J. Chosun Natural Sci.
Vol.16, No.4 (2023) pp.123-131
https://doi.org/10.13160/ricns.2023.16.4.123

An *In Silico* Drug Repositioning Strategy to Identify Specific STAT-3 Inhibitors for Breast Cancer

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Abstract

Breast cancer continues to pose a substantial worldwide health challenge, thereby requiring the development of innovative strategies to discover new therapeutic interventions. Signal Transducer and Activator of Transcription 3 (STAT-3) has been identified as a significant factor in the development of several types of cancer, including breast cancer. This is primarily attributed to its diverse functions in promoting tumour formation and conferring resistance to therapeutic interventions. This study presents an in silico drug repositioning approach that focuses on identifying specific inhibitors of STAT-3 for the purpose of treating breast cancer. We initially examined the structural and functional attributes of STAT-3, thereby elucidating its crucial involvement in cellular signalling cascades. A comprehensive virtual screening was performed on a diverse collection of drugs that have been approved by the FDA from zinc15 database. Various computational techniques, including molecular docking, cross docking, and cDFT analysis, were utilised in order to prioritise potential candidates. This prioritisation was based on their predicted binding energies and outer molecular orbital reactivity. The findings of our study have unveiled a Dihydroergotamine and Paritaprevir that have been approved by the FDA and exhibit considerable promise as selective inhibitors of STAT-3. In conclusion, the utilisation of our in silico drug repositioning approach presents a prompt and economically efficient method for the identification of potential compounds that warrant subsequent experimental validation as selective STAT-3 inhibitors in the context of breast cancer. The present study highlights the considerable potential of employing computational strategies to expedite the drug discovery process. Moreover, it provides valuable insights into novel avenues for targeted therapeutic interventions in the context of breast cancer treatment.

Keywords: Breast cancer, cDFT, Drug repurposing, FDA drugs, Molecular docking

(**Recceived** September 4, 2023; **Revised** October 15, 2023; **Accepted** November 1, 2023)

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1. Introduction

One in eight women will get breast cancer, which is one of the deadliest kinds of cancer in the world and accounts for 10.4% of all cancer cases[1]. Breast tissue cells undergo genetic and epigenetic changes that give rise to the complex and heterogeneous disease known as breast cancer. For the creation of efficient treatments, it is essential to comprehend the underlying molecular mechanisms and signaling pathways involved in the occurrence and spread of breast cancer[2]. One of the main breast cancer-related genes, STAT-3, a transcription factor that controls gene expression, has been identified as a possible target for treatment. A family of transcription factors called Signal Transducers and Activators of Transcription (STAT) is essential for controlling how genes are expressed in response to extracellular inputs. STAT-1, STAT-2, STAT-3, STAT-4, STAT-5a, STAT-5b, and STAT-6 are the seven members of the STAT family. Among these, STAT-3 has received substantial research due to its critical function in the onset and spread of cancer. When STAT-3 is exposed to numerous cytokines, growth factors, and other stimuli, phosphorylation causes STAT-3 to become active[3]. As soon as it is activated, STAT-3 moves into the nucleus and binds to particular DNA sequences, controlling the production of a number of genes important in cell growth, differentiation, and survival. Recent research has shown the activationof STAT-3 increases the production of genes that support cell survival, proliferation, and metastasis. Breast cancer formation and progression have both been linked to dysregulation of the STAT-3 pathway, making this pathway a prospective target for therapeutic intervention[4].

This work describes an in silico drug repositioning method to identify specific STAT-3 inhibitors for breast cancer. 1600 FDA drugs from Zinc15 database was downloaded and docked with STAT-3 protein using Autodock Vina on a high-performance computer. Ten compounds with high binding affinity as possible selective STAT-3 inhibitors were segregated. Followed by cross docking between all STAT proteins (STAT1, STAT2, STAT4, STAT5a, STAT5b, STAT6) and STAT-3 to check the intra-family selectivity of FDA drugs towards the target. Finally cDFT analysis was done to check the reactivity of compounds based on their outer molecular orbitals.

