molecular informatics
models – molecules – systems

Supporting Information

Identification of lead compound as inhibitors of STAT3: design, synthesis and bioactivity.

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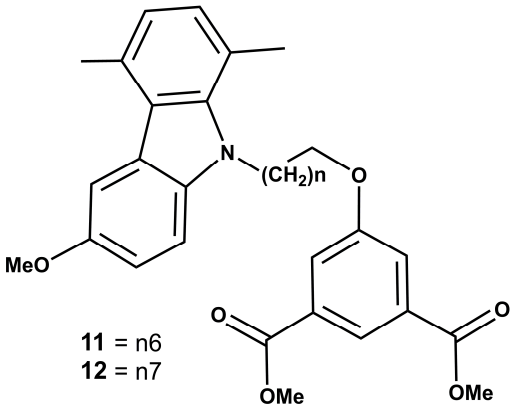

Figure S1. Molecular structures of compounds **11** and **12**.

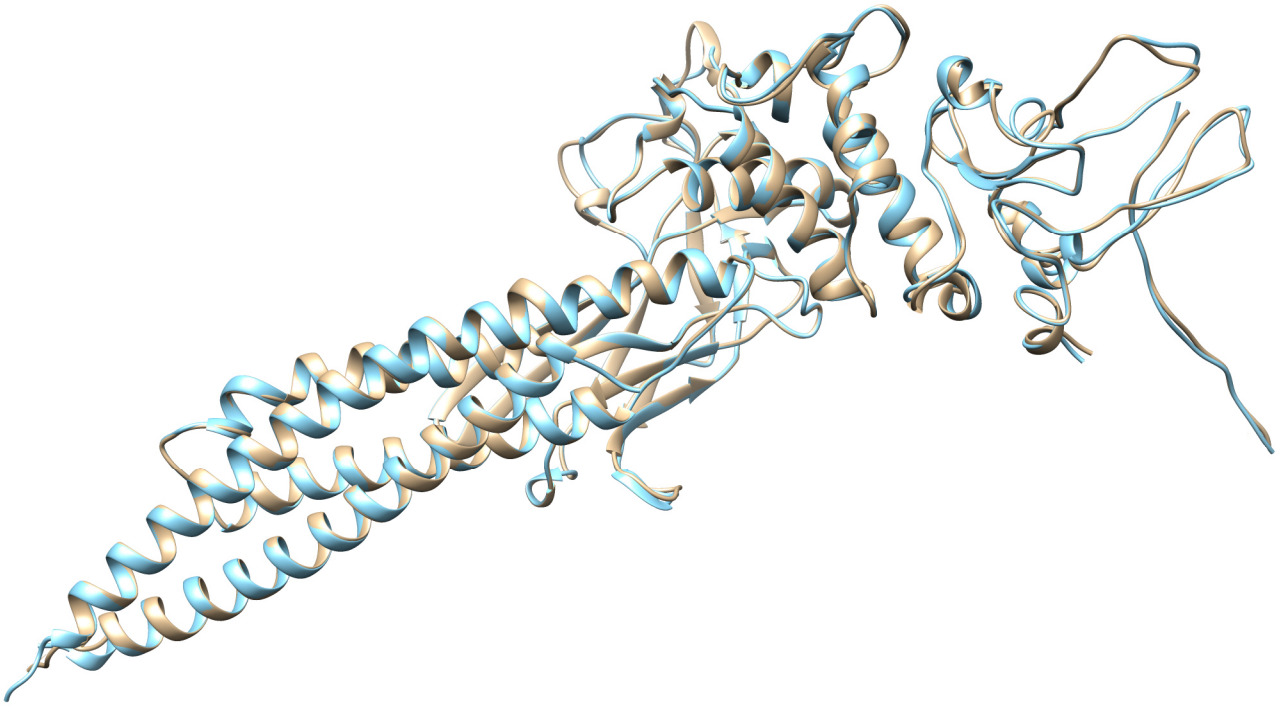

Figure S2. Superimposition (RMSD 0.43 Å) of uSTAT3 (tan) and pSTAT3 (cyan).

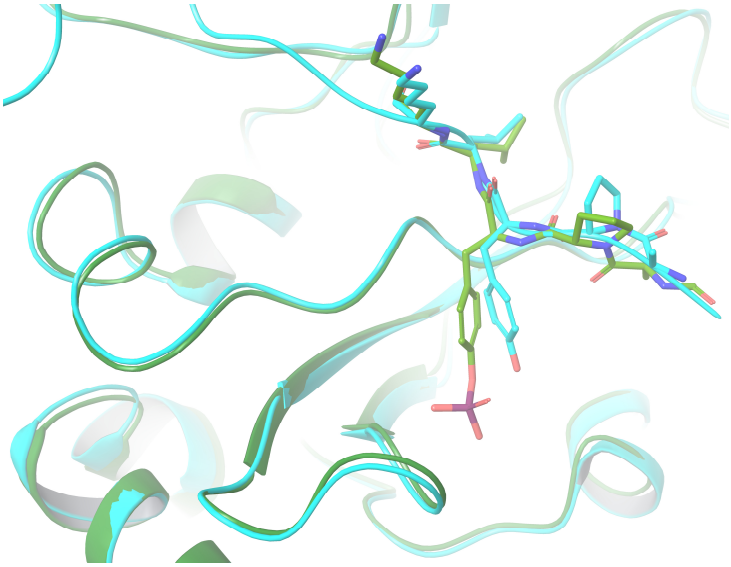

Figure S3. Superimposition of uSTAT3 (cyan) and pSTAT3 (grey), which shows the 2.0 Å shift of Tyr705 respect to pTyr705.

