Structural Insights and Inhibitor Identification Against Mitogen-Activated Protein Kinase Kinase 7 (MAP2K7) Obtained in Microgravity Environment

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Abstract: Mitogen-activated protein kinase kinase 7 (MAP2K7) plays a central role in the c-Jun N-terminal kinase (JNK) signaling pathway, governing stress and inflammatory responses. It is a promising target for drug discovery in severe conditions such as arthritis, hepatoma, and cardiac hypertrophy. However, due to the poor structural resolution, the challenge of developing MAP2K7 inhibitors with specificity against other kinases remains significant. Proteins crystallize under microgravity in a space environment yield a high-resolution structure and help in the drug discovery process. In the present study, we have developed an automated approach to expedite the screening of chemical compounds utilizing the AutoDock Vina software. This approach was further used to identify potential inhibitors against the MAP2K7 protein crystallized in a microgravity environment. More than 5200 compounds were obtained from the Zinc20 database for virtual screening against this protein, out of which 5 candidates were selected for further screening. Furthermore, molecular dynamics simulations highlighted the protein's flexibility, with significant fluctuations mainly observed in the Gly-rich loop region. These findings provide valuable insights into the structural attributes of MAP2K7 and lay the groundwork for the development of specific inhibitors against this protein. Crystals yielding superior reflections, unattainable on Earth, can be acquired in space, thereby enhancing their utility in structure-based drug design.

1. INTRODUCTION:

MAP2K7, also known as mitogen-activated protein kinase kinase 7, is a protein that controls cell functioning like cell growth, cell division, and cell death. [1] This protein functions as a middleman that transports outside signals/messages to inside the cell. These messages can be when the cell needs to grow, divide, or die which depends on the outside environment. In simple language, these proteins function as a traffic signal controller in cells. First, it receives signals from outside the cell, and based on the signal, it decides the cell should grow, divide, or die. Chemical compounds that can inhibit the activity of MAP2K7 are called MAP2K7 inhibitors. [1] This will inhibit the signaling pathway of the protein, which can be used to control the cell growth in cancers and inflammatory diseases. The 3D structure of the protein is shown in Figure 1 and 2. Based on this structure, the protein contains two parts: N-lobe and C-lobe. The two lobes are connected to a flexible hinge region, which controls the flexibility of the protein and is related to the ligand binding activity of the protein. The active site of this protein is located close to the hinge region. In the current work, we have targeted this binding site and have predicted inhibitors that competitively bind to this site. [1]

Computational chemistry/biology is a field in which computers simulate biomolecules. Molecular docking is a popular technique for finding receptor (proteins) and ligand interactions. In this technique, the 3D structure of the protein is taken, and a ligand (chemical compound) is docked on the protein. [2] The software predicts the best binding site based on the ligand's size, shape, and interaction with the protein. Molecular docking is a popular technique in drug discovery and is used at the early stage of computer-aided drug design (CADD). Various software is available for docking, and in this current work, we have used AutoDock software to perform docking. [3]

Although there are many treatment strategies available that are effective, they have shown drug resistance, serious adverse effects, and are expensive. Therefore, there is a need to novel effective therapy that can make the current therapeutic strategy more effective. In the current work, molecular docking simulations have been performed to find chemical compounds that can

