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Targeting Serine-Arginine Protein Kinase 1 (SRPK1): Roles in Cellular Processes and Regulation through Selective Inhibitors

Soha Aggarwal

The Cathedral and John Connon School, Bombay

Abstract: ***Protein kinases are a family of attractive enzyme targets for drug design, with significant relevance to various diseases, including cancer. Serine-arginine protein kinase 1 (SRPK1) is specifically responsible for the phosphorylation of serine/arginine (SR)-rich proteins, such as Alternative Splicing Factor/Splicing Factor 2 (ASF/SF2), which is involved in mRNA processing. Overexpression of ASF/SF2 has been observed in various diseases and plays a crucial role in promoting cell survival. The phosphorylation of SR proteins by SRPK1 is a significant regulatory mechanism in cancer progression. In the search for potential anticancer agents to inhibit SRPK1 activity, we utilized in-silico methods to screen natural and drug-like compound databases for specific inhibitors of SRPK1. Five promising inhibitors were identified, with SRPKIN-1 emerging as the most potent. Structural analysis of the SRPK1-SRPKIN-1 complex revealed that SRPKIN-1 uniquely binds to the ATP-binding site, potentially blocking SRPK1 activity and disrupting its recruitment. These findings suggest that SRPKIN-1 could serve as a lead compound paving the way for the development of potent and specific inhibitors of SRPK1 for designing of novel potential anticancer inhibitor is inferred from the current studies.***

Keywords: Serine arginine protein kinase 1 (SRPK1), Splicing, Phosphorylation, Protein kinase, Structural analysis

1. Introduction

Serine/arginine (SR)-rich proteins are a recognized group of splicing factors responsible for removing intervening sequences from primary transcripts and generating multiple mRNA isoforms through processes known as constitutive and alternative splicing (1-2). There are three characterized serine-arginine protein kinases (SRPKs), including SRPK1, SRPK2, and SRPK3, SRPK1: A well-investigated SRPK that shows a role in various cellular functions, including cell proliferation, tumor growth, and alternative splicing, SRPK 2: Involved in metabolic signaling and transcriptional regulation and SRPK3: A less understood SRPK 3 in this study we are focusing on the SRPK. Serine/arginine-rich protein kinase-1 (SRPK1) is an enzyme responsible for phosphorylating splicing factors that contain serine and arginine-rich domains. SRPK1 is structurally made up of two kinase domains that are separated by stretched divergent spacer sequences, its catalytic domain is approximately 250 amino acids long, and the overall molecular weight is 287.93 kDa. SRPK1 gene located on chromosome 6, as an oncoprotein, SRPK1 contributes to tumor development and plays a role in the EGFR/AKT signaling pathway. Its expression is linked to various cancers and other diseases (3). In addition to their role in splicing, SR proteins are involved in other cellular functions, including transcription, mRNA transport, stability, and protein translation (4). Changes in the expression levels of splicing regulatory factors have been noted in various cancers and other diseases, which can affect the splicing patterns of numerous genes involved in specific biological pathways related to cancer progression. SRPK1 is a member of the SRPK family and is categorized alongside Cdc-2-like kinases. It primarily phosphorylates ASF/SF2 in the cytoplasm, while Cdc-2-like kinases perform this function in the nucleus (**figure 1**). It was discovered in 1994 and is recognized for its strong specificity for phosphorylating serine residues within the RS domain of ASF/SF2, achieving this with prominent efficiency and accuracy (5).

Serine/arginine-protein kinase 1 (SRPK1) regulates alternative splicing it is important kinases that specifically phosphorylate SR proteins to regulate their distribution and activities in the central pre-mRNA splicing and other cellular processes (6), SRPK1 has been investigated in clinical material of various cancers, SRPK1 as a prognostic factor and potential therapeutic target in cancer and its expression has been correlated with prognostic factors (7) (e. g., staging, grading, and molecular subtypes) and survival SRPK1 high expression is found in most human cancers; this is shown in the analysis of publicly available TCGA data accessed through the UALCAN website (http: //ualcan. path. uab. edu/cgi-bin/Pan-cancer. pl?) (8).

Protein kinases are enzymes that transfer phosphate groups to specific target proteins, selectively modifying them by covalently attaching phosphates. It plays a crucial role in signal transduction and various cellular processes by transferring a phosphoryl group from ATP to target proteins (9). Phosphorylation is critical in various cellular pathways, including metabolism, cell signaling, protein regulation, cellular transport, and secretory processes. By phosphorylating molecules, kinases can enhance or inhibit their activity and modulate their interactions with other molecules. This regulation is essential for normal cellular function and for maintaining cellular and physiological processes (10). Serine arginine protein kinase 1 (SRPK1) is responsible for phosphorylating serine/arginine (SR)-rich proteins, such as Alternative Splicing Factor/Splicing Factor 2 (ASF/SF2), which plays a key role in mRNA processing. ASF/SF2 is often overexpressed in various diseases and cancers, where it is critical for cell survival. Phosphorylation of ASF/SF2 is responsible for the cancer-related functions. Disruption of its activity causes altered metabolism affecting the splicing patterns of many genes that involved in cancer-related processes, such as cell cycle progression, proliferation, migration, and other key pathways. SRPK1 is frequently overexpressed in several types of cancer, making

it a promising target for cancer drug development (6). Targeting SRPK1 could offer a potential therapeutic strategy for cancer treatment. This study aims to identify inhibitors that that could selectively interrupt the binding of SRPK1 activity.

