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HOMOLOGY MODELING OF CYP1A1, CYP1B1 AND ITS SUBSEQUENT MOLECULAR DOCKING STUDIES WITH RESVERATROL AND ITS ANALOGUES USING AUTODOCK TOOLS 4.0

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ABSTRACT – CYP1A1 and CYP1B1 are the inducible forms of cytochrome P450 expressed in extrahepatic tissues, which are responsible for the biotransformation of numerous exogenous compounds including carcinogens, xenobiotics and drugs. To test the interaction of exogenous compounds and CYP1A1 and CYP1B1, the unavailability of 3-D structures prompted us to construct them. Three-dimensional structures of CYP1A1 and CYP1B1 were constructed by using the software Modeller 9v9, taking the crystal structure of CYP1A2 (PDB_ID: 2HI4) as template and submitted the obtained model in the Protein Model Database (PMDB_ID of CYP1A1: PM0076866, PMDB_ID of CYP1B1: PM0076868). The validity of the CYP1A1 and CYP1B1 models was evaluated using PROCHECK, ERRAT, PROVE and WHATCHECK from Structural Analysis and Verification Server which indicated that the constructed models are respectively reliable with 95.1 % and 91.5% of the residues in the core regions of the Ramachandran's plot. The obtained models were tested and validated with Resveratrol and its analogues. The current models of CYP1A1 and CYP1B1 resulted in complete agreement with the wet lab data from these compounds. These structures will be extremely useful for in silico screening study of any compounds expected to be metabolized by these CYP isozymes.

Key words : CYP1A1, CYP1B1, Homology modeling, AutoDock Tools, Resveratrol.

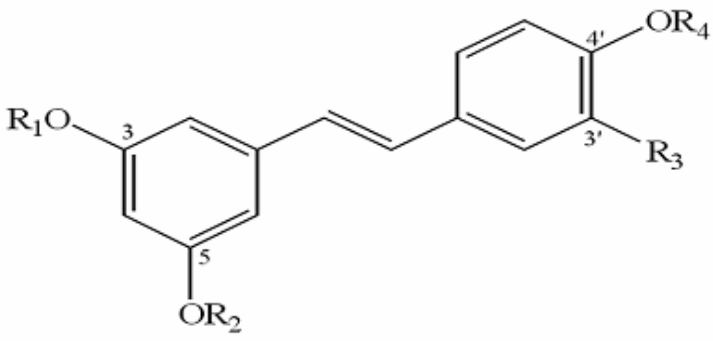
INTRODUCTION

A variety of approaches have been considered in anticarcinogenesis studies, including inhibition of the activation of procarcinogens, enhancement of detoxication pathways, interception of reactive products of carcinogens, antioxidants, and alteration of cellular signaling pathways. Several members of the P450 superfamily in conjunction with epoxide hydrolase have been shown to catalyze the metabolism of prodrugs and carcinogens. In extrahepatic tissues, CYP1A1 and CYP1B1 are thought to be the most important enzymes in catalyzing the formation of mutagenic intermediates from benzo[a]pyrene (BP) and a number of other PAHs and many compounds. CYP1B1 appears to be more active than CYP1A1 in the conversion of a number of PAHs to genotoxic intermediates (Shimada *et al*, 1996). In the presence of epoxide hydrolase, both CYP1A1 and CYP1B1 catalyze the conversion of BP to its 7,8-dihydrodiol, and both enzymes can in turn metabolically activate this BP metabolite to a mutagenic form (Shimada *et al*, 1996; Shimada *et al* 1999 and Kim *et al*, 1998). Generally the substrate specificities of the two isozymes towards various pro-carcinogens and pro-mutagens are found to be very similar, even though recombinant human CYP1A1 and CYP1B1 in their region and stereochemical selectivity for the activation of certain compounds e.g. dibenzo[*a,l*]pyrene (Shimada and Fujii-Kuriyama, 2004; Shimada *et al*, 1996 and Luch *et al*, 1999). Resveratrol has been shown to be a potent anticancer compound mainly metabolized by CYP1B1 (Chang et al, 2000). Grapes and blueberries are also significant sources of pterostilbene (Zern and Fernandez, 2005) which was demonstrated to have cancer chemopreventive activity similar to resveratrol (Auger *et al*, 2005). Resveratrol, the most studied stilbene derivative, was shown to inhibit the growth of tumor cells in several *in vitro* and *in vivo* systems (Jang *et al*, 1997 and Carbo *et al*, 1999) and was established as a chemopreventive agent in preclinical rodent models (Ignatowicz and Baer-Dubowska, 2001; Ulrich *et al*, 2005). Resveratrol's influence on the CYPs responsible for the procarcinogens biotransformation was also widely studied. These two isozymes of CYPs are responsible for the metabolism of numerous compounds and are the most important of all the CYP isozymes in activation of exogenous foreign compounds. The 3-D structures of these isozymes were not available in the PDB databases which prompted us to construct and validate these structures by testing the well known anticancer compound resveratrol and its analogues. The 3-D structures of these CYP isozymes were generated