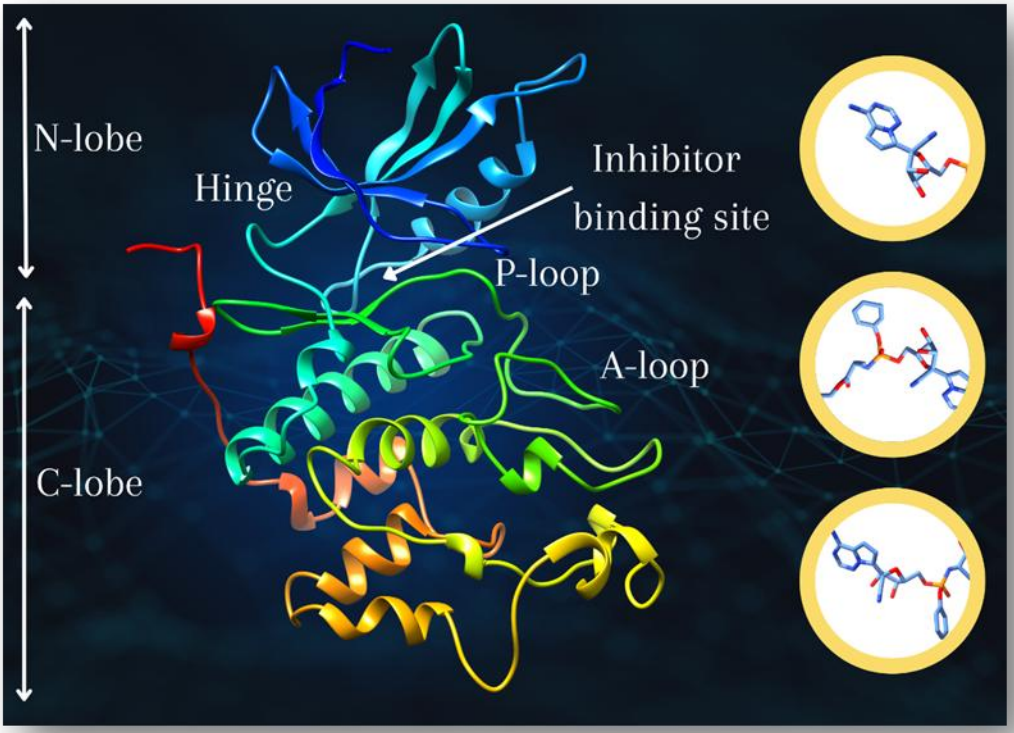

Fig. 1. Ribbon-shaped image of MAP2K7 protein. The inhibitor binding site is shown in the center of the protein, with three inhibitors displayed on the right-hand side of the image.

bind and inhibit the protein. Our research will help design novel therapies against the disease and will help address the global burden of the disease.

2. METHODS AND MATERIALS

The three-dimensional structure of the MAP2K7 protein was obtained from the Protein Data Bank. [4] Ligands were down- loaded from the Zinc20 Database, and approximately 5000 com- pounds were selected for virtual screening. The following are the Zinc Database Tranches settings that were used to download the ligands files: (1) only 3D models were selected; (2) in the reactivity section, "Standard" and "Exclusive" were chosen; (3) in purchasability, "In-Stock" and "Exclusive" options were se- lected; (4) Reference (R) pH was chosen; (5) Charge: "0"; and (6) "Lead-Like" compounds were selected. In the next step, ligands were selected based on LogP values. Since, chemical compounds with LogP=2 have good oral and intestinal absorption only these compounds were selected for virtual screening. Finally, the com- pounds were downloaded in pdbqt file format. The docking was performed using AutoDock Vina 1.5.6 software. [3]

Data Analysis: Autodock scoring method was used to select the top five candidates that bind strongly to the protein. The scoring criteria is that the ligand should fit in the binding site and form strong interactions with the protein. Protein Ligand Interaction Profiler (PLIP) web server was used to find the protein-ligand interactions. [5] The ChimeraX and PyMol software were utilized to visualize and analyze the protein-ligand complexes. [5, 6] The pharmacological and carcinogenic properties of the compounds were assessed with the aid of ADMET AI. [7]

3. RESULTS AND DISCUSSION

The binding site on the protein surface has a higher tendency to bind with other molecules. The tool ChimeraX was used to visualize and obtain the electrostatic surface potential (ESP) The electrostatic surface potential of the protein dictates which substances can bind in specific areas based on the charge of (it has the opposite charge compared to the protein).

In the current work we have performed molecular docking simulations to predict inhibitors against the MAP2K7 protein. Based on our simulation we have proposed five ligands (Ligand I, II, III, IV, and V) that bind strongly to the MAP2K7 protein. The AutoDock software was used for molecular docking simulations. With these simulations, it is possible to observe which ligand binds the strongest with the MAP2K7 protein.

