

# STUDY OF PHENOTHIAZINE ON P53 CORE DOMAIN MUTANT Y220C: FINDING THE ANTI-TUMOR ACTIVITY OF PHENOTHIAZINE

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# Abstract

The tumor suppressor protein p53 is a transcription factor that plays a key role in the prevention of cancer development. The p53 cancer mutation Y220C induces formation of a cavity on the protein's surface that can accommodate stabilizing small molecules. We have attempted with the help of virtual screening and molecular docking approach using Lamarckian Genetic Algorithm to elucidate the extent of specificity of p53 cancer mutation Y220C towards different class of Phenothiazines (an anti-cancer agent).

The 393 residue p53 tumor suppressor protein exists in a dynamic equilibrium to form homotetramers.

Each chain comprises several functional domains. The N terminal part of the protein consists of the trans-activation domain (residues 1–63) followed by a proline rich region (64– 92). The central (core) domain (p53 core domain) is responsible for binding. The C terminal part of p53 contains the tetramerization domain (residues 326–355) and the negative regulatory domain at the extreme C terminus (363–393), which contains phosphorylation and acetylation sites and regulates the DNA binding activity of p53. With help of

The docking result of the study of 2,000 Phenothiazines demonstrated that the binding energies were in the range of -10.54 kcal/mol to -1.14 kcal/mol, with 8 molecules showing hydrogen bonds with the active site residues (Lys 164). All the selected 2000 inhibitors were selected on the basis of the structural specificity to the enzyme towards its substrate and inhibitors. Our research provides a blueprint for the design of more potent and specific drugs that rescue p53-Y220C.

**Publication:** The project has been selected for publication and poster presentation at ISCB Africa ASBCB Conference on Bioinformatics which is going to be held in Cape Town, South Africa, in March 2011

# Introduction

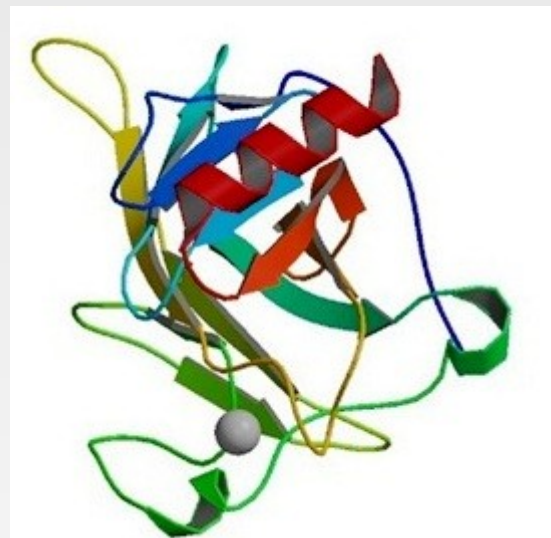
- ▶ **p53** (also known as **protein 53** or **tumor protein 53**), is a tumor suppressor protein that in humans is encoded by the *TP53* gene.
- ▶ The tumor suppressor protein p53 is a transcription factor that plays a key role in the prevention of cancer development.
- ▶ p53 is important in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumor suppressor that is involved in preventing cancer.
- ▶ Human p53 is 393 amino acids long and has seven domains

## P53- Y220C

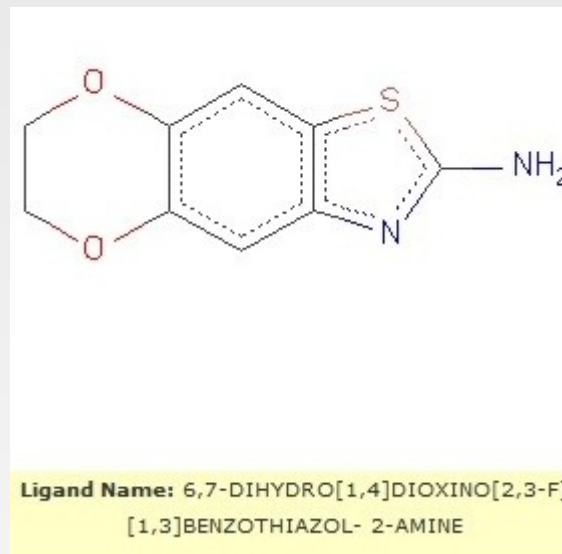
- ▶ The oncogenic Y220C mutation in the tumor suppressor p53 is among the most frequent genetic alterations in human cancers.
- ▶ The mutation markedly lowers the stability of the protein and, notably, induces formation of a cleft on the protein's surface at the mutation site.
- ▶ The p53 cancer mutation Y220C induces formation of a cavity on the protein's surface that can accommodate stabilizing small molecules.
- ▶ This site, which is away from the DNA-binding site of p53, could potentially accommodate a small-molecule drug that, in principle, could reverse the thermodynamic consequences of the mutation.