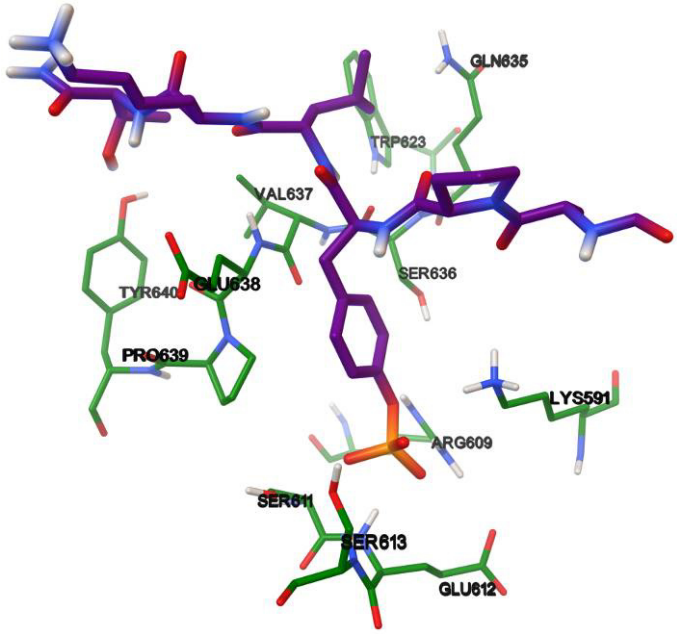

Figure S4. Three dimensional model of interactions between STAT3 and Pro-pTyr-Leu-Lys-Thr-Lys. The protein and peptide are represented by tube (coloured: C, green for the protein; C, purple for the peptide; polar H, white; N, blue; O, red; P, orange).

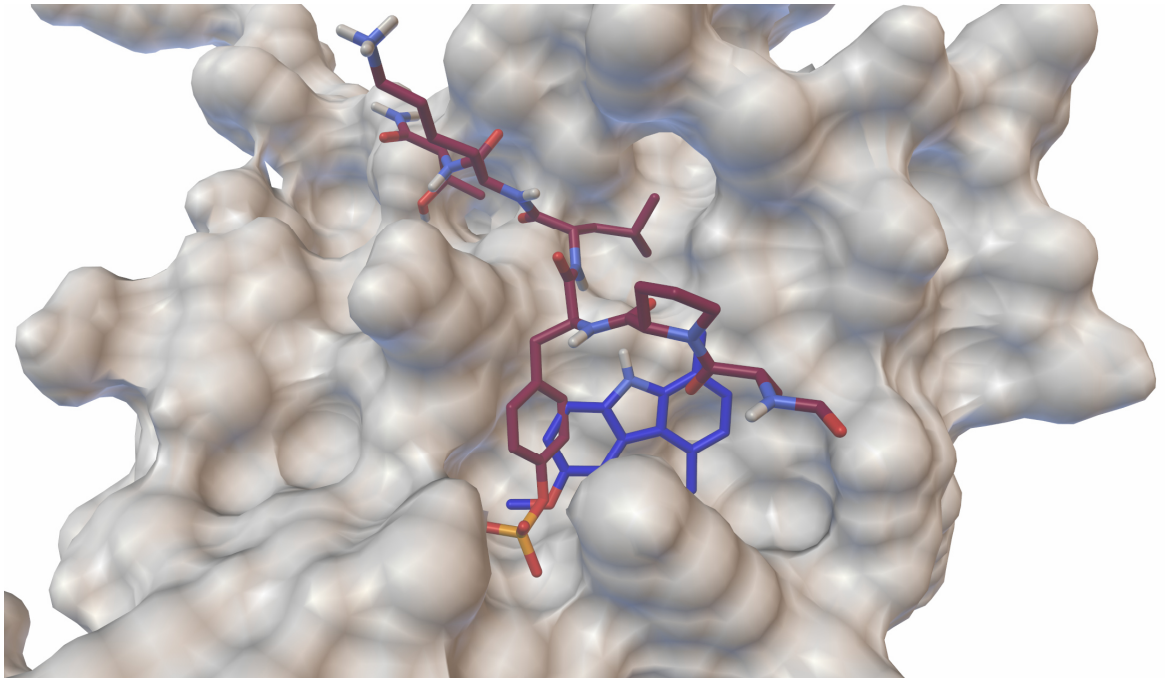

Figure S5. Superimposition between **3** and Pro-pTyr-Leu-Lys-Thr-Lys in the binding site of STAT3. The protein is depicted by molecular surface, whereas compound **3** and peptide are represented by tube (coloured: C, dark blue for **3**; C, plum for the peptide; polar H, white; N, blue; O, red; P, orange).

