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Discovery of Novel Selective Wee1 Kinase Inhibitors as Anti-Cancer Agents by Using Computational Methods

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ABSTRACT

This study outlines a comprehensive in silico approach to identify potential Wee1 kinase inhibitors for cancer therapy. A Targeted Oncology library of 6000 ASINEX compounds was screened, narrowing down to 81 based on drug-likeness and physicochemical properties. Molecular docking via AutoDock Vina identified the top 10 compounds, validated using eight clinical trial drugs that confirmed key binding residues (Asn376, Glu377, Cys379, Phe433). ADME and toxicity analyses highlighted compounds Y040-7562 and Y040-7522 for favourable pharmacokinetics, while compound 2248-0969 showed the best docking score and low toxicity. Molecular dynamics simulations confirmed the stability of the 2248-0969-Wee1 complex. Overall, the study presents a robust virtual pipeline and identifies 2248-0969 as a promising candidate for further development as a Wee1 kinase inhibitor.

INTRODUCTION

Cancer is a group of diseases marked by abnormal cell growth [1,6]. These cells can spread from their origin to other parts of the body—a process called metastasis, which is the most dangerous feature of cancer. A tumour is an abnormal tissue swelling, and the term comes from Latin. Tumours are classified as **benign** or **malignant**. **Benign tumours** are usually enclosed in a membrane, limiting their spread. Though mostly harmless and slow growing, they can grow large and cause serious issues if they compress vital tissues or nerves. Sometimes, they require surgical removal. If they occur in endocrine tissues, they may cause abnormal hormone levels. Chemotherapy is often used as a treatment, though it has side effects. **Malignant tumours** are cancerous and invade surrounding tissues. They grow uncontrollably and can spread (metastasize) via blood or lymph. These cells look abnormal and can be hard to distinguish from healthy ones. They often return after treatment. Types include carcinoma, sarcoma, leukaemia, lymphoma, and glioma. Diagnosis involves biopsy, CT, MRI, PET scans, and blood tests. There are many types of cancer—carcinoma, sarcoma, leukaemia, lymphoma, multiple myeloma, and melanoma [2]. Tumours can also be brain and spinal cord tumours, germ cell tumours, neuroendocrine tumours, and carcinoid tumours [2]. **Cancer cells** grow uncontrollably, resist death, invade tissues, and spread [1]. Their traits include:

1. Reduced dependence on external growth signals.
2. Resistance to anti-growth signals.

Normal cells have a limit to how many times they divide (Hayflick limit). Cancer cells bypass this and show **angiogenesis**—the formation of new blood vessels to support their growth. They also evade **apoptosis**, allowing damaged cells to multiply [1].

Wee1 kinase is a serine/threonine protein kinase involved in regulating the G2/M phase checkpoint[7] by phosphorylating CDK1 at Tyr15, thereby inhibiting its activity [3,15]. Located in the nucleus, Wee1 halts the cell cycle to allow DNA repair. When DNA damage is detected, Wee1 phosphorylates CDK, preventing premature mitosis. In cancer cells, especially those with p53 mutations, the G1/S checkpoint is dysfunctional, making the G2/M checkpoint crucial [8]. However, in the absence of Wee1 activation, CDK1/CDK2 remain unphosphorylated, allowing damaged cells to enter mitosis, leading to genomic instability and apoptosis. Wee1 is thus a promising target in cancer therapy. Inhibitors of Wee1, which abrogate the G2 checkpoint, can be used alone or with DNA-damaging agents to enhance cancer cell death [4,9,10]. Studies in fission yeast support its role in maintaining CDK1 phosphorylation until DNA repair is complete.

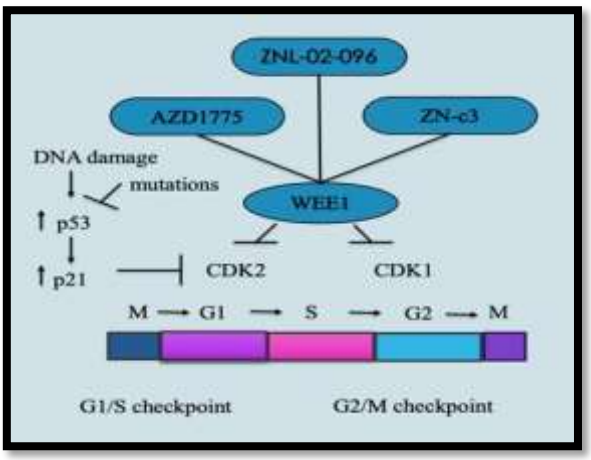


Figure 1. Wee1 pathway [1]

MATERIALS & METHODS

MATERIALS

UCSF CHIMERA for molecular docking, SwissADME for ADME studies[13], PROTOX-3.0 for toxicity studies, MOLVIEW to draw 3D structures, GROMACS for MD simulation[15], DATA WARRIOR for screening, DISCOVERY STUDIO for 2D interactions.

