MOLECULAR DOCKING STUDIES AND *IN-SILICO* ADMET SCREENING OF SELECTED PHYTOCONSTITUENTS AS STAT3 INHIBITORS

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Abstract

An important function of STAT3 is the maintenance and control of cell growth and function. Different solid and blood malignancies, as well as rheumatoid arthritis and pulmonary fibrosis, are associated with abnormal STAT3 activation and/or expression, making the search for STAT3 inhibitors an expanding area of research. The creation of chemotherapy and several commercially available medications, whether they are of natural origin or have undergone structural alteration, both benefited from the use of phytoconstituents in the process of drug discovery. More significantly, the STAT3 inhibitors found in natural products may offer a reliable foundation for developing novel inhibitors. The present computational study provides insights into the inhibition of stat3 by a few selected phytoconstituents. The results showed that all compounds possess better binding affinity compared to the standard drug (niclosamide). The involvement of amino acid residues like ILE-634, ARG-595, GLU-594, LYS-591, LYS-557, ARG-609, SER-636, and SER-613, whereas for standard hydrogen bond interaction is ARG 595. These interactions play an important role in binding phytocompounds with stat3 active sites. The in-silico ADMET analyses revealed all the compounds show properties within the permitted limits of physicochemical, pharmacokinetics, Lipinski rules, and drug-likeness profiles. This study is successful in identifying potent natural compounds for STAT3 inhibition.

Keywords: STAT3, phytoconstituents, *in-silico* studies, ADMET screen, STAT3 inhibition.

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1. Introduction:

One of the most important STAT family members, Signal Transducer and Activator of Transcription 3 (STAT3) act as an intermediary between the plasma membrane and the nucleus (following growth factors and cytokines)[1], and it is crucial for the regulation of cell development as well as survival[2]. The Src homology 2 (SH2) domain, one of seven functionally and structurally domains that are conserved in STAT3, is necessary for the activation cascade pathway to function. Phosphorylation of Tyr705 on STAT3 monomers through the three tyrosine kinases (Tyrosine-protein kinase, c-Src kinases, and Janus kinase) induces the dimer formation of stat3-stat3 via interaction of the pTyr–SH2 domain. By binding to particular DNA consensus sequences, the dimer complex enters the cell's nucleus as well as stimulates transcription of the target gene[2,3]. The aberrant modulation of cytokine receptors in the body's developmental factors, and Janus kinases leads to the constitutive activation of STAT3 in a wide range of human solid and blood-related cancers, resulting in uncontrolled cell development and survival[4], enhanced angiogenesis, and metastasis[5]. Notably, inhibiting STAT3 solely induced apoptosis in cell lines of cancer, while having no impact on healthy cells[6]. As a result of the importance of abnormal STAT3 expression and/or activation in the progression of cancer, as well as its implications in other ailments, such as atherosclerosis, psoriasis, rheumatoid arthritis, and inflammatory bowel disease, the pursuit of STAT3 inhibitors has evolved into an increasingly hot field in medicinal chemistry[7].

The discovery of extremely specific and efficient STAT3 antagonists has drawn a significant amount of interest due to the potential for drug development it offers[8]. A lot of studies have been done on biological mechanisms of action and new STAT3 inhibitors[9]. No STAT3-targeted drug has, however, been authorized for therapeutic use. Among the STAT3 inhibitor small molecules that have been described, napabucasin (also known as BBI608) and curcumin are examples of natural compounds (or) phytoconstituents[10,11]. These compounds have been very important in drug development, such as the production of chemotherapeutic and various commercially available drugs of natural or structurally modified origin[12,13]. Due to their complex biological and chemical diversity, natural compounds are being utilized significantly to investigate the biologically useful chemical region[14,15]. Importantly, natural product STAT3 antagonists may serve as a firm foundation for the design of new inhibitors.