by homology modeling using Modeller 9v9 taking the crystal structure of CYP1A2 (PDB_ID: 2HI4) as template. The newly generated 3-D structures resulted in the total alignment of wet lab data from resveratrol and its analogues. Our group has already validated similar studies with the help of Aglycones. (Akhtar *et al*, 2011).

MATERIAL AND METHODS

Homology Modeling : The sequence of CYP1A1 (Uniprot_ID: P04798, 512 Amino acids) and CYP1B1 (Uniprot_ID: Q16678, 543 Amino acids) was obtained from ExPASy proteomic server (http://www. expasy.org). The initial model of CYP1A1and CYP1B1 was built by Homology Modeling method using Modeller9v9 on window based operating system (Sali *et al,* 199*3*); a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as Probability Density Functions (pdfs) for the features restrained which includes: (i) Homology derived restraints on the distances and dihedral angles in the target sequence extracted from its alignment with template structure. (ii) Stereochemical restraints such as bond length and bond angles preferences obtained from the CHARMM22 molecular mechanics force field (MacKerell *et al* 1998). (iii) statistical preferences for dihedral angles and non-bonded inter atomic distances, from a representative set of known protein structures (Sali *et al,* 1994) and (iv) optional manually curated restraints, such as those from NMR spectroscopy, rules of secondary structure packing, cross linking experiments fluorescence spectroscopy, image reconstruction from electron



	R1	R2	R3	R4
Resveratrol	H	H	H	H
Deoxyrhapontigenin	H	H	H	CH3
Pinostilbene	H	CH3	H	H
Pterostilbene	CH3	CH3	H	H

Fig. 1 : Chemical structure of resveratrol and its analogues tested in molecular docking study with CYP1A1 and CYP1B1.

Table 1 : Docking studies of Selected Compounds with CYP1A1.

	$Wet Lab results		Insilico results	
Compounds	**IC_{50} value (µM)**	**Ki value (µM)**	**#Binding Energy (kcal/mol)**	***Ki (µM)**
Resveratrol	23	9.00	-10.58	0.15
Deoxyrhapontigenin	2.20	0.13	-9.20	1.80
Pinostilbene	1.25	0.16	-10.23	0.22
Pterostilbene	3.60	0.57	-8.70	2.80

#Calculated free energy of binding (∆G) in kcal/mol.
*Calculated inhibition constant Ki from AutoDock.
$R. Mikstacka *et al*.

Table 2 : Docking studies of Selected Compounds with CYP1A1.

	$Wet Lab results		Insilico results	
Compounds	**IC_{50} value (µM)**	**Ki value (µM)**	**#Binding Energy (kcal/mol)**	***Ki (µM)**
Resveratrol	1.40	0.80	-9.10	0.20
Deoxyrhapontigenin	2.60	2.06	-8.50	2.80
Pinostilbene	0.80	0.90	-10.50	0.19
Pterostilbene	2.10	0.91	-8.40	2.95

#Calculated free energy of binding (∆G) in kcal/mol.
*Calculated inhibition constant Ki from AutoDock.
$R. Mikstacka *et al*.