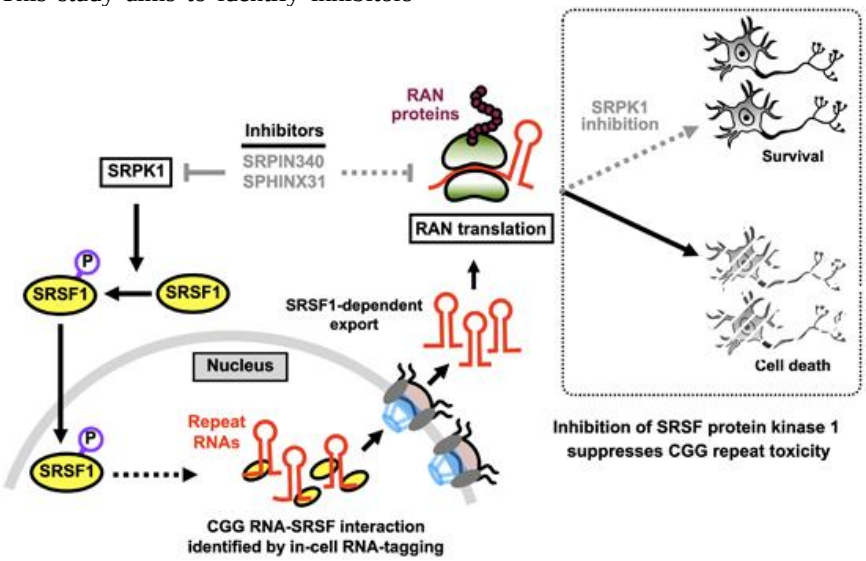


Figure 1: SRPK1 inhibition disrupts the movement of SRSF proteins from the cytoplasm to the nucleus, altering splicing and RAN translation, thereby affecting key cellular functions (11)

Protein kinases are key regulators of cell growth and proliferation and play significant roles in immune responses. Due to their significant role in these signaling mechanisms, they are important therapeutic targets in cancer and other diseases, so the deregulation can drive pathological conditions. The human genome contains 518 protein kinases, making them the largest protein family involved in regulating various biological processes. Among all of them one important protein kinase is Serine-arginine protein kinase 1 (SRPK1), which phosphorylates serine/arginine-rich (SR) proteins that are critical for splicing and gene expression (12). Since the discovery of kinase inhibitors in the 1980s, the FDA has approved 37 therapeutic molecules targeting various kinases for treating cancer and other inflammatory diseases. Overall, protein kinases are essential for numerous cellular functions, and their regulation through specific inhibitors presents significant therapeutic potential for treating various diseases. The present study emphasizes the development and use of more selective inhibitors of Serine-arginine protein kinase 1 (SRPK1) for the prevention and treatment of various diseases. SRPK1 is a critical enzyme involved in the phosphorylation of SR proteins, which are essential for pre-mRNA splicing and subsequent gene expression. Given its significant role in various cellular processes, targeting SRPK1 with selective inhibitors holds therapeutic potential in multiple pathological conditions.

The extensive research on SRPK1 has provided valuable insights into its essential roles in cellular function and its potential as a target for anti-cancer therapies. Numerous studies have focused on designing synthetic antagonists for SRPK1 (13-15), with many of these compounds targeting the ATP binding site of the protein. Since most inhibitors tend to interact with the highly conserved ATP binding pockets of kinases and other cellular enzymes, developing a therapeutic drug that selectively targets only SRPK1 poses significant challenges. To address this, we investigated potential compounds from natural and drug-like databases that could bind to alternative sites on SRPK1, effectively disrupting its interactions and inhibiting ATP hydrolysis needed for activation. A high-throughput structure-based virtual screening was performed, “Molecular docking tries to predict the structure of the intermolecular compound formed between two or more constitute molecule” using docking scores we identify promising lead compounds. We also evaluated the chemical stability of the top compounds, selecting the most stable and potent candidates for further physicochemical and pharmacokinetic analyses (16). This study identified novel, specific inhibitors of SRPK1 from a library of natural compounds and Drug like compounds, which could be valuable in developing safe and effective anti-cancer agents they may helpful to control the splicing pattern along with the alteration of SRPK1 activity. This analysis provided valuable information about the critical residues involved in the SRPK1 complex formation and utilized in designing specific and effective anticancer inhibitors of SRPK1 for cancer therapy. According to this study, these compounds could be developed as anticancer agents targeting SRPK1 as a potential candidate for further study.

2. Materials and Methods

Molecular docking is a kind of bioinformatic modelling which involves the interaction of two or more molecules to give the stable adduct. Depending upon binding properties of ligand and target, it predicts the three-dimensional structure of any complex. Docking was performed on commercial software like SwissDock and AutoDock Vina etc. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational drug design. Significance of molecular docking, docking technique was used to suggest the binding energy, free energy and stability of complexes (17). Moreover, molecular docking has major application in drug designing and discovery (18).

The accession of the target protein and ligands, along with the crystal structure coordinates of the target protein (SRPK1), protein-inhibitor complexes, and protein-substrate complexes (PDB ID: 5MY8), were retrieved from the PDB database (https: //www.rcsb. org/). Structures of small organic compounds were obtained from various databases (ZINC12, PubChem, and ChEMBL) (https: //pubchem. ncbi. nlm. nih. gov/, https: //zinc12. docking. org/). Some natural compounds and drug-like compounds were selected from the literature, and the compounds were downloaded in 2-dimensional SDF format from the databases.

2.1 Preparation of protein

The three-dimensional crystal structure of the SRPK1 complex protein was obtained from the Protein Data Bank (www.rcsb. org) with the PDB ID: 5MY8. This structure was prepared for docking studies using the graphical interface of Auto-Dock Tools. The resolution of the selected protein structure is 1.70 Å, determined through X-ray diffraction. The protein was in complex with the substrate, for docking preparation, all co-crystallized heteroatoms, including water molecules and ligands, were removed from the SRPK1 coordinates. Kollman charges, polar hydrogen atoms, and solvation parameters were then added to the protein (19). Gasteiger charges were applied exclusively to the protein, as the ligands used were non-peptides. To optimize the structure for docking, nonpolar hydrogen were merged with the protein.

