MOLECULAR INSIGHTS INTO TOXIN-ENZYME INTERACTIONS: DOCKING STUDIES ON CYP1A2 (2HI4) AND GST (1GTA): AN IN-SILICO APPROACH

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I am Gaurav Boruah, Roll Number 23/MSCBIO/77 student of M.Sc. Biotechnology hereby certify that the work which is being presented in the thesis entitled "**MOLECULAR INSIGHTS INTO TOXIN-ENZYME INTERACTIONS: DOCKING STUDIES ON CYP1A2 (2HI4) AND GST (1GTA): AN IN-SILICO APPROACH**" in partial fulfilment of the requirements for the award of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from **May 2024** to **June 2025** under the supervision of **Dr. Kriti Bhandari**.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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Certified that GAURAV BORUAH **(**23/MSCBIO/77) has carried out their research work presented in this thesis "**MOLECULAR INSIGHTS INTO TOXIN-ENZYME INTERACTIONS: DOCKING STUDIES ON CYP1A2 (2HI4) AND GST (1GTA): AN IN-SILICO APPROACH**" for the award of **Master of Science** from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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ABSTRACT

This study applies molecular docking methods to predict interactions of environmental toxins with two vital human detoxification enzymes, cytochrome P450 1A2 and glutathione S-transferase. Through simulation of the binding dynamics of environmental toxins to these enzymes, the aim is the prediction of binding affinities, potential inhibitory activities, and detoxification efficiencies. Docking simulation results show that some toxins exhibit high binding affinities at the active sites of CYP1A2 and GST, suggesting potential competitive inhibition or metabolic transformation. For instance, benzo[a]pyrene-like compounds exhibit high interactions with CYP1A2, suggesting potential metabolic activation or inhibition, while other environmental toxins exhibit favourable binding to GST, suggesting efficient detoxification pathways. These findings emphasize the applicability of molecular docking in the prediction of toxicological effects of environmental toxins and the efficacy of enzymatic detoxification processes. Such computational tools may be applicable in the risk assessments and guide the development of measures to limit toxin exposure. Future studies should aim to relate these in silico observations with experimental verifications to advance our understanding on toxin-enzyme interactions and their implications to human health.

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LIST OF ABBREVIATIONS

GST	Glutathione-S-Transferase
GTA	Glutathione
PAH	Polycyclic Aromatic Hydrocarbons
CYP	Cytochrome P450
TCDD	TetraChloroDibenzo-p-Dioxin
PDB	Protein Data Bank
ADME	Adsorption Distribution Metabolism and Excretion
VOC	Volatile Organic Compounds

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

In today's world, marked by rapid growth of industrialization and urbanization, environmental toxins have become a significant concern for public health and ecological sustainability. These toxic compounds deriving from numerous sources such as industrial waste, agricultural practices, and vehicular emissions emits serious risks to both human health and environmental integrity. Most of these toxins are xenobiotic substances meaning when they enter to the biological systems, they can cause a variety of deleterious effects. The human body's ability to counter these threats depends on its detoxifying enzymes, primarily cytochrome P450 enzymes and glutathione S-transferases [1,2]. Understanding the molecular interactions between these enzymes and environmental toxins is crucial for elucidating the detoxification mechanisms and developing strategies to mitigate the effects of these harmful substances.

Enzyme	Function
Cytochrome P450s (e.g., CYP1A2)	Oxidation of xenobiotics, increasing their solubility for excretion
Glutathione S-transferases (GSTs)	Conjugation of toxins with glutathione, facilitating their removal
UDP-glucuronosyltransferases	Addition of glucuronic acid to substances, enhancing water solubility
Sulfotransferases	Sulfation of compounds, aiding in detoxification
N-acetyltransferases	Acetylation of arylamine and hydrazine drugs, reducing their toxicity

Table 1: Key Detoxifying Enzymes and Their Functions

Environmental toxins are harmful substances that majorly affect human health and the ecosystem. Origination of these toxins includes industrial processes, agricultural practices, and household products. Therefore, understanding the sources and implications of these toxins and creating awareness is crucial for public health and environmental sustainability.

One of the main sources of environmental toxins is industrial activity. Toxic metals are dispersed into the land and water bodies through industrial effluents, organic wastes, and power generation [3]. Large disposal of heavy metals such as lead, mercury, and cadmium are the most common byproducts of industrial operations. Consuming these meat foods can accumulate and cause harmful diseases. For instance, mercury contamination in fish (Minamata disease) can lead to neurological disorders in those who consume them.

