ANTIFUNGAL DRUGS

A.Tandon[1] , A.Naqvi[1] , N.Chitranshi [2], S.Sinha [3]
[1]Amity University, Lucknow, India
[2]Rajashri Rananjay Singh College of Pharmacy, Amethi, India
[3]ACS-bioINFORMATICS, Lucknow, India

ABSTRACT

Crystal Structure of cytochrome p450 2B4 has 476 amino acids, through docking approach we have attempted to explain the specificity of CYP2B4, total 28 imidazole drug were used for the studies as antifungal drugs in which bound bifonazole (reference) shows the binding energy of -8.67 kcal/mol .Compound Miconazole shows the minimum binding energy of -10.45 kcal/mol. The 2B4-bifonazole structure identified 10 residues (ALA 298, GLY 299, GLU 301, THR 302, ILE 363, VAL 292) within 6.5 Å of the active site of bifonazole. GLU 301, THR 302 are also located in 6.5 Å of the bound ligand in 2B4 structure. Due to the presence of the multiple binding substrates in cytochrome p450, it acts as the major target of many drugs in xenobiotic metabolism.

INTRODUCTION

Cytochrome P450, family of enzymes plays a major role in xenobiotic metabolism in all classes of living beings. Cytochrome P450 (EC 1.14.14.1), mainly involved in the biotransformation of many drugs, environmental pollutants, steroids, fatty acids, bile acids, and, frequently, also in activation of carcinogens. Cytochromes P 450 (CYPs) are super family of heme protein enzymes which differ in their substrate specificity and are regulated by numerous factors including age, sex , and exposure to certain CYP inducers. P450s range in size from 40-50 KDa and contain a single HEME group. The HEME iron catalyzes cleavage of O-O bond leaving an iron linked oxygen atom that provides potent oxidant. This can perform regio and stereo selective hydroxylation of a wide variety of endogenous and exogenous organic molecule. The striking feature of these enzymes is their ability to bind ligands of various sizes and shapes.
Cytochrome P450 is an electron donor protein for several oxygenase enzymes found on the endoplasmic reticulum of most eukaryotic cells. These oxygenases include the cytochromes p450-enzyme involved in metabolism of drugs, heme oxygenase- involved in the degradation of heme to bilirubin, and squalene mono-oxygenase- involved in sterol biosynthesis.

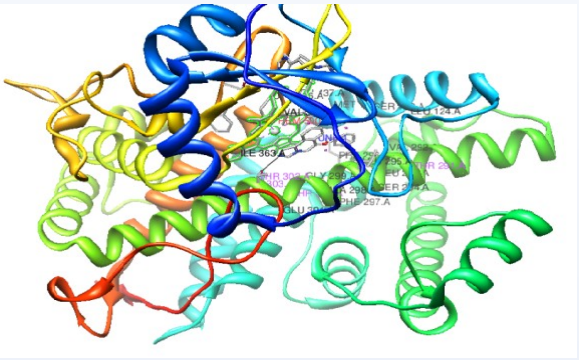


Fig1: 3D model of CYP2B4 with Heme residue form hydrophobic interaction with Thr302

MATERIAL & METHODS

To elucidate the specificity of the cytochrome p 450 docking approach have been attempted .Docking is used to predict the binding orientation of drug to their protein target in order to in turn predict the affinity & activity of drug which includes docking of ligand to a set of grids describing the target protein.
The imidazole drug were indentified using azole ring bind to their respective derivative. Total no of imidazole used for the studies are 28 in number having bifonazole as the reference.
The drug complying the Lipinski rule of five required in the pdb format were converted using the open Babel converter.
Protein have been modeled as per the binding pattern of the drug using Modeller which models three dimensional structures of proteins & their alignment by satisfaction of spatial restraints. Modeller implements an automated approach for comparative protein structure modeling, the input are from the PDB atom files of cytochrome p 450 2B4 & their alignment with the drug. The output is a model for the drug that includes all non hydrogen atoms. Modelled proteins were classified in two categories: 1) Modelled protein with HAEM E residue 2) Modelled protein with TMI & CM 5 residue.
AutoDock4 was used for the docking study combined with the Lamarckian genetic algorithm to search for the globally optimized conformation. The grid spacing was set to 0.375A in each spacing & each grid map consisted of a 60x 60x 60 grid point. For every protein, the center of the grid was set to the position of the HAEM E500.During each docking experiment 15 runs were carried out & the rest of the parameters were set as the default value. At the end of the docking experiment with multiple runs, a cluster analysis was performed. Docking solution with a ligand all-atom root mean square deviation with 0.1nm of each other were clustered together & ranked by lowest binding energy.
The compounds ranked as per the lowest binding energy are Miconazole, Sertaconazole, Ticonazole, Econazole, Isoconazole.