METHODS

Screening: A Targeted Oncology Library 6000 compounds were taken from ASINEX. These compounds were screened in DATAWARRIOR by applying the desired parameters. Compounds obtained from screening were used further for docking.

Docking:

- **Protein Preparation:** 3D structure of protein PDB: 2IN6 was downloaded from the RCSB protein data bank[11]. The protein structure is opened in UCSF CHIMERA. Unwanted water molecules and residues are removed from the protein. Then, hydrogen atoms and Gasteiger charges are added to the protein structure.
- **Ligand Preparation*:*** 3D structure of the ligand is drawn in MARVIN SKETCH. Ligand structure is opened in UCSF CHIMERA. Unwanted water molecules and residues are removed from the ligand. Then, hydrogen atoms and Gasteiger charges are added to the ligand structure.
- **Grid Box Generation:** Coordinates for grid box generation are synthesized from referenced homology modelling study. A grid box is generated around the active protein binding site.

- **Docking Process:** Docking is done via Autodock Vina and the dock score is obtained[12]. The obtained dock score is analysed and documented.
- **Clashes and contacts:** The docked complex is then subjected to contact formation. The ligand is assigned as the first set, and the protein is assigned as the second set. The contacts are developed, and the amino acid interactions can be seen in overlaps file. Further enhancement in the complex can be done for publication purpose.

ADMET Studies

- SwissADME steps- Top 10 docked compounds were shortlisted based on dock score. These 10 compounds were uploaded on SwissADME software and desired parameters were chosen. The produced Excel sheet of 50 compounds with ADME properties is saved.
- Toxicity Steps- Top 10 docked compounds were shortlisted based on dock score. These compounds were uploaded to PROTOX 3.0 software. The toxicity prediction studies start by choosing desired parameters. The obtained results of toxicity prediction are documented.

Molecular Dynamic Studies

System Preparation- The protein-ligand complex structure (e.g. complex.pdb) was obtained from docking studies. Hydrogen atoms were added, and missing residues or atoms were corrected if required. The appropriate protonation states were assigned at physiological pH (~7.4).

Force Field Selection- A suitable force field (e.g. AMBER ff14SB, CHARMM36, GROMOS96) was selected for the protein. Ligand parameters were generated using tools like ACPYPE (for GROMACS), Antechamber (for AMBER), or CGenFF (for CHARMM), with charges computed using RESP or AM1-BCC methods. The complex was placed in a periodic box of water molecules (e.g. TIP3P or SPC water model). A buffer distance (e.g., 10 Å) was maintained between the complex and box edge. Counterions (Na^+, Cl^-) were added to neutralize the system. Additional ions were added to mimic physiological salt concentration (e.g. 0.15 M NaCl). The system was minimized to remove bad contacts or steric clashes using a steepest descent or conjugate gradient algorithm. NVT Equilibration (Constant Number, Volume, Temperature): The system was heated gradually (e.g. from 0 K to 300 K) while restraining the heavy atoms. Pressure equilibration was performed to stabilize density and box volume. A production run was carried out for 200 PS (Picoseconds) under constant temperature and pressure. Constraints (e.g. LINCS algorithm) were applied to bond lengths involving hydrogen atoms. RMSD, RMSF, Radius of Gyration (Rg), Hydrogen Bonds. The binding stability and conformational changes were evaluated over the simulation period.

OBSERVATION

Screening - Targeted Oncology Library of 6000 compounds was downloaded from ASINEX online library. Desired parameters were applied in DataWarrior. These 6000 compounds were screened in DataWarrior. From these 6000 compounds, 81 compounds were obtained. Filters used for screening are Target Specific Criteria, Physiochemical Filters, ADMET Filters, and Structural Filters.

Docking studies- 81 compounds were docked, from which 10 compounds were shortlisted based on docking score. Firstly, the protein and ligands were prepared by adding Gasteiger charges and hydrogen atoms. Now, a grid box was generated around the binding site of the protein. AutodockVina was run to start the dock, and the dock score was obtained. Important interacting amino acids crucial for docking are followings

(ASN376, GLU377, CYS379, and PHE433)[5,10].

Comparison of Dock Scores with Clinical Trial Drugs

Compounds which are under clinical trial as wee 1 inhibitor were docked against protein PDB: 2IN6. Dock score of compounds (AZD1775, ZN-C3, CJM-061, DEBIO-0123, PD0407824, PYRIDOPYRIMIDINE, SC0191) is listed below. This study is performed to identify the binding site and better understanding of the known crucial interactions of binding.