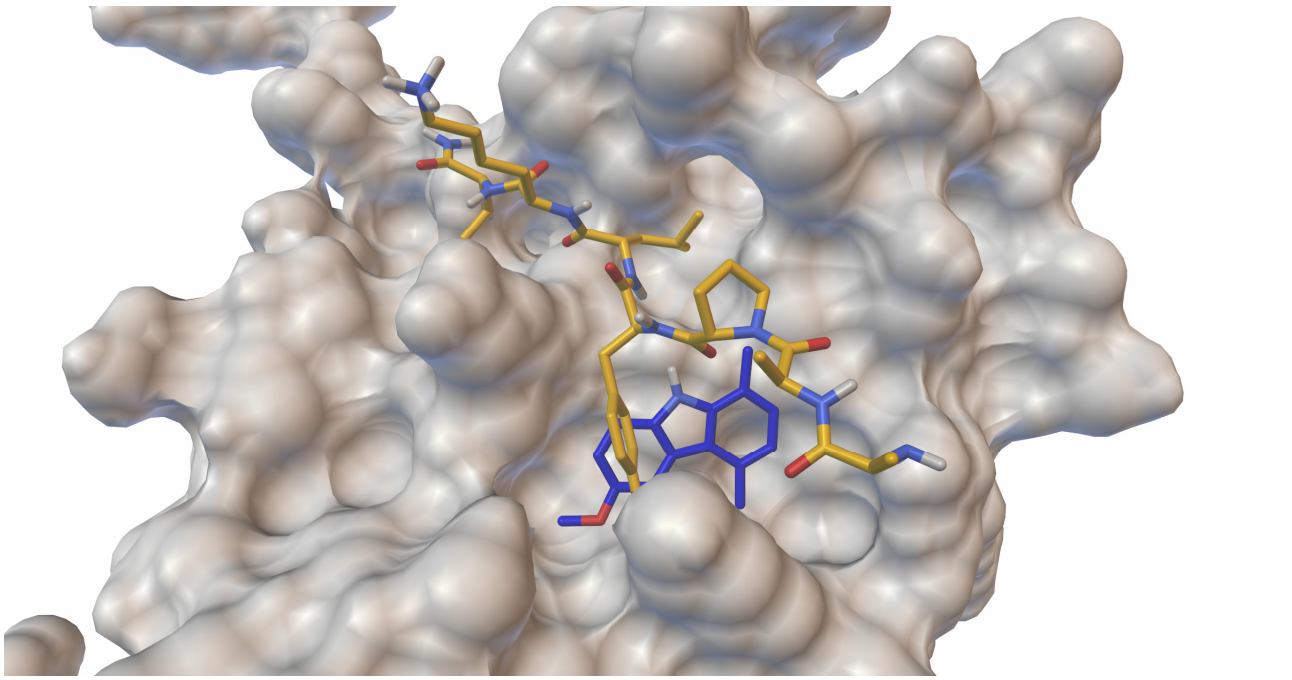

Figure S6. Superimposition between **3** and Pro-Tyr-Leu-Lys-Thr-Lys in the binding site of STAT3. The protein is depicted by molecular surface, whereas compound **3** and peptide are represented by tube (coloured: C, dark blue for **3**; C, yellow for the peptide; polar H, white; N, blue; O, red; P, orange).

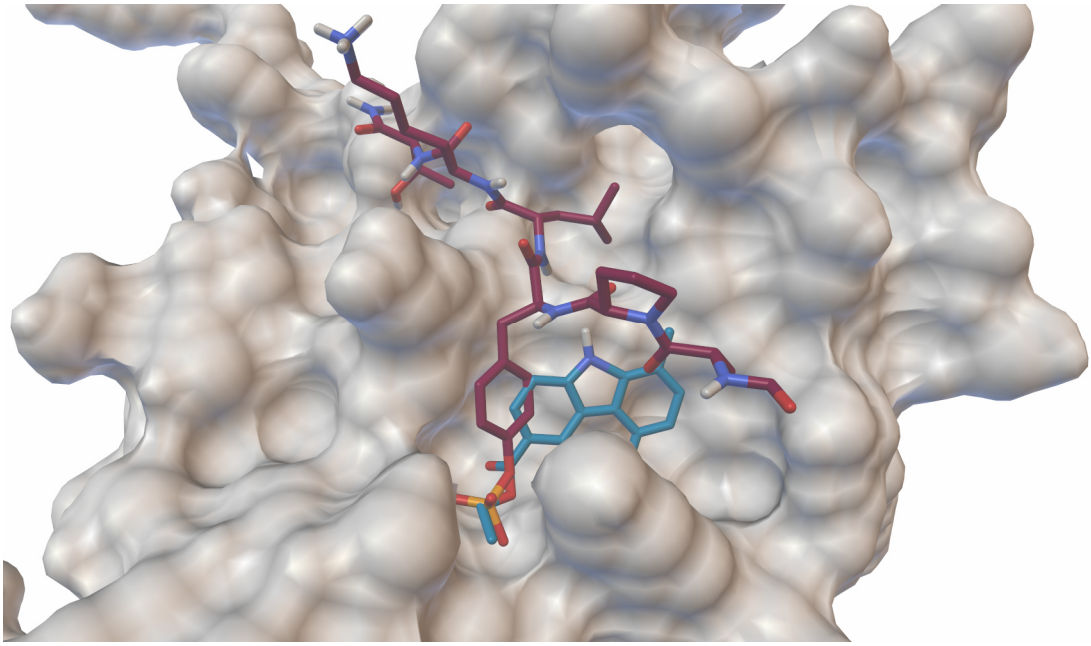

Figure S7. Superimposition between **4** and Pro-pTyr-Leu-Lys-Thr-Lys in the binding site of STAT3. The protein is depicted by molecular surface, whereas compound **4** and peptide are represented by tube (coloured: C, indigo for **4**; C, plum for the peptide; polar H, white; N, blue; O, red; P, orange).