2.2 Structure based Drug like compounds screening and ligand preparation

Structure-based virtual screening is a key computational technique used to evaluate a vast number of compounds from chemical libraries, aiming to identify specific and promising virtual hits. This process narrows down the compounds to a more manageable set for biological testing against the target protein, playing a crucial role in modern drug discovery. All the ligands used in this study were retrieved from the ZINC Database, a freely accessible database of commercially available compounds designed for virtual screening. It was created by the Department of Pharmaceutical Chemistry at the University of California, San Francisco (20-21). Here we have used Zinc^{12} library of drug like compounds. This study chose drug like compound library. A total of 1200 compounds were obtained in 2-dimensional Structure Data File (SDF) format and then converted to Protein Data Bank (PDB) coordinates by utilizing the Open Babel converter (22).

2.3 Control validation

The docking method's competence to identify and screen potential novel SRPK1 inhibitors from the particular compounds was evaluated by using it on SRPIN31, which were derived from the co-crystal structure of the SRPK1 complex with its known inhibitor.

2.4 Molecular docking-based virtual screening and receptor grid generation

Molecular docking is a widely used technique for predicting the optimal binding orientation and affinity of small molecules, such as drugs or substrates, to a protein receptor (23). In this study, molecular docking was employed to identify the most favorable conformational poses and binding affinities of various compounds with SRPK1. The stability of the protein-ligand complexes depended largely on polar interactions. To carry out protein-ligand docking, the automated tool Auto Dock was used to predict how small molecules interact with SRPK1 at its target binding site. The top ligand compounds were selected based on their predicted binding affinity values.

Auto Dock Vina 1.5 was used for the docking process, utilizing a blind search space defined by a grid centered at X: 64.07, Y: 58.87, and Z: 70.54. This grid was designed to cover all the heavy atoms of the protein, enabling the ligands to move freely and locate their optimal binding sites (24). A filtered library of compounds was screened through structure-based molecular docking to identify those with higher binding affinities for SRPK1. After identifying the compounds with the best docking scores, all possible docked conformers were generated and split for further analysis. These conformers were then examined using PyMOL and Discovery Studio to explore their interactions with SRPK1.

2.5 ADME prediction

In addition to the docking analysis, used to analyze the Physicochemical properties of compounds the Swiss ADME and PKCSM servers were employed to assess the compounds' physicochemical properties, pharmacokinetics, drug-likeness, and ADMET characteristics (25-26). The compounds were filtered according to Lipinski's Rule of Five to ensure they met the necessary criteria for drug-like behavior (27). Additionally, the PAINS filter was applied to eliminate compounds containing substructures that are likely to bind to multiple targets, reducing the chance of false positives. Lastly, the compounds were further filtered based on their ADMET profiles, selecting only those with favorable drug-like characteristics and a high likelihood of success in biological applications. These factors were carefully considered to ensure the chosen compounds met the required standards for further investigation.

2.6 Biological activity predictions

After filtering the compounds based on their physicochemical and ADMET properties, the selected candidates were evaluated for potential biological activity using the PASS (Prediction of Activity Spectra for Substances) online server (28). PASS predicts the biological activity of compounds based on their chemical structure and estimates the likelihood of these compounds exhibiting specific activities. The results from PASS analysis are expressed in two values: "Pa" and "Pi". A higher "Pa" value suggests a greater probability that the compound will demonstrate the predicted biological activity, while a higher "Pi" value indicates a lower probability of exhibiting that activity.

2.7 Interaction analysis

The interactions between the ligands and SRPK1 were examined by analyzing the bonds and forces involved in binding. PyMOL (29-30) and BIOVIA Discovery Studio

version 4.5 (Discovery Studio Modeling Environment, Release 4.5.2015. BIOVIA, Dassault Systèmes, San Diego) were utilized to produce the docking results for the filtered compounds, allowing for the exploration of their possible conformations. Following this analysis, we focused on compounds that demonstrated significant interactions with the key residues within the SRPK1 substrate binding pocket for further investigation.

2.8 Protein-Protein Interactions

The server requires two protein structures in PDB format and it produces a ranked list of up to 1000 docking predictions. Several FFT-based docking programs have also been made available as web servers e. g. ClusPro, GRAMM-X, ZDOCK, Clustal Omega for MSA or PyMOL/Chimera for structural superimposition. Protein docking is the task of calculating the 3D structure of a protein complex from its unbound or model-built subunits. Proteins to be docked were rigid, but they sample densely all possible rigid-body orientations in the 6D search space. This is based on a rigid-body geometric hashing algorithm, to much more computationally intensive approaches incorporating models of flexibility (31). Knowledge of one or both protein binding sites used to focus and shorten the calculation. The first 20 predictions accessed individually, and a single file of all predicted orientations downloaded as a compressed multi-model PDB file (31).

- Sequence Retrieval: Obtain the amino acid sequences of SRPK1, SRPK2, and SRPK3 from a database such as UniProt or NCBI.
- Alignment Tool: Use a tool like Clustal Omega, MUSCLE, or T-Coffee to align the sequences. These tools will align based on sequence similarity and will highlight conserved residues and functional motifs, such as the kinase domain and ATP binding sites.