# Our Protein : P53- Y220C

Structure of the p53 core domain mutant Y220C bound to a 2-amino substituted benzothiazole scaffold PDB ID- 2X0U was taken as a protein.



(A)



(B)

FIGURE . A, Structure of the p53 core domain mutant y220c bound to a 2-amino substituted benzothiazole scaffold. B, The chemical formula of X0U(6,7-DIHYDRO[1,4] DIOXINO[2,3-F][1,3]BENZOTHIAZOL-2-AMINE) is shown above.

# Ligands used: Phenothiazine Derivatives

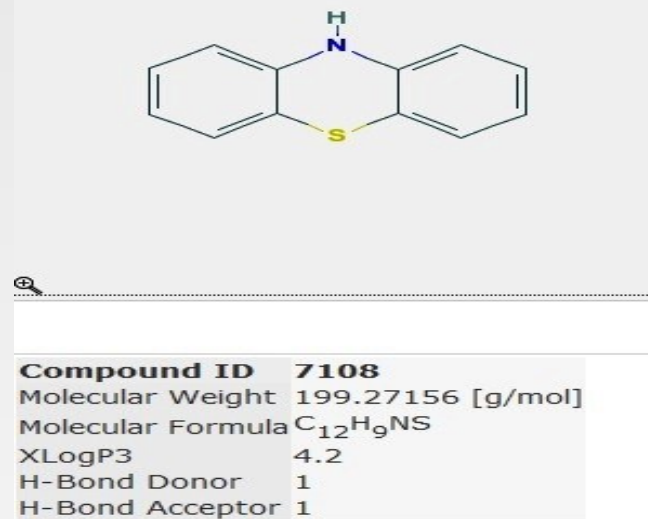


FIGURE A & B- Structure of Phenothiazine

- ▶ Organic sulphur & nitrogen containing compounds.
- ▶ The compound is related to the thiazine-class of heterocyclic compounds.
- ▶ Phenothiazine derivatives has shown a wide range of chemotherapeutic activities, one of it is antitumor.
- ▶ Large activity data set available.
- ▶ Set of 2000 phenothiazine containing molecules were chosen.

# Materials and methods

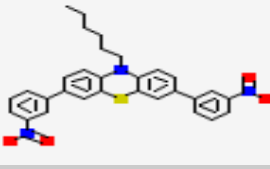
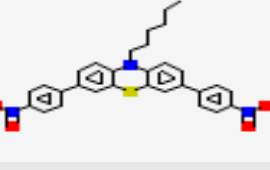
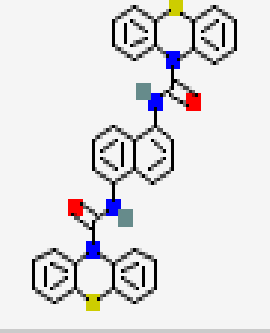
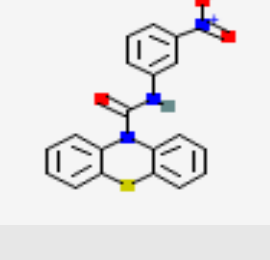
- ▶ Structure Search for protein (from PDB), target identification for active sites (By UCSF Chimera)
- ▶ Virtual screening of ligands: using ChemBank and PubChem Compound home
- ▶ Structure of ligands drawn using Chems sketch, converted using OpenBabel converter
- ▶ Docking performed using Autodock4 (MGL tools).
- ▶ Lamarckian Genetic Algorithm used for docking.

