there is a great need for potent and cell-permeable STAT3 inhibitors. In this letter, we present our structure-based design, synthesis, and biochemical evaluation of peptidomimetic inhibitors of STAT3. Incorporation of Freidinger lactams into our designed compounds has reduced their peptidic character and afforded potent STAT3 inhibitors.

We previously used the phosphorylated peptide (pYLKTKF) derived from STAT3 as the basis of our design of a macrocyclic peptidomimetic inhibitor of STAT3.[8] One significant drawback of this phosphopeptide is that it has a relatively weak affinity to the STAT3 protein (K_i = 25.9 μM)[8] and consequently, in this study, we employed a gp130 protein derived phosphopeptide pYLPQTV, which binds to STAT3 with a much higher affinity than the STAT3 phosphorylated peptide.[7,8,14]

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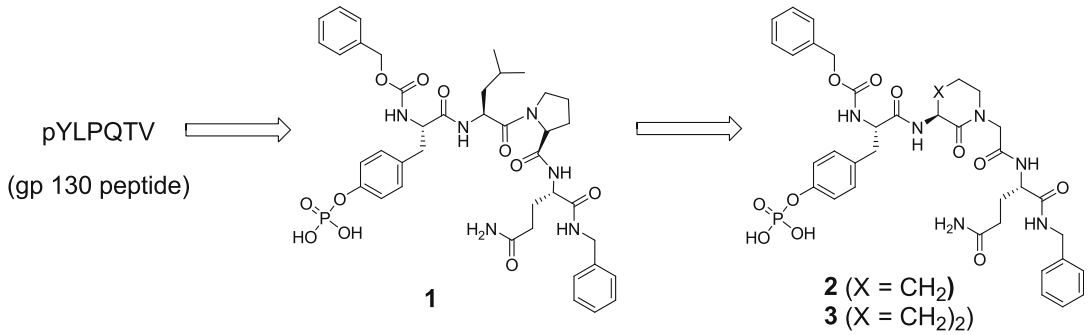


Figure 1. Design of STAT3 peptidomimetics by incorporating Freidinger lactams.

To quantitatively determine the binding affinity of our designed compounds to STAT3, we developed a fluorescence-polarization (FP) based binding assay which is similar to our previously reported FP assay.[14] The gp130 pYLPQTV peptide binds to STAT3 with a high affinity,[7,8,14] and accordingly we incorporated this sequence into our fluorescently labeled probe. We synthesized peptide, β(Ala)-β(Ala)-G-pYLPQTV, and labeled it at the N-terminus with a fluorescent tag, 5-carboxyfluorescein (5-FAM). A saturation experiment determined that this fluorescently tagged gp130 peptide binds to recombinant human STAT3 protein (residues 127–722) with K_d = 35 ± 2.3 nM.

It has been shown that the threonine and valine residues in the gp130 phosphopeptide can be replaced by a benzyl amide without a significant loss in its STAT3 binding affinity.[6] Accordingly, we first designed and synthesized compound **1**, which represents the shortened gp130 sequence (Fig. 1). Our competitive FP binding assay shows this compound binds to STAT3 with K_i = 350 nM. Thus, although compound **1** binds to STAT3 less potently than the gp130 pYLPQTV peptide, it still has an appreciable binding affinity to STAT3 and is a good lead structure for further optimization.

The crystal structure of mouse STAT3 homodimer bound to DNA has been determined (PDB entry: 1BG1),[5] but no crystal structure has been reported for any gp130 based phosphopeptide in a complex with STAT3. To assist our design efforts, computational docking was performed to predict the binding mode of compound **1** in a complex with STAT3. The mouse and human STAT3 protein sequences differ by just one residue: Glu760 in human STAT3 is replaced by Asp760 in mouse STAT3. This residue is at the N-terminus of STAT3, distant from the phosphopeptide binding site within STAT3's SH2 domain. We have therefore used the 1BG1 crystal coordinates in our modeling studies as an acceptable approximation.