2.9 Ramachandran Plot

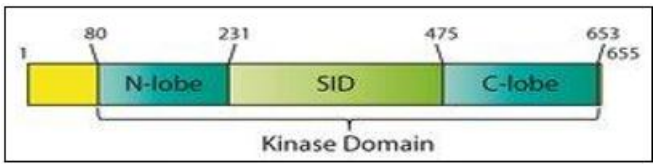
G N Ramachandran used computer models of small polypeptides to systematically vary phi and psi with the objective of finding stable conformations. A Ramachandran plot is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. A Ramachandran plot can be used in two somewhat different ways. One is to show in theory which values, or conformations, of the ψ and φ angles are possible for an amino-acid residue in a protein.

The Ramachandran plot is a two-dimensional graph of the phi (f) and psi (y) backbone angles for each amino acid residue of a protein; it is a simple method of assessing the quality of a protein structure. In a protein chain three dihedral angles are defined as φ (phi), ψ (psi) and ω (omega). The planarity of the peptide bond usually restricts ω to be 180° (the typical trans case) or 0° (the rare cis case). Ramachandran recognized that many combine ions of angles in a polypeptide chain are forbidden because of steric collisions between atoms (https: //Ramachandran-plot. html).

3. Results and Discussion

3.1 Domain Analysis

SRPK1 has two main functional domains that are common in kinases, in addition to some specialized regions unique to its function as an SR protein kinase: Catalytic Domain (Kinase Domain): Serine/Threonine Protein Kinase Domain, its function in the core domain responsible for the transfer of phosphate groups from ATP to the serine or threonine residues in target proteins (typically in SR proteins). The kinase domain consists of two lobes-N-lobe is a primarily involved in ATP binding another one is C-lobe-that is responsible for substrate recognition and phosphorylation. ATP Binding Site: It is a conserved glycine-rich loop that binds ATP and Catalytic Loop-it contains conserved motifs such as DFG (Asp-Phe-Gly) that help in catalysis by positioning ATP and the substrate apart form that some other domains are present (**Figure.1**) in the SRPK1 structure such as Spacer Domain (Linker Domain) and RS Domain Interaction Site (Target-Binding Domain).



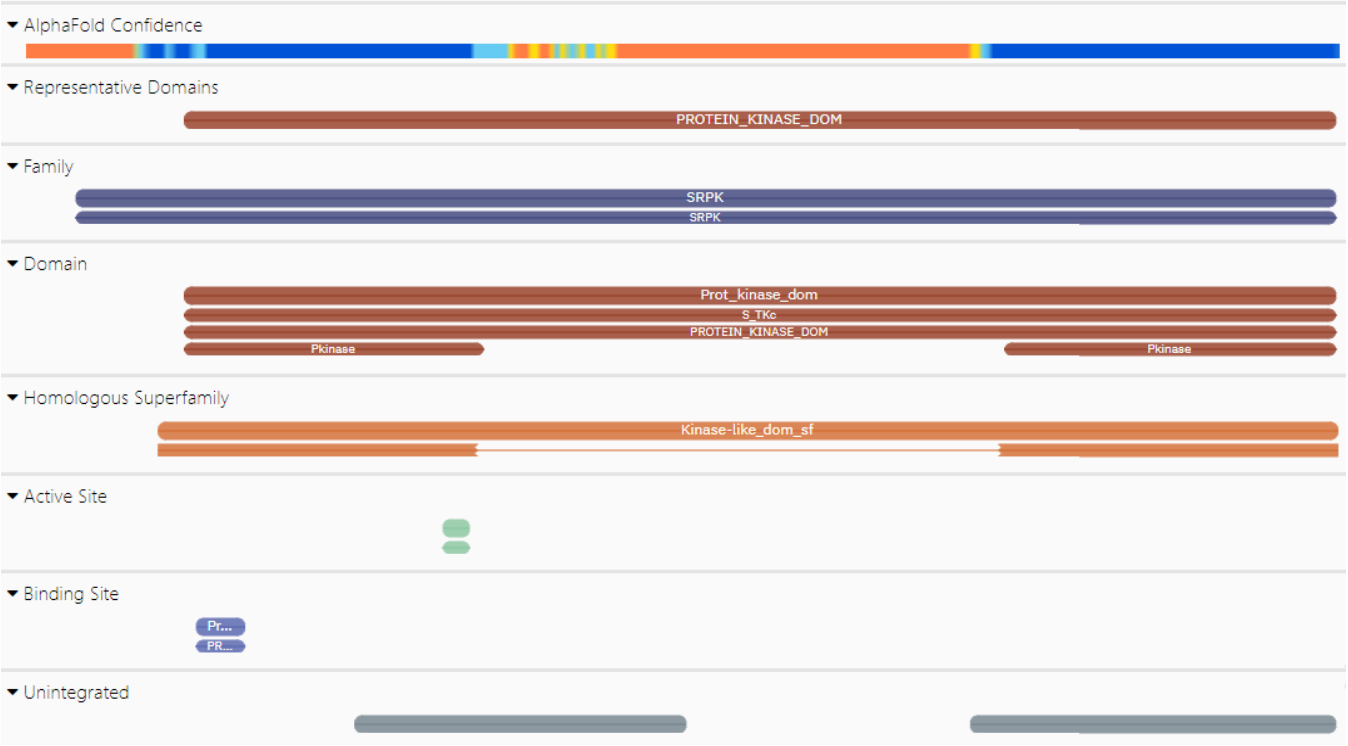


Figure 2: Structures of SRPK1, implicated in alternative mRNA processing, representation of the functional domains of the splicing factor kinases SRPK1 and CLK1