SR.NO	NAME OF COMPOUNDS	DOCK SCORE	INTERACTING AMINO ACID WITH BOND LENGTH
1	AZD1775	-6.8	ASN376-H21 2.519 ASN376-C24 2.514 ASN376-C24 3.165
2	ZN-C3	-10.4	ASN376-O2 2.322 GLU377-H28 1.983 GLU377-C23 2.973 CYS379-N8 2.220 PHE433-N2 3.168
3	CJM-061	-7.9	ASN376-C24 2.543 ASN376-C24 3.185 PHE433-H26 2.582
4	DEBIO-0123	-9.1	PHE433-O1 2.353
5	PD0407824	-12.5	CYS379-O1 1.894 CYS379-O3 2.703 CYS379-O1 2.834 GLU377-N2 2.974 GLU377-H4 2.239 ASN376-O2 2.326
6	PYRIDOPYRIMIDINE	-9.5	PHE433-H22 1.720
7	SC0191	-12.4	CYS379-N8 2.027 CYS379-H32 1.917 CYS379-N8 2.977 CYS379-N7 2.924
8	WEE1-IN-3	-10.5	ASN376-H9 1.101 ASN376-C8 1.988 ASN376-H9 1.984 ASN376-C8 2.973 CYS379-N4 3.148 CYS379-C16 2.795 CYS379-N4 2.166 CYS379-H16 1.751

Table No.1. Clinical Trial Drug

Top 10 Docked Compounds with their Key Binding Interaction

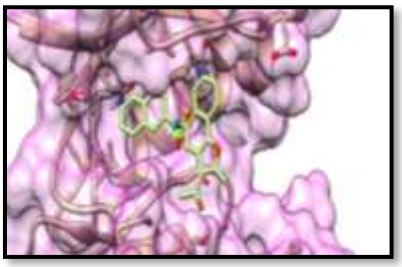
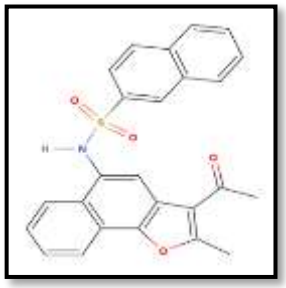
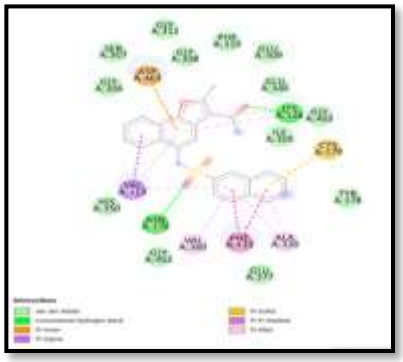
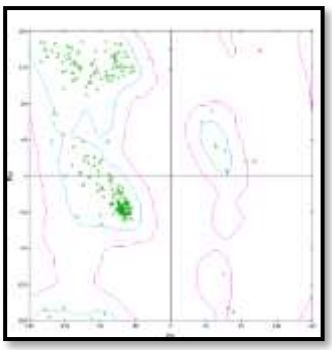
Here are the top 10 compound as per dock score. As per previous studies crucial amino acids of Wee 1 binding site are (**ASN376, GLU377, CYS379, and PHE433)**[5].

SR.NO.	COMPOUNDS ID	DOCK SCORE	INTERACTING AMINO ACID WITH BOND LENGTH
1	2248-0969	-12.0	CYS379-C25 2.459 CYS379-H19 1.811
2	2081-1164	-11.4	CYS379-H12 1.990 CYS379-C18 2.955
3	2154-0349	-11.4	ASN376-H6 1.833 CYS379-C18 2.965 CYS379-H12 1.994
4	2072-0767	-11.2	CYS379-H12 2.015 CYS379-C18 2.981
5	2153-0115	-11.2	ASN376-H5 1.810 CYS379-C23 2.946 CYS379-H15 1.968
6	Y040-7562	-11.1	ASN376-O3 2.323 PHE433-H1 2.388
7	2081-0527	-11.1	CYS379-H12 1.963 CYS379-C18 2.914
8	2081-0529	-10.9	CYS379-H15 2.009 CYS379-C23 2.973
9	K788-6913	-10.9	CYS379-H15 2.010 CYS379-C23 2.965 ASN376-H7 1.830
10	Y040-7522	-10.8	CYS379-H16 1.530 CYS379-C17 2.430 ASN376-O3 2.863 ASN376-O3 1.862