In addition, we have also developed an automated molecular docking process that will help make the docking process automated and faster. In addition, it will also make the process error-free. The protocol of the current process is shown in Figure 2. All the programming is done in Python. We have named this software FastDock. The software is user-friendly and requires basic knowledge of python. It involves input files (protein.pdbqt, ligand.pdbqt, conf.txt). In addition, prerequisite docking files are required to be built into Autodock vina software (vina.ese, vina_split.ese, vina_license.rtf). The Python program scripts are as follows (1.pl, 2.pl, 3.pl, analysis_1.pl, analysis_2.pl, vina_windows.pl). The output files are *MODEL_log.log and

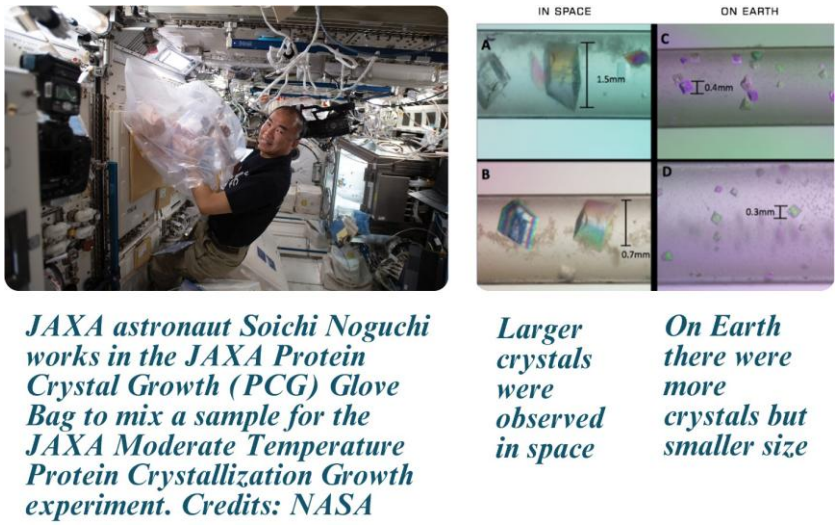

Fig. 2. JAXA astronaut Soichi Noguchi conducts the Moderate Temperature Protein Crystallization Growth experiment aboard the ISS, mixing samples in microgravity to enhance crystal quality. Comparative microscopy shows that protein crystals grown in space (left) were significantly larger (up to 1.5 mm) than those grown on Earth (right), which were more numerous but smaller in size (~0.3–0.4 mm). This demonstrates the advantage of microgravity in producing high-quality protein crystals, as used in the MAP2K7 structural study for inhibitor identification.

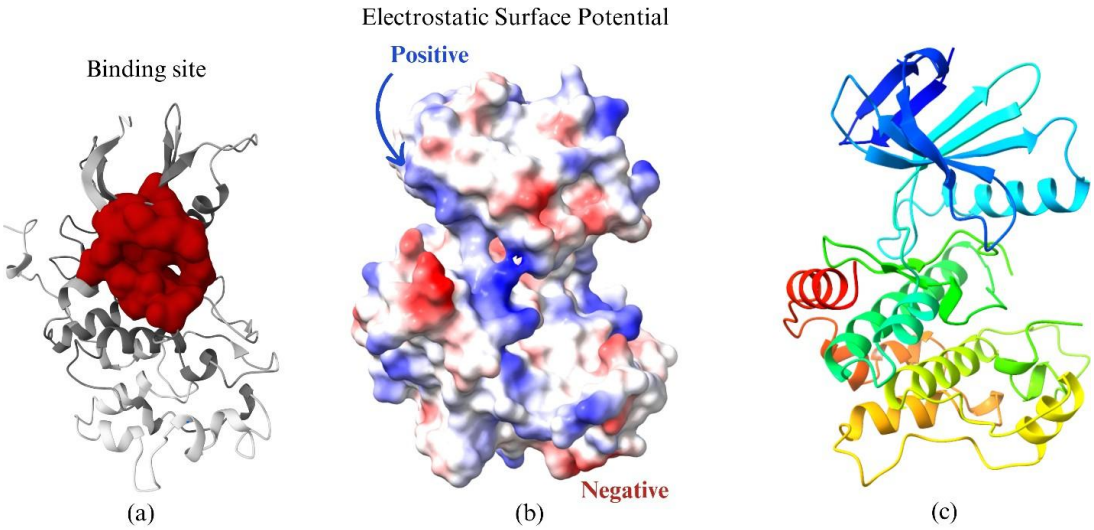

Fig. 3. (a) Binding site of the protein obtained from ScanNet Web Server (Deep Learning page method used to obtain the binding site of the protein); (b) Electrostatic surface potential of the protein. The blue represents a positive charge while the red represents a negative charge; (c) Reference Structure of Protein (MAP2K7)

*MODEL_out.pdbqt file. The data can be analyzed using PYmol and ChimeraX software.