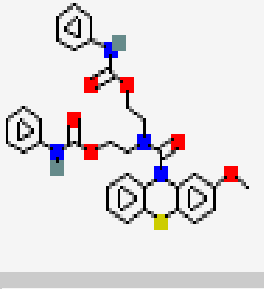
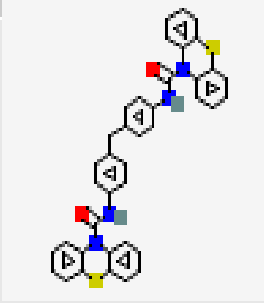
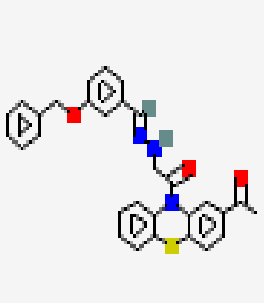
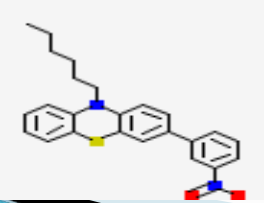
# Analysis of data generated

- ▶ Data Set generated, Binding energy and cluster information used for selection.
- ▶ Visualization of docked proteins, using UCSF Chimera.
- ▶ Analysis of H-bonds using structure analysis tools (UCSF chimera).



# Select Ligands

Ligands	Structure	Binding Energy (Kcal/mol)	Rule of Five	H- Bond	Cluster RMSD
10-hexyl-3,7-bis(3-nitrophenyl)ph		-10.54	Yes	2, 1, 2 GLN100,LYS101, ,GLN167,SER166	0.35
10-hexyl-3,7-bis(4-nitrophenyl)ph		-9.03	Yes	1,1,1,1 LYS164,SER99, LYS101,GLN100	0.80
N-[5-(phenothiazine-10-carbonylamino)naphthalen-1-yl]phenothiazine		-8.18	Yes	2 GLN 100	0.68
N-(3-nitrophenyl)phenothiazine-10-carboxamide		-7.90	Yes	2, 2, 1 SER99,LYS101, GLN100	0.80

Ligands	Structure	Binding Energy (Kcal/mol)	Rule of Five	H- Bond	Cluster RMSD
2-[(2-methoxyphenothiazine-10-carbonyl)-[2-(phenylcarbamoyloxy)ethyl]amino]ethyl N...		-7.88	Yes	2 GLN 165	0.58
N-[4-[[4-(phenothiazine-10-carbonylamino)phenyl]methyl]phenyl]phenothiazine		-7.87	Yes	2 SER 166	0.41
1-(2-acetylphenothiazin-10-yl)-2-[(2E)-2-[(3-phenylmethoxyphenyl)methylidene]hydrazinyl]etha...		-7.77	Yes	1, 2, 1 GLN165,SER166, LYS164	0.35
10-hexyl-3-(3-nitrophenyl)phenothiazine		-7.71	Yes	1, 1 GLN100,LYS101	0.80

# Results

- ▶ The logP value of the ligands fall within prescribed range.
- ▶ Ligands follow Lipinski's rule of five.
- ▶ Residues LYS 164, SER 99, LYS 101 and GLN 100 verified as the most important substrate recognition residues in the active site pocket (using PAR- 3D and SCF Bio) of the protein.
- ▶ 8 phenothiazine derivatives shows H-Bond with active site residues.