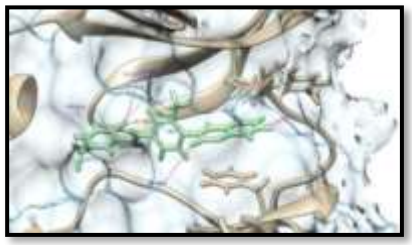
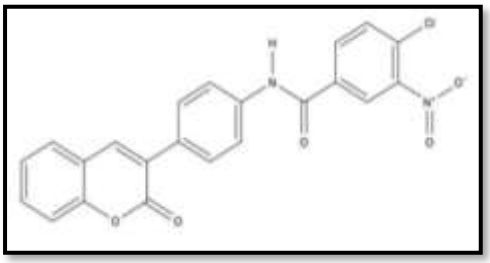
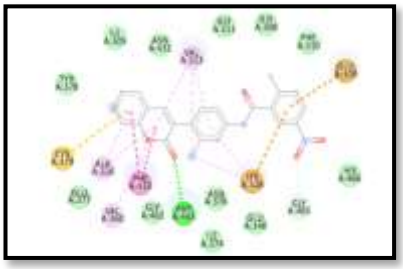
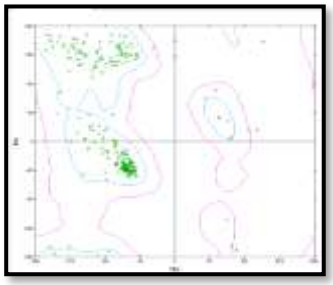
Table No.2. Top 10 Docked Compounds with Their Key Binding Interaction

We used drugs that are currently in clinical phase trials to verify & validate the process done for docking, the interacting amino acids in the drugs obtained through screening. Refer to Table no.1 & Table no.2.

1}2248-0969

 **Docked molecule**	 **3d structure**
 **3d interaction**	 **Ramchandran plot**

2}2081-1164

 **Docked molecule**	 **3d structure**
 **3d interaction**	 **Ramchandran plot**

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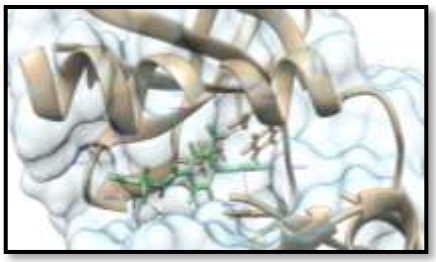
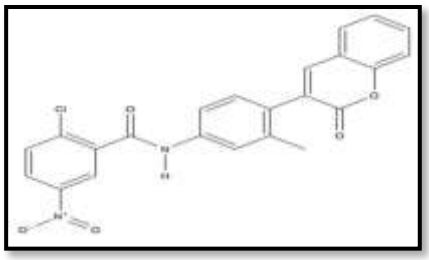
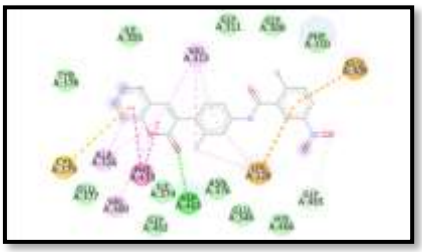
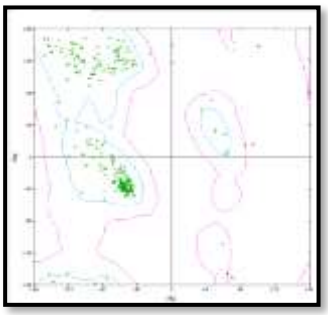
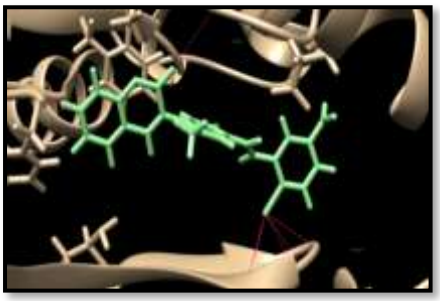
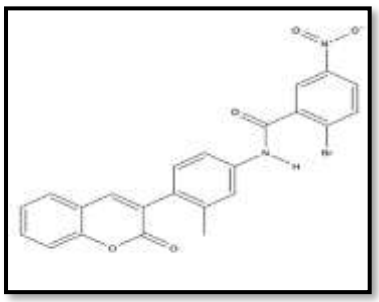
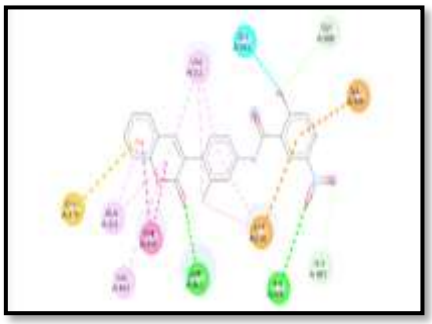
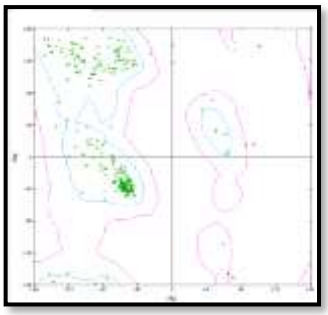
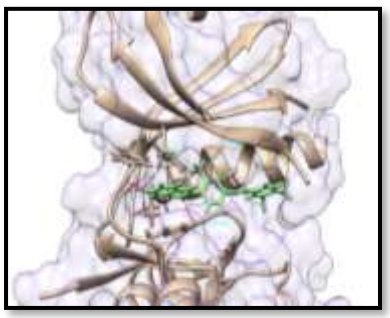
 **Docked molecule**	 **3d structure**
 **3d interaction**	 **Ramchandran plot**

