Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(1):756-763

Elucidation and Validation of Metabolic Activation of Human Mammary Carcinogen Dibenzo[*a,l*] pyrene and its Metabolites Using *In silico* Approach

M. Kalim Ahmad Khan[1], Salman Akhtar[1], [1,3]Mohammed Haris Siddiqui, Othman A. Al-Sagair[2] and Jamal M. Arif[1, 2,*]

[1]*Department of Biotechnology, Microbiology and Bioinformatics, Integral University, Lucknow, India*
[2]*University of Hail, Hail P.O. Box 2440, Hail, KSA*
[3]*School of Life Sciences, Jawaharlal Nehru University, New Delhi*

ABSTRACT

The routine testing of any carcinogen are generally performed in the cell free, cell culture and animal models, however, due to involvement of specific isozymes of metabolic activation and detoxification enzymes, these tests become extremely time consuming and expensive in testing metabolic activity of any carcinogens or drugs. The emergence of the In silico approach offers easier, quick, relatively inexpensive and authentic tools to predict potent analogs of a compound, however, it needs validation of the wet lab elucidated specific pathways for activation and detoxification. Dibenzo [a, l] pyrene (DBP), the most potent human carcinogen requires metabolic activation to its reactive diol-epoxides by cytochrome P450 (CYP) isozymes and epoxide hydrolase. The present study tested interactions of DBP, DBP-11, 12-diol (DBPD) with various isozymes of CYP in order to elucidate and validate the wet lab findings using the simple In silico approach. The CYP1A1, 1B1, 2C9 and 2B6 reported in wet lab studies to actively metabolize DBP which also showed strong binding energies (Kcal/mol) of -11.50, -10.67, -10.37 and -9.76, respectively with inhibition constants ranging between 0.005-0.070 µM. The CYP3A4 showed less binding energy (-9.51 Kcal/mole) which is in alignment with the wet lab reports. Further, better affinity of CYP1A1 and CYP1B1 with the DBPD might be indicative of their involvement in its subsequent conversion to DBPDE. Our results for the first time has In silico validated the metabolic pathways of DBP reported in the wet lab studies and provides a novel platform to study and elucidate the metabolic pathways and mechanism(s) of carcinogens, pharmaceutical drugs and xenobiotics in quick, reliable and economic manner.

INTRODUCTION

For any newly isolated and synthesized compounds or drugs, elucidation of their biological potentials is exclusively important. Phase I and Phase II drug metabolizing enzyme systems are largely involved in metabolism of almost all the exogenous compounds entering into the

cells [1]. For any carcinogen or drug to exert their mutagenic, toxic or carcinogenic effect, the activation of phase I and suppression of phase II enzymes occurs.[2] Due to diverse classes of drugs, xenobiotics, mutagens and carcinogens, a variety of specific isozymes of CYPs were evolved in human and other living organisms [3].

Currently used routine approaches such as high throughput screening, cell free system, animal and human cell lines and animal models in testing any compounds are extremely time consuming and require expensive infrastructure which may restrict testing of large number of compounds in mechanistic details. Further, a number of new drugs have to be continuously developed to combat multidrug resistance. In addition, for cancer treatment, there is also a need of new and customized anticancer drugs with the least associated risk. In the past several decades, there have been numerous drugs, compounds, toxicants, xenobiotics and carcinogens which have been well studied with elucidation of their mechanism(s) of activation and detoxification in detail [4]. Different biological activities of a compound could well be simultaneously screened using the *In silico* approach if the metabolic activation and detoxification pathways have been *In silico* elucidated and validated. Thus far, no study can be found in the literature which thoroughly elucidated and validated the wet lab studied pathways for any compound using *In silico* tools which recently has been emerged as an excellent substitute to the wet lab studies.

