Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 09, pp.129-134, September, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DETERMINATION OF BINDING MODE OF 1, 3, 4- THIADAZOLE ON CYTOCHROME P450 2B4: A VIRTUAL SCREENING AND MOLECULAR DOCKING STUDY

[1]S. Tanwar, [2]N. Kumar, [2]L. Kumar, [3] Hameed Mohammad, [4]M. Osman, [4]M. Ishaq and [5]S. A. H. Naqvi

[1]Amity University, (Lucknow, IN); [2]Anand Engineering College, (Agra, IN); [3]Bharat Insitute of Engineering & Technology (Hyderabad, IN); [4]Sri Indu College of Engineering & Technology (Hyderabad, IN); [5]Bio Discovery-Solutions for future (Agra, IN).

ARTICLE INFO

Article History:
Received 25th June, 2012
Received in revised form
19th July, 2012
Accepted 15th August, 2012
Published online 30th September, 2012

Key words:
Cytochrome P450 2B, 1, 3, 4-Thiadazole,
Antifungal,
Antibacterial,
Anti- inflammatory,
Anticancer,
Anti-tubercular,
Antiviral ,
Anti-parkinsonism.

ABSTRACT

The human genome encodes more than fifty-seven functional Cytochrome P450 proteins and mainly five isomers (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) account for 90% of drug metabolism. This accounting changes from the liver to intestinal and other extra-hepatic organs. Most are found on the endoplasmic reticulum of eukaryotic cells, but few are localized primarily in mitochondria. The majority of these are involved in metabolism for biotransformation of many drugs, environmental pollutants, steroids, fatty acids, bile acids, fat soluble vitamins as well as in activation of several carcinogens. Cytochrome P450 isomers are a super family of HEME protein enzymes which differ in their substrate specificity. They are influenced by numerous factors including age, sex, nutrition as well as exposure to certain CYP inducers. The HEME iron catalyzes cleavage of O-O bond leaving an iron linked oxygen atom that provides potent oxidant. The special features of CYP family of enzymes are ability to metabolize multiple substrates which differ in size, shape and stereochemistry. 1, 3, 4 Thiadiazole and its derivatives represents one of the most biological active classes of compound possessing a wide spectrum of activities. Literature survey shows that the 1,3,4 Thiadiazole nucleus is associated with diverse pharmacological activities such as antifungal, antibacterial, anti- inflammatory, anticancer, anti-tubercular, antiviral and anti-parkinsonism. We have attempted with the help of Virtual Screening and Molecular Docking approach to study the binding mode of 1,3,4 Thiadiazole on Cytochrome P450. A study of around 3500 derivatives has been conducted and the binding energies were in the range of -9.13 kcal/mol to -2.63 kcal/mol. As of structure analysis 20 molecules showed better binding affinities with the active site Thr^{302}. Our study gives an idea about the interaction between the active site residues and the substrate which is explained on the basis of size & hydrophobicity of the binding pocket. The study provides hints for future design of drugs with higher potency and specificity.

Copy Right, IJCR, 2012, Academic Journals. All rights reserved.

INTRODUCTION

During recent years, there have been intense investigations on 1, 3, 4 Thiadiazole and its derivatives represents one of the most biological active classes of compound possessing a wide spectrum of activities. Literature survey shows that the 1,3,4 thiadiazole nucleus is associated with diverse pharmacological activities such as antifungal (1), antibacterial (2, 3), antiinflammatory (4, 5, 6), anticancer (7,8), anti-tubercular (9,10), antiviral (11, 12) and anti-parkinsonism(13, 14). Moreover, much interest has also been focused on the cardiotonic, diuretic and herbicidal activities displayed by compounds incorporating this heterocyclic system. Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme cofactor and, therefore, are hemeproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions.