Computational docking was performed using the GOLD program (version 3.1).[16,17] The binding site for STAT3 was centered at Glu638 and given a radius of 13 Å, sufficient to cover the entire binding pocket. The docking simulations were terminated after 10

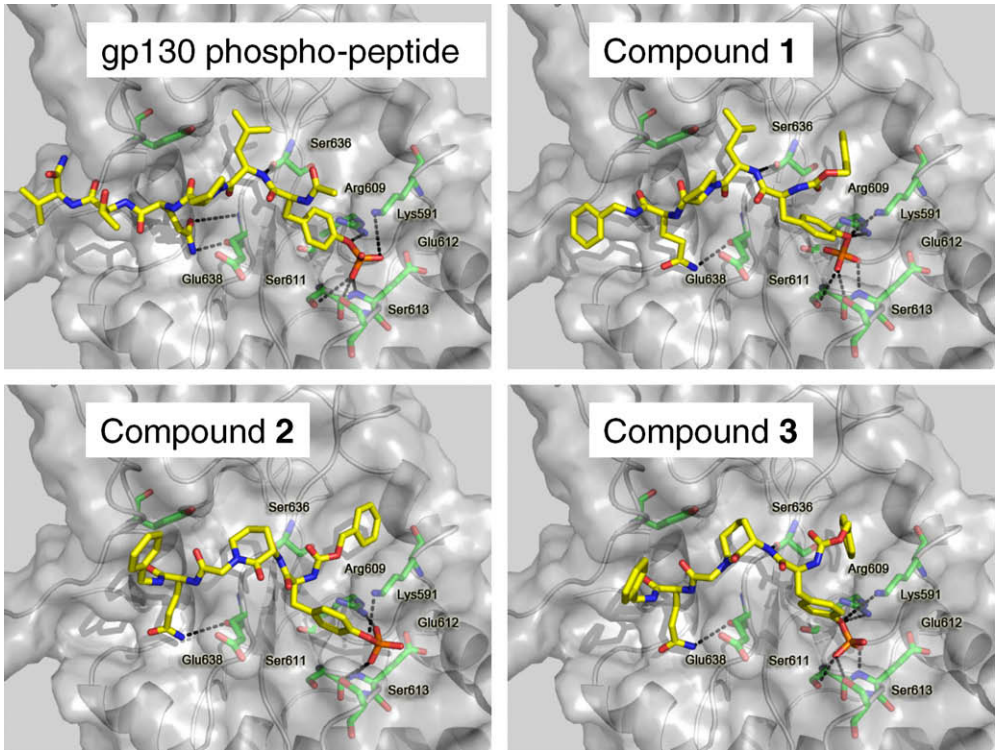
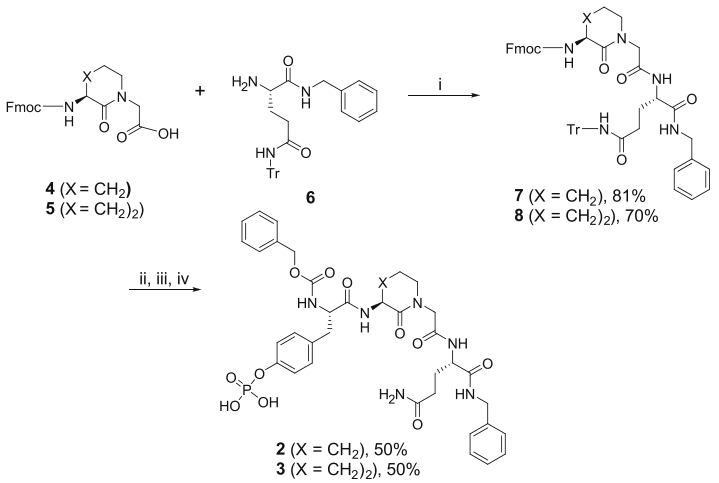


Figure 2. Predicted binding models of gp130 phospho-peptide, compounds **1**, **2**, and **3** in complex with STAT3. STAT3 protein is shown in surface model, with key binding residues displayed in stick model and colored in green for carbon atoms, blue for nitrogen atoms, and red for oxygen atoms, respectively. STAT3 ligands were shown in stick model and colored in yellow for carbon atoms, blue for nitrogen atoms, red for oxygen atoms, and orange for phosphorous atoms, respectively. Figures were generated using pymol (http://www.pymol.org/).



Scheme 1. Synthesis of compounds **2** and **3**. Reagents and conditions: (i) EDCI, HOBt, DIPEA; (ii) DEA/CH_3CN; (iii) Cbz-phosphotyrosine, di-*tert*-butyl ester, EDCI, HOBt, DIPEA; (iv) 1:1 TFA/DCM, TES.

runs for each compound. GoldScore, implemented in Gold 3.1 was used as the fitness function with which to evaluate the docked conformations. The conformation ranked highest by the fitness function in each run was saved as potential docking modes. The highest ranked conformation of each compound among the 10 runs was reported as the predicted binding model (Fig. 2).