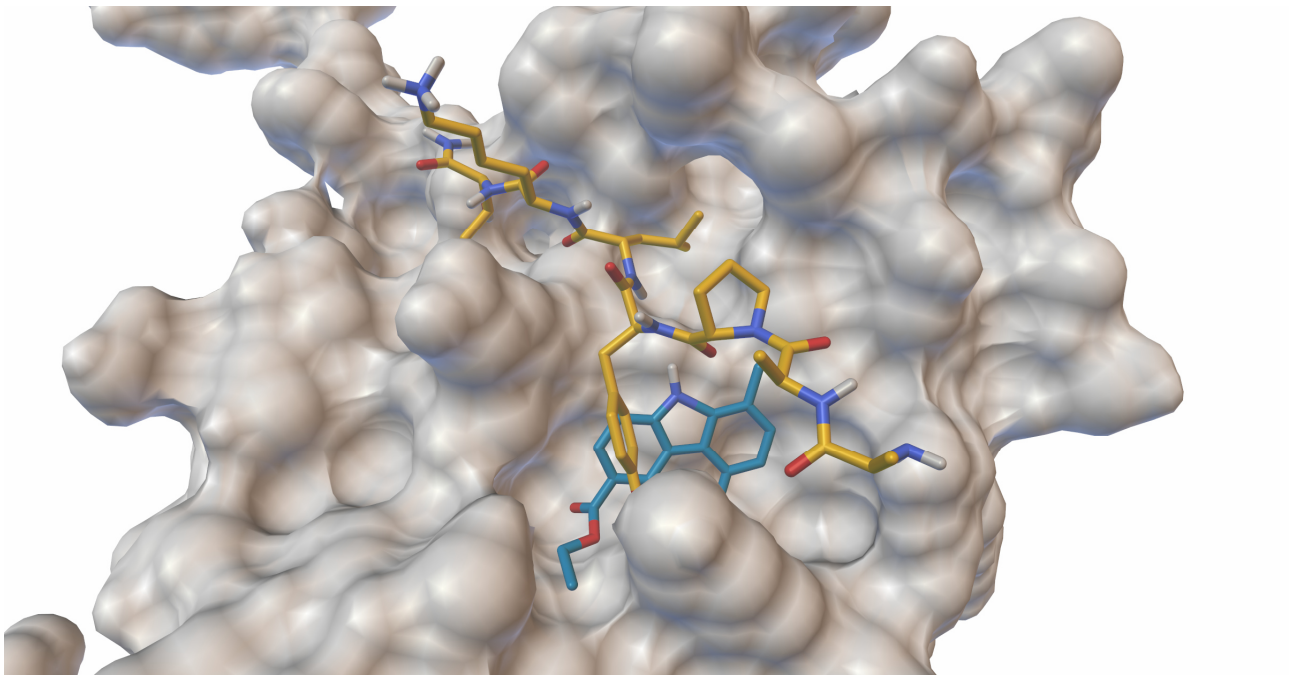

Figure S8. Superimposition between **4** and Pro-Tyr-Leu-Lys-Thr-Lys in the binding site of STAT3. The protein is depicted by molecular surface, whereas compound **4** and peptide are represented by tube (coloured: C, indigo for **4**; C, yellow for the peptide; polar H, white; N, blue; O, red; P, orange).

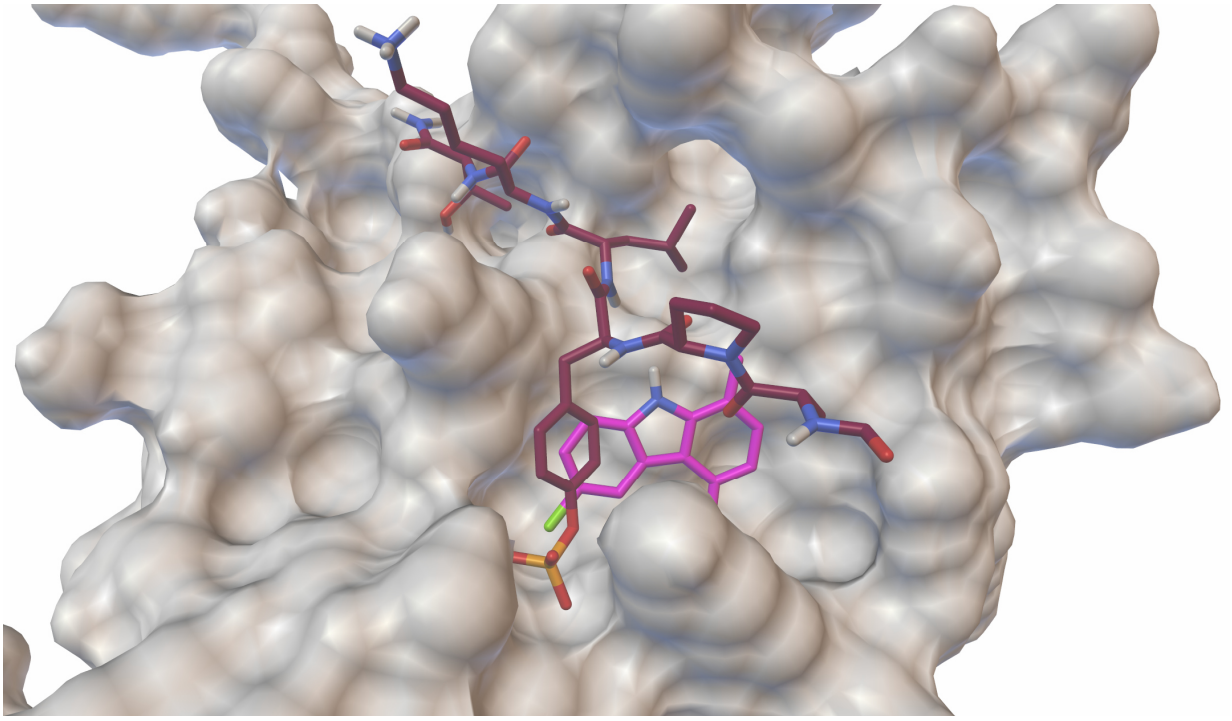

Figure S9. Superimposition between **5** and Pro-pTyr-Leu-Lys-Thr-Lys in the binding site of STAT3. The protein is depicted by molecular surface, whereas compound **5** and peptide are represented by tube (coloured: C, pink for **5**; C, plum for the peptide; polar H, white; N, blue; O, red; P, orange).