Agricultural practices are also significantly contributing to environmental toxins. The use of pesticides and herbicides in farming is widespread, which aims at protecting crops from pests and diseases. However, these chemicals can percolate into the soil and waterways, affecting non-target species and disrupting ecosystems. Additionally, the overuse of fertilizers can cause greater threat as nutrient runoff leads to algal blooms in water bodies, which increases the BOD and harm aquatic life [4].

Household products are another source of environmental toxins. Many everyday items, such as cleaning agents, paints, and personal care products, contain toxic chemicals such as Volatile organic compounds (VOCs) which can evaporate into the air, contributing to indoor air pollution and prolonged exposure can lead to respiratory issues, headaches, and other health problems [5,6].

Exposure to environmental pollutants has wide-ranging effects. Individuals who are exposed to excessive quantities of toxins may suffer from acute or long-term health problems, such as cancer, reproductive troubles, and childhood developmental impairments. Particularly at risk are vulnerable groups like the elderly, pregnant women, and newborns. Public health systems may also be strained by the financial burden of medical expenses related to diseases caused by toxins.

On a larger scale, environmental pollutants can cause ecosystem deterioration and biodiversity loss. Decreases in species populations can be caused by contaminated

environments, upsetting ecological equilibrium and food webs. These pollutants have the potential to modify the environment over time, making it less adaptable to alterations like climate change.

In conclusion, environmental pollutants are a serious issue that originate from a number of sources, such as household goods, agricultural methods, and industrial operations. Because of the significant effects these harmful substances have on both the environment and human health, more awareness and actions are required. In order to promote safer practices and safeguard the environment and public health, governments, businesses, and individuals must work together to reduce exposure to environmental toxins.

Environmental Toxins: Sources, Mechanisms of Action, and Health Impacts

The term "environmental toxins" refers to a broad and diverse category of chemicals that are present throughout our environment. They come from a variety of sources, including synthetic chemicals made by industrial processes and naturally occurring substances made by living things.

Natural Environmental Toxins:

Phytotoxins (pyrrolizidine alkaloids, solanine) found in plants, marine toxins (saxitoxin, brevetoxin) produced by algae, bacterial toxins (botulinum toxin, tetanus toxin), and mycotoxins (ochratoxins, aflatoxins) produced by fungi are examples of natural toxins. By discouraging competitors or predators, these substances frequently safeguard the species that produce them. However, a wide range of harmful effects can be induced in humans or other creatures upon exposure. Aflatoxin, for example, is a widespread contamination of food crops like peanuts and corn. It is a strong hepatocarcinogen that needs CYP metabolic activation to cause genotoxicity. The liver bioactivates pyrrolizidine alkaloids, which are significant in many plant species, producing highly reactive pyrrolic esters that can lead to veno-occlusive liver disease (VOD).

Anthropogenic Environmental Toxins:

Many of the synthetic chemicals that are released into the environment as a result of the industrial revolution and the ensuing technological developments are poisonous, persistent, and bio-accumulative. These include:

- **Persistent Organic Pollutants (POPs):** For example, dioxins (like TCDD), polychlorinated biphenyls (PCBs), and organochlorine insecticides (like DDT). These substances build up in the food chain, have a high resistance to degradation, and have been linked to cancer, developmental, and reproductive consequences.
- **Heavy Metals:** Lead, mercury, cadmium, arsenic, and chromium are common environmental pollutants that come from mining, industry, and land use. By causing cellular damage, producing reactive oxygen species (ROS), and interferes with enzyme function, exhibiting toxicity.
- **Pharmaceuticals and Personal Care Products (PPCPs):** Many medications and their metabolites, though necessary for health, end up in the environment through wastewater, where they may affect non-target creatures and pose ecotoxicological concerns.
- **Air Pollutants:** Air pollution is mostly caused by particulate matter, nitrogen oxides, ozone, and polycyclic aromatic hydrocarbons (PAHs), which can lead to cancer, heart disease, and respiratory disorders. Since many PAHs are carcinogenic, they must be activated by enzymes.
- **Plastics and Microplastics:** In their bulk form, plastic additives like bisphenol A and phthalates are not dangerous, but they can leak out and cause endocrine disruption. Other adsorbed contaminants are also carried by microplastics.

