SUMMARY AND CONCLUSION

Cytochrome P450 26A1 (CYP26A1) gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum protein acts on retinoids, including all-trans-retinoic acid (RA), with both 4-hydroxylation and 18-hydroxylation activities. This enzyme regulates the cellular level of retinoic acid which is involved in regulation of gene expression in both embryonic and adult tissues. CYP26A1 plays a key role in retinoic acid metabolism. It acts on retinoids, including all-trans-retinoic acid (RA) and its stereoisomer 9-cis-RA. Capable of both 4-hydroxylation and 18-hydroxylation and is responsible for generation of several hydroxylated forms of RA, including 4-OH-RA, 4-oxo-RA and 18-OH-RA. Cytochrome P450 (CYP450) enzymes are a diverse group of catalysts that contain 57 members in humans. CYPs are usually membrane-bound and are localized to the inner mitochondrial or endoplasmic reticular membrane. CYPs have oxygenase activity and commonly catalyze redox reactions, involving the oxidation of the substrate and reduction of water. This group of enzymes contain a heme ion within the active site, which is essential for catalytic activity. CYPs have been found in all organisms tested and are ubiquitously expressed. They are found at high levels in the liver, where they have an important role in metabolism of drugs and endogenous toxic compounds (for example bilirubin). Most CYPs can metabolize numerous substrates and this accounts for their major role in drug interactions. CYPs also have functions in steroid hormone synthesis, cholesterol synthesis and vitamin D metabolism.

The aim of the present study to analyze *in silico* structural and functional characterstics of retinoic acid metabolizing protein CYP26A1 by using different bioinformatics tools and software. The study of biodiversity and evolution of CYP26A1 was performed in order to gather information about the phylogentic relationships of CYP26A1 and its isoforms. BLAST tool was used to find homologs for individual protein with stringent parameters. The multiple sequence alignment was carried out through guide tree approach using ClustalW program on protein level.

Finally the phylogenetic tree was greated by Neighbor joining approach. This is followed by construction of stable protein models and prediction of active site point for blocking by a specific inhibitor molecule to stop the protein activation at a disease stage. Modeling of structures and its different conformations were carried out by homology modelling using Modeller and validated by SAVS. The stability of modelled CYP26A1 protein structure was validated using moleclular dynamics through GROMACS software. The library of anticancer drugs for *in silico* virtual screening was prepared on the basis of literature retrieved from the database NCI diversity set III by using AutoDockVina software. Further the target and drug molecules interaction was studied by using Hex software. The interaction maps between target protein and putative drug molecules were constructed by Ligplot+ software.

Predicted 3D optimized structure of Cytochrome P450 26A1 have 89.5% accuracy. Ramachandran map analysis and trajectory analysis of molecular dynamics simulation (energy minimization, radius of gyration, RMSD and RMSF) indicated the CYP26A1 protein model constructed was in stable conformation. The chemical molecules were screened on the basis of Lipnski's rule of 5, Ambiguity analysis, ADME/Toxicity analysis and also through docking which is one of most important phases of drug design. Docking was helpful in the study of protein – ligand interaction at different position of residues (mainly active site). Docked structure helps in study of the potency of drug at the specific target sites, and it's a still a challenge to identify the specific target position for drug binding at minimum cost.

Life on earth depends on water, on hydrogen bonds, and on hydrophobic interactions. DNA and proteins are held together in their defined three-dimensional structures primarily by hydrogen bonds. The double helix of DNA, RNA structures, peptide and protein secondary structures, like α-helices, βsheets, β- and γ-loops, and the tertiary structures of proteins are formed by hydrogen bonds (enthalpic contributions) and by hydrophobic contacts (primarily entropic contributions). With a few exceptions, *e.g.*, the binding of retinol to RBP and of some ligands to the aromatic-hydrocarbon (Ah) receptor, also the formation of ligand-protein complexes depends on hydrogen bonding. It is pointed out that

the following two factors are primarily involved to influence binding conformation between a ligand and a protein. These are binding energy and hydrogen bonding, following criteria can be used to compare the binding affinity of ligands to receptor protein as presented in the current study.

The present study data characterize **ZINC01568793 (8-[4-[4-(7,9-dioxo-8-azaspiro[4.4]nonan-8-yl)-3-methylphenyl]-2-methylphenyl]-8-azaspiro[4.4]nonane-7,9-dione)** act as a potent, orally active inhibitor of oncogenic retinoic acid metabolism CYP26A1 and capable of enhancing RA levels and displaying retinoidal actions. After bioassay test, the study found the obtained chemical compound is one of the anticancer drugs. This study might be useful in exploring the potent drug inhibitor of oncogenic RA metabolizing enzyme CYP26A1.