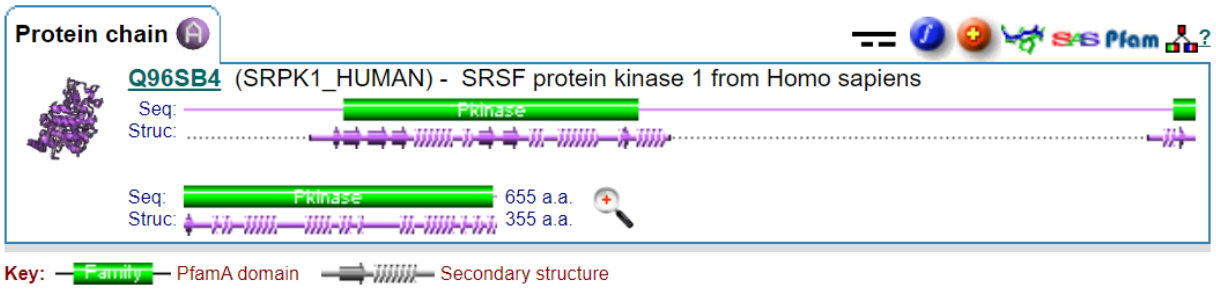


Figure 3: Protein Chain of SRPK1 (Serine/Arginine-Rich Protein-Kinase 1)

3.2 Crystal structure of SRPK1

- This compound is identified as CRYSTAL STRUCTURE OF HUMAN SRPK1 IN COMPLEX WITH SPHINX31
- This structure was solved by X-RAY DIFFRACTION.
- This structure was solved at 1.70 Å resolutions.
- 1 chain (s) is/are present [1 unique chain (s)]
- A total of 335 residues are present.
- Protein mainchain and sidechains are present.
- 16 Protein residues have alternative confirmations
- No explicit hydrogen atoms are included.
- 420 hetero group (s) is/are present.
- Refinement was carried out in PHENIX 5.8.0131.
- R = 0.164; Rfree = 0.200
- 0 PDBv2.3 atoms were found. Proceeding assuming PDBv3 formatted file.

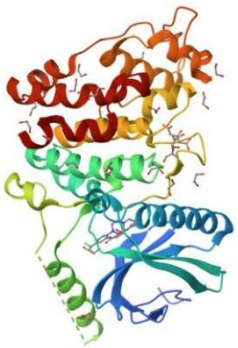


Figure 4: Crystal structure of SRPK1 PDB ID (5MY8) (https: //www.rcsb. org/structure/5MY8)

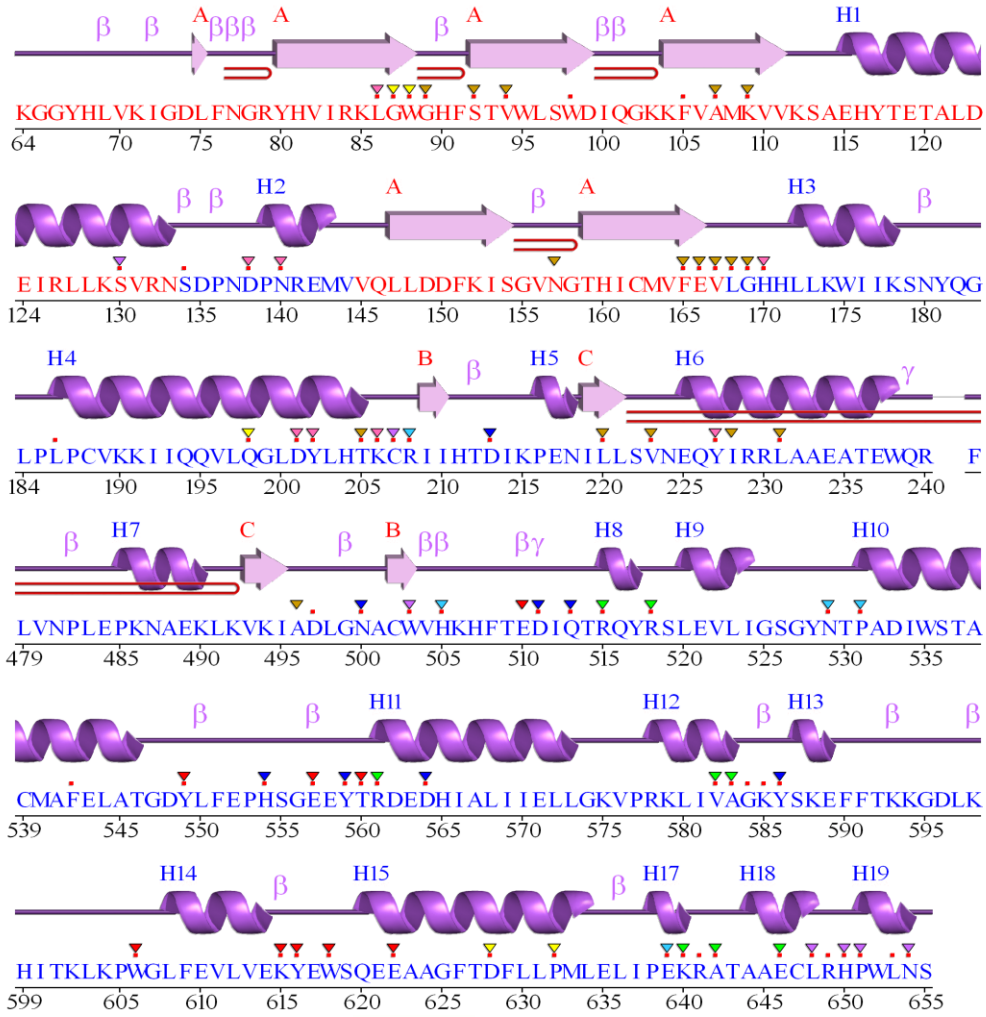


Figure 5. Secondary Structure of SRPK1 PDB Helices labelled H1, H2,. ,. . . and strands by their ID (5MY8): sheets A, B,. . .Helix Strand

Motifs

β beta turn

γ gamma turn

beta hairpin

Residue contacts

to ligand

PDB SITE records

AC1 AC2 AC3 AC4 AC5
AC6 AC7 AC8 AC9 AD1
AD2 AD3 AD4 AD5 AD6
AD7 AD8 AD9 AE1 AE2
AE3 AE4

3.3 Ramachandran Plot

The main-chain bond-lengths and bond angles are compared with the Engh & Huber (1991) ideal values derived from small-molecule data. Therefore, structures refined using different restraints may show apparently large deviations from normality.