***Corresponding author:** mdhameed19@gmail.com

Cytochrome P450 is a family of isozymes responsible for the biotransformation of several drugs. Drug metabolism via the Cytochrome P450 system has emerged as an important determinant in the occurrence of several drug interactions that can result in drug toxicities, reduced pharmacological effect, and adverse drug reactions. Recognizing whether the drugs involved act as enzyme substrates, inducers, or inhibitors can prevent clinically significant interactions from occurring. Avoiding co-administration or anticipating potential problems and adjusting a patient's drug regimen early in the course of therapy can provide optimal response with minimal adverse effects. The active site of Cytochrome P450 contains a heme iron center. The iron is tethered to the P450 protein via a thiolate ligand derived from a cysteine residue Human CYPs are primarily membrane-associated proteins, located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells. CYPs metabolize thousands of endogenous and exogenous chemicals. Some CYPs metabolize only one

(or a very few) substrates, such as *CYP19* (aromatase), while others may metabolize multiple substrates. Both of these characteristics account for their central importance in medicine. Cytochrome P450 enzymes are present in most tissues of the body, and play important roles in hormone synthesis and breakdown (including estrogen and testosterone synthesis and metabolism), cholesterol synthesis, and vitamin D metabolism. Cytochrome P450 enzymes also function to metabolize potentially toxic compounds, including drugs and products of endogenous metabolism such as bilirubin, principally in the liver. The apparent pharmacokinetic effect of a mechanism-based inhibitor of Cytochrome p450 would be a function of its K(I), k(inact) and partition ratio and the zero-order synthesis rate of new or replacement enzyme. The inactivators for Cytochrome p450 can be inducers and P-gp substrates/inhibitors, confounding in vitro-in vivo extrapolation. The clinical significance of Cytochrome p450 inhibition for drug safety and efficacy warrants closer understanding of the mechanisms for each inhibitor. Furthermore, such inactivation may be exploited for therapeutic gain in certain circumstances. The special features of CYP family of enzymes are ability to metabolize multiple Substrates which differ in size, shape and stereochemistry (Bathelt *et al.*, 2002; Poulos *et al.*, 2003; Olavi*et al.*, 2008; Meunier *et al.*, 2004)

MATERIALS AND METHODS

In silico screening

3500 compounds from different chemical database were screened, including the PubChem&ChemBank.They were docked into the active-site **Thr^{302}** of cytochrome P450 2B (pdb ID 2BDM (12)) using the program AutoDock4 (13).

Substrate selection

Firstly top 3500 drug structures most 2D-similar to1, 3, 4-Thaidazole (Fig 1) were chosen based on screening from the ChemBank (14) .The chosen ligands have conformational stability and structural diversity in relation to the bound ligands of the Thiadiazole crystal structure. The ligand structures used in docking were obtained from ChemBank compound database. Ligands were identified as per the pharmacokinetic parameter and solubility. The active site i.e. Thr^{302} in the protein interacts with ligand of the substrate and gives rise to the catalytic activity to test ligands that helps in determining the binding pattern of the ligands to the active site of CYP 450 2B(PDB; 2BDM).

Synonyms: 1, 3, 4-Thiadazole N- (5- (2- methylphenyl) - 1, 3, 4- thiadiazol- 2- yl) cyclopropanecarboxamide

Docking setup

Docking was performed using AutoDock 4, which combines energy evaluation through grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein (Morris *et al.*, 1998). While docking, polar hydrogen's were added to ligands using the hydrogen's module in AutoDock tool and thereafter, Kollman united atom partial charges were assigned (La Motta *et al.*, 2007). Docking of HIV-PR to ligands was carried out using LGA with standard docking protocol on the basis a population size of 150 randomly placed individuals; a