The predicted binding model for compound **1** (Fig. 2) showed that phosphotyrosine has significant charge–charge interactions with Lys591, Arg609, Ser611, and Ser613, highlighting the critical importance of the phosphate for binding to STAT3. While the hydrophobic side chain of the Leu residue has no specific contacts with STAT3, its backbone amide forms a hydrogen bond with the backbone carbonyl of Ser636 in STAT3. Although the Pro residue in compound **1** does not have specific interactions with the protein, it plays a key role in controlling the conformation of the peptide. The amide side chain of the peptide's Gln residue forms a hydrogen bond with the backbone carbonyl of Glu 638 within STAT3. This may be significant because the Gln residue has been shown to play a critical role in the specific recognition of gp130 based phosphopeptides by STAT3 over other STAT proteins and may also be important for high-affinity binding to STAT3.[10]

Analysis of the predicted binding model for compound **1** in complex with STAT3 also reveals that directed by the proline residue, it assumes a β-turn conformation. We sought to redesign compound **1** by incorporating a Freidinger lactam into the structure. The 6- and 7-membered Freidinger lactam structures assume a II′ β-turn conformation and have been extensively used in the design of conformationally constrained peptidomimetics.[15] This led to the design of compounds **2** and **3**, which in addition to being conformationally constrained also have reduced peptide character. Overlay of our predicted binding models of compounds **1**, **2**, and **3** showed that while compound **3** nicely mimics compound **1** in its binding to STAT3, compound **2** shows significant deviations.

The synthesis of compounds **2** and **3** is described in Scheme 1. Compounds **2** and **3** were synthesized in solution from the respective β-turn mimetic with standard (EDCI/HOBt/DIPEA) peptide chemistry. A di-orthogonal protection strategy was employed, where the N-termini were protected with base-labile Fmoc and side-chains were protected with acid-labile trityl and *tert*-butyl esters. The final products were obtained using known peptide chemistry (EDCI/HOBt/DIPEA; 1:1 DCM/TFA) for final deprotection.

The binding affinities of compounds **2** and **3** to STAT3 were determined using our FP-based, quantitative and competitive binding assay and the results are shown in Figure 3. Compound **2** containing a 6-membered Freidinger lactam binds to STAT3 with

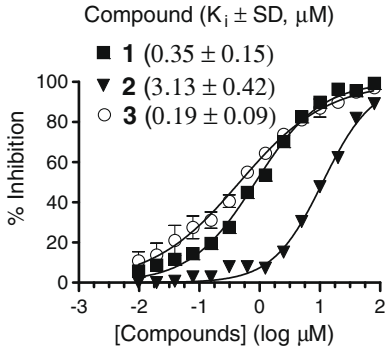


Figure 3. Binding affinities of designed peptidomimetics to STAT3, as determined using a fluorescence-polarization-based, competitive binding assay.

K_i = 3.13 μM, nine times less than compound **1**. By comparison, compound **3**, with a 7-membered Freidinger lactam binds to STAT3 with K_i = 190 nM, and is twice as potent as compound **1** and 18 times as potent as compound **2**. Thus, the binding data for compound **3** showed that incorporation of a 7-membered Freidinger lactam structure into compound **1** results in a novel peptidomimetic compound with a high binding affinity to STAT3. The data also suggest that the hydrogen bond between the Leu main chain amino group in compound **1** and the carbonyl group of Ser 636 in STAT3 is not required for high-affinity binding since compound **3** no longer has the corresponding carbonyl group in its structure but still binds to STAT3 with a high affinity.

In summary, using the STAT3 recruitment sequence from the gp130 protein as our basis, we have carried out structure-based design of conformationally constrained peptidomimetic inhibitors of STAT3, which incorporate Freidinger lactams. While compound **2**, containing a 6-membered lactam structure, is much less potent than compound **1**, compound **3** containing a 7-membered lactam structure binds to STAT3 with K_i = 190 nM. Compound **3** has reduced peptide character compared to compound **1** and the gp130 phosphopeptide, and is a promising lead compound for further design and optimization toward developing potent and cell-permeable STAT3 inhibitors as a new class of anticancer drugs.

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