Therefore, it becomes imperative to analyze the phytochemical components of a plant so that the full pharmacological potential could be exploited. In order to save money and time, we utilized in-silico methodologies to identify novel ideas or hits. For docking calculations, a variety of instruments and software are available today. Among them, BIOVIA Discovery studio is the most up-to-date and extensively used version for virtual screening[16]. Here, we report the binding capacity values of compounds derived from a collection of open-source molecular docking approaches, as well as the ADMET profiles of selected compounds. This article concentrates on natural compounds for the identification of new drug possibilities for the STAT3 inhibition target. Using molecular tethering over the SH2 domain of a STAT3 monomer, we investigate the interaction of selected phytoconstituents in pursuit of a potential lead molecule for inhibiting STAT3 dimerization.

2. Materials and Methods

2.1Preparation of Protein

The Protein Data Bank (https:// www.rcsb. org/ structure/1BG1) has been utilized to derive the three-dimensional crystal structure of a STAT3 beta homodimer bound to DNA with PDB ID: 1BG1[17]. The Protein Structural Preparation for the Macromolecule Protocol was performed with the default parameters in BIOVIA Discovery Studio. The complexes bound to the receptor molecule were deprived of all heteroatoms and non-essential water molecules, hydrogen atoms were then added, and 3D protonation of the target protein was minimized.

2.2Ligand Preparation

A total of 10 phytochemicals was extracted from the literature review and the TCIM database. The canonical grins were saved in.csv format, structures were created with the Data Warrior tool (https:// openmolecules.org/datawarrior/), and all phytoconstituents were saved in SD format. Using the CHARMm force field in the small molecule protocol, all ligand structures were energy minimized and different conformers were generated. The generated output file (Best conformations of the ligands) was subsequently utilized for docking research.

2.3Molecular Docking Studies using BIOVIA Accelrys Discovery studio

Molecular docking was used to uncover molecular

interactions between phytoconstituents and the target STAT3 (PDB ID: 1BG1). All of the ligands were docked using Discovery Studio version 3.5 and the Libdocker program. The protein structures were obtained from the protein data library, and protein processing and reduction were formed using the default settings of BIOVIA Discovery Studio. The ligand-binding sites form the active site sphere selected in the Define and Edit Binding sites section of the Receptor-Ligand Interaction tools. The binding site sphere, Docking Tolerance of 0.25, and Docking Preferences of High Quality have been specified based on previously published data binding interactions against the target protein. Using Discovery Studio Visualizer, 3D and 2D interactions were obtained after analyzing the results.

2.4*In Silico* ADMET and Drug-Likeness Prediction

The *in silico* ADMET screening and drug-likeness evaluation of the proposed compounds were determined by using the free web tools Swiss ADME, and ADMET lab 2.0 [18-21]. That tools gave information about the simple physicochemical properties such as molecular weight (MW), the number of hydrogen bond acceptors, the number of hydrogen bond donors, the number of rotatable bonds, molar refractivity (MR), total polar surface area, Log S (solubility), Log P o/w (octanol-water partition coefficient) were computed. Pharmacokinetic parameters were computed, such as GI absorption, BBB permeation, cytochrome P450 isomers inhibition, and log Kp (Skin permeation). The Drug-likeness prediction was made using selected rules such as Lipinski's, Ghose's, and Veber's, as well as bioavailability scores. For a molecule to be considered an active drug candidate, it must meet the Lipinski rule of five and other requirements. Synthetic accessibility is a method that can characterize molecules and score between 1 (very easy to synthesize), and 10 (complex to synthesize). Molecular weight ≤ 500, hydrogen bond donor 0-6, hydrogen bond acceptor 2-20, number of rotatable bonds ≤ 9, molar refractivity ≤ 140, Total polar surface area range between 20 to 130Å, Log S range lies above -4, and Log P ≤ 5, satisfy the rule of five, Log K_p -7.4 to -5.35, Bioavailability score is ≥ 0.55.

3. Results and Discussion

3.1Protein target's structural details

Transcription factor STAT3 B/DNA complex (1BG1) has been a primary therapeutic target. STAT3 Receptor's three-dimensional structure, which was determined by X-Ray crystallography with a resolution of 2.25, was visualized using Pymol and was taken from the Protein Data Bank with PDB ID: 1BG1. Its total structure weight is 73.66 kDa. Figure 1 illustrates this point.