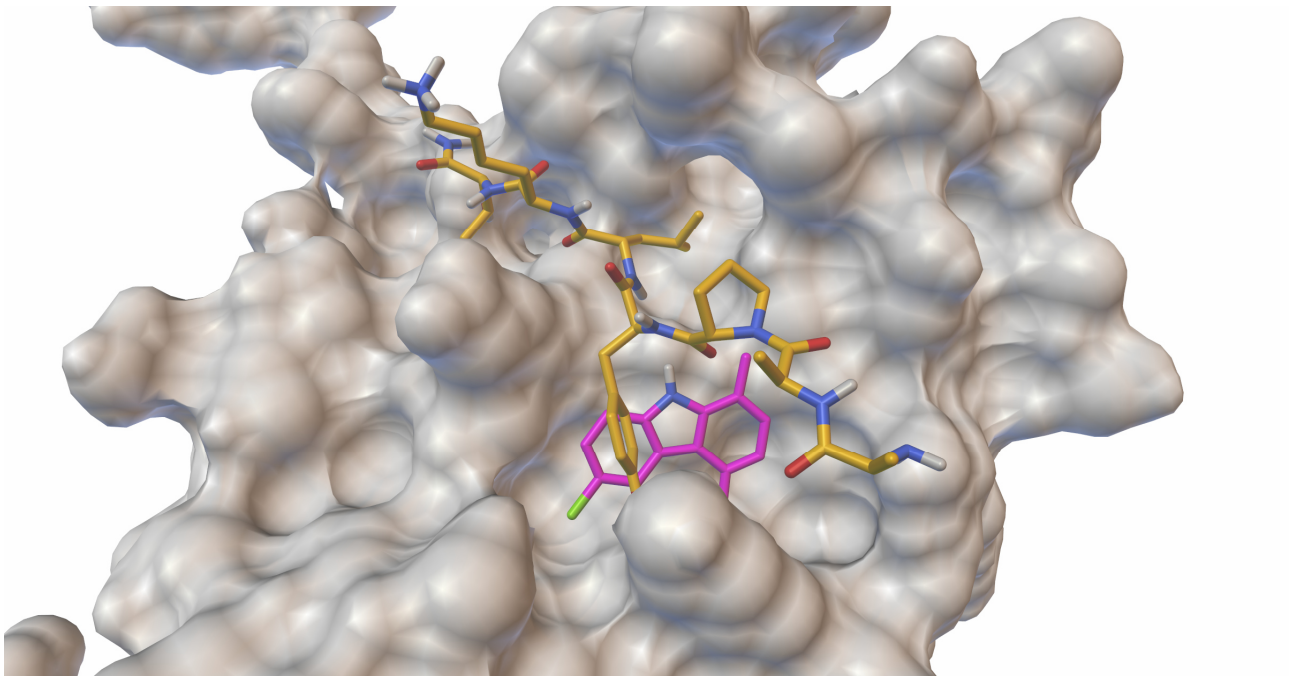

Figure S10. Superimposition between **5** and Pro-Tyr-Leu-Lys-Thr-Lys in the binding site of STAT3. The protein is depicted by molecular surface, whereas compound **5** and peptide are represented by tube (coloured: C, pink for **5**; C, yellow for the peptide; polar H, white; N, blue; O, red; P, orange).