4}2072-0767

 **Docked molecule**	 **3d structure**
 **3d interaction**	 **Ramchandran plot**

5}2153-0115



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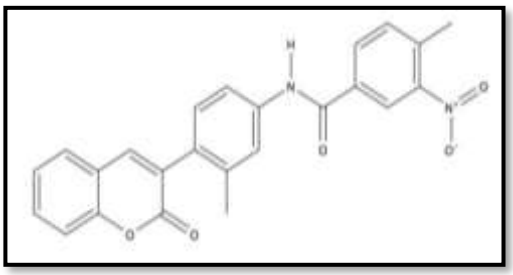

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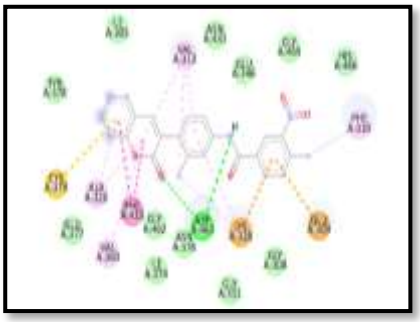

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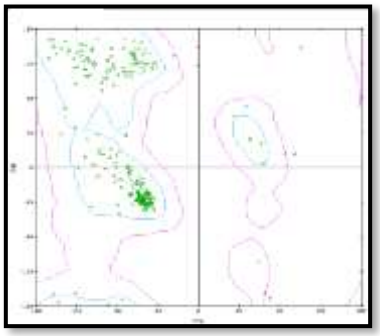
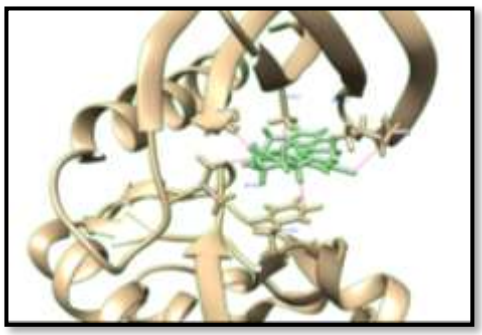


Ramchandran plot

6}Y040-7562



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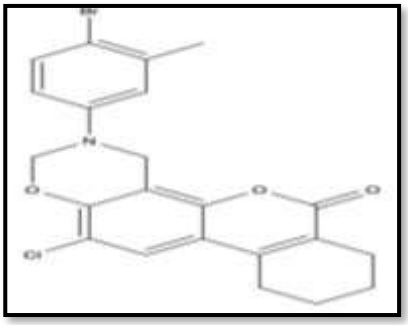

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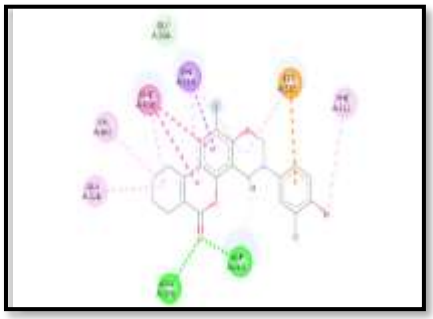