Total number of residues 355 and Most favoured regions 93.3%

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and *R*-factor no greater than 20.0 it is a good quality model expected to have over 90% in the most favoured regions.

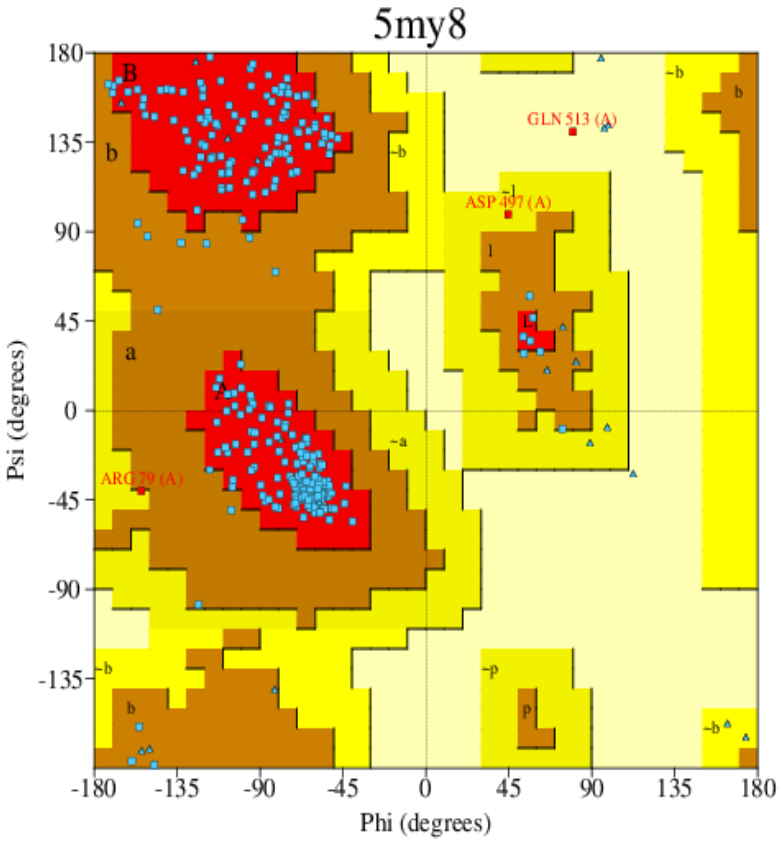


Figure 6: Ramachandran Plot of SRPK1 PDB ID 5MY8

3.3 Molecular docking-based virtual screening

To identify potential inhibitors of SRPK1with high binding affinities, we conducted a structure-based virtual screening of the filtered compounds using molecular docking (Beg et al., 2019). Our analysis revealed that several compounds had notable binding affinities to SRPK1. From the 1200 compounds, top 5 hits were selected based on their higher binding affinities to SRPK1. The selected compounds exhibit a binding affinity range of-8.5 to-11.2 kcal/mol towards SRPK1 (Table 1). The study showed that the illuminated compounds in our study show higher binding affinity than the SRPK1 reference inhibitor SPHINX31. Using PyMOL and the Discovery Studio Visualizer, we thoroughly studied these 5 hits along with its substrates of SRPK1, namely ASF/SF2 for their particular interactions with the SRPK1 binding site. Increased inhibition results from lower binding energy due to higher binding efficiency.

Table 1: Lists the various Docked compounds increased inhibition results from lower binding energy scores of the top five compounds

S. No.	Compounds	IUPAC Name	Residues involved in interactions	Binding energy
1.	SRPKIN-1	9-ethyl-8-iodo-6, 6-dimethyl-11-oxo-6, 11-dihydro-5H-benzo [b]carbazole-3-carbonitrile	PRO-515, SER-519 and SER-531	- 11.5
2.	SPHINX	5-methyl-N-[2-morpholin-4-yl-5 (trifluoromethyl) phenyl]furan-2-carboxamide	ASN-271, SER-352, GLN-408 and VAL-414	- 9.4
3.	SRPIN803	2-hydroxy-4-methy-6-(methylthio) pyrimidine	GLN-408, THR-412, SER-352 and LEU-351	- 9.1
4.	SRPIN340	N-(2-piperidin-1-yl)-5-(trifluoromethyl) phenyl) isonicotinamida	THR-412, GLN-408, ASN-415, SER-352 and LEU-351	- 8.4
5.	MSC-1186	N-[3-[[[2-(6-Chloro-7-fluoro-1H-benzimidazol-2-yl)-4-pyrimidinyl]amino]	LYS-675 and THR-673	- 7.2

A. Molecular Docking With AutoDock Vina V 1.5.6

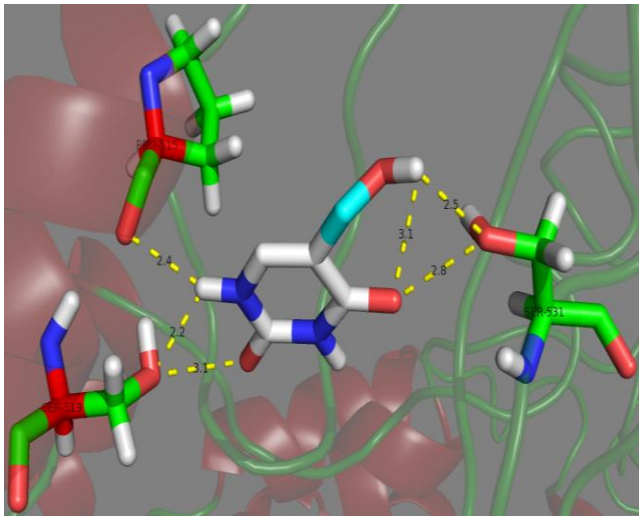


Figure 7: Interaction between SRPK1 backbone atoms active sites residues (PRO-515, SER-519 and SER-531) complex with SRPKIN-1 and binding affinity-11.5

