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Identification of Novel STAT3 Dimerization Inhibitor Through Structure-Based Virtual Screening for Cancer Management

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Abstract

Background: Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that controls cell proliferation, differentiation, angiogenesis, and immunological responses. In many human malignancies, abnormal STAT3 activation promotes tumor growth via oncogenic gene expression, resulting in tumor malignancy. Many drugs with clinically authorized analogues that are used as STAT3 inhibitors for cancer therapy have several drawbacks in terms of stability and toxicity.

Methods: This study used PyRx 0.8 tool to screen the Traditional Chinese Medicine (TCM) database of about 32,364 commercially available natural compounds in order to identify new STAT3 inhibitors. Physicochemical and ADME properties of selected compounds were estimated using Datawarrior and SwissADME.

Result: The top 20 compounds were initially chosen based on their strong binding affinities with STAT3. Lipinski and Vaber tools were used to filter the top 20 compounds, yielding the top 6 compounds. The compounds ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537 were passed through these filters. These compounds were found to interact with active site STAT3 residues and have several amino acid interactions in common with the control compound (STX0119).

Conclusion: This study suggests that the compounds ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537 could be used as a lead for the development of novel STAT3 inhibitors. However, further experimental validation is required to optimized them as STAT3 inhibitors.

Introduction

The characteristics of cancer include sustaining proliferative signaling, improper replication with a lack of growth suppressors, initiating angiogenesis, and increasing invasion and metastasis in carcinogenesis [1]. Transcription factors (TFs) are signaling nodes and are among the most commonly affected genes in cancer [2,3]. Signal transducer and activator of transcription (STAT) is a cytoplasmic TF family (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) that is responsible for the transfer of signals from various receptors and non-receptor related kinases to the nucleus [4]. STAT3 activity has been found to be increased and dysregulated in a variety of cancer cell lines [5], indicating that this protein might be a prospective target for anticancer drug development [6-8]. STAT3 controls a number of genes involved in cell proliferation, differentiation, metastasis, and immunity [9,10]. In addition, STAT3 overexpression has been linked to a poor prognosis in some malignancies [11]. Conferring the murine STAT3β crystal structure, pTyr705, located at the boundary of the SH2 and transactivation domains of one STAT3 monomer, binds to the SH2 domain of the other [12]. Because dimerization via reciprocal phosphotyrosine-SH2 interactions is a critical step in STATs activation, small compounds that impair dimer formation make the protein incapable of forming dimers, binding DNA, and activating gene transcription [13]. Disrupting, for example, STAT3 dimer formation provides an effective therapeutic strategy in cancer by inhibiting its abnormal signaling hyperactivity and pro-oncogenic consequences [14].

The time and cost of researching new pharmaceuticals has increased dramatically in past decades. In typically, it takes 15 years and up to $800 million to turn a promising novel molecule into a commercially available drug [15]. Various in silico methods for virtual screening (VS) compounds from virtual chemical spaces, as well as structure and ligand-based methodologies, allow for improved profile analysis, faster removal of nonlead compounds, and therapeutic molecule selection at lower costs [16]. This work employed bioinformatics tools to uncover new possible hits from the Traditional Chinese Medicine (TCM) database that may be used to suppress STAT3 in order to fight cancer.

Methods

Retrieval and preparation of the 3-D structure of STAT3

The RCSB protein data bank was queried to acquire the three-dimensional structure of the target protein STAT3 (PDB ID: 1BG1). Since 1BG1 is a homodimer attached to DNA, the DNA and other heteroatoms were deleted, leaving a clean STAT3 for docking experiments. Energy minimization and geometry optimization were performed using Dock Prep, a built-in tool in UCSF Chimera for creating protein 3D structures. The energy was minimized for the 1000 steepest descent steps using a root mean square gradient of 0.02, and an Amber ff12SB force field, and the prepared protein was saved in .pdb format.

Natural compounds library preparation

ZINC database was utilized to construct a collection of natural compounds, integrating information from the Traditional Chinese Medicine (TCM) database, which encompasses 32,364 commercially accessible natural compounds. This library comprises TCM compounds represented in 3D file formats, specifically .sdf, that accurately depict the processed chemical structures of these compounds.