In our approach we have chosen the most potent human breast carcinogen, dibenzo[a,l]pyrene (DBP) (Fig 1A) which has been extensively studied in various in vitro and in vivo studies for its genotoxic, mutagenic and carcinogenic potentials. [5-7] Bioactivation of DBP to DNA-reactive species involves metabolic activation to either diolepoxides through cytochrome P450 (CYP)-dependent monooxygenases and epoxide hydrolases or to reactive metabolites by the one-electron oxidation pathway. [8-12] DBP is a hexacyclic aromatic hydrocarbon, having both a bay region and a hindered bay region (Fjord region), because of an additional aromatic ring compared with benzo[*a*]pyrene (BP), and therefore, it can be metabolized into three different kinds of sterically hindered diolepoxides, the 11,12-diol-13,14-epoxides, the 1,2-diol-3,4-epoxides and the 3,4-diol-1,2-epoxides. [13] However, the 11,12-diol-13,14-epoxide was the most reactive of all the epoxides known so far. [14,15] DBP has been shown to be activated by CYP1A1, 1B1, 2B6 and 2C9 in cell free as well as MCF-7 cells to diastereomeric ll,12-diol-13,14-epoxides.[16-18]

Our goal here was to systematically investigate DBP metabolism by various CYP isozymes using molecular docking studies. The promising results obtained for the first time have opened up future gateways for quick, reliable and inexpensive testing of biological potential of any compound, drug, xenobiotics and carcinogens.

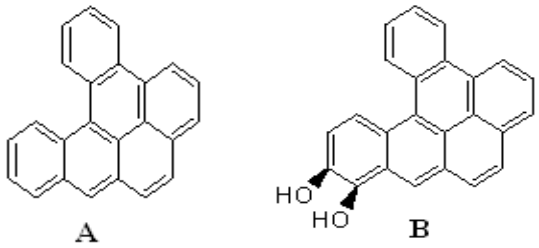

Fig 1 (A) Chemical structures of DBP (B) Chemical structures of DBPD

EXPERIMENTAL SECTION

Docking Simulations:
Docking experiments were performed using the AutoDock Tools 4.0 [28,29,30] the most commonly cited docking program in the scientific literature[19] 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: Structures of CYP's [(CYP1A1homology modeling (submitted in PMDB, PM0076866), CYP1B2 homology modeling (submitted in PMDB, PM0076868), CYP1A2 2HI4, CYP2B6 3IBD, CYP 2C9 1R9O, CYP3A4 2V0M, were retrieved from BrookHaven Protein Data Bank (www.pdb.org)[20] and SwissModel Repository [21]. The missing residues were corrected and the complexes bound to receptor molecule were removed using Accelrys Discovery Studio Visualizer 2.5.5. The PDB files were energy minimized using GROMACS [22]. The non-essential water molecules were removed and polar hydrogen's were merged.

Ligands: The molecular formula and SMILES notations for the DBP and its diol metabolite DBPD (Fig1B) was obtained from Pubchem database[23] (http://pubchem.ncbi.nlm.nih.gov). The 3D structures were built using the online demonstration of CORINA [24] 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's, the active-site regions for the comparative AutoDock simulations of DBP and its metabolites 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's were generated using the Accelrys Discovery Studio Visualizer 2.5.5.

Approach: BP was shown to be metabolically activated to DBP-11, 12-diol (DBPD) which is subsequently oxidized to DBPDE by the CYP isozymes in various in vitro and in vivo models. Our approach was to dock various CYP isozymes with the DBP and DBPD.

RESULTS AND DISCUSSION

The *In silico* approach is becoming an essential tool in the inexpensive preliminary analysis of novel drugs, compounds, analogs against various molecular targets. However, this approach is still in its infancy and requires the elucidation and validation of numerous biological pathways using well identified molecular targets of specific pathways. We have taken a lead in this direction by elucidating and validating the *In silico* pathways for various

molecular mechanism(s) involved in the process of carcinogenesis and cancer chemoprevention.[25]The present study is also the first report where the interactions of DBP and its diol (DBPD) with various isozymes of CYPs validated the wet lab studies of DBP activation in various model systems.

