ORIGINAL ARTICLE

Targeting Cytochrome P450 1A1 Enzyme with Crucifer Phytocomponents: An *In Silico* Approach for Chemopreventive Drug Design against Lung Cancer

L. Inbathamizh[1], K. Shyamala Devi[2], S. Sri Vaishnavi Taarikaa[1], P. Mithula[1], K. N. Vennela[1]

[1]Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India, [2]Department of Zoology, Presidency College, Chennai, Tamil Nadu, India

Abstract

Introduction: Metabolism of carcinogens plays a key role in cancer. Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) is one of the main cytochrome P450 enzymes. It is involved in the activation of compounds such as polycyclic aromatic hydrocarbons with carcinogenic properties associated with lung cancer. Inhibition of enzymes that activate carcinogenesis is a major strategy of chemoprevention. Thus, CYP1A1 is selected as the target protein. Crucifer vegetables offer a promising source of phytochemicals that are emerging as strong contenders in the arena of cancer chemoprevention and thus can be analyzed as ligands to the selected target. **Aim:** The study is an attempt to find an *in silico* solution to lung cancer by CYP1A1 inhibition-based chemoprevention using crucifer phytocomponents. **Materials and Methods:** Bioinformatics databases and tools are used. The study involves the structural analysis of CYP1A1 and its interaction with crucifer ligands using Modeller 9V2 and AutoDock, respectively, as the prominent software. Pharmacokinetic properties of the ligands are also predicted with ACD/I-Lab 2.0 modules. **Results:** The findings indicated effective active site interactions of CYP1A1 with the crucifer phytocomponents. Glucosinolate was found to be the best inhibitory ligand with docking energy −16 KJ/mol and 9 hydrogen bonds. The compounds also exhibited preferable drug properties. Indole-3-carbinol and 3, 3'-Diindolylmethane seemed to possess more druglikeness comparatively. **Conclusion:** From the study, it can be concluded that crucifer phytocomponents can act as natural, safe, and potent drug candidates in anti-lung cancer drug design.

Key words: Chemoprevention, crucifer phytocomponents, CYP1A1, drug design

INTRODUCTION

Lung cancer is the prevalent type of cancer afflicting both men and women. It has turned out to be the most common cause of several million cancer-related deaths worldwide. The World Health Organization has stated that prolonged exposure to tobacco smoke and carcinogenic polycyclic aromatic hydrocarbons (PAHs) poses to be the major cause of lung cancer.

Cytochrome P450s constitute a category of haem-containing enzymes.[1] The family 1 enzymes of this category play a major role in the activation of PAHs.[2] Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) is a member of the cytochrome P450 superfamily of enzymes.[3] CYP1A1 is involved in Phase I xenobiotic and drug metabolism. It is also known as aryl hydrocarbon hydroxylase (AHH) whose expression is regulated by a ligand-activated transcription factor, aryl hydrocarbon receptor (AHR).[4] Earlier reports have related the expression of both AHR and CYP1A1 to smoking in lung adenocarcinoma patients.[5]

Address for correspondence:
L. Inbathamizh, Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Chennai - 600 119, Tamil Nadu, India. E-mail: inbathamizh.l@gmail.com

Received: 05-12-2019
Revised: 24-01-2020
Accepted: 02-02-2020

Polymorphisms in CYP1A1 have been associated with more highly inducible AHH activity and positively correlated with the occurrence of cancer. These polymorphisms include substitution of A→G at nucleotide 2455, resulting in an amino acid change of isoleucine to valine at codon 462.[6] Similarly, amino acid change of threonine to asparagine at codon 461 is due to C→A substitution at nucleotide 2453.[7]

CYP1A1 catalyzes the conversion of various PAHs to highly reactive products that can cause oncogenic mutations and carcinogenesis.[8,9] Oxidation of Benzo[a]pyrene, a completely ubiquitous PAH found in tobacco smoke, is catalyzed by CYP1A1 to Benzo[a]pyrene-7,8-epoxide. This can be further converted to form Benzo[a]pyrene-7,8-dihydrodiol by Epoxide hydrolase. Ultimately, this intermediate is catalyzed by CYP1A1 to Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide, which is the consequent carcinogen.[10]