Virtual screening

VS sorts of compound databases using computer technology and drug design theory-based software. Potentially valuable compounds are screened in large numbers in order to find novel leads for further experimental evaluation. The prepared compound library was screened against the STAT3 using PyRx 0.8 tool [17].

In silico Physicochemical and ADME Prediction

To estimate the physicochemical and ADME properties of the selected compounds, Datawarrior and SwissADME (http://www.swissadme.ch) were employed. These software tools facilitated the assessment and calculation of crucial characteristics of their performance in biological systems and overall pharmacokinetic profiles.

Results

This study used molecular docking-based VS to screen 32,364 TCM compounds for possible hits with significant STAT3 binding scores. The top 20 compounds were initially chosen based on their strong binding affinities with STAT3 (Table 1). The process of filtration to the initial set of top 20 compounds was applied using Lipinski and Veber's tools. As a result, the top six compounds were identified and chosen: ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537. These six compounds passed Lipinski and Veber's rigorous criteria, suggesting their potential as prospective candidates for drug development. Table 2 presents a brief assessment of the physicochemical and drug-likeness values of these compounds. Based on the estimated values, these compounds exhibit a favorable

drug-likeness and drug score, suggesting their potential as lead compounds in pharmaceutical development.

S. No.	Ligand	Binding affinity (kcal/mol)
1.	ZINC95910488	-10.6
2.	ZINC85628521	-9.7
3.	ZINC85542836	-9.6
4.	ZINC85569460	-9.6
5.	ZINC85593523	-9.6
6.	ZINC85571153	-9.5
7.	ZINC85593528	-9.5
8.	ZINC85593537	-9.5
9.	ZINC85867410	-9.5
10.	ZINC04098720	-9.4
11.	ZINC70450906	-9.4
12.	ZINC85594055	-9.4
13.	ZINC85594051	-9.3
14.	ZINC85531373	-9.2
15.	ZINC85542844	-9.2
16.	ZINC85543599	-9.2
17.	ZINC85547121	-9.2
18.	ZINC85547170	-9.2
19.	ZINC85867431	-9.2
20.	ZINC86034711	-9.2
21.	STX0119 (control)	-8.3

Table 1: Top 20 compounds resulted from virtual screening.

An intra-molecular interaction analysis was conducted on the top six compounds. ZINC85593528 interacted with Thr456, His457, Asp237, Asp242, Arg245, Ala241, Lys244, Asn485, Pro487, Gln248, Leu459, Gln247, Trp243, Gln326, Val322, and Val323 residues of STAT3 (Figure 1a); while Glu455, Lys318, His457, Ser319, Thr456, Val322, Phe321, Gln247, Val323, Trp243, Gln248, Arg245, Asn485, Ala241, Leu459, Pro487, Gln326, Ser458, and Lys244 residues of STAT3 interacted with ZINC85593537 (Figure 1b). ZINC85542844 was found to interact with Glu455, Lys318, Ser319, Thr236, Asp237, Ala241, Lys244, Asn485, Asn486, Pro487, His457, Lys488, Leu438, Thr440, Asp371, Lys370, and Ser372 residues of STAT3 (Figure 1c). ZINC4098720 interacted with Pro487, Lys244, His457, Phe321, Thr456, Glu455, Leu454, Lys318, Ser319, Val322, Val323, Trp243, Gln247, Gln326, and Leu459 residues of STAT3 (Figure 1d). ZINC85543599 interacted with Gln247, His457, Lys244, Thr456, Phe321, Glu455, Asp237, Ala241, Arg245, Asn485, Leu459, Pro487, Val322, Val323, Trp243, and Gln326 residues of STAT3 (Figure 1e). Furthermore, ZINC85593523 interacted with Asp237, Glu455, Ser319, Lys318, Val322, Trp243, Gln326, Val323, Gln247, Leu459, Thr456, His457, Pro487, Lys244, Thr236, Ala241, Asn486, and Asn485 residues of STAT3 (Figure 1f).