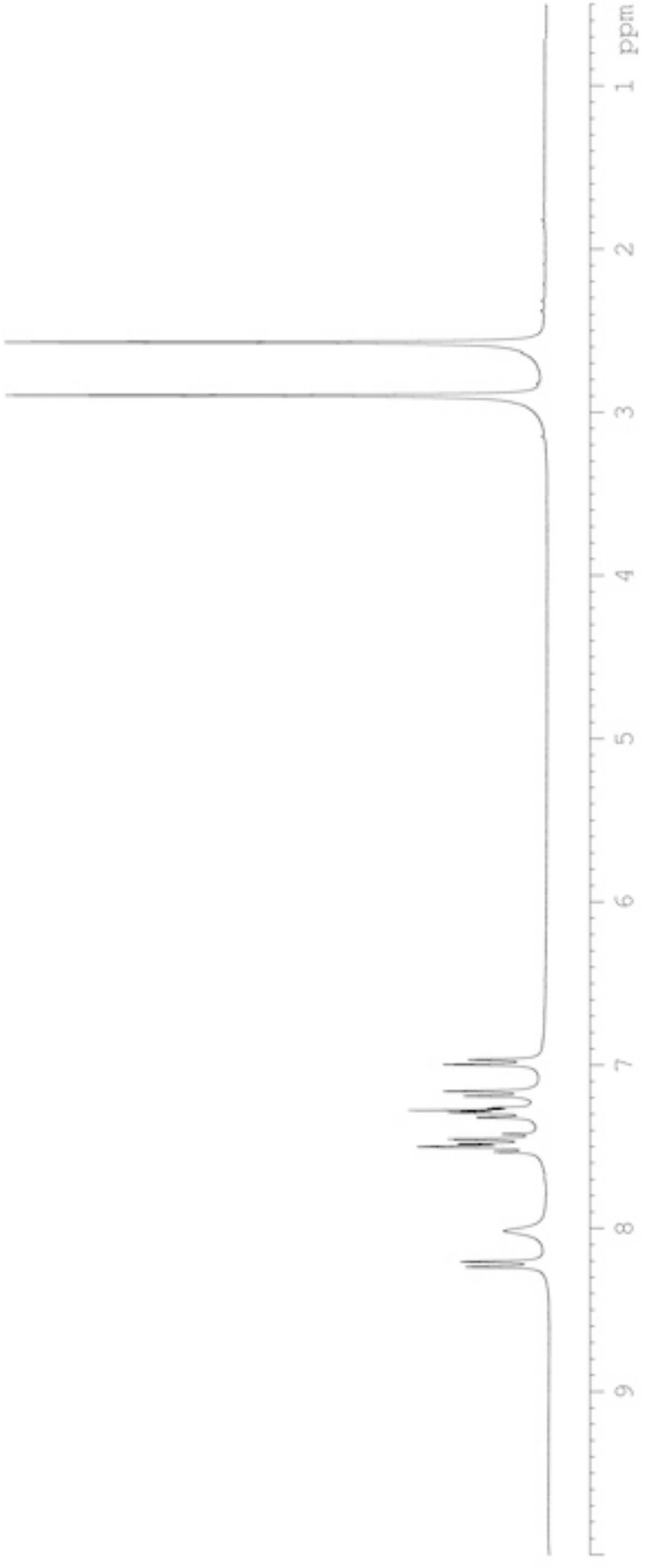

Figure S11. ^{1}H NMR spectrum of compound **18**.

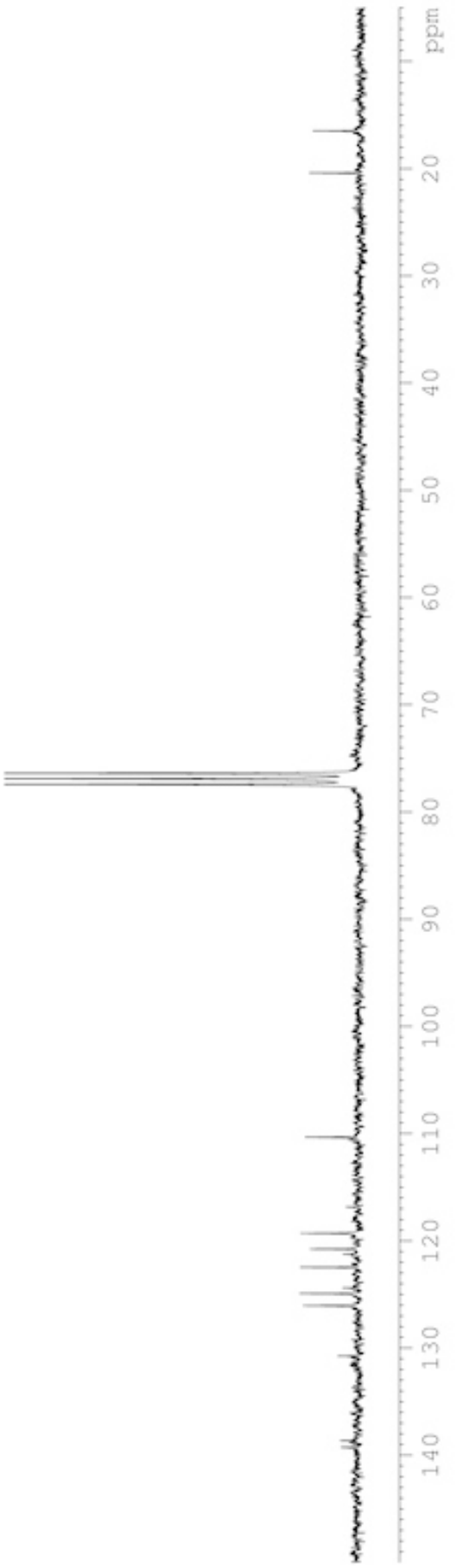

Figure S12. ^{13}C NMR spectrum of compound **18**.