Carcinogenic potential of CYP1A1 in the activation of PAHs has been well established both *in vitro* and *in vivo* investigations. The role of CYP1A1 in the activation of aflatoxin B1, a carcinogenic mycotoxin present in foodstuffs, to its corresponding 8, 9-epoxide in rabbit lung and liver has been documented. Studies in transgenic strains have demonstrated the role of CYP1A1 in Benzo[a]pyrene-induced carcinogenesis with an increase in CYP1A1 expression following PAHs treatment.[11-13] CYP1A1 is also involved in catalyzing hydroxylation of heterocyclic amines such as 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, the most abundant heterocyclic amine in cooked meat and fish,[14] and tobacco-related N-nitrosamines that induce carcinogenesis.[15,16] CYP1A1 with such immense carcinogenic potential serves as an effective target in designing significant anticancer drugs and hence chosen for the study.

Recently, cancer prevention and treatment through naturally available phytoconstituents is receiving significant attention. Cruciferous vegetables that include broccoli, Brussels sprouts, cabbage, cauliflower, kale, turnips, collard greens, kohlrabi, and mustard rutabaga have a number of nutrients and phytochemicals with cancer chemopreventive properties. Besides providing basic nutrition, they add substantial health benefits.[17-19] A positive correlation is found to exist between cancer prevention and consumption of cruciferous vegetable bioactives.[20]

In silico tools that are fast, safe, and cost efficient are of great value to investigate the impact of such compounds at an early stage of drug development. The current study focuses on the application of such tools and databases in target-based therapy for lung cancer, with CYP1A1 as the target, and its interaction with crucifer bioactives, for designing potent chemopreventive drugs.

MATERIALS AND METHODS

Structural analysis of the target protein

Biological databases and bioinformatics tools were used for the study.[21,22] Human CYP1A1 protein was chosen as the drug target after studying its role in carcinogenesis. The sequence of the target protein (Accession No: P04798) was retrieved from UniProt database.[23] The 3D structure analysis for CYP1A1 was carried out using BLAST[24] and modeled by Modeller 9V2[25] with the programs Align2d and Model-default. The predicted structure was visualized by RasMol,[26] validated by Procheck module of structural analysis and verification server.[27] Mutations of threonine to asparagine and isoleucine to valine were created at codons 461 and 462, respectively, in the validated 3D target structure using the tool Swiss PDB Viewer,[28] as shown in Figure 1. The active site of the mutated CYP1A1 protein was determined by Q-SiteFinder.[29]