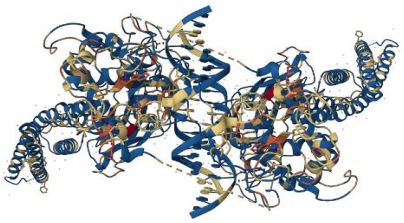


Figure 1: Three-dimensional structure of STAT3 receptor (1BG1)

3.2Molecular docking

Molecular docking was performed using selected protein and ligands in Discovery Studio. The higher the LibDock score, the higher the probability of ligand-protein binding. The docked compounds puerarin, nodakenin, quercetin, luteolin, naringenin, apigenin, kaempferol, 6,8,3-tri hydroxy-3,7,4- tri methoxy flavone, biochanin A, genistein, as well as the standard compound niclosamide have LibDock scores of 121.61, 120.45, 100.03, 98.31, 97.92, 93.96, 93.91, 91.79, 90.17, 89.77, and 89.32, respectively. In comparison to the standard compound, the first three compounds exhibited a high docking score, indicating a high binding affinity for these hit compounds. The top three substances were evaluated for interaction criteria. Table 2 summarises the interaction data. All the substances exhibited hydrogen bond interactions, e.g., puerarin exhibited ILE 634, ARG 595, GLU 594, and LYS 591 interactions; nodakenin exhibited LYS 557, ARG 609, and SER 636 interactions; and quercetin exhibited SER 613 and SER 636 interactions, whereas the interaction for the standard drug is ARG 595. All substances were discovered to exhibit Pi- cation interactions i.e., LYS 591, ARG 609, GLU 594, except nodakenin. And Pi-Pi stacking interaction PRO 639 is also found in all compounds.

S. No	Phytoconstituents	LibDock score (kcal/mol)
1	Puerarin	121.61
2	Nodakenin	120.45
3	Quercetin	100.03
4	Luteolin	98.31

5	Naringenin	97.92
6	Apigenin	93.96
7	Kaempferol	93.91
8	6,8,3-tri hydroxy-3,7,4-tri methoxy flavone	91.79
9	Biochanin A	90.17
10	Genistein	89.77
11	Niclosamide (standard)	89.32

Table 1: Results of Docking score of selected Phytoconstituents in the active site of STAT3 (1BG1)

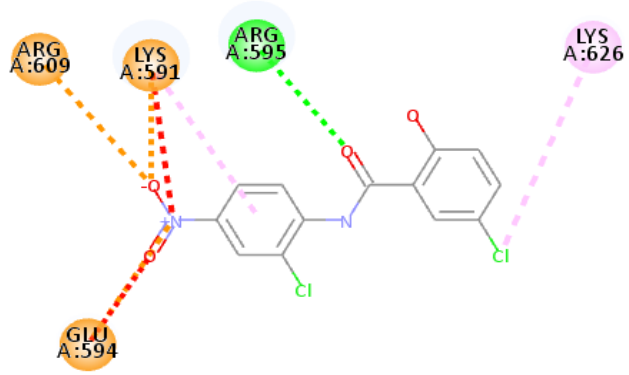
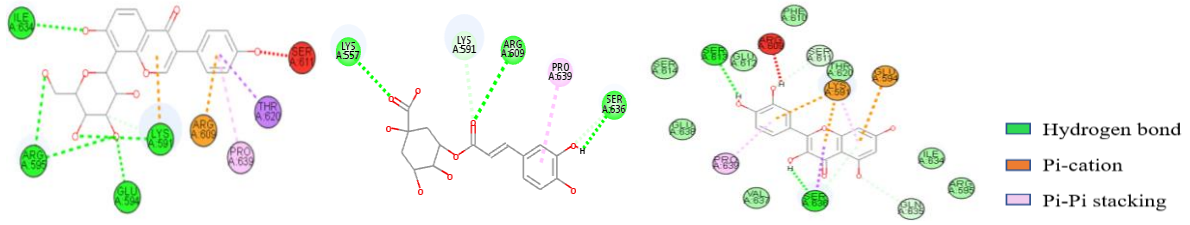


Figure 2: Diagrammatic illustration of interactions between the active site of STAT3 and Niclosamide