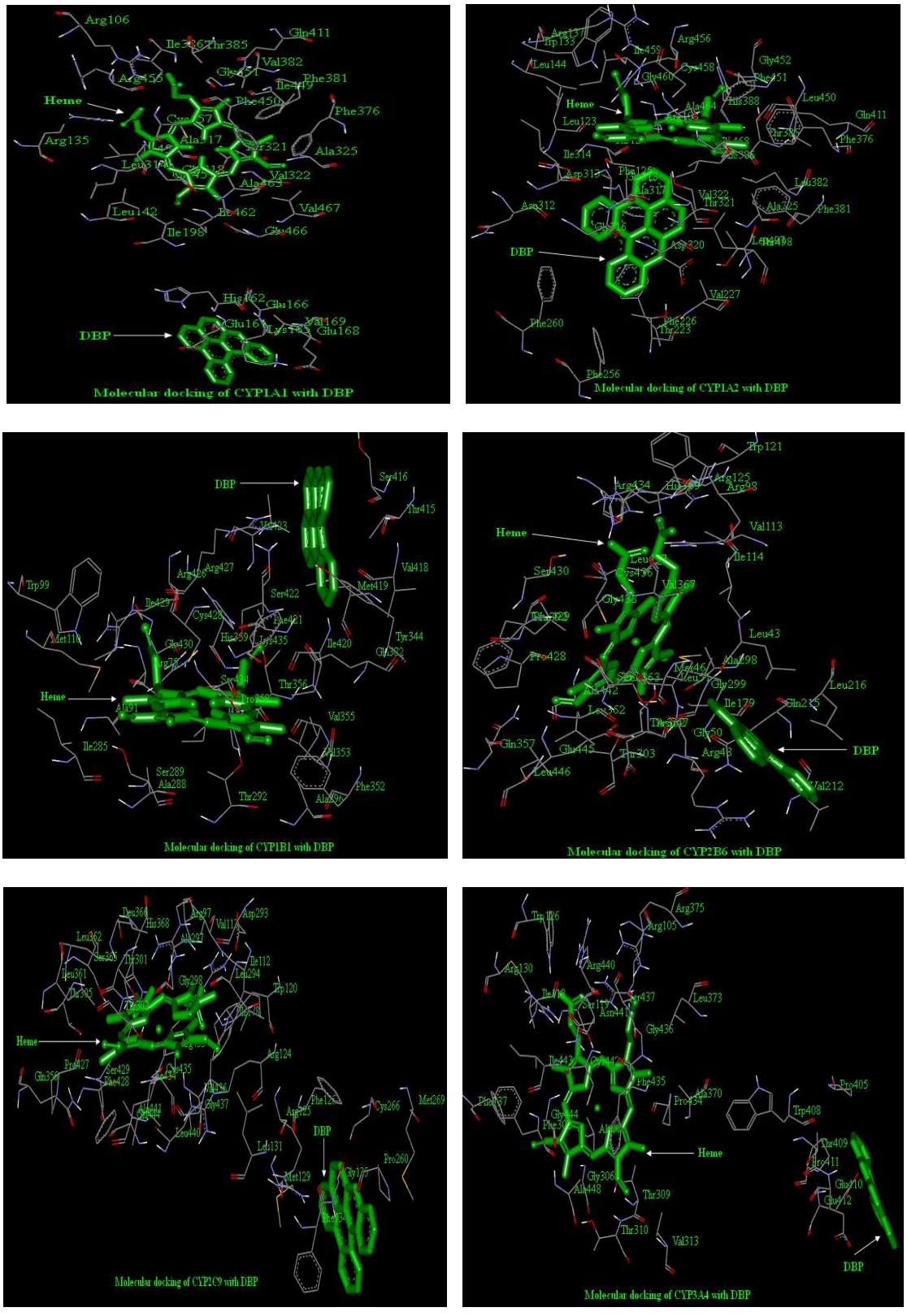

Fig 2 Docked structures of DBP with CYP's

CYP 1A1 [18] is reported to emerge as the most active form in the metabolism of DBP, in comparison to other isozymes. The work further stated the order of activity in the formation of DBPD as 1A1>2C9>1A2>2B6>3A4>others [18]. In consensus to this, our insilico results were able to produce exactly similar results, with DBP interacting 1A1 with maximum binding energy of -11.50 kcal/mol followed by 2C9 (-10.37 kcal/mol) and at last 3A4 with minimum binding energy of -9.51 kcal/mol. Another wet lab data (Schober et.al.) quotes the similar EC50 values of CYP 1A1 and CYP 1B1 in human cells [26], which was even visible in our results with 1B1 producing the binding energy of -10.67kcal/mol, close to CYP 1A1. Further the inhibition constants for DBP activation by CYP1A1 and 1B1 of 0.005 and 0.015 µM confirms the above reports. The docked complexes further helped us to analyze the key amino acids of CYP' s to be involved in the interaction with DBP. As for example DBP was found to be docked near the heme group of CYP1A1 surrounded by HIS162, GLU161, LYS165 and VAL169 amino acid residues. Similarly, DBP was found surrounded with THR321, VAL322, ASP320 and PHE125 amino acid residues in interaction with CYP1A2. DBP which is in least interaction with CYP3A4 was also found to be docked in close vicinity to PRO405, TRP408, THR404 and GLU412 amino acid residues. Moreover, DBP owing to its closed structure was involved in none of the Hydrogen bond interactions with any of the CYP's.