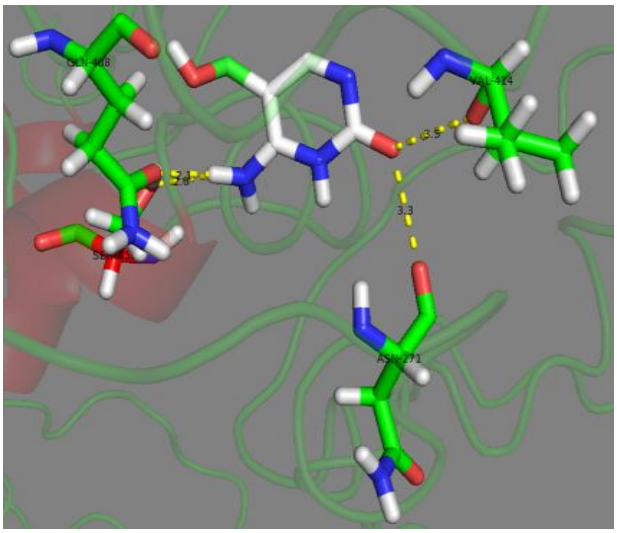


Figure 8: Interaction between SRPK1 protein backbone atoms active sites (ASN-271, SER-352, GLN-408 and VAL-414) complex with SPHINX and binding affinity-9.4

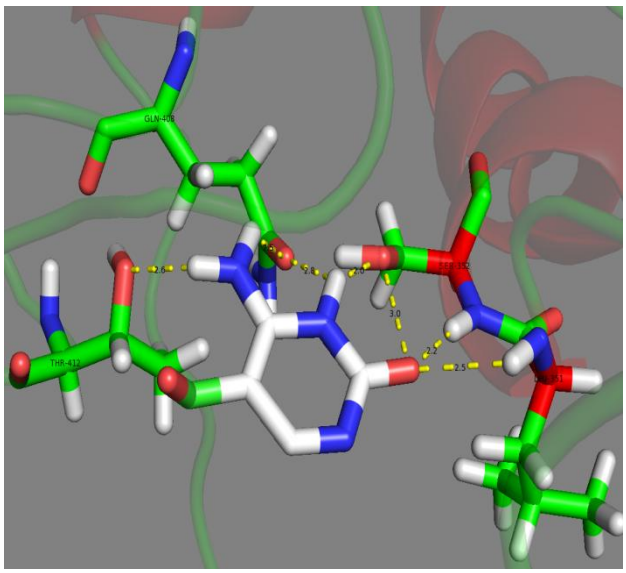


Figure 9: Interaction between SRPK1 protein backbone atoms active sites (GLN-408, THR-412, SER-352 and LEU-351) complex with SRPIN803 and binding affinity-9.1

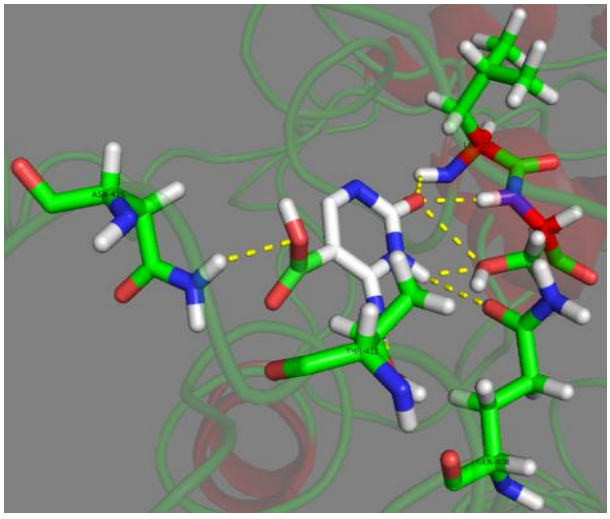


Figure 10: Interaction between SRPK1 protein backbone atoms active sites (THR-412, GLN-408, ASN-415, SER-352 and LEU-351) complex with SRPIN340 and binding affinity-8.4

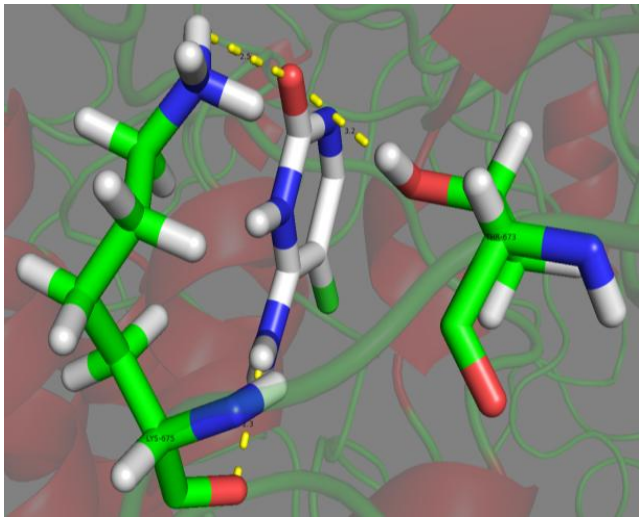


Figure 11: Interaction between SRPK1 1st conformant result of docking, Protein backbone atoms active sites (LYS-675 and THR-673) complex with MSC-1186 binding affinity-7.2