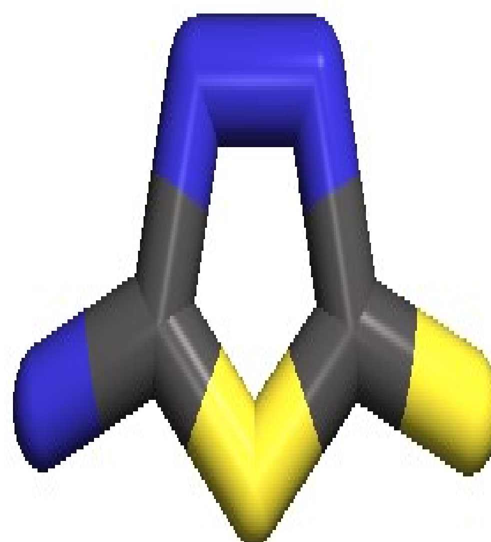

Fig.1 Structure of 1,3,4-thiadiazole

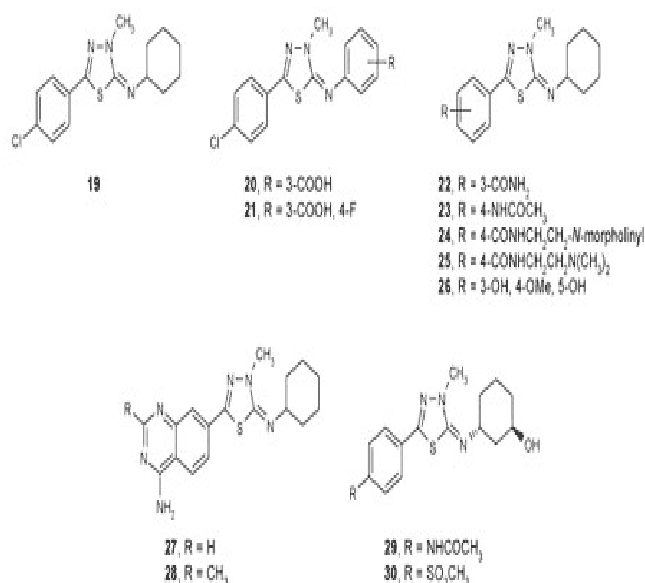

Fig. 2. Derivatives of 1,3,4-thiadiazole

maximum number of 2.5 *107 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Fifteen independent docking runs were carried out for each ligand and results were clustered according to the 1.0 Ǻ rmsd criteria. The grid maps Representing the proteins were calculated using auto grid and grid size was set to 60*60*60 points with grid spacing of 0.375 Ǻ. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera (15) (http://www.cgl.ucsf.edu/chimera) within 6.5 Ǻ region.

RESULTS AND DISCUSSION

Docking was carried out for 1, 3, 4 -Thiadiazole with Cytochrome P450 2B showed a minimum binding energy in the range of -9.13 kcal/mol to -2.63 kcal/mol. On docking of 3500 molecules with **Thr^{302}** (as an active site) residue according to the minimum binding energy generated by autodock4 the best results were shown by following compounds.

Table 1. Binding Energy of Compounds obtained from ChemBank

S.No.	COMPOUNDS NAME	BINDING ENERGY (MIN)	BINDING ENERGY (MAX)
1	N- (5- (2- methylphenyl) - 1,3,4- thiadiazol- 2- yl)cyclopropanecarboxamide	-7.26	-6.9
2	N- (2- chlorophenyl) - 3- ((5- methyl- 1,3,4- thiadiazol- 2- yl) thio)propanamide	-7.44	-6.74
3	2- ((5- amino- 1,3,4- thiadiazol- 2- yl) thio)- N- pyridin- 3- ylpropanamide	-6.32	-5.21
4	2- oxo- N- 1,3,4- thiadiazol- 2- yl- 2H- chromene- 3- carboxamide	-7.53	-6.78
5	1- (5- ethyl- 1,3,4- thiadiazol- 2- yl) - 3- (3- methylphenyl) urea	-4.96	-4.02
6	N- (2,2- dimethylpropyl) - 5- methyl- 1,3,4- thiadiazole- 2- sulfonamide	-4.95	-3.86
7	3- phenyl- N- (5- (propylthio) - 1,3,4- thiadiazol- 2- yl)propanamide	-5.72	-5.48
8	2- hydroxy- N- (5- isopropyl- 1,3,4- thiadiazol- 2- yl) benzamide	-6.8	-5.45
9	N- (5- pyridin- 3- yl- 1,3,4- thiadiazol- 2- yl) cyclobutanecarboxamide	-6.46	-4.8
10	5- (2- (1H- benzimidazol- 2- ylthio) ethyl)- 1,3,4- thiadiazol- 2- amine	-7.3	-5.59