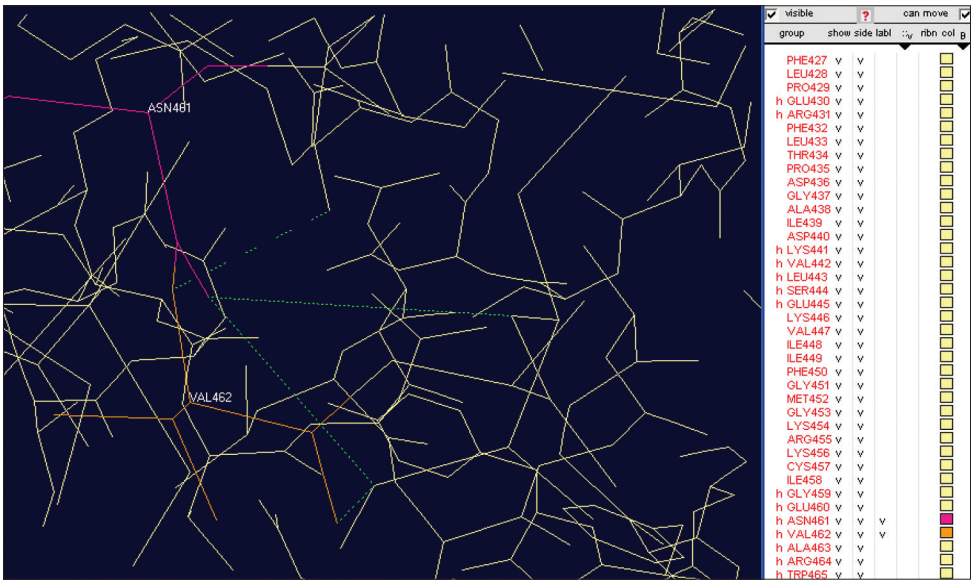

Figure 1: Mutations in CYP1A1 at 461 and 462 using Swiss PDB Viewer

Ligand preparation

The phytoconstituent bioactive compounds of cruciferous vegetables, namely, ascorbic acid, 3,3'-diindolylmethane, glucosinolate, and indole-3-carbinol, as provided in the literature were selected as the ligands for the target protein CYP1A1.[19,20,30,31] The structures of the ligands were obtained from PubChem compound.[32] The 3D structure of the ligand was drawn by the drawing tool ACD ChemSketch.[33] Molecular file converter was used for file conversions.[34,35]

Interaction studies

The docking analysis was carried out for the mutated CYP1A1 protein with the ligand, using AutoDock software.[36] The steps involved were editing of macromolecule, preparing the ligand, preparing the macromolecule, preparing the grid parameter file, starting Autogrid, running AutoDock, docking with the best conformation, and analyzing the docking result. The results were visualized by WebLab Viewer.[37]

Pharmacokinetic studies

Lipinski had explained pharmacokinetics of a drug in terms of its molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, topological polar surface area (TPSA), and number of rotatable bonds.[38] These properties of the crucifer drug candidates were analyzed using the basic Physchem properties module of ACD/I-Lab 2.0.[39] The absorption, distribution, metabolism, and excretion (ADME) module was utilized to analyze the bioavailability, volume of distribution (Vd), and partition coefficient between n-octanol and water (logP).

RESULTS AND DISCUSSION

Structure of the drug target

The alignment of the template 2HI4 A chain of crystal structure of human microsomal P450 1a2 with CYP1A1 resulted in 73% identity, 85% positives of similar amino acids, and 0% gaps. The Pfam showed single-domain cytochrome P450 in the target protein, as shown in Figure 2.

The PAP file generated by the program align2d of the Modeller indicated significant alignment between the template and the target. The modeled 3D structure when visualized by RasMol as flat ribbon-shaped model showed the presence of alpha-helix, sheet, loop, and turns, as shown in Figure 3.

The Ramachandran plot for the predicted structure in Figure 4 showed 93.7% residues in the most favored region and no residues in the disallowed region.

Red shaded – the most favored region, dark yellow shaded – additional allowed region, light yellow shaded – generously allowed region, white – disallowed region.

The root-mean-square deviation as calculated by Swiss PDB Viewer was found to be 0.29A°. The value was found to be <1A°. This suggested that the template and the model superimposed well.

The active site prediction result of CYP1A1 showed the pocket with the best site 1 among the 10 binding sites obtained from Q-site finder. The site 1 was highly conserved and the most favorable site for docking. The active site residues were found be:

LEU96, ARG106, MET121, SER122, TRP131, ARG135, LEU142, VAL197, ILE198, ASP313, LEU314, ALA317, GLY318, PHE319, THR321, VAL322, ALA325, PHE376, PHE381, VAL382, PHE384, THR385, ILE386, HIS388, GLN411, ILE449, PHE450, GLY451, MET452, LYS454, ARG455, LYS456, CYS457, ILE458, GLY459, GLU460, ASN461, VAL462, ALA463, GLU466, VAL467, LEU496.