∆

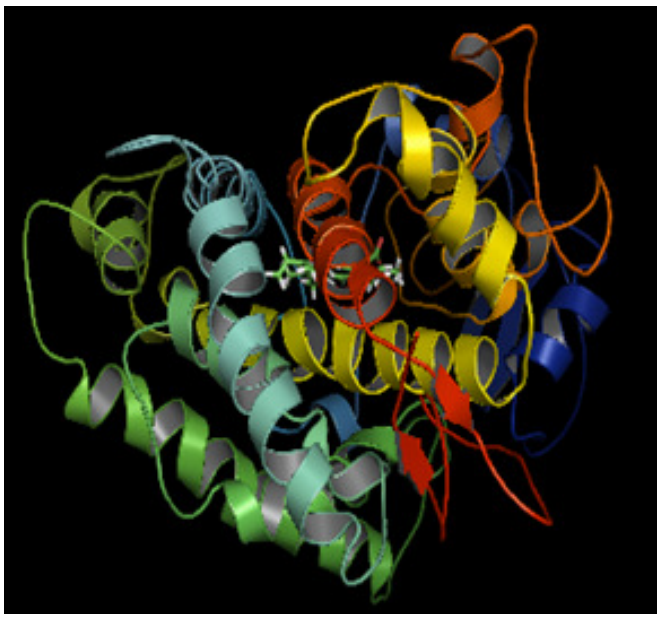


Fig. 2 (A) : Cartoon Structure of Modeled CYP1A1visualized in PyMol.

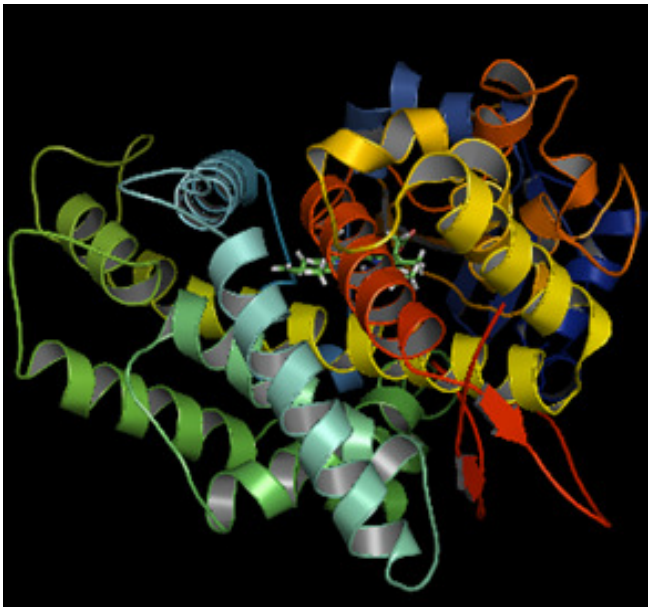


Fig. 2 (B) : Cartoon Structure of Modeled CYP1B1visualized in PyMol.

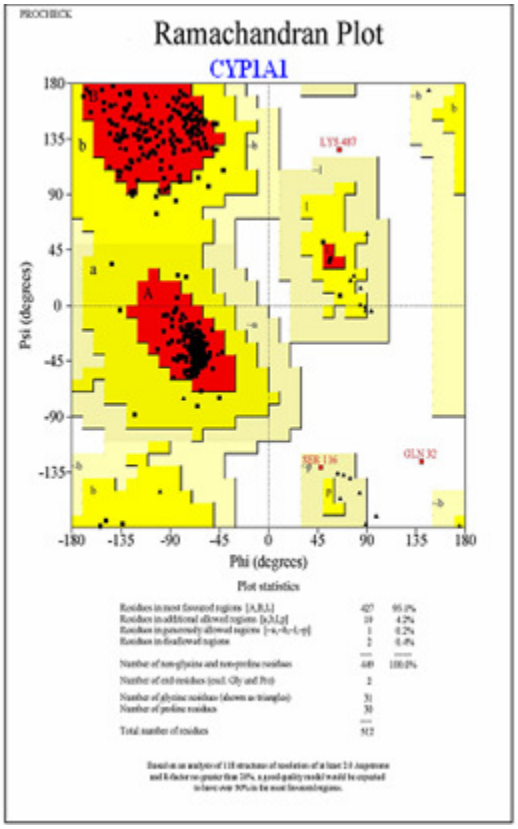


Fig 2 (C)