Table 2. Hydrogen Bonds and compounds that follow Lipinski's rule of 5

COMPOUNDS	MIN	MAX	HYDROGEN BONDS
N- (5- (ethylthio) - 1,3,4- thiadiazol- 2- yl)- 4- propylbenzamide	-7.43	-4.02	THR 302.A0G1-UNK 102.668Å
2- (benzylamino) - 5H- (1,3,4)thiadiazolo(2,3- b)quinazolin- 5- one	-8.9	-3.86	THR 302.A0G1-UNK 102.668Å
2- ((5- amino- 1,3,4- thiadiazol- 2- yl) thio)- N- (4- fluorophenyl) propanamide	-7.49	-4.8	THR 302.A0G1-UNK 103.259Å
N- (5- (4- chlorophenyl) - 1,3,4- thiadiazol- 2- yl)cyclopropanecarboxamide	-7.35	-5.78	THR 302.A0G1-UNK 103.259Å
2- ((5- amino- 1,3,4- thiadiazol- 2- yl) thio)- N- (2- cyanophenyl) acetamide	-7.38	-4.49	THR 302.A0G1-UNK 102.668Å
2- ((5- methyl- 1,3,4- thiadiazol- 2- yl) thio)- N- (1- phenylethyl) acetamide	-7.47	-5.36	THR 302.A0G1-UNK 102.668Å
N- (4- methylbenzyl) - 2- ((5- methyl- 1,3,4- thiadiazol- 2- yl) thio)acetamide	-7.52	-4.4	THR 302.A0G1-UNK 102.668Å
2- (benzylthio) - 7- methyl- 5H- (1,3,4)thiadiazolo(3,2- a)pyrimidin- 5- one	-8.0	-6.3	THR 302.A0G1-UNK 102.668Å

N- (5- (ethylthio) - 1,3,4- thiadiazol- 2- yl)- 4- propylbenzamide
2- (benzylamino) - 5H- (1,3,4)thiadiazolo(2,3- b)quinazolin- 5- one
2- ((5-amino- 1,3,4- thiadiazol- 2- yl) thio)- N- (4- fluorophenyl) propanamide
N- (5- (4- chlorophenyl) - 1,3,4- thiadiazol- 2- yl)cyclopropanecarboxamide
2- ((5- amino- 1,3,4- thiadiazol- 2- yl) thio)- N- (2- cyanophenyl) acetamide
2- ((5- methyl- 1,3,4- thiadiazol- 2- yl) thio)- N- (1- phenylethyl) acetamide
N- (4- methylbenzyl) - 2- ((5- methyl- 1,3, 4- thiadiazol- 2- yl) thio)acetamide
2- (benzylthio) - 7- methyl- 5H- (1, 3,4)thiadiazolo(3,2- a)pyrimidin- 5- one

Fig: 2a- H-Bond with active site residue

Fig: 2b- H-Bond with active site residue

The corresponding ligands which we have find out are matched for the Lipinski's Rule of 5 parameter and the results showed that all from the list of 3500 compounds follow Lipinski's rule of 5 (table 2). Our result indicates that the ligand have varied binding pattern with substrate binding site i.e. **Thr**^{302} along with various other active sites (table 2). The structure of the protein-ligand complex reveals that Cytochrome P450 2B partially occupies the active-site (**Thr**^{302}) providing a framework for the design of compounds with potential therapeutic applications. 9 compounds

demonstrate better binding patterns with protein in terms of hydrogen bonds with the various residues of the protein.