# CLUSTERING AROUND THE ACTIVE SITE

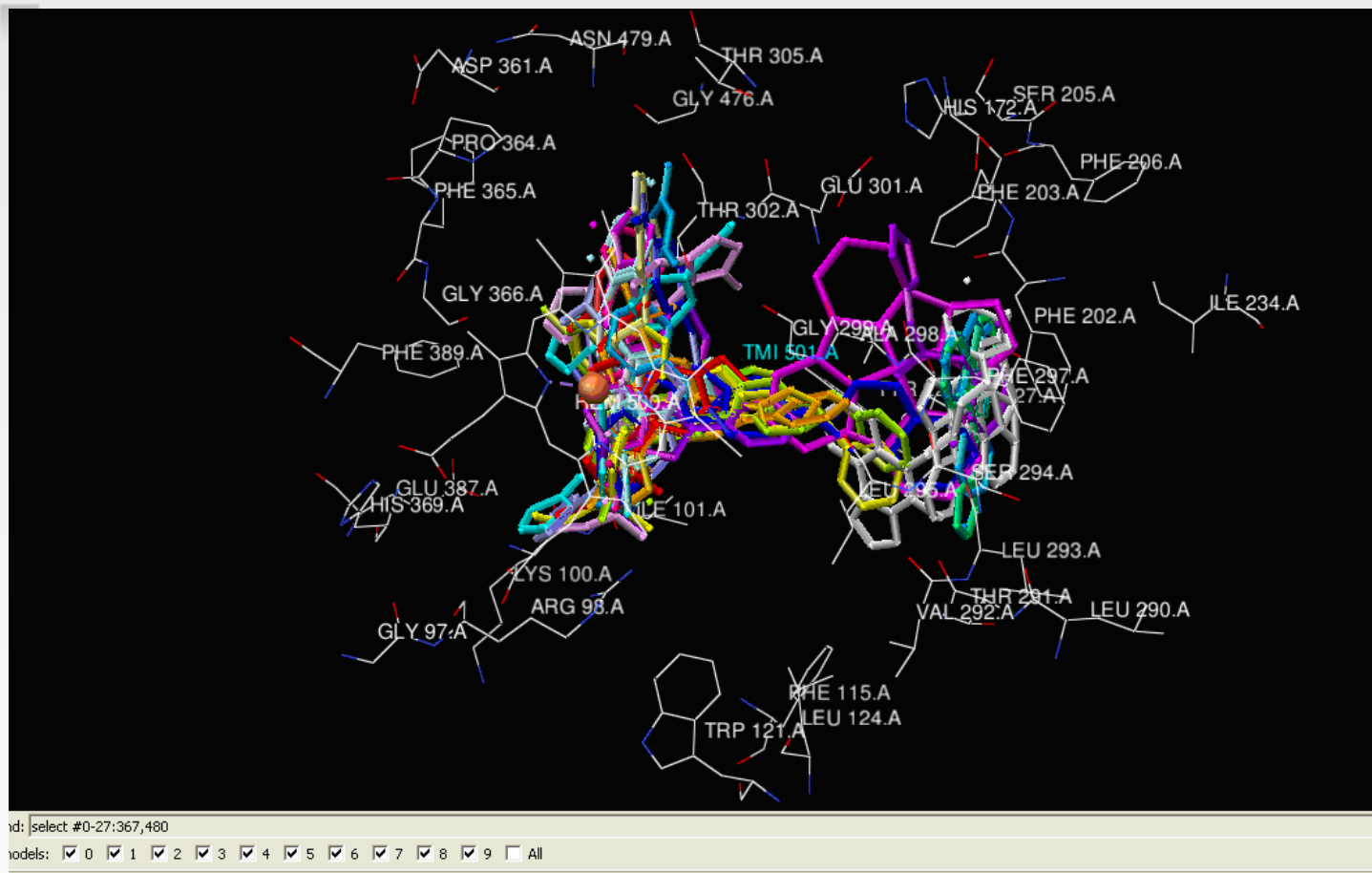


Figure: Clustering of Ligands at the active site of the protein.