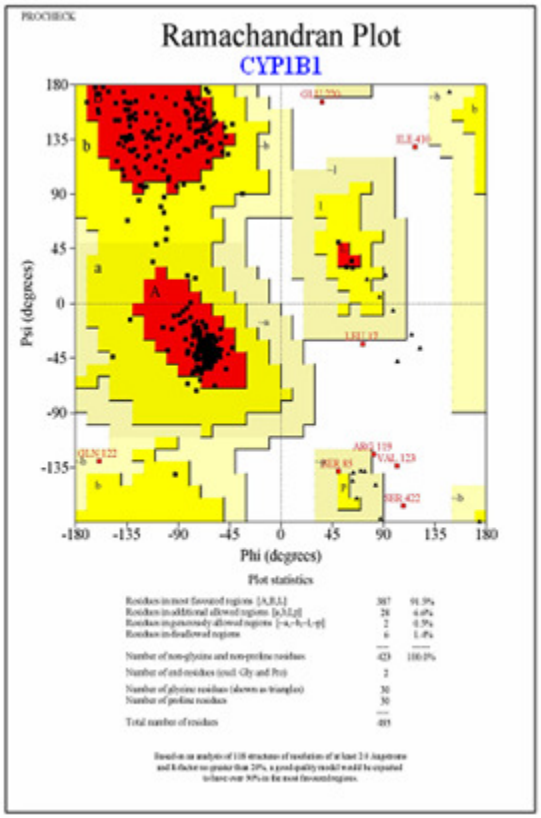


Fig 2 (D)

Modeled CYP1A1 and CYP1B1 showing 95.1% and 91.5% amino acid residues in core region of Ramachandran Plot respectively.

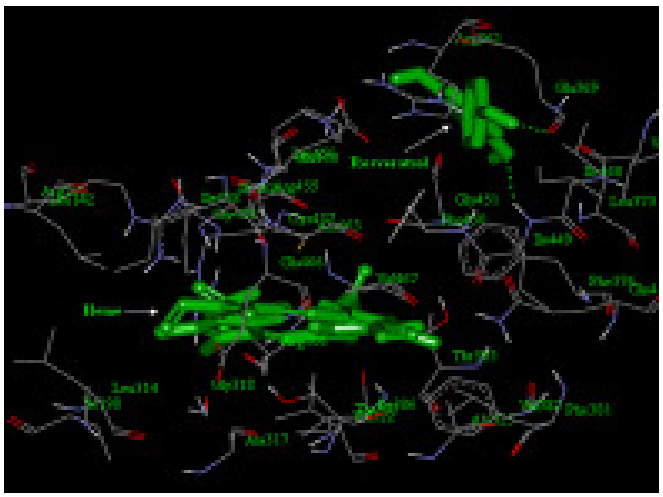


Fig 3 (A) : ILE449 and GLU369 form H-bonds with Resveratrol shown in (**…..**) line.

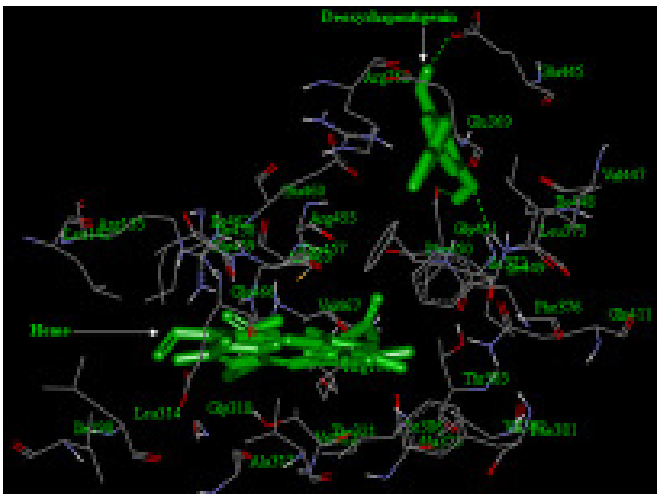


Fig 3 (B) : ILE449 and GLU369 form H-bonds with Resveratrol shown in (**…..**) line.

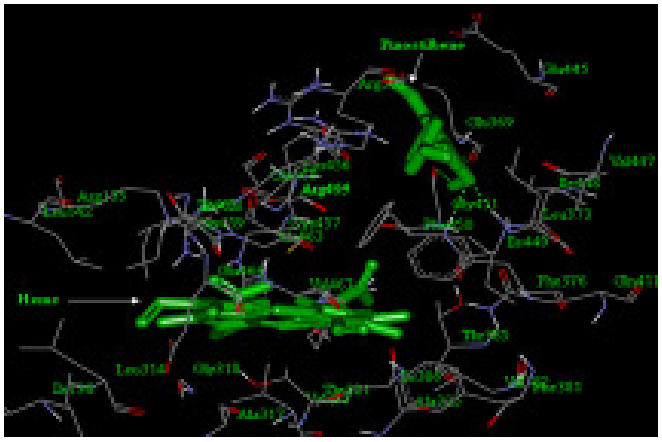


Fig 3 (C) : GLU369, ILE449, PHE450 and GLY451 form H-bonds with Pinostilbene shown in (**…..**) line.