Mechanisms of Toxin Action:

Environmental toxins exert their detrimental effects through various molecular mechanisms. These can be broadly categorized as:

- **Direct Macromolecular Damage:** In order to cause mutations and carcinogenesis, toxins can covalently attach to DNA to produce adducts that interfere with transcription and replication. Additionally, they have the ability to attack lipids, causing lipid peroxidation and cell membrane damage, or target proteins, disrupting their function.

- **Enzyme Inhibition/Dysregulation:** By attaching themselves to and blocking important enzymes, many toxins interfere with metabolic processes. Organophosphates, for instance, cause neurotoxicity by inhibiting acetylcholinesterase. In enzymes, heavy metals can attach to sulfhydryl groups, leading to widespread malfunction. Toxins, on the other hand, might cause abnormal enzyme activation, which can result in unchecked metabolism or signalling.
- **Receptor Modulation:** Certain toxins interfere with signalling pathways by imitating or opposing endogenous ligands for cellular receptors. One such example is endocrine-disrupting chemicals (EDCs), which cause anomalies in reproduction and development by interfering with the synthesis, transport, binding, or function of hormones. As an example, dioxins activate the aryl hydrocarbon receptor (AhR), which sets off a series of alterations in gene expression, including CYP1A2 upregulation.
- **Oxidative Stress Induction:** Super-oxides, hydrogen peroxide, and hydroxyl radicals are among the most common reactive oxygen species (ROS) produced by a variety of toxins. ROS can damage DNA, proteins, and lipids throughout the cell, which can lead to inflammation, aging, and the development of illness.
- **Mitochondrial Dysfunction:** In order to cause energy depletion and cell death, toxins can harm mitochondrial DNA, disrupt mitochondrial respiration, or uncouple oxidative phosphorylation.
- **Immunotoxicity:** Increased vulnerability to infections or autoimmune disorders can result from certain toxins' suppression or overstimulation of the immune system.
- **Neurotoxicity:** Certain poisons alter neurotransmission, neuronal growth, or neuronal survival, which can lead to deficits in cognition, motor function, or sensory perception.

  The various modes of action demonstrate how complicated environmental toxicology is and how strong detoxification systems are necessary to mitigate these negative consequences.

1.2 OBJECTIVE OF THE STUDY

1. To investigate the protein-ligand interactions.
2. To study the structural dynamics of the proteins in relation to ligands.
3. Analysis of these interactions based on docking values for further research.

CHAPTER 2
LITERATURE REVIEW

2.1 Detoxifying Enzymes: Roles and Mechanisms of Xenobiotic Metabolism

Protein 2HI4 (cytochrome P450 1A2) include CYP1A2, a cytochrome that is endemic to the cytochrome P450 superfamily [7]. It is essential for both the manufacture of endogenous substances like cholesterol, bile acids, and asteroid hormones as well as the detoxification of external substances. Flutamide, lidocaine, olanzapine, theophylline, triamterene, and zolmitriptan are among the drugs that it significantly adds to the liver's drug metabolism process. As a result, it frequently serves as a target for drug discovery.

As a member of the supergene family of metabolic enzymes, protein 1GTA (glutathione-S transferases) catalyzes the conjugation of reactive chemical intermediates with glutathione (GSH), which aids in the phase II detoxification process and intermediates' removal [8]. Therefore, GSTs assist in the effective elimination of a variety of potentially reactive electrophilic molecules by conjugating them to GSH, which shields cells from oxidative stress and chemical attacks like pesticides [9]. Eight classes are distinguished among human cytosolic GSTs based on the homology of their amino acid sequences: GST-α, GST-μ, GST-θ, GST-π, GST-ζ, GST-σ, GST-κ, and GST-ω [10].

To deal with the continual assault of external xenobiotics and endogenous waste products, biological systems have developed complex and incredibly effective enzymatic machinery. As the first line of defense against exposure to the environment, this detoxification system is mainly found in the liver but is also found in the kidneys, lungs, intestines, and skin. There are two main stages to the detoxification process: Phase I (functionalization) and Phase II (conjugation), which are frequently followed by Phase III (efflux) procedures.

2.1.1 Phase I Metabolism: Functionalization Reactions

By adding polar functional groups (such as hydroxyl, amino, or carboxyl) to xenobiotic compounds, Phase I enzymes increase their polarity and provide sites for Phase II conjugation processes. Among Phase I enzymes, the Cytochrome P450 monooxygenases are the most well-known and adaptable family.