Table 1: Docking study of DBP with CYP isozymes

	#Binding Energy (Kcal/mole)	***Inhibition Constant (µM)**	**No. of H-bonds**
DBP_CYP1A1	-11.50	0.005	NIL
DBP_CYP1A2	-9.72	0.079	NIL
DBP_CYP1B1	-10.67	0.015	NIL
DBP_CYP2B6	-9.76	0.070	NIL
DBP_CYP2C9	-10.37	0.025	NIL
DBP_CYP3A4	-9.51	0.073	NIL

#Calculated free energy of binding (ΔG) in kcal/mol.
**Calculated inhibition constant Ki from AutoDock Tools 4.0.*

Further, the DBPD with the help of specific CYP isozymes subsequently oxidized to the ultimate carcinogenic DBPDE[27]The insilico results showed the similar interaction of DBPD and CYP1A1 with the maximum binding energy of -10.56 kcal/mol, followed by 1B1 which showed -10.42 kcal/mol. The CYP3A4 again showed the least affinity with minimum binding energy of -8.71 kcal/mol.

Table 2. Docking study of DBP-11,12-diol (DBPD) with CYP isozymes

	#Binding Energy (Kcal/mole)	***Inhibition Constant (µM)**	**No. of H-bonds**
DBPD _CYP1A1	-10.56	0.023	01
DBPD _CYP1A2	-9.01	0.249	NIL
DBPD _CYP1B1	-10.42	0.023	02
DBPD_CYP2B6	-8.92	0.350	01
DBPD_CYP2C9	-9.30	0.153	02
DBPD_CYP3A4	-8.71	0.411	NIL

#Calculated free energy of binding (ΔG) in kcal/mol.
**Calculated inhibition constant Ki from AutoDock Tools 4.0.*

The inhibition constants of 0.023 µM for both CYP1A1 and 1B1 further showed that these two isozymes are actively involved in the activation of DBPD to the DBPDE. The docked

complexes further reveal DBPD present near the LEU420, TRP421, VAL422 and ASP440 amino acid residues of CYP1A1, with the formation of 1 hydrogen bond with ASP440. DBPD was also found surrounded with GLY58, TYR495, GLY233 and PRO235 amino acids in interaction with CYP1A2. Likewise, CYP1B1's LYS100, VAL101, ARG104 and SER266 amino acid residues were found to be involved in key interaction with DBPD. The binding mechanism and key amino acid interactions of the two CYP's with the compounds under study further helped us to gain the insight mechanism and validation of the insilico studies.