2. Materials and method

2.1 Preparing Ligands and the Target

STAT-3 (PDB ID: 6NJS) receptor binding domain (RBD) was taken as the protein target from the Protein Data Bank (https://www.rcsb.org/). The protein's crystallographic three-dimensional structure was concretely culled since the RBD is the most paramount location for targeting inhibitory compounds[5]. Utilizing PyMol (http://www.pymol.org), the co-crystal ligands and molecules which were affixed to protein were all optically discerned. The protein was further processed by integrating charges, abbreviating the protein's energy, and then converting it to .pdbqt format utilizing Autodock tools 1.5.7. The ligands' 3D

structures were acquired from the zinc15 database (https://zinc.docking.org/) from FDA subset. In-depth details about chemical compounds, including their 2D and 3D structures, properties, bioactivity, and safety, are contained in this chemical database. The ligands' SDF files are converted to PDB format utilizing the OpenBabel program (https://openbabel.org/).In order to create the ligand library the FDA drugs are additionally prepared by identifying the torsion root, rectifying the torsion angles, assigning charges, and converting them to .pdbqt format.

2.2 Selection of Binding Site and Molecular docking:

According toprevious research findings, it is suggested that several sites in close proximity to the ligand coordinates within the cocrystal region exhibit potential as favourable candidates for ligand binding [6]. Molecular docking is an in silicotechnique that prognosticates the interactions and relations between a protein and small molecules predicated based on the geometry and the scores. In order to investigate the activity in terms of binding affinity (Kcal/mol), we made use of Autodock 1.5.7. The results were then compared using the binding affinity score for the best-docked conformation. At a specified area of the protein (RBD), the interactions between the molecules will be precisely determined. In this study we specify this area with thehelp of cocrystal ligand and locate it using the Grid map option. The software ultimately predicts just the interactions and binding energies between the ligand molecule and the amino acids present in the GridBox. Thus, it's crucial to position the GridBox at the protein's binding site, active site, or other crucial areas. The docking calculations were carried out by utilising a force field that relies on empirical free energy, which is a commonly employed approach in molecular docking studies. Additionally, a conformational search was performed using a Lamarckian genetic algorithm, a well-established method in the field, with the default parameters as provided by the software. The observation of negative binding energies, as reported in reference, indicates a high degree of selectivity exhibited by the drug towards its intended target [6]. A careful selection was made to identify ten compounds exhibiting the lowest binding affinities, warranting further investigation. The software application PyMOL was employed in the generation of visual representations depicting the intricate interactions between proteins and ligands.

2.3 Cross docking:

The cross-docking method is a widely used computational technique to assess the binding affinity and selectivity of protein structures for different ligands. This method involves docking multiple ligands into a single protein structure or multiple protein structures, allowing for a comparative analysis of the ligand-protein interactions. Cross-docking can help you determine which protein structure exhibits the highest affinity for a particular ligand, aiding in understanding ligand selectivity across different protein conformations [7].

2.4 cDFT:

The application of density functional theory

(DFT) allows for the analysis of molecular and atomic structure by harnessing the energies associated with their respective molecular orbitals. This computational method proves to be valuable in providing meaningful insights into the relationship between molecular structure and activity. The Hohenberg-Kohn theorem serves as the foundational principle for this theory. The present investigation involves a visual analysis of the chemical behaviour of a molecule, employing relevant concepts derived from the field of conceptual Density Functional Theory (DFT), which is a specialized subfield within the broader domain of DFT. Based on a comprehensive set of ten distinct molecular descriptors the drugs were selected[5][6]

3. Results and Discussion

3.1 Molecular docking:

Based on the binding affinity ratings produced for various conformations of the docked poses, the molecular docking technique ultimately helps in determining the optimal inhibitors to a specific protein[8]. Finding the ligands in the binding pocket and the bonds formed with nearby residues is further assisted by visualization tools like PyMol. In this case, top 10 compounds based on the binding affinity were segregated. Among them, Ledipasvir, Irinotecan, Avapritinib, Dihydroergotamine, and Netupitant displayed the best binding affinity with the protein with scores of -9.6 kcal/mol, -9.5 kcal/mol, -9.4 kcal/mol, -9.3 kcal/mol, and -9.3 kcal/mol, respectively and they formed hydrogen bonding with residues Arg 609, Ser 613, Lys 658; Pro 639, Tyr 640; Ile 467; Asp 570; and Tyr 640 respectively.