1: Comparative analysis of molecular interactions across five protein-ligand complexes (IDs: 25, 401, 2423, 2663, 4301) reveals consistent hydrophobic interactions at Leu266, with additional key residues such as Thr146, Val150, and Ser218 frequently involved. Notably, hydrogen bonding with Ser218 and salt bridge formation with Lys221 and Lys165 are prominent in higher-affinity complexes, suggesting their potential role in ligand stabilization.

Conclusion: In the current molecular docking simulation, we have predicted inhibitors that bind to the Mitogen-activated protein kinase kinase 7 (MAP2K7). Inhibiting these proteins will help control stress and the inflammatory response. Based on our docking simulations, we have predicted ligands showing strong interactions with the protein.

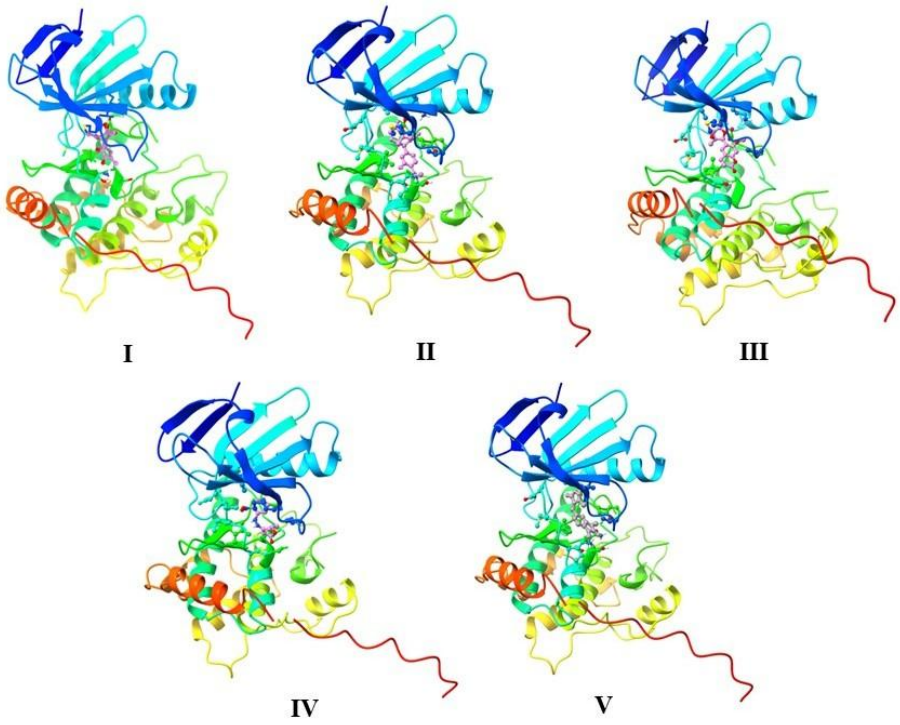

Fig. 4. 3D structural representations of protein-ligand docking complexes for five ligands (I–V) within the active site of the target protein. The protein backbone is shown in rainbow ribbon format (N-terminus in blue, C-terminus in red), while the docked ligands are displayed in stick representation (magenta). All ligands are positioned in the same binding pocket, allowing visual comparison of binding orientations and conformational adaptations across complexes. Ligand III demonstrates deeper pocket embedding, correlating with its higher number of stabilizing interactions observed in prior analysis.