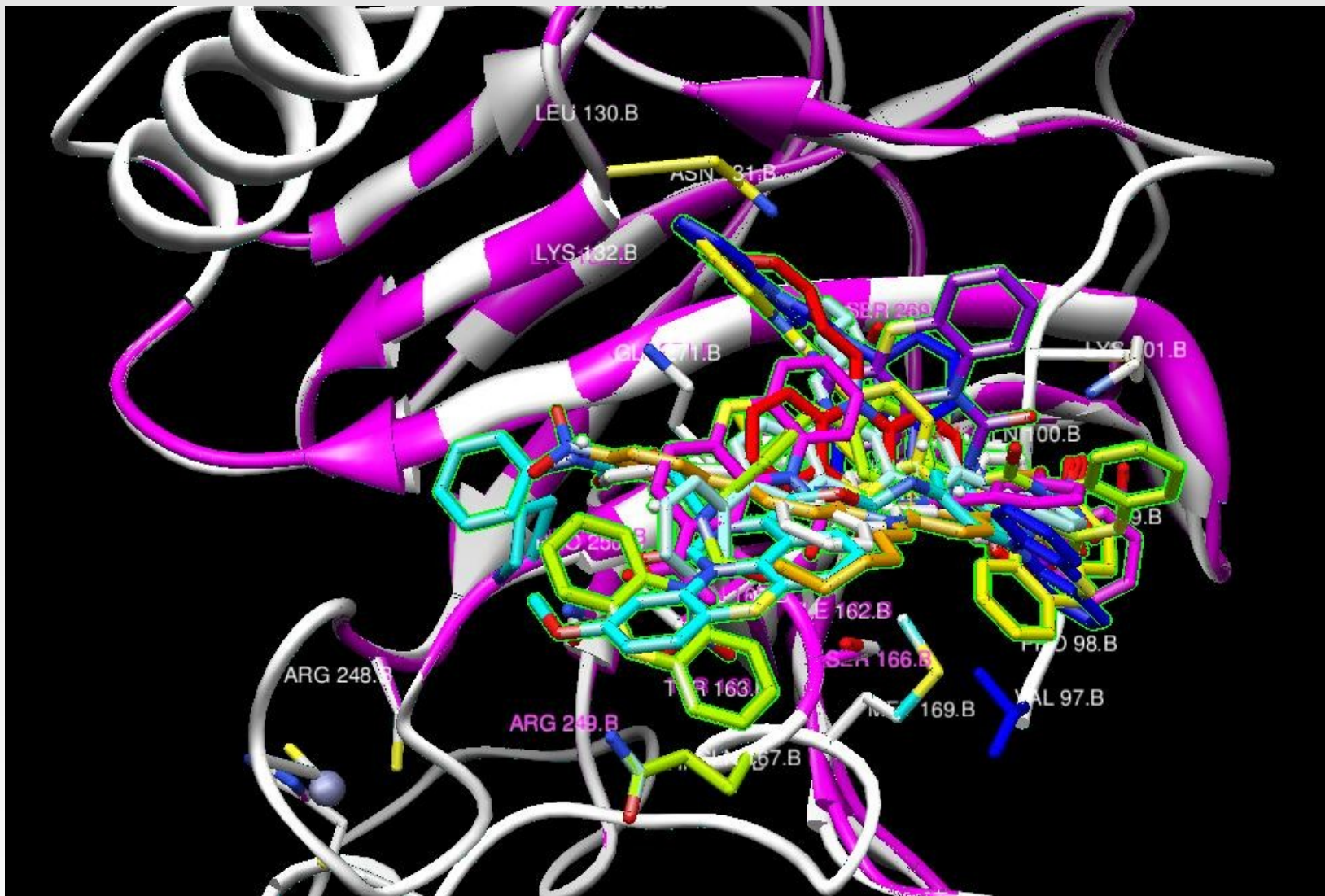
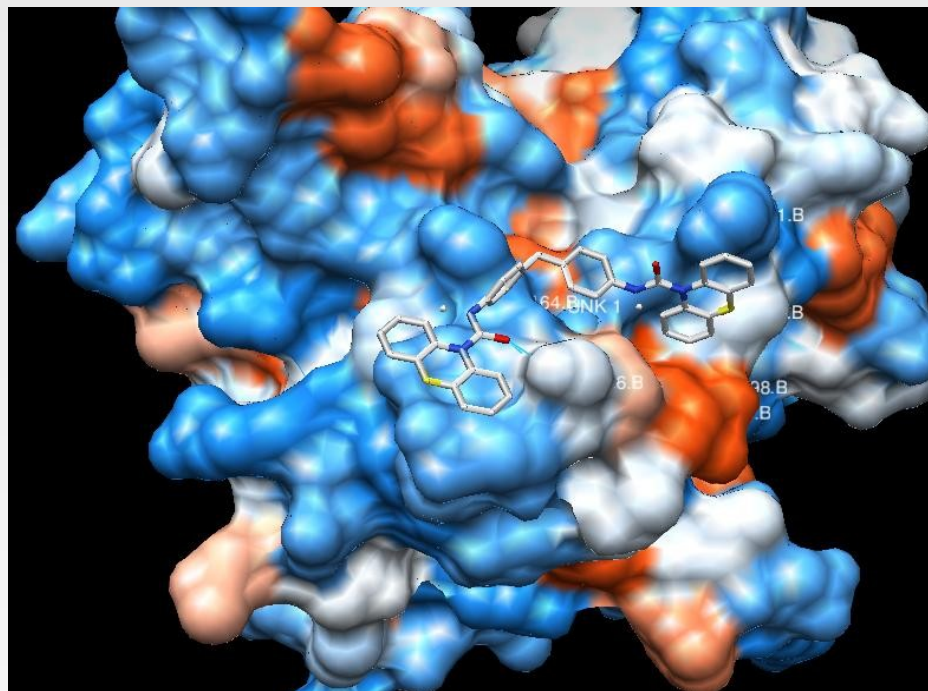