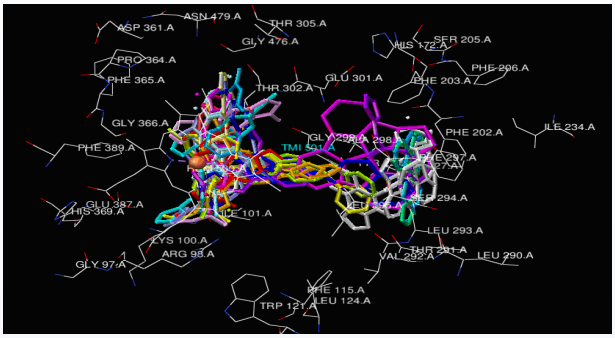


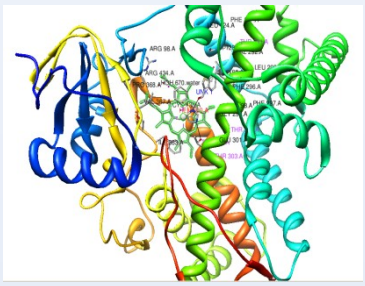
Fig2: Docked confirmation of azole compounds

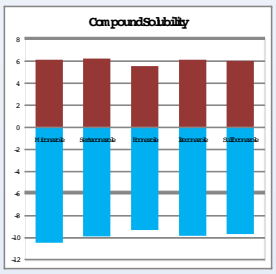
Ligands	Structure	Binding Energy (Kcal/mol)	Rule of Five	Elimination t1/2 values	Cluster RMSD
Fenticonazole		-9.26	Yes	10-20 hrs	0.35
Miconazole		-8.69	Yes	20-30 hrs	0.80
Sertaconazole		-8.67	Yes	20-30 hrs	0.68
Isoconazole		-8.25	Yes	20-40 hrs	0.80
Sulconazole		-7.97	Yes	20-30 hrs	0.58
Tioconazole		-8.05	Yes	10-20 hrs	0.41

RESULT

Correlation was established between the docked score of the tested molecule with there pharmacokinetic parameter, solubility. As the electronegativity present on the benzene ring form the ionic integration with the hydrophobic moiety of Thr 302.. The embedded Heme in the protein interact with azole derivative gives the catalytic activity to the tested azole molecule.

The structure of the cytochrome p 450 was taken from the PDB file 2BDM (resolution 2.30 A R value 0.200).Docked conformations are rated by a scoring functions that include terms for Vander walls, hydrogen bond & electrostatic interactions plus internal energy of ligands. The solubility of the docked compound were related with the binding energy with the help of the log P value





CONCLUSION

The compound Sertaconazole & Isoconazole showed the hydrogen bonding with the THR 302 & GLY 301 which also act as the substrate binding site for the PDB 2BDM . The drug was also analyzed as per the reference Bifonazole, the Bifonazole was analyzed with the modified PDB & there was no major differences found in the drug in interaction with heme ring. The drug was further analyzed for the solubility with the reference of Log P & minimum binding energy, the drug having the higher co-ordinate of the graph for the both log P and minimum binding energy was chosen as the best soluble drug ie. Miconazole showed the best solubility. All the 28 Imidazole drug were docked using the Auto Dock4 and were visualized in UCSF Chimera as shown above have the same orientation which further validate our Docking Result

REFERENCES

1.Coon M .J.(2005) Annu. Cytochrome P 450 flexibility Rev. Pharmacol. Toxicol. vol 100 23
2.Emily E. Scott (2003) Mark A. White An open conformation of mammalian cytochrome PNAS 112 76-89
3.Yonghong Zhao (2005) B.K. Muralidhara, James R. Halpert Structure of microsomal Cytochrome P450 2B4. J. biol. Chem. 281 5973-77
4. Kollman, P. A. Free Energy Calculations: Applications to Chemical and Biochemical Phenomena. Chem. Rev. 1993, 93, 2395−2417

ACKNOWLEDGEMENT

We thankful to Department of Bioinformatics, Biotechnology Park, Lucknow and Dr. B. N. Mishra, Institute of Engineering & Technology, Uttar Pradesh Technical University, Lucknow for their kind support and all ACS staff members for helpful comments on this poster and simulating discussion and practical help. We also thank Mr. Manish Jha for his invaluable support.

Email: arpit.tandon@ymail.com