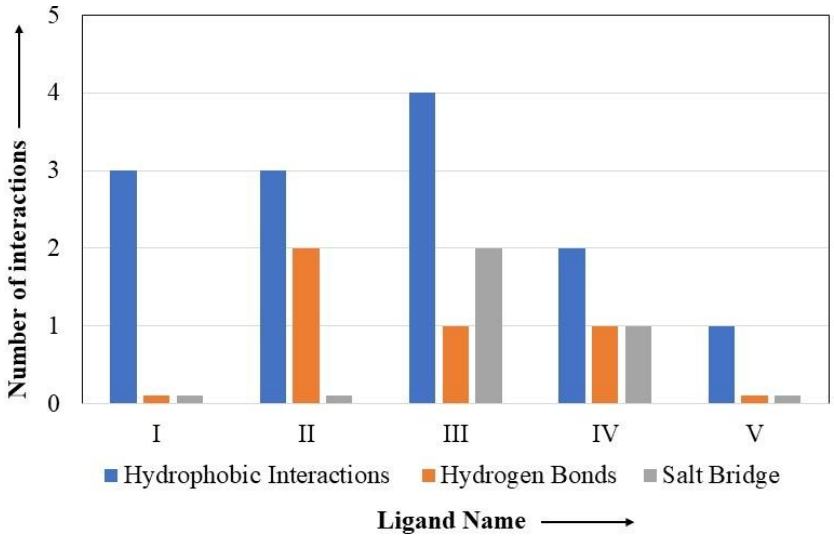

Fig. 5. Bar graph depicting the number of hydrophobic interactions, hydrogen bonds, and salt bridges formed by five different ligands (I–V) with the target protein. Ligand III exhibits the highest total number of interactions, including the greatest number of hydrophobic contacts (4) and the presence of both hydrogen bonds and salt bridges. Ligands I and II show comparable hydrophobic interactions, but Ligand II forms more hydrogen bonds. Ligands IV and V show fewer overall interactions, with Ligand V forming the least. These variations suggest differential binding affinities and stabilization patterns among the ligands.

Table 1. Protein-ligand interactions.

25		
	Residue	**Distance (Å)**
Hydrophobic Interactions	Thr146	3.74
	Val150	3.34
	Leu266	3.17, 3.75
401		
	Residue	**Distance (Å)**
Hydrophobic Interactions	Thr146	3.55
	Val150	3.71
	Ala163	3.87
Hydrogen Bonds	Lys165	2.84
	Ser218	2.31, 2.33, 2.91
2423		
	Residue	**Distance (Å)**
Hydrophobic Interactions	Thr146	3.58
	Val196	3.80
	Met215	3.85
	Leu266	3.06, 3.67
Hydrogen Bonds	Ser218	2.36
Salt Bridges	Lys165	4.73
	Lys221	5.16
2663		
	Residue	**Distance (Å)**
Hydrophobic Interactions	Leu266	3.73
	Phe278	3.75
Hydrogen Bonds	Ser218	2.45
Salt Bridges	Lys221	4.92
4301		
Hydrophobic Interactions	**Residue**	**Distance (Å)**
	Leu266	3.56

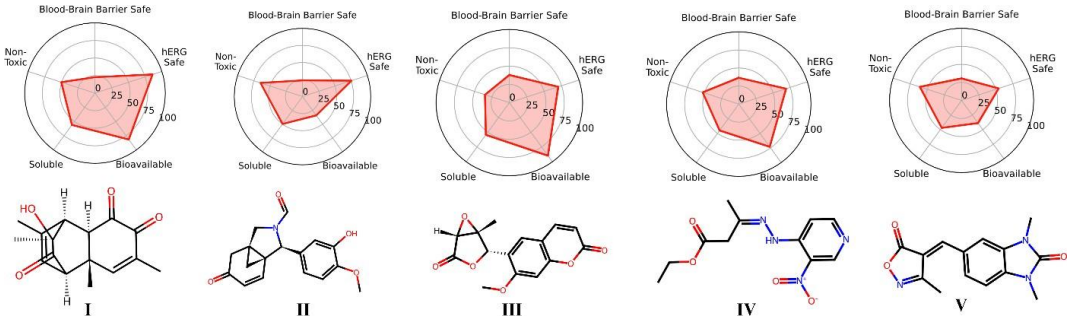

Fig. 6. Radar plot displaying pharmacokinetic and safety profiles of a compound. The axes represent key drug-likeness parameters: Blood-Brain Barrier safety, hERG safety, Bioavailability, Solubility, and Non-Toxicity. The filled red region indicates the compound's relative score (out of 100) in each category, with strong performance in solubility and bioavailability, moderate blood-brain barrier safety, and lower scores in hERG safety and non-toxicity.

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