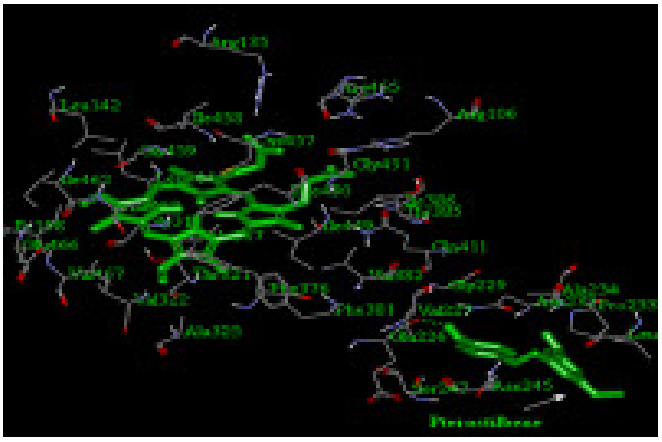


Fig 3 (D) : GLU226 form H-bond with Pterostilbene shown in (**…..**) line.

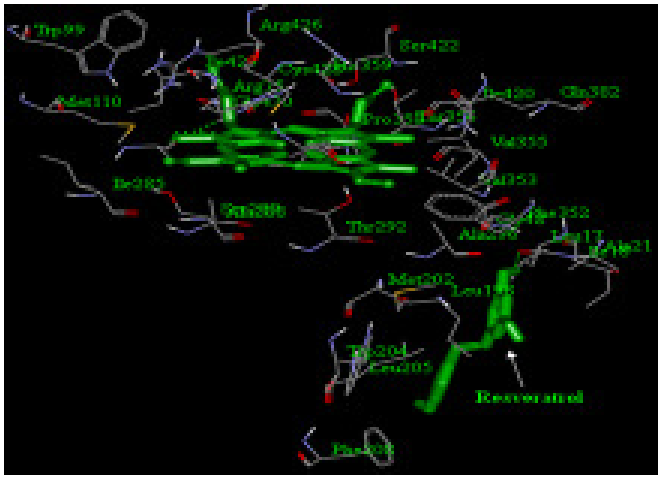


Fig 4 (A) : LEU17 form H-bond with Resveratrol shown in (**…..**) line

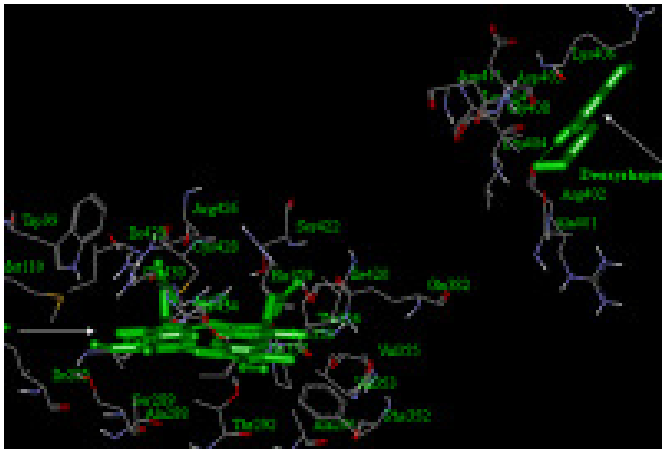


Fig 4 (B) : ARG402 form H-bond with Deoxyrhapontigenin shown in (**…..**) line

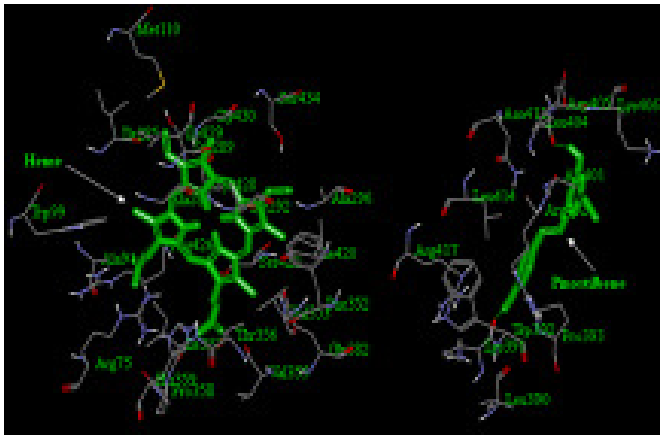


Fig 4 (C) : LYS391 form H-bond with Pinostilbene shown in (.....) line.