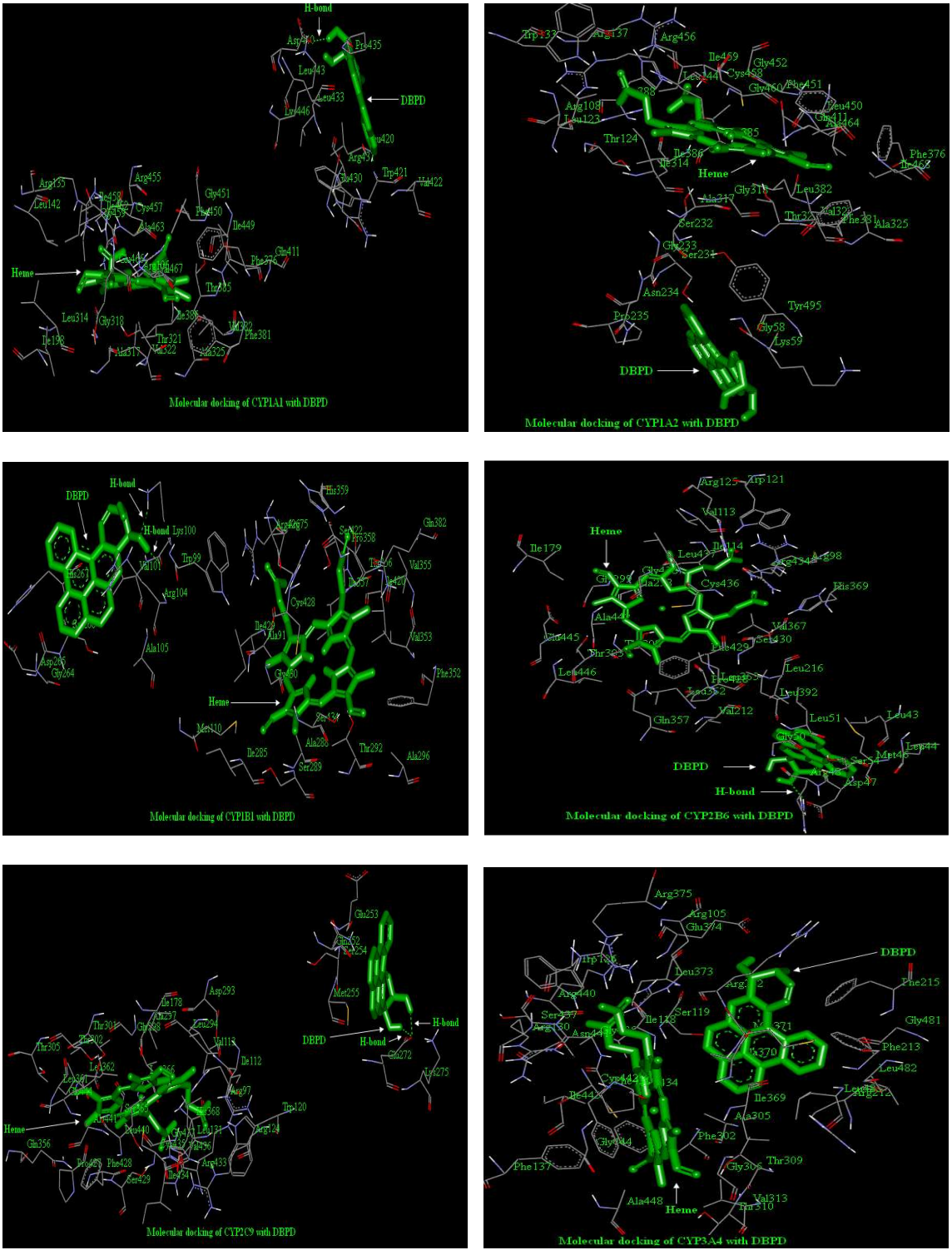

Fig 3 Docked structures of DBPD with CYP's

The insilico results obtained in our study are in complete agreement with the reported wet lab studies in the activation of DBP to its metabolites. The validation of the wet lab results on the insilico platform not only strengthens the novel hypothesis to use the *In silico* approach studying the metabolic activation of various carcinogens, xenobiotics and other drugs but also opens the gateway for the study of other similar carcinogens. The proposed study of the selective affinity of different carcinogens to various receptors on insilico platform may not only reduce the time and cost of the expensive experimental set up but will also provide the opportunities to validate other cellular pathways important in the screening of various drug candidates. Our group is actively engaged in elucidation and validation of other cellular pathways to study carcinogens and newly isolated or synthesized medicinal compounds.

Acknowledgments
The authors would like to acknowledge Prof. S. W. Akhtar, Vice Chancellor of the Integral University, Lucknow, India for providing the infrastructure facility to carry out this study. Thanks to Dr M. Imran Siddiqui, Scientist, Central Drug Research Institute, Lucknow, India for the valuable and critical comments.