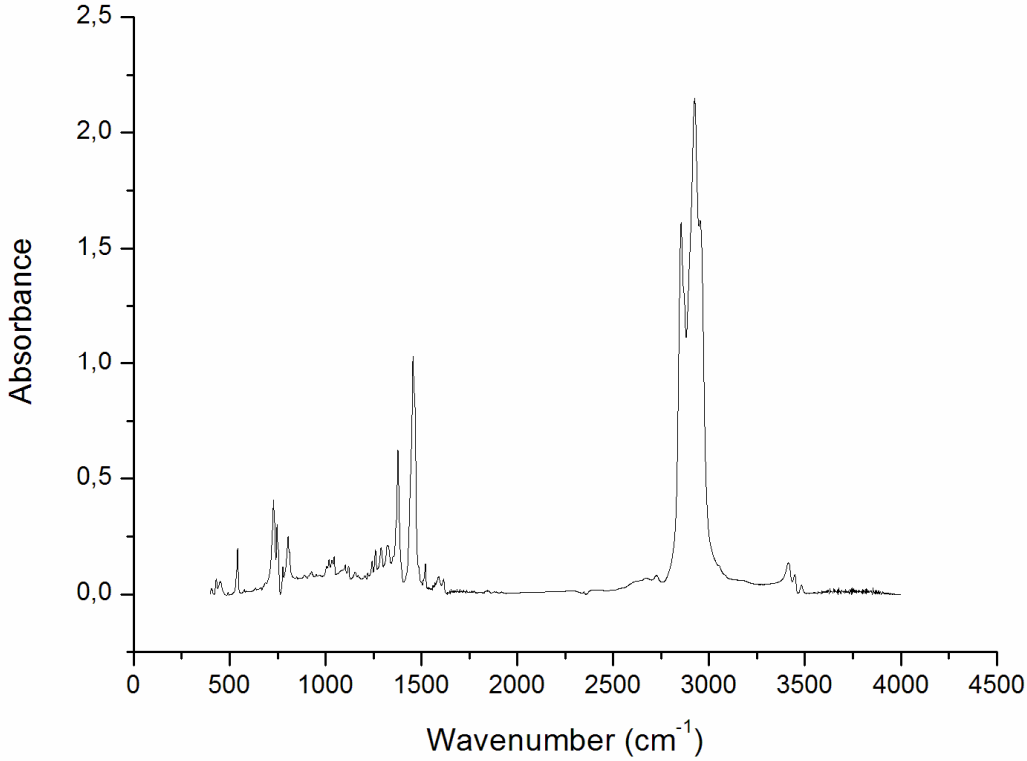

Figure S13. FT-IR spectrum of compound **6**.

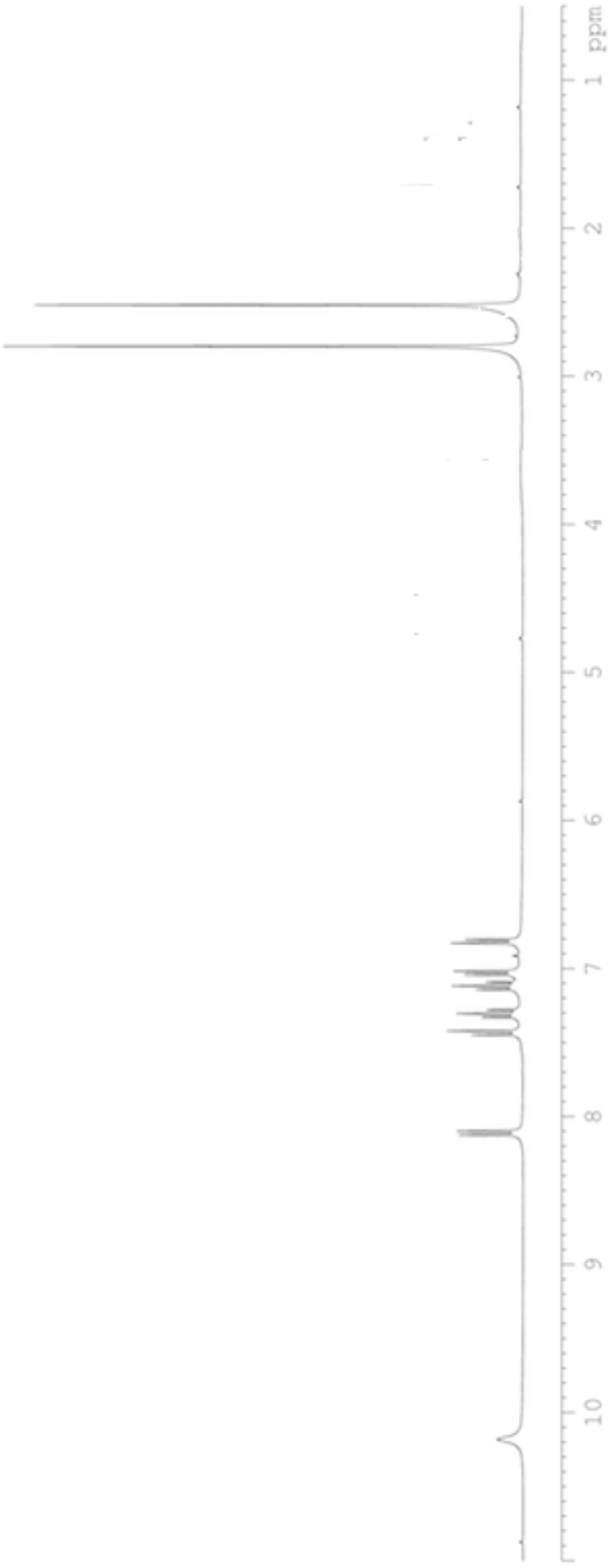

Figure S14. ^{1}H NMR spectrum of compound **6**.