CONCLUSION

- **Cytochrome P450 26A1** is a protein that in humans is encoded by the *CYP26A1* gene. This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum protein acts on retinoids, including all-trans-retinoic acid (RA), with both 4-hydroxylation and 18-hydroxylation activities. This enzyme regulates the cellular level of retinoic acid which is involved in regulation of gene expression in both embryonic and adult tissues. Two alternatively spliced transcripts and one alternative catalyst activity variant of this gene, which encode the distinct isoforms have been reported.

- The primary structure analysis classified the protein CYP26A1 as an unstable protein and secondary structure has mean accuracy of 64.4% for three state prediction. The phylogenetic or molecular evaluation of CYP26A1 shows the close relationship with primates and in its study with specific model organisms, CYP26A1 shows a close relationship with *Macca mulatta*. The function domain of CYP26A1 protein ranges from position 242-318.

- Homology models were built, using a multiple template approach. The three human P450 crystal structures CYP3A4, CYP2C8 and CYP2C9 were selected as the templates for constructing CYP26A1 models. The stereo chemical properties of model were checked from SAVS server and shows 89.5% accuracy.

- Based on intrinsic dynamics, structural stability and the improved relaxation of modeled protein, the energy of the structure, the radius of gyration, RMSD and RMSF, the protein modeled was in stable conformation. It is interesting to note that the amino acid change due to altered catalyst activity blocked the over expression of CYP26A1 in the active site of protein.

- On the basis of Virtual screening and docking analysis found top four inhibitors ZINC01607786, ZINC01568793, ZINC05462666 and ZINC03916235 have lower lower docking energy scores. From the overall analysis of ADME, toxicity and ambiguity one potent inhibitor ligand molecule was selected.

- The ligand molecule, ZINC01568793, is the effective chemical molecule to inhibit function of retinoic acid catabolism, which will in turn arrests the process of cell growth and proliferation of the cancerous cells. Further, this ligand molecule can be incorporated into the drug development and research.

Future scope of the study

- For CYP26A1, although important DNA elements have been mapped and tested for functionally, the regulation of the gene at the level of complex chromatin structure has not been determined. Given the very strong regulation of this gene by at-RA, CYP26A1 it appears to be an ideal model for future studies at the chromatin level.

- Evidence suggests that CYP26A1, CYP26B1 and CYP26C1 fulfill different functions, based on development studies and on non identical tissue distributions in the adult vertebrate, but their specific functions are not well defined. The discovery that CYP26B1 is essential in germ cell development provides strong evidence that CYP26B1 serves a different purpose from the other CYP26 family genes, including CYP26A1, even though enzyme studies have demonstrated similar catalytic activities of CYP26A1 and CYP26B1 toward at-RA, and they are co-expressed in some tissues (e.g. liver, lung). Moreover, studies of gene expression have been conducted mainly at the organ / tissue level. Further research is needed to understand the cell-specific expression and regulation of the CYP26 genes.

- Many members of the Cytochrome P450 superfamily have been shown to utilize substrates other than those for which they are named, and it cannot be concluded that the CYP26 family exclusive metabolizes RA; thus, additional enzymes studies using other substrates as competitors, especially structurally similar substrates such as long-chain fatty acids, should be conducted.

- Primary and Secondary sequence analysis gives annotation and characterization of Cytochrome P450 26A1 by using various Bioinformatics approaches so that using this study it could be understand their role in various biological mechanisms and also useful in formation of crystallographic structure of CYP26A1.

- Evidence suggest there is considerable person to person variability in the ability of liver microsomes to oxidize at-RA. Polymorphisms are known, but population genetic studies and the effects of polymorphism on vitamin A requirements or RA used pharmacologically should be conducted. And since RA is used clinically, and CYP2C genes (e.g. human CYP2C8) are well known to metabolize many clinically important drugs and can also oxidize RA, the potential for retinoic acid-drug interactions seems imminent. Thus,

additional studies with patient samples and/or in vitro studies to investigate retinoid-drug interactions and the effects of CYP26 gene polymorphism are likely to yield important new information.

- The wet lab validation of growth inhibition of CYP26A1 by inhibitor ZINC01568793 can be established by the qualitative and quantitative biological techniques.