REFERENCES

[1] Xu,C., Li,C.Y., Kong,A.N.,*Arch Pharm Res.*,28(3):249-68.
[2] Arif, J.M., and Gupta, R.C. **(1996)**,*Int. J. Oncol* .,8: 681-685
[3] Nagata,K.and Yamazoe,Y.**(2002)**,*Drug Metab.Pharmacokinet.*,17:167-189
[4] Shimada T.**(2006)**, *Drug Metab Pharmacokinet.*,21(4):257-76.
[5] Arif, J.M., Smith, W.A. and Gupta, R.C.**(1997)**,*Mut. Res.*,378: 31-39
[6] Gupta, R.C., Arif, J.M. and Gairola, C.G.**(1999)**, *Mut. Res.*, 425: 195-205
[7] Smith, W.A., Arif, J.M. and Gupta, R.C.**(2001)**, *Int. J. Cancer.*,91: 132-136
[8] J.L. Bolton, M.A. Trush, T.M. Penning, G. Dryhurst, T.J.**(2000)**,*Chem. Res. Toxicol*., 13:135–160.
[9] Ralston,S.L., Lau,H.H.S., Seidel,A., Luch,A., Platt,K.L. and Baird,W.M.**(1994)**,*Cancer Res.*, 54, 887–890.
[10] Shou,M., Krausz,K.W., Gonzalez,F.J. and Gelboin,H.V. **(1996)**,*Carcinogenesis*., 17, 2429–2433.
[11] Arif,J.M. and Gupta,R.C.**(1997)**,*Carcinogenesis.*, 18, 1999–2007.
[12] Li,K.-M., Todorovic,R., Rogan,E.G., Cavalieri,E.L., Ariese,F., Suh,M., Jankowiak,R. and Small,G.J. **(1995)**,*Biochemistry.*, 34, 8043–8049.
[13] Ralston,S.L., Seidel,A., Luch,A., Platt,K.L. and Baird,W.M. **(1995)**, *Carcinogenesis.*, 16, 2899–2907.
[14] Krzeminski,J., Lin,J.-M., Amin,S. and Hecht,S.S. **(1994)**,*Chem. Res. Toxicol*., 7, 125–129
[15] Gill,H.S., Kole,P.L., Wiley,J.C., Li,K.-M., Higginbotham,S., Rogan,E.G. and Cavalieri,E.L.**(1994)**,*Carcinogenesis*, 15, 2455–2460.
[16] Peter,G.F, Chun,Y.J., Kim,D., Gillam,E.M., Shimada,T.,**(2003)**,*Mutat Res*.,523-524:173-82.
[17] Arif,J.M.,Hazzani,A.A.A.,Kunhi,M., and Khodairy,F.A.**(2004)**,*J Biomed Biotechnol*., 2004(2): 93–98.
[18] Shou,M., Krausz,K.W.,Gonzalez,F.J., Gelboin HV.**(1996)**,*Carcinogenesis*.,17(11):2429-33.
[19]Sousa, S.F.,Fernandes, P.A., Ramos, M.J.**(2006)**,*Proteins*.,65:15-26.
[20] The Brookhaven Protein Data Bank website. http://www.rcsb.org [23 September 2010]

[21]SWISS-MODEL Repository website. http://swissmodel.expasy.org/repository/ [22 October 2010]
[22]GROMACS, Version 3.3; Groningen, Netherlands, 2007.
Available from http://www.gromacs.org/
[23] Wang, Y., Xiao, J., Suzek, T.O., Zhang, J., Wang, J., Bryant, S.H.**(2009)**, *Nucleic Acids Res*.,37:W623-633.
[24]The 3D structure generator CORINA is available from Molecular Networks GmbH, acErlangen, Germany (http://www.molecular-networks.com). [25 March 2010].
[25] Akhtar, S.,Al-Sagair, O.A. and Arif, J.M.**(2010)**,*Letters in Drug Design & Discovery*, [BSP/LDDD/E-Pub/00116]
[26] Schober, W.,Luch, A., Soballa, V.J., Raab, G., Stegeman, J.J., Doehmer, J., Jacob. and Seidel,J.A.**(2006)**, *Chemico-Biological Interaction*., 161:37-48
[27]Ralston,S.L., Coffing,S.L., Seidel,A., Luch,A., Platt,K.L., Baird,W.M.**(1997)**,*Chem Res Toxicol*., 10 (6):687-93.

Abbreviations

DBP: Dibenzo[a,l]pyrene.
DBPD: Dibenzo[a,l]pyrene-11,12-diol.
DBPDE: Dibenzo[a,l]pyrene-11,12-diol-13,14-epoxide..
CYP: Cytochrome P450.