Dutasteride, Selpercatinib, Ergotamine, Exatecan and Paritaprevir which were also docked for comparison purposes, scored -9.2 kcal/mol, -9.2 kcal/mol, -9 kcal/mol, -9 kcal/mol, and -9 kcal/mol respectively.

Information about hydrogen bonds, hydrophobic interactions, and docking scores are tabulated in Table 1. Hydrophobic interactions include Ile 467, 569, 653; Cys 466; Met 470, 648; Val 563, 637; Lys 573. Since the ligands in the binding pocket are surrounded by a lot of hydrophobic residues, interactions between these residues may help keep the complex stable.

3.2 Cross docking:

We performed Cross docking between allSTAT proteins (STAT1, STAT2, STAT4, STAT5a, STAT5b, STAT6) and STAT-3 to check the intra-family selectivity of drugs towards the target. The compounds showed the highest binding affinity towards STAT-3 compared to all the other STATs.

3.3 cDFT analysis:

To first estimate the molecular characteristics of the drugs using Fukui's molecular orbital theory, the B3LYP function with a 6-31G (d) basis in Gaussian 16[9] was used to optimizethe drugs. Calculations were made for molecular orbital energies such the HOMO energy (EHOMO) and LUMO energy (ELUMO). EHOMO and ELUMO, which stand for a molecule's capacity to give and accept electrons, respectively, are significant descriptors[5]. The statistics of DFT-based molecular descriptors of top 10 drugs are shown in the Table 3. The order of

increasing reactivity corresponds with decreasing energy gap values.A molecule's dipole moment directly correlates with its chemical reactivity[6]. Dipole moment of the drugs were in the range of 13.5 - 2.02 Debye respectively. Electronegativity measures a compound's electron-accepting ability. It indicates molecule inhibition efficiency. Lower electronegativity increases inhibition efficiency [5]. Dihydroergotamine has the highest electronegativity index (-5.24). This index for other drugs was almost in the range of -5.24 to -0.1. It is significant to note that the energy gap is inversely connected with the reactivity of the molecules, which is corroborated by the molecules' change from HOMO to LUMO. It is known that the higher the activity of the molecules, which is connected with the change of the molecules from the HOMO to the LUMO, the smaller the energy gap (ΔE) is. The range of ΔE was between -0.02 to 4.43.

Maps representing the density of electrons in different regions of the molecules at HOMO and LUMO were generated and analyzed Fig. 2. Dihydroergotamine and Paritaprevirbe showed better values for all the descriptors. The results of conceptual DFT are in agreement with the docking results.

4. Conclusion

In this study, a virtual screening approach was employed to evaluate FDA-approved drugs from the FDA subset of the zinc15 database. Subsequently, molecular docking techniques were utilised to assess the binding affinity of these drugs. As a result of this analysis, a selection of the top 10 drugs with the most promising docking scores was identified. The docking studies have provided evidence suggesting that the molecular interactions between the drugs approved by the FDA and the STAT3 protein are potentially facilitated, at least in part, by hydrogen bonds and hydrophobic interactions. Based on the results obtained from conceptual Density Functional Theory (cDFT) and molecular docking analyses, it is highly recommended that compounds Dihydroergotamine and Paritaprevirbe considered as potential candidates for anti-cancer drug development. These compounds exhibit promising characteristics and therefore warrant further investigation, particularly through in vitroexperiments.

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Table. 1. H-bond and hydrophobic interactions between STAT3 (PDB ID: 6NJS) and the top ten FDA-approved drugs.

S.NO	FDA Drugs	Binding affinty (kcal/mol)	H-bond interactions	Hydrophobic interactions
1	Ledipasvir	-9.6	Arg 609, Ser 613, Lys 658	Ile 467, 569, 653; Cys 466; Met 470,648; Val 563, 637; Lys 573
2	Irinotecan	-9.5	Pro 639, Tyr 640	
3	Avapritinib	-9.4	Ile 467	
4	Dihydroergotamine	-9.3	Asp 570	
5	Netupitant	-9.3	Tyr 640	
6	Dutasteride	-9.2	Asn 567	
7	Selpercatinib	-9.2	Asp 334, Arg 335, Cys 468, Dsp 570	
8	Acetyldigitoxin	-9	Tys 640, Ser 613, Glu 612	
9	Ergotamine	-9	Lys 574	
10	Paritaprevir	-9	Ile 467, Asp 566, Asn 567	

Table. 2. Cross docking between all STAT proteins (STAT1, STAT2, STAT4, STAT5a, STAT5b, STAT6) and STAT3 to check their interactions with the top 10 FDA drugs.