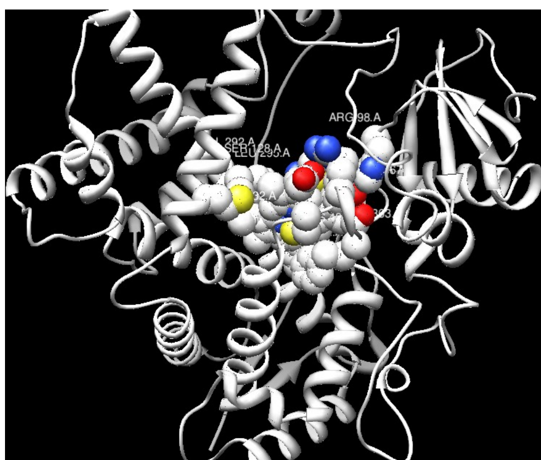

Fig: 2c-H-Bond with active site residue

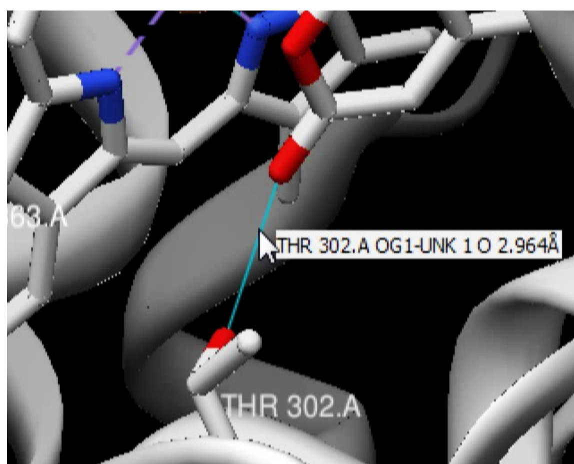

Fig: 2d-H-Bond with active site residue

In summary, based on the molecular docking we found that 9 molecules as of structure similarity from ChemBank showed better binding affinities with the active site pocket (**Thr^{302}**) of the Cytochrome P450 2B (Fig- 2a, b, c, d). Our study gives an idea about the interaction between the active site residues and the substrate which is explained on the basis of size & hydrophobicity of the binding pocket. The molecules from ChemBank that showed less binding energy and showed better interactions with protein are not yet tested in the laboratory and the autoflourescence data for these molecules is not available. The extent of the work stretches to the Insilico approach for determining the binding mode. Further there is need to generate in vitro and in-vivo activity of the generated data to synthesize and test so to design drug with better specificity and metabolism.

ACKNOWLEDGEMENT

Firstly, we express our gratitude to Almighty ALLAH with great pleasure and with due decorum. We are very grateful to our trainer Mr.Syed Asif Hussain Naqvi at Biodiscovery-Solutions for future, Agra for his valuable guidance throughout our research project. We also thank Biodiscovery staff for their guidance in the research project work presented in this dissertation. It was mainly due to his effort that the current research project made possible. It would be a failure in discharging our duties if we don't express a word for our parents and siblings whose love and support has been of Monumental Proportion And all my friends, who had been a source of inspiration to us at all steps in this study.

REFERENCES

Bailey DG, Dresser GK (2004). "Interactions between grapefruit juice and cardiovascular drugs". *Am J Cardiovasc Drugs* 4 (5): 281–97. doi:10.2165/00129784-200404050-00002. PMID 15449971.

Demirayak S, Mohsen U A and Karaburun A C*, Eur J Med Chem*., 2002, 37, 255.

Domanski, T. L., Liu, J., Harlow, G. R., and Halpert, J. R. (1998) *Arch. Biochem. Biophys.* 350, 223–232

Emily E. Scott (2003) Mark A. White An open conformation of mammalian cytochrome PNAS 112 76-89. from coordinates of small molecules. *J Comput-Aided Mol Des* 10:255–262.

Goodford PJ. A computational procedure for determining energetically favorable binding sites on biologically important macromolecules. J Med Chem 1985; 28(7):849-857.

Guengerich FP (January 2008). "Cytochrome p450 and chemical toxicology". *Chem. Res. Toxicol.* 21 (1): 70–83. doi:10.1021/tx700079z. PMID 18052394. (Metabolism in this context is the chemical modification or degradation of drugs.)

Guengerich FP *et al*. *Biochem Biophys Res Commun*. 2005 338: 465 (PMID: 16126164)

Hayes C L, Spink D C, Spink B C, Cao J Q, Walker N J and Sutter T R 1996 17 beta-estradiol hydroxylation catalyzed by human cytochrome P450 1B1; *Proc. Natl. Acad. Sci. U S A* 93

Hosea, N.A. *et al*. (2000) Elucidation of distinct ligand binding sites for cytochrome P450 3A4. Biochemistry 39, 5929–5939 *J. Biol. Chem.* 273, 17036–17049

Jarvis R A and Patrick E A1973 Clustering using a similarity measure based on shared nearest neighbors; *IEEE Trans. Comput.* 22 1025–1034.

Johnson, E.F. (1992). Mapping determinants of the substrate selectivities of P450 enzymes by site directed mutagenesis. Trends pharmacol. Sci. 13:122-126.

Bathelt, C., Schmid, R.D., Pleiss, J. (2002). Regioselectivity of CYP2B6: homology modeling, molecular dynamics simulation, docking. J. Mol. Model. 8:327-335.

Jones G, Willett P, Glen R C, Leach A R and Taylor R 1997 Development and validation of a genetic algorithm for fl exible docking; *J. Mol. Biol.* 267 727–748

Joshi S H and Thaker M K, *Indian J Chem,* 2005, 44B, 410-412

Kaplan W and Littlejohn T G 2001 Swiss-PDB viewer (Deep View); *Brief. Bioinform.* 2 195–197.

Kataky J C S and Harzarika J, *Indian J Hetetrocycl Chem.,* 1999, 61, 293-296.Calmodulin- August 2003 Molecule of the Month- by Shuchismita Dutta and David S. Goodsell. Kollman, P. A. Free Energy Calculations: Applications to Chemical and Biochemical Phenomena. Chem. Rev. 1993, 93, 2395–2417.