Docking results

Hydrogen (H) bonds and the residues involved in bonds between the target protein and the crucifer ligands, in the

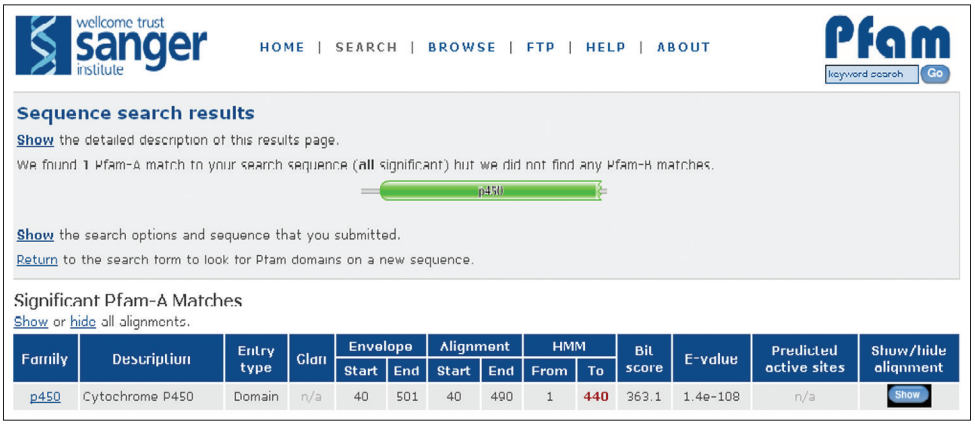

Figure 2: Pfam result of CYP1A1 with single-domain cytochrome P450

Figure 3: 3D cartoon model of CYP1A1 as deciphered by RasMol, showing alpha-helix (pink), sheet (yellow), loop (white), and turns (blue)

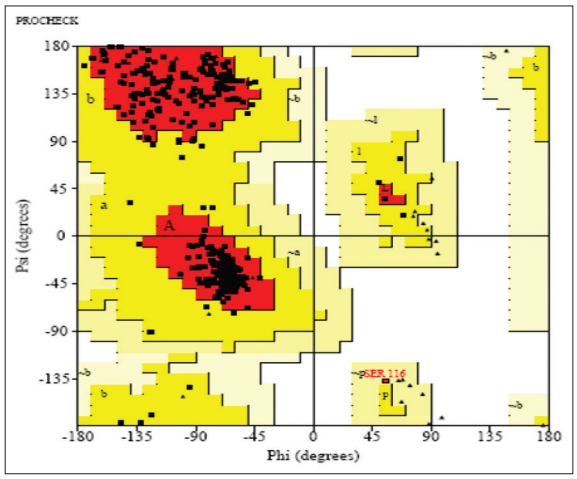

Figure 4: Ramachandran plot of the modeled CYP1A1

docked models with their docking energies are obtained in Table 1. The docked conformations, as illustrated by WebLab Viewer, are shown in Figure 5. The final docked conformations of the target with the ligands were evaluated based on the docking score and the number of H-bonds formed. The conformation with minimum docking value and maximum number of H-bonds was found to be stable. Thus, glucosinolate seemed to be the best-docked ligand. However, on the whole, all the four crucifer ligands showed significant docking results. The effective docking of these compounds with CYP1A1 suggested their intact blocking of the active site of the target, thereby inhibiting the enzyme and thus preventing carcinogenesis.

Drug properties and anticancer potential of ligands

Adverse drug reactions have become the prominent cause for the failure of drugs. Thus, it is highly necessary to analyze the toxicity of drugs along with their physicochemical and ADME properties.[40] Drug properties of the crucifer compounds studied, as shown in Table 2, were found to be favorable.

Table 1: Docking results of CYP1A1 – Crucifer ligand complexes

Crucifer ligand	Docked residues (target ligand)	Total number of H-bonds	Docking energy (KJ/mol)
Ascorbic acid	ARG106-HH11 ARG135-HE PHE450-O ARG455-NE LYS456-O ILE458-N (2)	7	−6.49
3,3'-Diindolyl methane	ARG106-HH11 ARG455-HE (2) ARG455-HH22	4	−10.0
Glucosinolate	SER122-N TRP131-HE1 THR385-OG1 ILE386-O ILE386-N ARG455-HE ARG455-O (2) ILE499-O	9	−16.0
Indole-3-carbinol	ARG135-HE ARG135-NE ARG455-HH22 ARG455-HE ARG455-NE LYS456-O	6	−6.91