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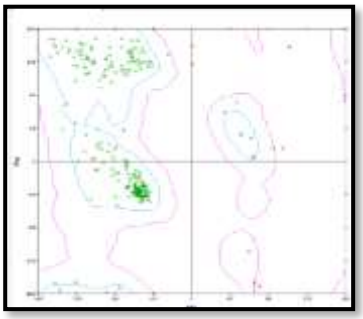
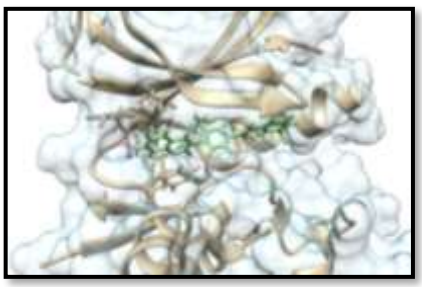
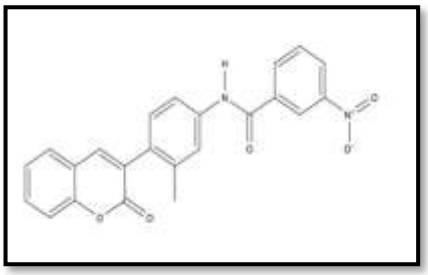
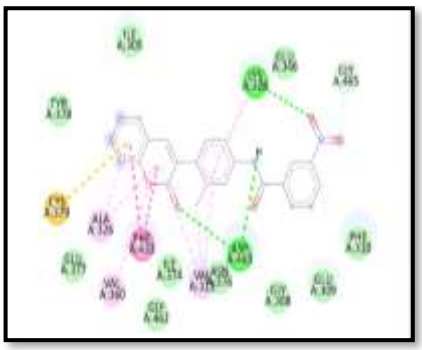
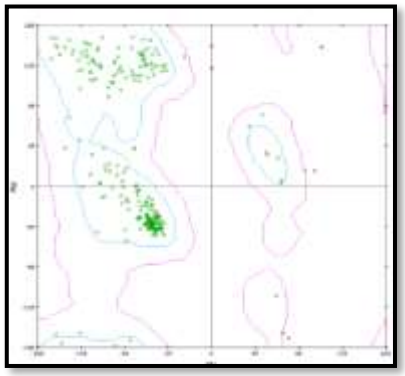


Ramchandran plot

7}2081-0527

 Docked molecule	 3d structure
 3d interaction	 Ramchandran plot

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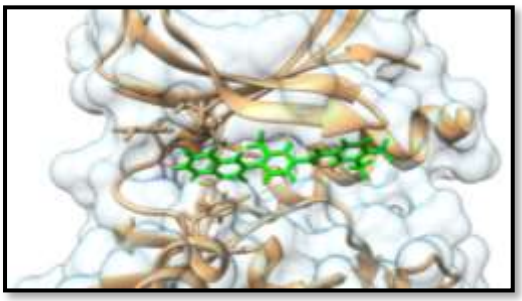
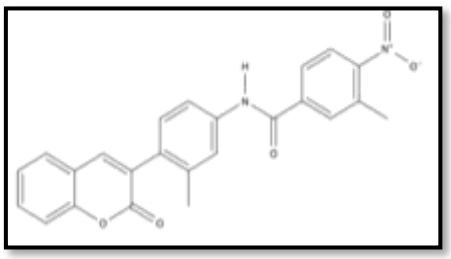
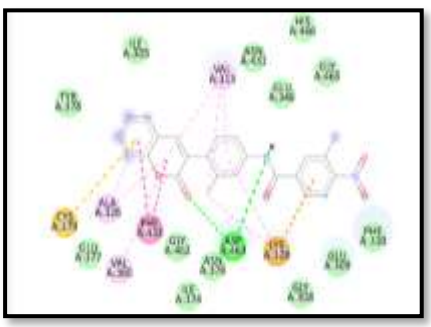
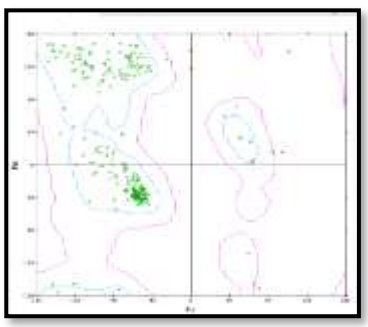
 Docked molecule	 3d structure
 3d interaction	 Ramchandran plot

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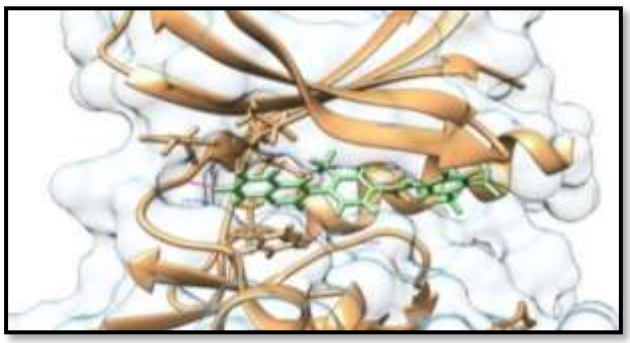

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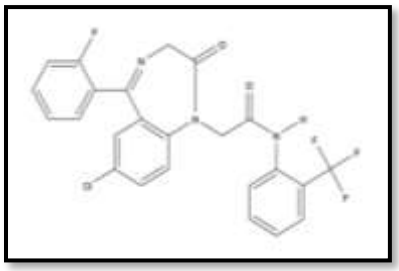