Puerarin Nodakenin Quercetin

Figure 3: Diagrammatic illustration of the Top 3 compounds' interactions with the active site of STAT3

S. No	Compounds	No. of interacting residues	H-bond	Pi-cation	Pi-Pi stacking
1	Puerarin	7	ILE 634, ARG 595, GLU 594, LYS 591	LYS 591, ARG 609	PRO 639
2	Nodakenin	4	LYS 557, ARG 609, SER 636	-	PRO 639
3	Quercetin	5	SER 613, SER 636	LYS 591, GLU 594	PRO 639
4	Niclosamide (standard)	4	ARG 595	LYS 591, ARG 609, GLU 594	-

Table 2: Interaction data of top 3 compounds with the active site of STAT3

3.3Characteristics of the compounds that were chosen in terms of their physicochemical constitution

Table 3 provides a listing of the fundamental physical and chemical characteristics of the compounds that were selected based on the docking outcome. The selected compounds' molecular weights ranged from 270.24 to 416.38. The acceptors of hydrogen bonds varied between 5-9. The donors of hydrogen bonds varied between 0 to 6. The number of rotatable bonds lies between 1 and 7. The molar refractivity ranged from 71.57 to 106.87. The total polar surface ranged from 79.90 Å to 130.36 Å except for puerarin which showed a significantly higher value (160.82 Å). Log S values varied between -4.243 and -3.440. which are slightly near the ideal value. Log P values varied between 0.69 and 3.307. The results, therefore, suggest that all compounds have acceptable physicochemical properties.

3.4ADME parameters (or) Pharmacokinetics

The computed ADME characteristics of absorption in the gastrointestinal tract (GI), permeability

across the blood-brain barrier (BBB), glycoprotein (P-gp) substrate, and cytochrome P450 system inhibition, cytochrome P450 inhibition, and Log K_p (Skin Permeation) of mentioned compounds are presented in Table 4. All of the substances demonstrated substantial gastrointestinal absorption, according to the findings. BBB permeation potential was not shown. Only the Naringenin compound showed the potential as a P-gp substrate. However, the majority of the substances inhibited the CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4 isomers of cytochrome P450. Skin permeation is also achieved by all compounds. Puerarin compound showed slightly higher skin permeability.

3.5Drug-Likeness and medicinal chemistry parameters

Tables 5 and 6 show the compounds that met the criteria for inclusion in the study together with their predicted drug-likeness, medicinal chemistry, and lead-likeness features. According to the results, Puerarin showed one violation of Lipinski, Veber, Egan, and 2 violations of Muegge. The remaining compounds did not violate any of the 5 filters (Lipinski, Ghose, Veber, Egan, and Muegge). The bioavailability score of all the compounds examined was 0.55, indicating that they all had promising drug-like properties. The findings showed that all of the compounds had excellent drug-likeness scores and had never broken any drug-likeness guidelines for research. In addition, the PAINS, Brenk, and Lead-likeness methods were employed in order to isolate troublesome portions that may produce erroneous biological results. According to the findings of this screening investigation, absolutely no compounds exhibited this sort of segment. A few compounds i.e., quercetin and luteolin showed one alert of PAINS and BRENK. Whereas, for Lead-likeness, puerarin, nodakenin, and 6,8,3-tri hydroxy-3,7,4-tri methoxy flavone showed 1 violation. The rest of the compounds did not show a violation. The outcomes of the test for synthetic accessibility showed that the compounds' scores ranged from 2.87 to 4.98. The acquired results showed that it was simple to synthesize the chemicals.

Compounds	M.W	nHA	nHD	nROT	M. Rfy	TPSA (Å)	Log S	Log P
Puerarin	416.38	9	6	3	104.59	160.82	-3.924	0.694
Nodakenin	402.39	8	0	7	106.87	85.59	-4.243	2.888
Quercetin	302.24	7	5	1	78.03	130.36	-3.671	2.155
Luteolin	286.24	6	4	1	76.01	111.13	-3.629	2.902
Naringenin	272.25	5	3	1	71.57	86.99	-3.876	2.562
Apigenin	270.24	5	3	1	73.99	90.90	-3.606	3.307
Kaempferol	286.24	6	4	1	76.01	111.13	-3.624	2.656
6,8,3-tri hydroxy-3,7,4-tri methoxy flavone	360.31	8	3	4	93.47	118.59	-3.801	2.693
Biochanin A	284.26	5	2	2	78.46	79.90	-3.478	3.081
Genistein	270.24	5	3	1	73.99	90.90	-3.440	2.506