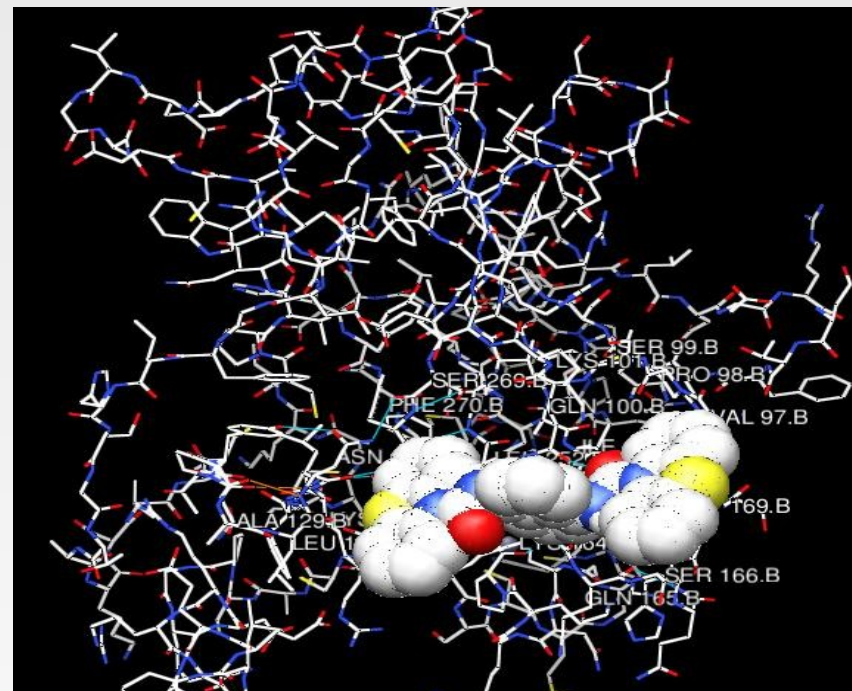