Oral bioavailability denotes the extent to which the active principle of the drug molecule can enter the systemic circulation and reach the target site after oral administration. Lipophilicity, degree of ionization of the molecule and many other factors determine the rate of diffusion. Vd indicates the distribution of drug in the body to that in the blood at the same time. It depends on the relative affinity of the drug for the blood and tissues.[41] Bioavailabilities of ascorbic acid and glucosinolate were found to be <30% and were dependent on their lipophilicities. Indole-3-carbinol showed maximum bioavailability. The greater value of 3, 3'-Diindolylmethane supported that Vd values could be larger for very hydrophobic drugs.[42,43]

According to Lipinski's Rule of Five, a good drug compound should have molecular weight <500 Daltons, partition coefficient between n-octanol and water (logP) <5, H-bond donors <5, and H-bond acceptors <10, all being multiples of 5.[38] It is found that the molecular weight range within 180–500 Daltons seems to be closely associated with druglikeness.[44] In the current study, all the four crucifer compounds obeyed Lipinski's rule, though indole-3-carbinol's molecular weight exhibited lesser value. Among the compounds studied, all except glucosinolate had H-bond donors <5. Again, glucosinolate seemed to be the only compound with H-bond

Table 2: Drug properties of crucifer compounds

Ligand	Bioavailability	Vd (L/kg)	Physicochemical property	LogP
Ascorbic acid	<30%	0.34	Molecular weight: 176.12 No. of hydrogen bond donors: 4 No. of hydrogen bond acceptors: 6 TPSA: 107.22 No. of rotatable bonds: 2	0.04
3, 3'-Diindolylmethane	30–70%	2.53	Molecular weight: 246.31 No. of hydrogen bond donors: 2 No. of hydrogen bond acceptors: 2 TPSA: 31.58 No. of rotatable bonds: 2	4.54
Glucosinolate	<30%	0.30	Molecular weight: 448.47 No. of hydrogen bond donors: 6 No. of hydrogen bond acceptors: 11 TPSA: 215.58 No. of rotatable bonds: 7	1.04
Indole-3-carbinol	more than 70%	1.40	Molecular weight: 147.17 No. of hydrogen bond donors: 2 No. of hydrogen bond acceptors: 2 TPSA: 36.02 No. of Rotatable Bonds: 1	1.25

TPSA: Topological polar surface area, Vd: Volume of distribution

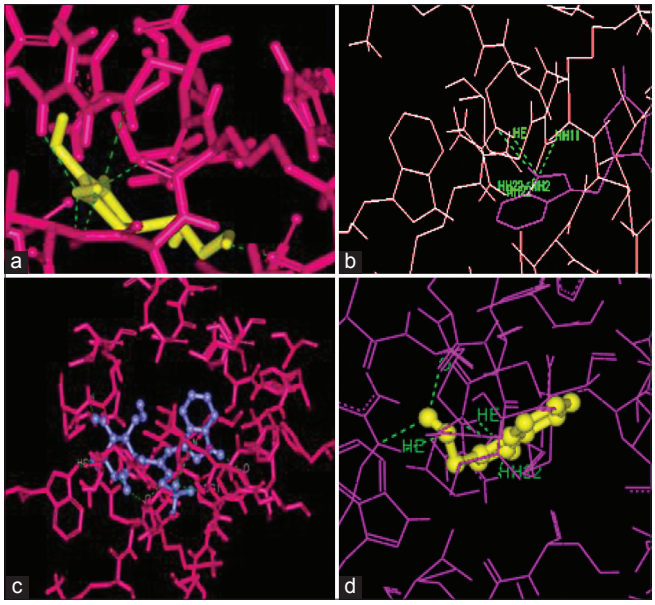

Figure 5: CYP1A1 interactions with (a) ascorbic acid, (b) 3,3'-Diindolylmethane, (c) glucosinolate, (d) indole-3-carbinol. H-bonds are indicated by dotted lines

acceptors more than 10. It is the presence of greater number of H-bond donors and acceptors in this compound that accounts for the greater number of H-bond formation with the target.