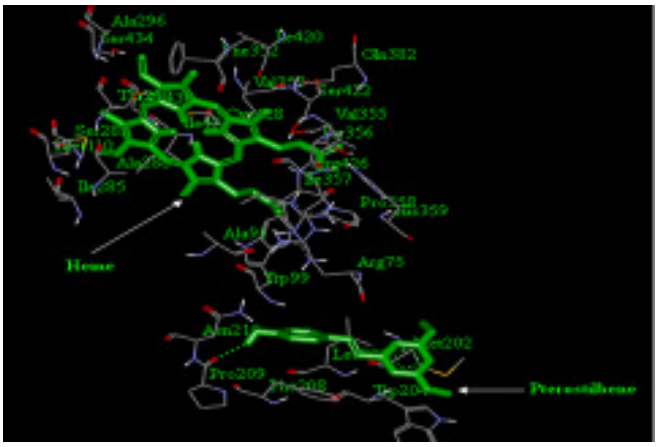


Fig 4 (D) : PRO209 form H-bond with Pterostilbene shown in (.....) line

microscopy, side directed mutagenesis and intuition. The pdfs restrain Cá- Cá distances, main chain N-O and main chain-side chain dihedral angles. The 3-D model of protein is obtained by optimization of molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as combination of pdfs restraining individual spatial feature of the whole molecule. The optimization procedure is variable target function method that applies the conjugate gradients algorithm to positions of all non hydrogen atoms. The query sequence CYP1A1 and CYP1B1 was searched to find out the related protein structure to be used as template by the BLAST (Altschul *et al*, 1990 and Altschul *et al*, 1997) against PDB (Protein Data Bank). Sequence that showing maximum identity with high score and less E-value with related family was aligned and used as reference structure to build a 3-D model of CYP1A1 and CYP1B1 respectively. The co-ordinates structurally conserved regions for CYP1A1 and CYP1B1 were assigned from the template (PDB_ID: 2HI4) using pairwise sequence alignment based on the Needleman-Wunsch algorithm (Needleman *et al*, 1970 and Thomson *et al*, 1994). After construction of 3-D model of CYP1A1 and CYP1B1 heme group was introduced into the protein to occupy the same position as heme of the template protein CYP1A2. Finally energy minimization of the constructed structures was performed until the energy gradient was lower than 0.1Kcal/mole Å using CharMM force field. The validity of the CYP1A1and CYP1B1 models was evaluated using PROCHECK, ERRAT, PROVE and WHATCHECK from Structural Analysis and verification Server (http://nihserver.mbi.ucla/edu/SAVES/).

Docking Simulations :

Docking experiments were performed using the AutoDock Tools 4.0 (Morris *et al*, 1996; Morris *et al*, 1998; Goodsell and Olson 1990) the most commonly cited docking program in the scientific literature (Sousa et al, 2006) using genetic algorithm approach to find the preferred binding conformations of the ligand in the receptor. The Docking methodology involved the preparation of receptor and ligand molecules, docking using a Search algorithm and analysis of the binding conformation using a scoring function.

Receptors: Modeled structure of CYP1A1 (submitted in PMDB, PM0076866) and CYP1B1 (submitted in PMDB, PM0076868) were used as receptors. The missing residues were corrected and the PDB files were energy minimized using GROMACS (http://www.gromacs.org/). Further, Kollman charges were added and polar hydrogen's were merged.

Ligands: The molecular formula and SMILES notations for the resveratrol and its analogues deoxyrhapontigenin, pinostilbene and pterostilbene were obtained from PubChem database (Wang *et al*, 2009). The 3D structures were built using the online demonstration of CORINA (http://www.molecular-networks.com) for generating 3D coordinates. The ligand files were minimized and converted into .pdb format.