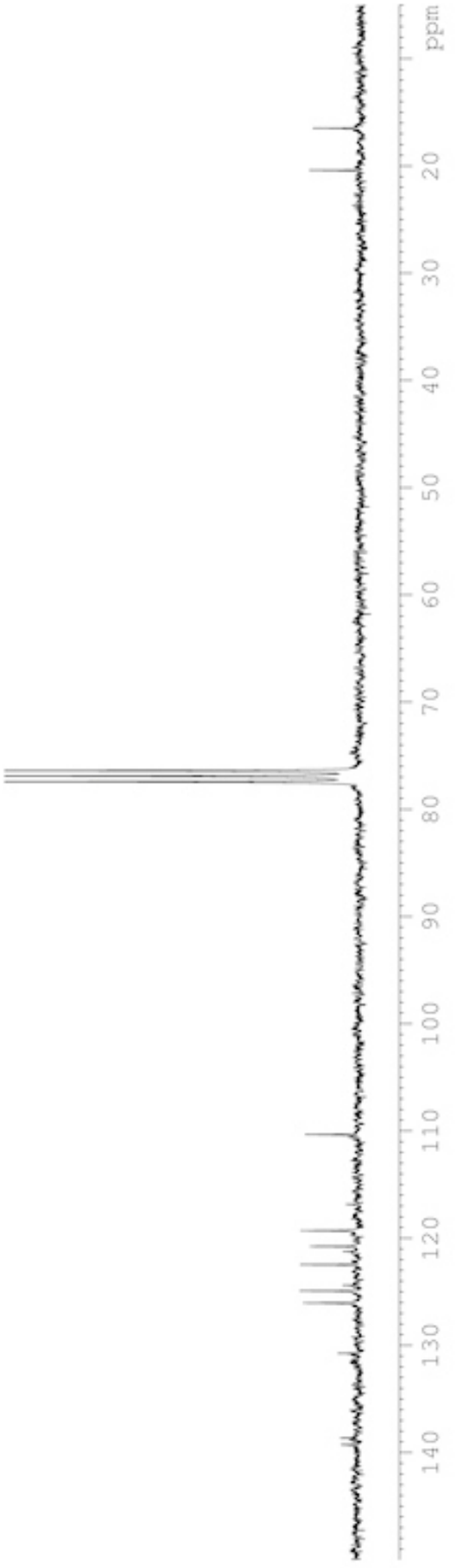

Figure S15. ^{13}C NMR spectrum of compound **6**.

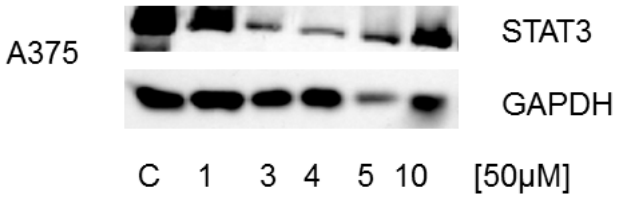

Figure S16. Representative Western blot of STAT3 expression level in A375 cells treated with selected compounds (50 μM).

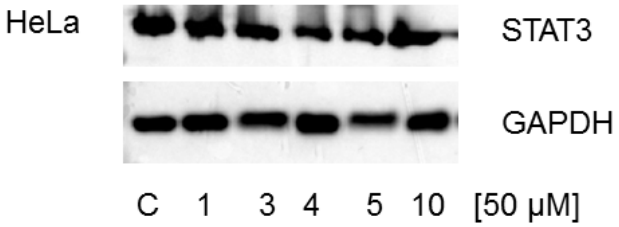

Figure S17. Representative Western blot of STAT3 expression level in HeLa cells treated with selected compounds (50 μM).

Detailed interactions between 1-10 and STAT3

From our theoretical calculations, we observed that the compound **1** is not able to establish interactions where the pTyr705 takes place (Figure 3a). The docked poses of **2**-**10** are superimposable in the binding SH2 domain of STAT3, where Pro-Tyr/pTyr-Leu-Lys-Thr-Lys peptide binds (Figures 3a,c). The carbazole ring is in close contacts with Glu594, Ser636, Pro639, Lys591, Gln635 (Figure 3a,b). The methyl groups at positions C-1 and C-4 extend the van der Waals contacts of the carbazole (Figure 3a,c). In particular the methyl at position 1 interacts with Gln635, whereas the methyl at C-4 interacts with hydrophobic portion of side chain of Lys591 and Glu594 (Figure 3a,c). The Lys591 forms a Cation-π interaction with the carbazole. The hydroxyl group of Thr620 faces the electron π system of carbazole (Figure 3a,c). The different substituents at position 6 of **2**-**10** occupy the site of phosphate group of Tyr705, establishing key interactions with Arg609 (Figure 3). Moreover, only the NH of carbazole core of **2**-**6** is hydrogen bonded to backbone CO of Ser636 (Figure 3a). Compound **2** forms two hydrogen bonds with Arg609 by the hydroxyl group. The same interactions are made by **3** through its methoxy group (Figure 3a). Compound **3** gives a further contact by the methyl group with the side chain of Ser611 (Figure 3a). **4** presents at C-6 an ethyl ester, and is hydrogen bonded by the two oxygen of the ester group with Arg609 (Figure 3a). The OMe of the ester group is hydrogen bonded with the Lys591. The ethyl contributes to the binding giving van der Waals interactions with hydrophobic moiety of Lys591, Ser611 and Glu612 (Figure 3a). It is interesting the insertion of the chlorine at C-6 (**5**, Figure 1). We observed that the guanidine group of Arg609 is nearly normal to Cl—C-6 (Figure 3a) bond donating a hydrogen bond toward the negative belt of the 6-chloro moiety. Further contacts could be established with Ser611 and Ser613. Compound **6** is engaged in H-bonds with Lys591 and Arg609, and with NH backbone of Glu612 by the sulfon group (Figure 3a). The NH_2 of sulfonamide establishes two hydrogen bonds with Ser611 and Ser613 (Figure 3a). The introduction of nitro group at position 3 in compounds **7** and **8** allows the interaction with Arg595 (Figure 3b).

Moreover, we observe that the methoxy group interacts with Arg609. On the contrary, **8** looks to interfere with Tyr705/pTyr705-protein interactions better than **7**, thank to the ester group. The docked poses of **7** and **8** show the carbazole ring not superimposable with the tricyclic system of **1**-**6**, **9** and **10** (Figure 3). As found for **3**, the methoxy group of **9** and **10** gives a hydrogen bond with Arg609 and a van der Waals interaction with Ser611 (Figure 3c). The compounds **9** and **10** structurally differ from **3** for the alkylation of the carbazole nitrogen. This chemical modification causes the loss of a hydrogen bond with backbone CO of Ser636 (Figure 3c), but present van der Waals contacts with side chain of Trp623 and Val637. The **10** presents the ethyl group parallel to the hydrophobic portion of Glu638 side chain, giving van der Waals contacts.