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

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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]

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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. Similarly, amino acid change of threonine to asparagine at codon 461 is due to C→A substitution at nucleotide 2453.

CYP1A1 catalyzes the conversion of various PAHs to highly reactive products that can cause oncogenic mutations and carcinogenesis. 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-dihydriodiol by Epoxide hydrolase. Ultimately, this intermediate is catalyzed by CYP1A1 to Benzo[a]pyrene-7,8-dihydriodiol-9,10-epoxide, which is the consequent carcinogen.

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. 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, and tobacco-related N-nitrosamines that induce carcinogenesis. 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. A positive correlation is found to exist between cancer prevention and consumption of cruciferous vegetable bioactives.

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. 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. The 3D structure analysis for CYP1A1 was carried out using BLAST and modeled by Modeller 9V2 with the programs Align2d and Model-default. The predicted structure was visualized by RasMol, validated by Procheck module of structural analysis and verification server. 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, as shown in Figure 1. The active site of the mutated CYP1A1 protein was determined by Q-SiteFinder.

![Figure 1: Mutations in CYP1A1 at 461 and 462 using Swiss PDB Viewer](image-url)
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 a 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 0.29Å°. The value was found to be <1Å°. 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 to be:

LEU96, ARG106, MET121, SER122, TRP131, ARG135, LEU142, VAL197, ARG313, LEU314, ALA317, GLY318, PHE319, THR321, VAL322, ALA325, PHE376, PHE381, VAL382, PHE384, THR385, HIS388, GLY411, ILE449, PHE450, GLY451, MET452, LEU496.

Docking results

Hydrogen (H) bonds and the residues involved in bonds between the target protein and the crucifer ligands, in the
The 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. Drug properties of the crucifer compounds studied, as shown in Table 2, were found to be favorable.

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. 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.

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. It is found that the molecular weight range within 180–500 Daltons seems to be closely associated with druglikeness. 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

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

![Figure 4: Ramachandran plot of the modeled CYP1A1](image)

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

Table 1: Docking results of CYP1A1 – Crucifer ligand complexes
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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. Compounds with TPSA equal to or less than 140 Å² and 10 or fewer rotatable bonds are associated with good oral bioavailability. TPSA of all the compounds studied except glucosinolate was <140 Å². 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. 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. 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. 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.

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

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

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.
They highlight the cancer chemoprotective attributes of this bioactive compound besides its fungicidal, bacteriocidal, nematocidal, and allelopathic properties.\[131\]

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.\[109\]

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.\[330\]

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 phytoconstituents 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**

Inbathamizh, et al.: Targeting CYP1A1 with crucifer compounds for chemopreventive drug design


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