Figure: Docking conformation of all the ligands

# RESULTS

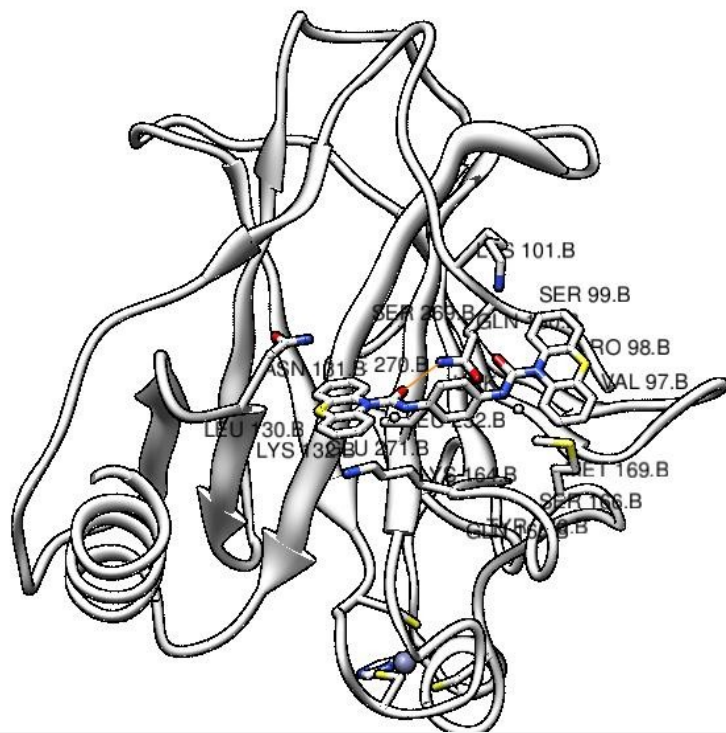


(a)

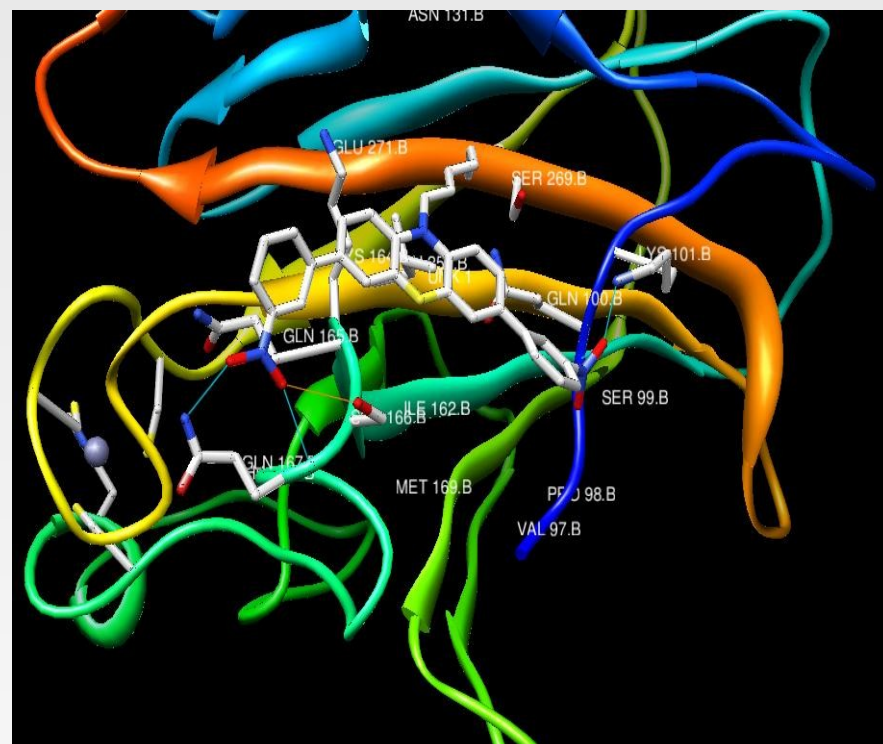


(b)

Figure- **a, b**: Showing selected ligand docked.



(c)



(d)

Figure- **c, d**: Showing selected ligand docked.





# Discussion/Conclusion

- ▶ Protein chosen (P53-Y220C) shows good initial results with computational approach.
- ▶ The docking result of the study of 2,000 Phenothiazines demonstrated that the binding energies were in the range of -10.54 kcal/mol to -1.14 kcal/mol
- ▶ The study showed the druggability of P53- Y220C on the ligand interaction sites within the mutational cavity.
- ▶ Elucidation of the binding mode of hits yielded a clear picture of how a drug might dock in the cavity.

- ▶ The study supports the importance of considering flexibility of the cavity in screening for optimized ligands.
- ▶ Our findings provide a blueprint for the design of effective drugs that rescue P53-Y220C.
- ▶ The in-silico data gives hints for future design of drugs with better specificity and activity.

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THANK YOU