STX-0119 interacted with Val323, Trp243, Gln326, Gln247, Val322, Leu459, Pro487, Thr236, Ser319, Lys244, Phe321, Asp453, Lys318, Leu454, Glu455, Thr456, and His457 residues of STAT3 (Figure 2).

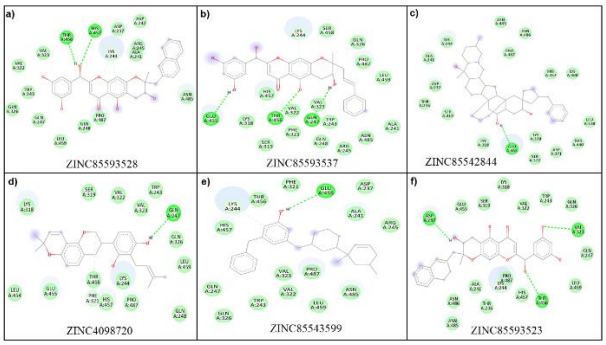

Figure 1: 2D interacting residues of STAT3 protein with ZINC85593528 (a), ZINC85593537 (b), ZINC85542844 (c), ZINC4098720 (d), ZINC85543599 (e), and ZINC85593523 (f).

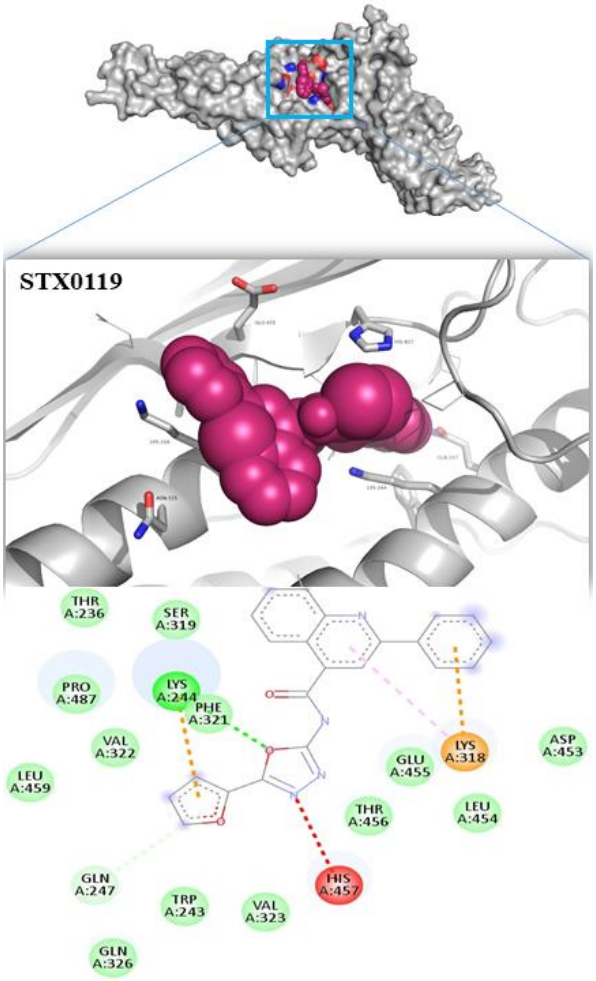

Figure 2: 3D and 2D interaction of STX-0119 in the STAT3 protein binding pocket.

The superimposition of representative structures revealed that the binding pattern and conformational alignment of hits (ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537) in the STAT3 active site were identical to that of STX-0119 (Figure 3).