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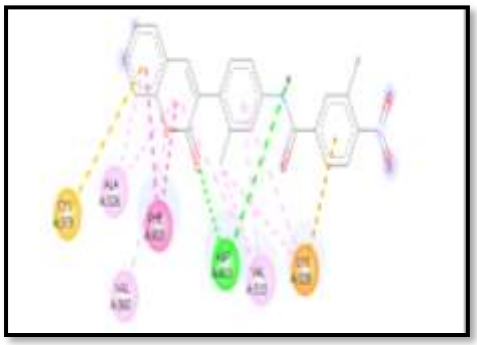
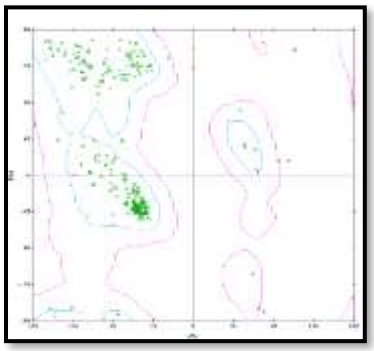
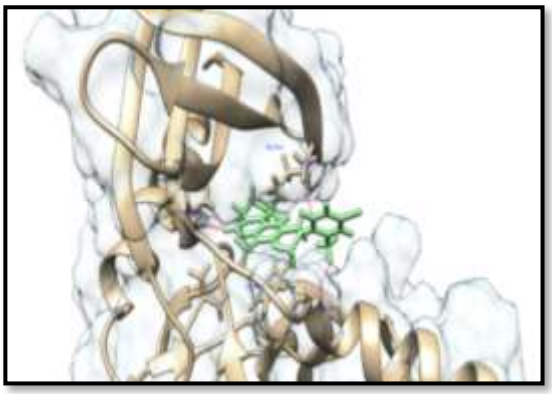


3d interaction

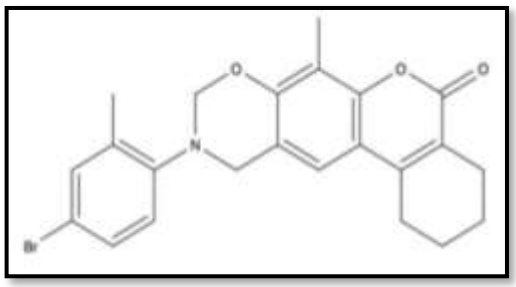


Ramchandran plot

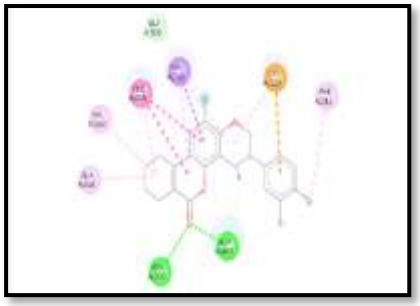
10}Y040-7522



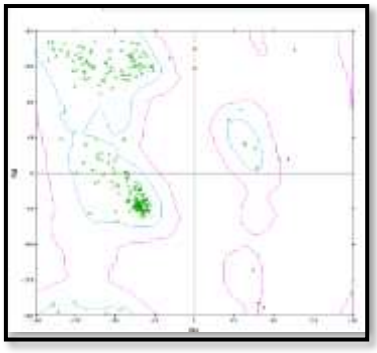
Docked molecule



3d structure



3d interaction



Ramchandran plot

ADMET Studies

In-silico ADME Studies

Top 10 compounds were uploaded on software (SwissADME) and desired ADME properties were selected. The obtained result was documented and evaluated.

SR.NO.	COMPOUND ID	TPSA	H-BOND ACCEPTOR	H-BOND DONOR	ESOLLOGS	BBB PERMEATION	LIPINSKI VIOLATION	CYP 3A4 INHIBITOR	CYP 2C9 INHIBITOR	CYP 1A2 INHIBITOR	CYP 2D6 INHIBITOR	GI ABSORPTION	ROTATABLE BONDS
1	2248-0969	84.76	4	1	-6.21	No	0	Yes	Yes	Yes	No	Low	4
2	2081-1164	105.13	5	1	-5.71	No	0	No	Yes	Yes	No	High	5
3	2154-0349	105.13	5	1	-6.01	No	0	No	Yes	No	No	High	5
4	2072-0767	105.13	5	1	-6.32	No	0	No	Yes	No	No	High	5
5	2153-0115	105.13	5	1	-5.72	No	0	Yes	Yes	No	No	High	5
6	Y040-7562	42.68	3	0	-6.83	Yes	1	No	Yes	Yes	No	High	1
7	2081-0527	105.13	5	1	-5.41	No	0	Yes	Yes	Yes	No	High	5
8	2081-0529	105.13	5	1	-5.72	No	0	Yes	Yes	No	No	High	5
9	K788-6913	61.77	7	1	-5.73	No	0	Yes	Yes	Yes	No	High	6
10	Y040-7522	42.68	3	0	-6.54	Yes	1	No	Yes	Yes	No	High	1

Table No.3. Data showing ADME properties of Top 10 Compounds after docking

***In-silico* Toxicity studies:**

Top 10 compounds were uploaded on software (PROTOX 3.0) and desired toxicity parameters were applied. The obtained result was documented and evaluated:

Sr. no.	Compound ID	Predicted LD50	Predicted toxicity class	Interpretation
1	2248-0969	6000mg/kg	6	Non-Toxic
2	2081-1164	1000mg/kg	4	Harmful if swallowed
3	2154-0349	1000mg/kg	4	Harmful if swallowed
4	2072-0767	1000mg/kg	4	Harmful if swallowed
5	2153-0115	1000mg/kg	4	Harmful if swallowed
6	Y040-7562	67mg/kg	3	Toxic if swallowed
7	2081-0527	1000mg/kg	4	Harmful if swallowed
8	2081-0529	1000mg/kg	4	Harmful if swallowed
9	K788-6913	1000mg/kg	4	Harmful if swallowed
10	Y040-7522	300mg/kg	3	Toxic if swallowed

Table No.4. Toxicity Of Top 10 Dock Compounds

Molecular Dynamics Result of Compound ID: (2248-0969)

RMSD graph

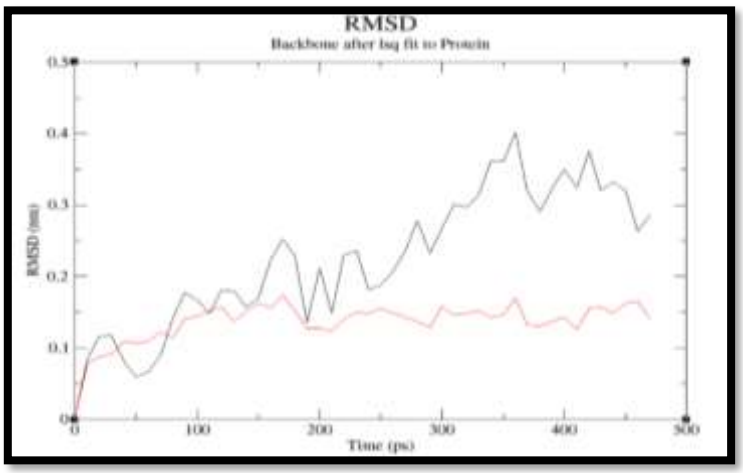


Figure 2. RSMD Graph of Compound ID (2248-0969)

In this RMSD graph, ligand and protein interaction can be seen with respect to time in nanoseconds. Between 200 ps, the ligand was found to be interacting with the protein; after that, there is little deviation. But the deviation is within an acceptable range.

RESULT

6000 compounds from the Targeted Oncology library were taken and screened in DATAWARRIOR, from which 81 compounds were shortlisted. 3D structures of all 81 compounds were drawn in molview.org. Now, these 81 compounds were screened based on the desired parameters. The compounds were further docked, and contacts were developed in UCSF CHIMERA. On the basis of the dock score, obtained top 10 compounds were shortlisted. Also, 8 compounds undergoing clinical trials were selected for validation of process, binding site and interacting amino acids; the structure of the same was drawn in mol.view.org. A comparative study on drugs in clinical trials and drugs obtained from screening was done. In this study, important interacting amino acids were identified, like Asn376, Glu377, Cys379, and Phe433. The top 10 compounds were then further tested for toxicity and ADME properties. The result of ADME and toxicity prediction was documented and well interpreted. Among these compounds, the compound with the highest dock score was shortlisted and used for MD simulations. Also, 2D interaction of the same was obtained. In MD simulations, various results were generated, like the RMSD graph, MD simulation movie, and different binding poses.

CONCLUSION

This research utilized a detailed in silico process to discover new and selective inhibitors of Wee1 kinase that may have anticancer effects. Starting with a library of 6000 ASINEX compounds, comprehensive screening, molecular docking, ADME profiling, and toxicity forecasting facilitated the discovery of leading candidates with significant binding affinity to crucial residues in the Wee1 binding site, especially Asn376, Glu377, Cys379, and Phe433. Validation with clinically studied Wee1 inhibitors confirmed the accuracy of the docking protocol and emphasized the significance of these conserved interactions.

Of the compounds screened, 2248-0969 stood out as the most promising candidate, displaying the highest docking score, advantageous drug-like properties, and the least expected toxicity. Molecular dynamics simulations also showed that the 2248-0969–Wee1 complex maintains structural stability during the simulation duration, reflecting strong binding and limited conformational changes.

This computational pipeline effectively recognized 2248-0969 as a promising lead compound for inhibiting Wee1 and offers a logical basis for future optimization based on structure. Additional in vitro and in vivo research is necessary to confirm its biological activity and evaluate its therapeutic potential in cancer models. The results support the current initiatives aimed at creating advanced Wee1 inhibitors that offer enhanced efficacy, selectivity, and safety.

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