Table 3: Physico-chemical properties of Phytoconstituents

Compounds	GI absorption	BBB penetration	P-gp	CYP1A2	CYP2D6	CYP3A4	Log K_p (cm/s)
Puerarin	High	No	No	No	No	No	-8.83
Nodakenin	High	No	No	No	No	Yes	-6.62
Quercetin	High	No	No	Yes	Yes	Yes	-7.05
Luteolin	High	No	No	Yes	Yes	Yes	-6.25
Naringenin	High	No	Yes	Yes	No	Yes	-6.17
Apigenin	High	No	No	Yes	Yes	Yes	-5.80
Kaempferol	High	No	No	Yes	Yes	Yes	-6.70
6,8,3-tri hydroxy-3,7,4-tri methoxy flavone	High	No	No	Yes	Yes	Yes	-6.52
Biochanin A	High	No	No	Yes	Yes	Yes	-5.91
Genistein	High	No	No	Yes	Yes	Yes	-6.05

Table 4: Pharmacokinetics of Phytoconstituents

Compounds	Lipinski (violations)	Ghose (violations)	Veber (violations)	Egan (violations)	Muegge (violations)	Bioavailability score
Puerarin	1	0	1	1	2	0.55
Nodakenin	0	0	0	0	0	0.55
Quercetin	0	0	0	0	0	0.55
Luteolin	0	0	0	0	0	0.55
Naringenin	0	0	0	0	0	0.55
Apigenin	0	0	0	0	0	0.55
Kaempferol	0	0	0	0	0	0.55
6,8,3-trihydroxy-3,7,4-tri methoxy flavone	0	0	0	0	0	0.55
Biochanin A	0	0	0	0	0	0.55
Genistein	0	0	0	0	0	0.55

Table 5: Drug Likeness Parameters of Phytoconstituents

Phytocompounds	PAINS (alerts)	Brenk (alerts)	Lead-likeness (violations)	Synthetic accessibility
Puerarin	0	0	1	4.98
Nodakenin	0	0	1	3.90
Quercetin	1	1	0	3.23
Luteolin	1	1	0	3.02
Naringenin	0	0	0	3.01
Apigenin	0	0	0	2.96
Kaempferol	0	0	0	3.14
6,8,3-trihydroxy-3,7,4-tri methoxy flavone	0	0	1	3.59
Biochanin A	0	0	0	2.89
Genistein	0	0	0	2.87

Table 6: Medicinal chemistry of Phytoconstituents

4. Conclusion

To foretell whether or not phytoconstituents or natural products will inhibit STAT3, we docked them over the SH2 domain, which is a component of a stat3 monomer, and studied their binding mechanisms. This computational analysis sheds light on how those specific Phytocompounds prevent stat3 dimerization. Molecular docking studies and ADMET screening showed that all phytoconstituents possess better binding affinity values compared to the standard drug (niclosamide) against the selected target protein STAT3. The presence of residual amino acids such as ILE-634, ARG-595, GLU-594, LYS-591, LYS-557, ARG-609, SER-636, and SER-613, whereas for standard drug hydrogen bond interaction is ARG 595. These interactions play an essential role in phytocompounds binding to the stat3 active site. Assessing the compounds for pharmacological impact is aided by the ADMET analysis shown here. These findings could provide a major boost to the understanding of the entire scope of computerized screening over the discovery of new molecules that have stronger biological activity and minimal to no toxicity. All the compounds show properties within the permitted limits of physicochemical, pharmacokinetics, Lipinski rules, and drug-likeness profiles. Now that these compounds have been further examined for in-vitro and in-vivo investigations and clear mechanisms of action, research may proceed on to clinical trials.

Conflict Of Interest

The authors declare that there are no conflicts of interest in this study. The authors are responsible for the content and writing of the papers.

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