Kroon LA (September 2007). "Drug interactions with smoking". *Am J Health Syst Pharm* 64 (18): 1917–21. doi:10.2146/ajhp060414. PMID 17823102.

Lai TY *et al. Invest Ophthalmol Vis Sci.* 2007 48: 5212 (PMID: 17962476)

Leclercq I, Desager JP, Horsmans Y (August 1998). "Inhibition of chlorzoxazone metabolism, a clinical probe for CYP2E1, by a single ingestion of watercress". *Clin Pharmacol Ther.* 64 (2): 144–9. doi:10.1016/S0009-9236(98)90147-3.

Li A *et al*. *Am J Hum Genet*. 2004 74: 817 (PMID: 15042513) Lin, H. L., Zhang, H., Waskell, L., and Hollenberg, P. F. (2003) *J. Pharmacol. Exp. Ther.*

Meunier B, de Visser SP, Shaik S (September 2004). "Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes". *Chem. Rev.* 104 (9): 3947–80. doi:10.1021/cr020443g. PMID 15352783.

Bishnoi A, Pandey V K, Saxena R, Joshi M N and Bajpai S K, *Indian J Chem*., 2002, 41(B), 1978-1979.

Meunier, B., de Visser, S.P., Shaik, S. (2004). Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes. Chem. Rev. 104:3947-3980.

Mishra P, Jakav V and Kashaw S K, *J Indian Chem Soc*., 2006, 83(1), 1157.

Morris CM, Goodsell DS, Halliday RS *et al*. Automated docking using a lamarckian genetic algorithm and and empirical binding free energy function. J Comput Chem 1998; 19:1639-1662.

Morris, G M. *et al.* (1998) Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comp Chem*. 19, 1639-1662.

Nakano M *et al. Drug Metab Dispos*. 2009 37: 2119 (PMID: 19661213)

Nelson D. "Cytochrome P450 Homepage". University of Tennessee. Retrieved 2010-05 04.

Nelson D. R. and Strobel H. W. (1988) On the membrane topology of vertebrate cytochrome P-450 proteins. J. Biol. Chem. 263: 6038–6050 of human microsomal cytochrome P450 3A4 determined by X-ray crystallography to 2.05-Å

Ortiz de Montellano, Paul R.; Paul R. Ortiz de Montellano (2005). *Cytochrome P450: structure, mechanism, and biochemistry* (3rd ed.). New York: Kluwer Academic/Plenum Publishers. ISBN 0-306-48324-6.

Pandey V K and Shukla A, *Indian J Chem.,* 1999, 38(B), 1381-1383.

Pelkonen, O., Turpeinen, M., Hakkola, J., Honkakoski, P., Hukkanen, J., Raunio, H. (2008). Inhibition and induction of human cytochrome P450 enzymes: current status. Arch. Toxicol. 82:667-715.

Bridges, A., Gruenke, L., Chang, Y. T., Vakser, I. A., Loew, G., and Waskel, L. (1998)

Poulos TL, Finzel BC, Howard AJ (June 1987). "High-resolution crystal structure of cytochrome P450cam". *J. Mol. Biol.* 195 (3): 687–700. doi:10.1016/0022-2836(87)90190-2. PMID 3656428.

Rendic, S. (2002). Summary of information on human CYP enzymes: human P450 metabolism data. Drug. Metab. Rev. 34:83-448.

Rittle J, Green MT (November 2010). "Cytochrome P450 Compound I: Capture, Characterization, and C-H Bond Activation Kinetics". *Science* 330: 933–937. doi:10.1126/science.1193478. PMID 21071661.

Roland Sigel; Sigel, Astrid; Sigel, Helmut (2007). *The Ubiquitous Roles of Cytochrome P450 Proteins: Metal Ions in Life Sciences*. New York: Wiley. ISBN 0-470-01672-8.

Sawhey S N, Gupta A and Sharma P K, *Indian J Heterocycl Chem.,* 1991, 1, 8-16.

Shabban M R, Saleh T S and Farag A M, *Heterocycl*., 2009, 78(1), 151-159.