S.NO	FDA Drugs	Binding affinity (kcal/mol)						
		STAT3	STAT1	STAT2	STAT4	STAT5A	STAT5B	STAT6
1	Ledipasvir	-9.6	-9.4	-9.2	-9.3	-9	-9.1	-8.8
2	Irinotecan	-9.5	-9.4	-9.3	-9.1	-9.2	-8.9	-9
3	Avapritinib	-9.4	-9.2	-8.8	-9	-8.9	-9.1	-9
4	Dihydroergotamine	-9.3	-9.1	-8.6	-9.2	-9	-8.8	-8.9
5	Netupitant	-9.3	-9.1	-9.2	-9	-8.6	-8.7	-8.6
6	Dutasteride	-9.2	-9	-8.9	-8.8	-8.1	-8.3	-8.5
7	Selpercatinib	-9.2	-9	-8.7	-8.9	-8.7	-9	-8.2
8	Acetyldigitoxin	-9	-8.9	-8.2	-8.5	-8.6	-8	-8.3
9	Ergotamine	-9	-8.9	-8.4	-8.7	-8.5	-8.2	-8.7
10	Paritaprevir	-9	-8.7	-7.9	-8.2	-7.8	-8.4	-8.1

Table. 3. Summary for conceptual Density Functional Theory (DFT) Molecular Descriptors of FDA-Approved Drugs.

FDA Drugs	Total Energy (E γ) (in eV)	Molecular dipole moment (Debye)	EHOMO	ELUMO	HOMO/ LUMO Gap (ΔE)	Absolute Hardness (η)	Global Softness (σ)	Electro-negativity (χ)	Chemical potential (μ)	Electrophilicity index (ω)
Ledipasvir	-2989.0	10.3	-2.01	-0.33	1.68	0.84	0.59	-1.17	1.17	-10.31
Irinotecan	-144446.1	8.5	-6.04	-2.25	3.79	1.89	-.26	-4.14	4.14	-3.92
Exatecan	-40289.99	10.98	-0.20	-0.18	0.02	0.01	44.20	-0.19	0.19	8.52
Dihydroergotamine	-52507.0	6.4	-5.55	-4.94	0.61	0.30	1.63	-5.24	5.24	-0.79
Netupitant	-55935.75	2.96	-0.21	-0.05	0.16	0.08	0.25	-0.13	0.13	0.16
Dutasteride	-51862.74	2.02	-0.24	-0.04	0.2	0.1	5	-0.14	0.14	0.2
Selpercatinib	-47143.20	3.87	-0.20	-0.06	0.14	0.07	7.14	-0.13	0.13	0.12
Avapritinib	-45014.7	7.1	-5.16	-1.14	4.02	2.01	0.24	-3.15	3.15	2.46
Ergotamine	-52473.3	7.0	-5.43	-1.0	4.43	2.2	0.22	-3.22	3.22	-3.56
Paritaprevir	-77754.3	13.5	-6.05	-2.45	3.60	1.80	0.27	-4.25	4.25	-3.83

Fig. 1. Top 10 FDA-approved drugs from Zinc15 database docked in the STAT-3 binding pocket (PDB ID: 6NJS). Red is used to indicate interacting residues.

