6. SUMMARY AND CONCLUSION:

P53 is an important tumor suppressor gene with a known role in the later stages of cancer. Since its discovery in 1979, p53 has become the focus of intensive cancer based research in laboratories around the world. Tumor suppressor gene p53 is an attractive cancer therapeutic target because it can be functionally activated to eradicate tumors.

Mdm2 has been identified as a p53 interacting protein towards represses p53 transcriptional activity. Mdm2 achieves this repression by binding to and blocking the N-terminal transactivation domain of p53. Mdm2 is a p53 responsive gene that is; its transcription can be activated by p53. When p53 is stabilized, the transcription of Mdm2 is also induced, resulting in higher mdm2 protein levels causing recognition and repair, transcription regulation problems leading to cancer. Thus inhibiting the mdm2- p53 interactions has been proven to be most promising approach for cancer therapy.

In present thesis an investigation was carried out on the mode of interaction between mdm2 and p53 at molecular level. Then a structure based virtual screening approach will be used to identify target specific mdm2 inhibitors by docking studies. The best compound will be subjected to molecular dynamic simulations for further validating the docking studies and to reveal interactions during the conformational changes. The identified compounds are compared to that of the FDA drug Nutlin compound that which has already proven.

In this work, we discovered several compounds that are potentially able to block the interaction between active residues of mdm2 and p53 complex, suggesting their capability to act as anti-cancer agents. Compound ZINC59256947 was found to be strong inhibitors for mdm2 protein. From our structure based virtual screening studies coupled with semi-flexible and flexible docking studies, we propose that the tested compounds have the capability to attenuate the mdm2 protein for plausible anti-cancer activity.

All the compounds we discovered in this work are found to bind the same active binding site of mdm2 where p53 peptide binds, by creating hydrogen bonds and various non-covalent interactions. We performed a detailed analysis of the atomic
interactions between the potential compound, and residues inside the mdm2 active sites to identify which residues interacted with the compound. We have shown that the interaction between the compound with the mdm2 active sites are facilitated by hydrogen bonds and non-covalent interactions, with atleast one of the active residues that are vital for mdm2-p53 complex formation.

On the other hand, our case study with the Hamelia patens leaf extract has shown that it has the anti-inflammatory and anti peroxidative effect on carrageenan induced rat paw oedema. Based on this anti-inflammatory effect the plant is significantly subjected to extract for its active constituents by Methanolic extraction method. To see whether any of the extracted compounds have the structural similarity and ability as to that of the Nutlin and identified Zinc compound to inhibit mdm2 protein. It has revealed that some promising results show similarity to that of FDA approved drug Nutlin compound and the identified novel compound ZINC59256947.

The present study provides a rationalization to the ability of present studied compounds as a valuable small ligand molecules with strong binding affinity towards mdm2 protein for plausible anti-cancer activity.

Our computational analysis evidenced that the large value of binding energy is involved in binding of present investigated five compounds with the mdm2 protein consolidating their complex’s thermodynamic stability; moreover predicted IC50 values further substantiated our hypothesis that these compounds have the potential to inhibit mdm2 protein.

Further, de novo simulations for 10 ns suggest that ligand interaction with the residues of mdm2, all or some of which fall under catalytic active site important residues for its structurally stability and/or functionality, could be critical for its inhibitory activity. The present information could be of high value for computational screening of mdm2 targeting drugs. A little Knowledge was gained through this study, that it would further enhance the discovery of mdm2 target specific drug compounds by understanding the molecular interaction basis between ligand and receptor.

The present study further demonstrated that mdm2 might be the drug target for the present investigated compounds for their anti-cancer properties. Results from the molecular dynamic simulations in water show that the trajectories of the protein complexed with ligands are quite stable over a time period of 10 ns, with the energies
of the complex being lowered in comparison to the un-docked protein, suggesting the compounds inhibitory potential along with thermodynamic.

Finally thesis will conclude by proposing a potent lead compound and an active alkaloid compound identified from the methanolic extraction of Hamelia patens leaf extract that which has an effect on carrageenan induced rat paw oedema for its inflammation has revealed some promising results that shows similarity to that of FDA approved drug Nutlin compound and the identified novel compound ZINC59256947 and these subjected for the further studies based on the above mentioned results.

In this we provided an overview of contemporary approaches to the discovery of small molecule cancer drugs, highlighting successes, current challenges and future opportunities. We focused particularly on four key steps: Target validation and selection; chemical hit and lead generation; lead optimization to identify a clinical drug candidate by using computational techniques. The Novel computational techniques have been developed to predict the interaction models of protein–protein (P53 –Mdm2 Interactions) interactions from medium to high resolution. The new developments in the quest for pharmacological p53 activators are reviewed with an emphasis on small-molecule inhibitors of the p53-MDM2 interaction inhibitors as a targeted therapy for cancer treatment. Collectively, these advances provide new opportunities to use macromolecular structures in pharmacogenomics and systems pharmacology.

The main aim of the present study is to identify a novel/similar/better drug like compound in comparison with that of the FDA approved drug Nutlin was supported with ZINC59256947 and Palmirine compounds from the Zinc database and from the Hamelia patens plant respectively through the Structure Based Virtual Screening, Docking and Molecular Dynamic Simulation studies reveals that the identified two compounds plays a crucial role in inhibiting the Mdm2- P53 interaction.

7. RECOMMENDATIONS:
Design of non-peptide, small-molecule inhibitors that block the MDM2-p53 interaction has been sought as an attractive strategy to activate p53 for the treatment of cancer and other human diseases. Major advances have to be made in the design of small-molecule inhibitors of the MDM2-p53 interaction for advanced preclinical development or clinical trials. This justifies the use of plant in traditional medicine practices. It is therefore recommended that more work be conducted to help optimally extract all the bioactive compounds in the plant and formulated into appropriate doses for the treatment.

The analogues reported in this study maybe further modified to increase their anticancer activity. Nonetheless, further investigations, including explorations into the specificity of inhibitors and in-vivo models, are needed to predict the ADME predictions of the identified compounds to analyze the pharmaceutical value of Hamelia patens plant-derived and Novel compounds such as Palmirine and ZINC59256947 respectively, as Mdm2-p53 interaction inhibitors for cancer therapy. Design of non-peptide, small-molecule inhibitors that block the MDM2-p53 interaction has been sought as an attractive strategy to activate p53 for the treatment of cancer and other human diseases. Major advances have to be made in the design of small-molecule inhibitors of the MDM2-p53 interaction for advanced preclinical development or clinical trials. This justifies the use of plant in traditional medicine practices. It is therefore recommended that more work be conducted to help optimally extract all the bioactive compounds in the plant and formulated into appropriate doses for the treatment.