TPSA indicates the van der Waals surface area of all nitrogen, sulfur, and oxygen atoms along with their attached H-atoms.[45] Compounds with TPSA equal to or less than 140 Å^2 and 10 or fewer rotatable bonds are associated with good oral bioavailability.[46] TPSA of all the compounds studied except glucosinolate was <140 Å^2. Number of rotatable bonds of all these compounds was found to be <10. In general, compounds with high molecular weight showed more number of rotatable bonds. A positive correlation existed between TPSA, H-bond count, molecular flexibility, and molecular weight.[46-48] That was why, glucosinolate with more molecular weight was found to exhibit more TPSA and more number of rotatable bonds compared to the other three compounds studied.

LogP is an important property that determines the druglikeness of a given molecule by measuring its lipophilicity.[49,50] LogP values of the compounds studied were all <5, obeying Lipinski's rule. This indicated that these compounds possessed good absorption and permeation capabilities. Among the four, 3,3'-Diindolylmethane exhibited maximum lipophilicity and ascorbic acid, the least. On the whole, indole-3-carbinol and 3, 3'-Diindolylmethane showed better drug properties compared to other two.

Scientific and epidemiological evidences further substantiate the observed results emphasizing the protective role of cruciferous vegetables against cancer. A number of mechanisms such as protection against reactive oxygen species, retardation of tumor growth, and induction of apoptosis have been reported.[51]

Indole-3-carbinol, occurring naturally in crucifers, is a powerful antioxidant that scavenges free radicals. *In vitro* and *in vivo* carcinogenesis models have demonstrated the chemopreventive role of this compound during initiation and promotion phases of cancer development.[20]

A wide collection of literature has addressed the presence of glucosinolates as a distinctive feature of crucifer vegetables.

They highlight the cancer chemoprotective attributes of this bioactive compound besides its fungicidal, bacteriocidal, nematocidal, and allelopathic properties.[31]

It has been reported that diindolylmethane, an essential dietary crucifer component, inhibits cell growth and migration by downregulating urokinase plasminogen activator associated with tumor progression and metastasis. Diindolylmethane is found to induce cell apoptosis either by downregulating antiapoptotic gene products or by upregulating proapoptotic proteins. It is also associated with anti-inflammatory activity, regulation of the redox status of the cells during oxidative stress, decreasing invasive properties of the tumor cells, altering gene expression, and reducing cell growth by inhibiting DNA methylation.[19]

Ascorbic acid, also an essential constituent of crucifer vegetables, generates significant quantities of hydrogen peroxide by autoxidation, to create oxidative stress targeting cancer cells. It also acts as a cofactor in the stimulation of the 2-oxoglutarate-dependent dioxygenase family of enzymes that regulate the hypoxic response in angiogenesis and metastasis.[30]

Although earlier literature suggests the possible mechanisms of the anticancer activity of the crucifer compounds, the current work unravels an unknown mechanism of these compounds, as a step forward for a novel anticancer therapy.

CONCLUSION

This study demonstrates the potential chemopreventive role of crucifer phytocomponents through their potential inhibitory interactions with the carcinogenesis inducing target CYP1A1. Their pharmacokinetic properties are also favorable, supporting their significance. The findings thus emphasize the promising scope of these natural drug candidates to combat lung cancer.