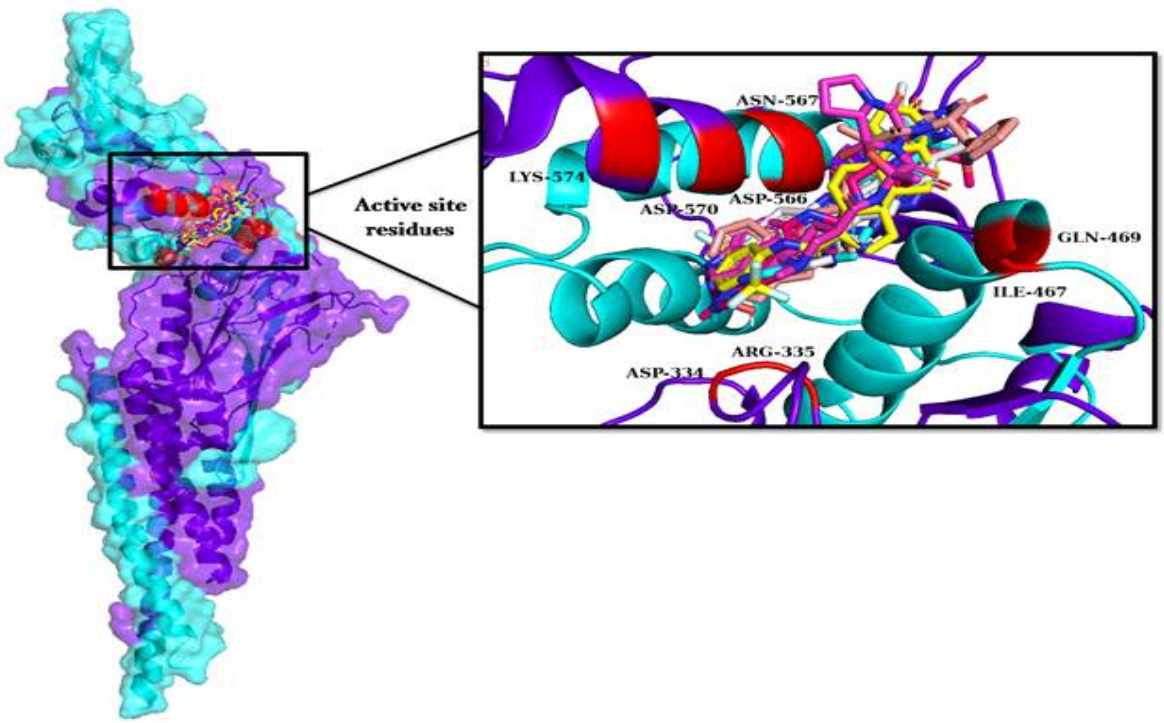
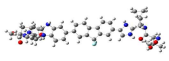
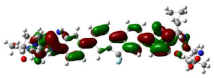
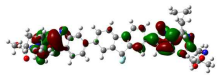
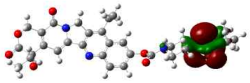
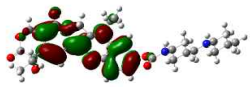
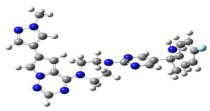
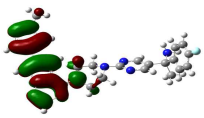
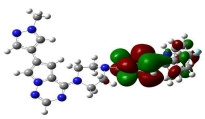
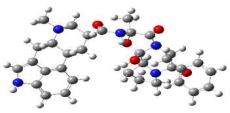
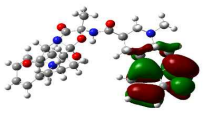
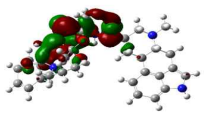
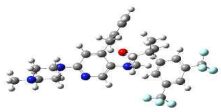
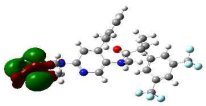
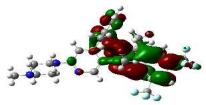
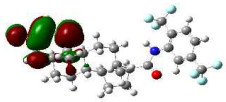
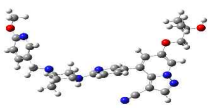
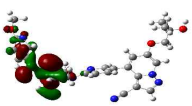
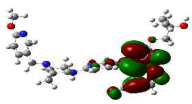
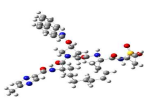
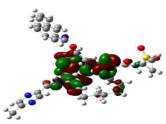
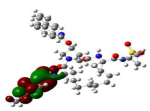
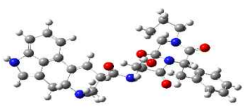
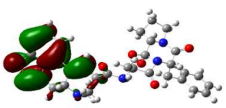
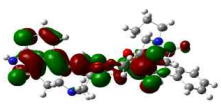


Fig. 2. Electron density maps of HOMO and LUMO of selected top 10 FDA drugs.

FDA drugs	DFT optimized structure	HOMO	LUMO
Ledipsavir			
Irinotecan			
Avapritinib			
Dihydroergotamine			
Netupitant			
Dutasteride			
Selpercatinib			
Paritaprevir			
Ergotamine			
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