Shen L, Shen J, Luo X, Cheng F, Xu Y, Chen K, Arnold E, Ding J, and Jiang H (2003) SteeredSingh, U.C., Peter, A., Kollman, P.A. (1984). An approach to computing electrostatic charges for molecules. J. comp. chem. 5:129-145.

Sippl MJ (1993) Recognition of errors in three-dimensional structures of proteins. *Proteins* Sligar SG, Cinti DL, Gibson GG, Schenkman JB (October 1979). "Spin state control of the hepatic cytochrome P450 redox potential". *Biochem. Biophys. Res. Commun.* 90 925–32. doi:10.1016/ 0006- 291X (79) 91916-8. PMID 228675.

Strandell J, Neil A, Carlin G (February 2004). "An approach to the in vitro evaluation of potential for cytochrome P450 enzyme inhibition from herbals and other natural remedies". *Phytomedicine* 11 (2-3): 98–104. doi:10.1078/0944-7113-00379.

Szklarz, G. D., He, Y. A., and Halpert, J. R. (1995) *Biochemistry* 34, 14312–14322

Carigo A, Lesueur B W, and Skibo E B, *J Med Chem*., 1999, 42, 3324.

Thomasco M L, Godwood C R, Weaver A E, Ochoada M J, Fard W C, Zurenko G E, Hamel C J, Stapert D, Moerman K J, Schadt D R and Yogi H B, *Bio Org Med Chem., Lett.,* 2003, 13(23),4193-4196.

Todeschini, R. and Consonni, V. (2000) Handbook of Molecular Descriptors. Wiley-VCH UCSF Chimera--a visualization system for exploratory research and analysis. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. *J Comput Chem.* 2004 Oct;25(13):1605-12.

van Aalten DM, Bywater R, Findlay JB, Hendlich M, Hooft RW, and Vriend G (1996)

Williams, P. A., Cosme, J., Ward, A., Angove, H. C., Matak-Vinkovic, D., and Jhoti, H.Wilson DJ *et al. Arch Ophthalmol*.1989 197: 213 (PMID: 2783846)

Wrighton, S.A. and Stevens, J.C. (1992) The human hepatic cytochromes P450 involved in drug metabolism. Crit. Rev. Toxicol. 22, 1–21

Yadav L D S, Singh S and Dubey S, *Indian J Chem,* 2001, 40B, 674-677.Yadav S D L and Singh S, *Indian J Chem B,* 2001, 40, 440-442.Yadav S L, Mohd Zaidi, H G and Singh N B, *Asian J Chem*., 2003, 15(3), 1805-1807.

Yonghong Zhao (2005) B.K. Muralidhara, James R. Halpert Structure of microsomal Cytochrome P450 2B4 J. biol. Chem .281 5973-77.

Zeratsky K (2008-11-06). "Grapefruit juice: Can it cause drug interactions?". *Ask a food & nutrition specialist.* MayoClinic.com. Retrieved 2009-02-09.

Zhang JW, Liu Y, Cheng J, Li W, Ma H, Liu HT, Sun J, Wang LM, He YQ, Wang Y,

Zhao, Y. *et al*. (2006) Structure ofmicrosomal cytochrome P450 2B4 complexed with the antifungal drug bifonazole: insight into P450 conformational plasticity and membrane interaction. J. Biol. Chem. 281, 5973–5981.

Chaudhary A, Willett KL (January 2006). "Inhibition of human cytochrome CYP 1 enzymes by flavonoids of St.

John's wort". *Toxicology* 217 (2-3): 194–205. doi:10.1016/j.tox.2005.09.010. PMID 16271822.

Coon.M.J.(2005) Annu. Cytochrome P 450 flexibility Rev. Pharmacol. Toxicol.vol 100 23

Danielson PB (December 2002). "The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans". *Curr. Drug Metab.* 3 (6): 561–97. doi:10.2174/1389200023337054. PMID 12369887.

De Groot, M.J. *et al*., (2004) In silico methods for predicting ligand binding determinants of cytochromes P450. Curr. Top. Med. Chem. 4, 1803–1824

Verma K, Jan K, Milan P and Jiri S, *Eur J Med Chem*., 2002, 37, 409-418.Wada Y *et al*. *Am J Ophthalmol*. 2005 139: 894 (PMID: 15860296)

Wang ZT, Yang L (2007). "Inhibition of human liver cytochrome P450 by star fruit juice". *J Pharm Pharm Sci* 10 (4): 496–503. PMID 18261370.

****