REFERENCES

1. Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: Wider roles in cancer progression and prevention. BMC Cancer 2009;9:187.
2. Rendic S, Guengerich FP. Contributions of human enzymes in carcinogen metabolism. Chem Res Toxicol 2012;25:1316-83.
3. Smith G, Stubbins MJ, Harries LW, Wolf CR. Molecular genetics of the human cytochrome P450 monooxygenase superfamily. Xenobiotica 1998;28:1129-65.
4. Ma Q, Lu AY. CYP1A induction and human risk assessment: An evolving tale of *in vitro* and *in vivo* studies. Drug Metab Dispos 2007;35:1009-16.
5. Chang JT, Chang H, Chen PH, Lin SL, Lin P. Requirement of aryl hydrocarbon receptor overexpression for CYP1B1 up-regulation and cell growth in human lung adenocarcinomas. Clin Cancer Res 2007;13:38-45.
6. Cosma G, Crofts F, Taioli E, Toniolo P, Garte S. Relationship between genotype and function of the human CYP1A1 gene. J Toxicol Environ Health 1993;40:309-16.
7. Kiyohara C, Hirohata T, Inutsuka S. The relationship between aryl hydrocarbon hydroxylase and polymorphisms of the CYP1A1 gene. Jpn J Cancer Res 1996;87:18-24.
8. Wei Q, Gu J, Cheng L, Bondy ML, Jiang H, Hong WK, *et al*. Benzo(a)pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. Cancer Res 1996;56:3975-9.
9. Buterin T, Hess MT, Luneva N, Geacintov NE, Amin S, Kroth H, *et al*. Unrepaired fjord region polycyclic aromatic hydrocarbon-DNA adducts in ras codon 61 mutational hot spots. Cancer Res 2000;60:1849-56.
10. Beresford AP. CYP1A1: Friend or foe? Drug Metab Rev 1993;25:503-17.
11. Shimada T, Fujii-Kuriyama Y. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci 2004;95:1-6.
12. Shimada T, Inoue K, Suzuki Y, Kawai T, Azuma E, Nakajima T, *et al*. Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice. Carcinogenesis 2002;23:1199-207.
13. Shimizu Y, Nakatsuru Y, Ichinose M, Takahashi Y, Kume H, Mimura J, *et al*. Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. Proc Natl Acad Sci U S A 2000;97:779-82.
14. Ma X, Idle JR, Malfatti MA, Krausz KW, Nebert DW, Chen CS, *et al*. Mouse lung CYP1A1 catalyzes the metabolic activation of 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). Carcinogenesis 2007;28:732-7.
15. Fujita K, Kamataki T. Predicting the mutagenicity of tobacco-related N-nitrosamines in humans using 11 strains of *Salmonella typhimurium* YG7108, each coexpressing a form of human cytochrome P450 along with NADPH-cytochrome P450 reductase. Environ Mol Mutagen 2001;38:339-46.
16. Fujita K, Kamataki T. Role of human cytochrome P450 (CYP) in the metabolic activation of N-alkylnitrosamines: Application of genetically engineered *Salmonella typhimurium* YG7108 expressing each form of CYP together with human NADPH-cytochrome P450 reductase. Mutat Res 2001;483:35-41.
17. Higdon JV, Delage B, Williams DE, Dashwood RH. Cruciferous vegetables and human cancer risk: Epidemiologic evidence and mechanistic basis. Pharmacol Res 2007;55:224-36.
18. Kristal AR, Lampe JW. Brassica vegetables and prostate cancer risk: A review of the epidemiological evidence. Nutr Cancer 2002;42:1-9.