Compound ID	Mol. Weight	ALogP	cLogP	cLogS	Total Surface Area	Relative PSA	Polar Surface Area	H-Acceptors	H-Donors	Number of Rotatable Bonds	Mol. Polar Surface Area	Drug likeness	Drug Score
ZINC85542844	602.909	5	6.6703	-7.351	432.19	0.099146	63.93	4	3	2	65.13	2.1393	0.183013
ZINC4098720	392.487	5.855	6.3296	-5.092	303.16	0.15239	58.92	4	2	3	58.92	-2.1685	0.047433
ZINC85543599	388.585	8.18	7.1722	-6.455	321.38	0.040762	20.23	1	1	5	20.23	-3.5036	0.151103
ZINC85593523	528.549	4.614	4.3213	-5.591	373.91	0.26354	136.68	8	5	4	136.68	1.783	0.222244
ZINC85593528	526.533	4.494	4.3876	-6.079	369.78	0.26648	136.68	8	5	4	136.68	2.833	0.214147
ZINC85593537	502.512	4.053	3.9787	-5.148	361.68	0.27245	136.68	8	5	5	136.68	1.5718	0.259739

Table 2: Physicochemical and drug-likeness evaluations of the top six compounds (ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537).

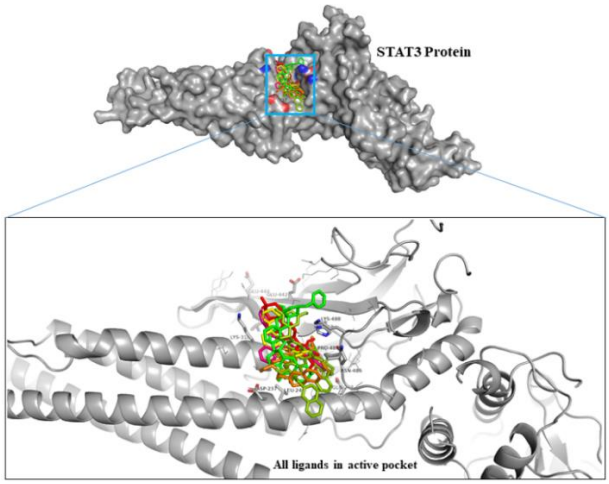

Figure 3: The superimposed conformation of reference inhibitor (STX-0119) and hits (ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537) in the STAT3 active site.

Discussion

VS is a popular computational tool for identifying potential compounds against pre-determined biological targets [18]. The study focused on screening TCM compounds against the STAT3 protein. Initially, the selection of the top 20 compounds was based on their robust binding affinities with STAT3. Subsequently, SMARTS tools were utilized to filter this group, resulting in the identification of the top 6 compounds: ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537. These compounds adhered to Lipinski and Veber's criteria and display potential for future drug development. Moreover, further analysis was conducted on these compounds for an in-depth analysis of their interactions, revealing their binding capabilities with crucial residues of the STAT3 protein. The active site residues of STAT3 have been determined as Lys244, Asp369, Arg382, Arg423, Leu438, Gly442, Thr443, Glu444, Thr456 and His457 [19]. Consistent with this the hits (ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537) were found to interact with these residues of STAT3 protein. STAT3 protein residues, suggesting that these compounds bind within the same binding pocket of the STAT3 protein, similar to STX-0119.

Computational drug discovery is an effective strategy for speeding up and streamlining the drug discovery and development pipeline, demonstrating efficacy in increasing efficiency and cost-effectiveness [21-23]. In docking analysis, if a chemical has a lower binding affinity than the control, it indicates that the molecule is more active [24,25]. Interestingly, hits (ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537) had higher negative binding affinity values relative to the control (STX-0119), indicating that these hits resulted in tighter binding and could be used as STAT3 inhibitors.

Natural products and structural counterparts have long played important roles in pharmacotherapy, particularly in the treatment of cancer and infectious disorders. With rising health risks, there is an urgent need to investigate preventive and treatment options, emphasizing the critical role of natural products in generating novel medicines and reducing disease spread and mortality. Plant-derived chemicals' therapeutic value has been recognized for decades, resulting in their widespread use in both households and clinics to treat a variety of illnesses [26].

This work used a structure-based drug discovery approach to identify natural compound-like inhibitors of STAT3. The hit compounds ZINC85542844, ZINC4098720, ZINC85543599, ZINC85593523, ZINC85593528, and ZINC85593537 demonstrated strong binding and stability with STAT3 and may serve as a lead for STAT3 inhibition to combat the cancers. However, further research is needed to optimize them as STAT3 inhibitors.

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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