Grid parameters file: Based on previously reported literature on structural information of CYP 1A1 and CYP 1B1, the active-site regions for the comparative AutoDock simulations of resveratrol and its analogues (Fig 1) were used for grid construction. The grids were sketched as such that the ligand was allowed to rotate freely inside the grid.

Docking parameter file: The preparation of dpf file involved the adjustment of the genetic algorithm parameters in terms of "Number of genetic algorithm runs", "Crossover frequency" and "Mutation rates" which were set to default values. The experiments were

repeated a number of times with 20 generations in each run to improve the precision level of result.

The generated docked structures were further minimized in the end. The interactions were finally studied in terms of binding energy (kcal/mol) and inhibition constant (μM) along with the number of hydrogen bonds formed with the surrounding amino acid residues. The figures of the best docked solutions of all ligands with CYP 1A1 and CYP 1B1 were generated using the Accelrys Discovery Studio Visualizer 2.5.5.

RESULTS AND DISCUSSION

The constructed models of CYP1A1and CYP1B1 are shown in Fig. 2A and B). The N-terminal regions of CYP1A1 (Met1-Pro31) and CYP1B1 (Met1- Gln42) were not constructed because the co-ordinates of the corresponding region of the CYP1A2 (Met1-Pro33) were not determined. We evaluated the model structure by the PROCHECK, ERRAT, PROVE and WHATCHECK from Structural Analysis and verification Server (http://nihserver.mbi.ucla/edu/SAVES/). A Ramachandran plot showed that 95.1 % of the residues in the CYP1A1 and 91.5 % of the residues in CYP1B1are in either the most favored regions Fig. 2C and D. The results were quite promising.

Furthermore, our study clearly brings out the specific interaction and inhibition potential of Resveratrol and its analogues with CYP1A1 and CYP1B1. The work further validates the inherent potential of AutoDock Tools in producing results parallel to wet lab data. Table 1 clearly shows the docking results of Resveratrol and its analogues with CYP 1A1, which were in consensus with the wet lab results (Mikstacka et al, 2007). Resveratrol produced maximum binding energy of -10.58 kcal/mol followed by Pinostilbene of -10.23 kcal/mol, Deoxyrhapontigenin of -9.20 kcal/mol and at last Pterostilbene of -8.70 kcal/mol (Table 1). The IC_{50} value obtained in the wet lab of all the four compounds were further very much similar to Ki values obtained in the docking results. The docked complexes when visualized through Accelrys DS visualizer showed the selected compounds present near the heme group of the protein and surrounded with GLU226, GLU369, GLU445, ILE449, PHE450 and GLY451 key amino acid residues Fig. 3A, B, C and D.

Similarly, when the same set of compounds were docked with CYP1B1, Pinostilbene emerged as the most potent inhibitor producing maximum binding energy of -10.50 kcal/mol, followed by Resveratrol of -9.10 kcal/mol, Deoxyrhapontigenin of -8.50 kcal/mol; Pterostilbene produced -8.40 kcal/mol binding energy (Table 2). Similarly, the Ki values obtained in our docking results were again found to be similar with wet lab results (Mikstacka *et al*, 2007]. The docked complexes further revealed the selected compounds again present near heme group of CYP1B1, surrounded by LEU17, PRO209, LYS391 and ARG402 amino acid residues from the top and MET202, LEU205, PHE205 amino acid residues from the bottom Fig. 4A, B, C and D.

The results obtained in our study purely correlates with the already available wet lab data. It further emphasizes on the key amino acid residues involved in the interaction of inhibitors with the CYP's under study. The binding potential/ inhibition potential of Resveratrol and its analogues against CYP1A1 and CYP1B1 have been also validated in our study. The work has additional advantage in the manner that though the interaction of Resveratrol and its analogues has been studied in wet lab, but due to the absence of crystallized structure of CYP1A1 and CYP1B1, the molecular level interactions were still been the subject of query.

Our homology modeled CYP1A1 and CYP1B1 structures provide us a useful interface for carrying out studies for quick screening of ever increasing library of newly isolated and synthesized compounds. Moreover the consensus in the results obtained in our insilico docking studies of model Resveratrol and its analogues with already available wet lab data further validates and signifies the importance of work in pharmaceutics. The aforementioned work opens the gateways for screening of newer compounds against CYP1A1 and CYP1B1.

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