19. Zhang WW, Feng Z, Narod SA. Multiple therapeutic and preventive effects of 3,3'-diindolylmethane on cancers including prostate cancer and high grade prostatic intraepithelial neoplasia. J Biomed Res 2014;28:339-48.
20. Murillo G, Mehta RG. Cruciferous vegetables and cancer prevention. Nutr Cancer 2001;41:17-28.
21. Inbathamizh L, Padmini PP. *In silico* studies on the enhancing effect of anti-cancer phytochemicals of *Moringa oleifera* on cellular prostatic acid phosphatase activity. Drug Invent Today 2011;3:186-92.
22. Inbathamizh L, Padmini E. *In silico* studies on the inhibitory effects of calcitriol and 5, 5'dithiobis -2-nitrobenzoic acid on human glucosaminyl N-acetyl transferase 1 activity. Asian J Exp Biol Sci 2012;3:14-21.
23. Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, *et al*. UniProt: The Universal Protein knowledgebase. Nucleic Acids Res 2004;32:D115-9.
24. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol 1990;215:403-10.
25. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, *et al*. Comparative protein structure modeling using Modeller. Curr Protoc Bioinformatics 2006;Chapter 5:Unit-5.6.
26. Sayle RA, Milner-White EJ. RASMOL: Biomolecular graphics for all. Trends Biochem Sci 1995;20:374.
27. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: A program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993;26:283-91.
28. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-pdbviewer: An environment for comparative protein modeling. Electrophoresis 1997;18:2714-23.
29. Laurie AT, Jackson RM. Q-SiteFinder: An energy-based method for the prediction of protein-ligand binding sites. Bioinformatics 2005;21:1908-16.
30. Vissers MCM, Das AB. Potential mechanisms of action for Vitamin C in cancer: Reviewing the evidence. Front Physiol 2018;9:809.
31. Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 2001;56:5-1.
32. Bolton E, Wang Y, Thiessen PA, Bryant SH. Pubchem: Integrated platform of small molecules and biological activities. Annu Rep Comput Chem 2008;4:217-41.
33. Li Z, Wan H, Shi Y, Ouyang P. Personal experience with four kinds of chemical structure drawing software: Review on chem draw, chem window, ISIS/Draw, and chem sketch. J Chem Inf Comput Sci 2004;44:1886-90.
34. Dalby A, Nourse JG, Hounshell WD, Aki G, Grier DL, Leland BA. Description of several chemical structure file formats used by computer programs developed at molecular design limited. J Chem Inf Comput Sci 1992;32:244-55.
35. Inbathamizh L, Padmini E. *Moringa oleifera* flower compounds as potent ligands to drug targets in PC3 cell lines an *in silico* analysis. Int J Pharm Pharm Sci 2013;5:377-83.
36. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK. Automated docking using a lamarckian genetic algorithm and empirical binding free energy function. J Comput Chem 1998;19:1639-62.
37. Perkins JA. A History of molecular representation part 2: The 1960s present. J Biocommun 2005;31:1-4.
38. Lipinski CA. Lead-and drug-like compounds: The rule-of-five revolution. Drug Discov Today Technol 2004;1:337-41.
39. ACD/I-LAB 2.0. Toronto on Canada: Advanced Chemistry Development Inc.; 2012. Available from: https://www.ilab.acdlabs.com/iLab2. [Last accessed on 2019 May 23].
40. Inbathamizh L, Padmini E. Quinic acid as a potent drug candidate for prostate cancer. Asian J Pharm Clin Res 2013;6:106-12.
41. van de Waterbeemd H. Which *in vitro* screens guide the prediction of oral absorption and volume of distribution? Basic Clin Pharmacol Toxicol 2005;96:162-6.
42. Seydel JK, Schaper KJ. Quantitative structure-pharmacokinetic relationships and drug design. Pharmacol Ther 1981;15:131-82.
43. Toon S, Rowland M. Structure-pharmacokinetic relationships among the barbiturates in the rat. J Pharmacol Exp Ther 1983;225:752-63.
44. Ghose AK, Viswanadhan VN, Wendoloski JJ. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. J Comb Chem 1999;1:55-68.
45. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. J Med Chem 2000;43:3714-7.
46. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 2002;45:2615-23.
47. Ajay A, Walters WP, Murcko MA. Can we learn to distinguish between "drug-like" and "nondrug-like" molecules? J Med Chem 1998;41:3314-24.
48. Waterbeemd H. Intestinal Permeability Prediction from Theory. Oral Drug Absorption. New York: Marcel Decker Inc.; 2000. p. 36-9.
49. Leeson PD, Springthorpe B. The influence of drug-like concepts on decision-making in medicinal chemistry. Nat Rev Drug Discov 2007;6:881-90.
50. Edwards MP, Price DA. Role of physicochemical properties and ligand lipophilicity efficiency in addressing drug safety risks. Annu Rep Med Chem 2010;45:381-91.
51. Keck AS, Finley JW. Cruciferous vegetables: Cancer protective mechanisms of glucosinolate hydrolysis products and selenium. Integr Cancer Ther 2004;3:5-12.

Source of Support: Nil. **Conflicts of Interest:** None declared.