3.4 Interaction analysis

To analyze the detailed interaction of the five specific compounds with the substrate-binding site of SRPK1, we extracted all possible docking conformations from their output files. Using PyMOL and Discovery Studio Visualizer, we evaluated the interaction patterns of the top five compounds: SRPKIN-1, SPHINX, SRPIN803, SRPIN340, and MSC-1186, focusing on their binding to crucial residues within the SRPK1 binding pocket (Figures 7-11). Figure 7 provides a magnified view of the protein-ligand interactions, showing that all five compounds are deeply bound within the SRPK1 binding pocket cavity. Based on this analysis, the compounds interacted with key residues such as PRO-515 and SER-519 (ATP-binding site) and SER-531 (active site), which are critical for SRPK1's catalytic function. These interactions highlight the compounds' potential as inhibitors, primarily targeting the ATP-binding pocket and obstructing SRPK1's enzymatic activity, thereby impeding substrate phosphorylation. A thorough assessment of each compound's

binding to SRPK1 ensured interactions with critical residues essential for the enzyme's functionality.

3.5 Protein –Protein Interaction

To perform a protein superimposition and a multiple sequence alignment (MSA) of SRPK1, SRPK2, and SRPK3, we would need to follow several steps to analyze their structural and sequence similarities. Here's an outline of how this is typically done, along with a conceptual overview of the results. MSA helps in comparing the sequences of SRPK1, SRPK2, and SRPK3 to identify conserved regions, insertions, deletions, and sequence divergence. This provides insight into how functionally important residues are conserved across these kinases.

Protein superimposition involves overlaying the 3D structures of SRPK1, SRPK2, and SRPK3 to visualize how similar their overall folds and active site geometries are. This analysis is key in understanding structural conservation and differences.

Protein superimposition result shows that the Kinase Domain Conservation, Differences in Spacer Domains, Similarity in Core Domains and Differences in Substrate Specificity.

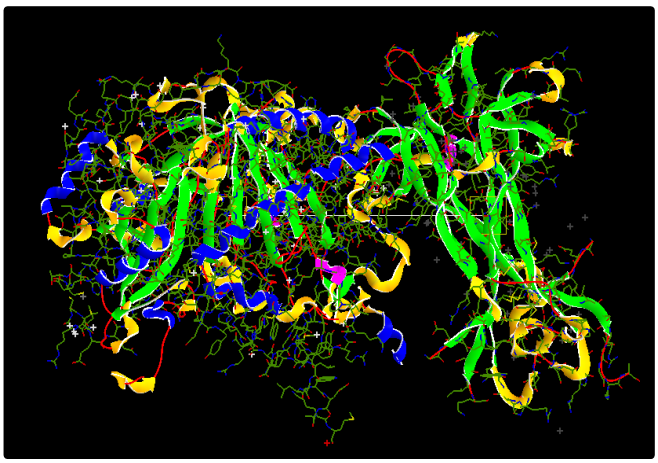


Figure 12: Protein superimposition involves overlaying the 3D structures of SRPK1, SRPK2, and SRPK3

Comparison of binding with other SRPK1 inhibitors

The reported synthetic inhibitors SRPIN31, Compound I, Sphinx340, and Alectinib occupy the same space within the ATP-binding site of SRPK1. Similarly, the five compounds identified in the present study also target this site, although with variable positioning within SRPK1. These compounds were selected based on their docking scores, which were higher than that of SRPIN31, used as the reference compound during the *in-silico* analysis. Structural analysis of the SRPK1 complexes reveals that all five compounds form a greater number of attractive interactions compared to SRPIN31. Notably, SRPIN31 and Compound 1 each form only one hydrogen bond with the main chain NH atoms, which is a common feature among other ligands. In contrast, the compounds identified in this study establish significantly more polar interactions in the form of hydrogen bonds and salt bridges than all other existing SRPK1 inhibitors (**Table 1**).

4. Discussion

SRPK1 is involved in tumor growth in case of several cancers and considered to be among attractive targets for the development of anti-cancer therapeutic compounds. The available designed inhibitors of SRPK1 recognize and fit into the ATP binding site. As ATP binding site is common in all kinases and other enzymes, the approach of targeting ATP binding site alone may not yield specific inhibitors of SRPK1, which may lead to binding to several other physiological targets resulting in non-desirous adverse physiological effects. Therefore, it is of great importance to find more selective inhibitors of SRPK1. Reported *In-silico* studies have inferred that that all the selected five compounds under study have good affinity for SRPK1. The SRPKIN-1 compound, in complex with SRPK1 (PDB ID: 5MY8), exhibits the highest binding affinity, with a score exceeding 11, surpassing the other four tested compounds as shown in Table 1. The table presents the docking scores and hydrogen bonding interactions with the SRPK1 protein (5MY8). Based on these docking scores, SRPKIN-1 was identified as the best candidate. It is likely that SRPKIN-1 can effectively inhibit SRPK1 activity, potentially normalizing the splicing patterns in cancerous cells. These results suggest that SRPKIN-1 could be a promising therapeutic agent for targeting SRPK1 in cancer treatment.

5. Future Prospects

The SRPKIN-1 and SRPK1 complex demonstrates a strong binding affinity, suggesting SRPKIN-1 potential as a therapeutic candidate for cancer and other disease models. *In-vitro* and *in-vivo* studies are warranted to validate its efficacy. Furthermore, SRPKIN-1 specificity could play a crucial role in the development of personalized medicine, tailored to individual patient profiles based on SRPK1 activity.

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