The general reaction catalysed by CYPs is:

$$\mathbf{RH + O2 + NADPH + H^{+} \rightarrow ROH + H2O + NADP^{+}}$$

where RH is the substrate (xenobiotic), and ROH is the hydroxylated product.

Due to their exceptional broad substrate specificity, CYPs can metabolize a vast range of structurally varied substances, such as steroids, fatty acids, prostaglandins, medicines, and environmental contaminants. Many CYPs' comparatively wide and flexible active sites, which may accept a variety of molecular configurations, are responsible for this broad specificity. Yet, a single CYP enzyme can metabolize several substrates due to its wide specificity, and numerous CYP enzymes can metabolize a single substrate.

2.1.1.1 Role of CYP1A2: A vital member of the CYP family, it is involved in the metabolism of several endogenous substances as well as xenobiotics, including a variety of environmental pollutants. It is extensively expressed in the liver and is induced by a number of environmental contaminants, including halogenated aromatic hydrocarbons (like dioxins) and polycyclic aromatic hydrocarbons (PAHs)[11]. Procarcinogens may become more bioactive as a result of this inducibility, which is a crucial adaptive reaction to chemical exposure. Substrates of CYP1A2 include:

- **Aromatic amines:** Usually found in cooked meat which are activated by CYP1A2 to genotoxic metabolites.
- **PAHs:** Many Polycyclic Aromatic Hydrocarbons (e.g., benzo[a]pyrene) are procarcinogens that are initially hydroxylated by CYP1A2 (and other CYPs) to form reactive intermediates like epoxides, which can then undergo further

metabolism or bind to DNA.

- **Caffeine:** CYP1A2 is the primary enzyme responsible for caffeine metabolism, making it a useful probe substrate for assessing CYP1A2 activity in pharmacokinetic studies.
- **Certain pharmaceuticals:** Including theophylline, clozapine, and imipramine.

CYP1A2's mechanism of action is similar to that of other CYPs in that it uses a heme iron prosthetic group to catalyse the insertion of an oxygen atom into a substrate.
The catalytic cycle involves several steps:

1. **Substrate Binding:** The xenobiotic binds to the active site, displacing water and causing a shift in the heme iron from a low-pin state to a high-spin state.
2. **Electron Transfer:** NADPH-cytochrome P450 reductase donates the first electron to the heme iron, reducing it from $Fe3^{+}$ to $Fe2^{+}$.
3. **Oxygen Binding:** Molecular oxygen (O2) binds to the reduced heme iron.
4. **Second Electron Transfer:** Another electron is transferred from NADPH-cytochrome P450 reductase to the heme-oxygen complex, forming a peroxy intermediate.
5. **Protonation and Water Release:** Two protons are added, leading to the breakdown of the oxygen-oxygen bond and the release of a water molecule, forming a highly reactive oxygen species, often termed "Compound I" (FeIV=O).
6. **Oxygen Insertion:** This highly reactive Compound I abstracts a hydrogen atom from the substrate, forming a substrate radical and a hydroxylated heme.
7. **Rebound:** The hydroxylated heme radical recombines with the substrate radical, leading to the hydroxylation of the substrate and regeneration of the $Fe3^{+}$ heme, ready for another catalytic cycle.

2.1.2 Phase II Metabolism: Conjugation Reactions

Phase II enzymes catalyse the conjugation of Phase I metabolites (or sometimes parent xenobiotics with suitable functional groups) with endogenous, polar molecules. This process further increases water solubility, reduces toxicity, and facilitates excretion via

urine or bile.

Glutathione S-Transferases (GSTs): They play a pivotal role in cellular detoxification by catalysing the conjugation of electrophilic xenobiotics and endobiotics with the tripeptide glutathione (GSH) [12]. This reaction yields a glutathione S-conjugate, which is more water-soluble and less reactive, and can then be further metabolized to mercapturic acids for excretion.

The general reaction carried out by GSTs is:

$$\mathbf{RX+GSH \rightarrow RS-G+HX}$$

where RX is the electrophilic xenobiotic or endobiotic, GSH is glutathione, RS-G is the glutathione S-conjugate, and HX is the leaving group.

2.1.2.1 Role of GST: It is a mammalian glutathione S-transferase with the particular GST structure. Although the precise isoform may differ (it frequently refers to a particular Mu or Alpha class GST), it typically reflects the structural characteristics and catalytic mechanism of cytosolic GSTs. GSTs are particularly important in detoxifying a wide range of electrophilic compounds, including:

- **Electrophilic metabolites from Phase I reactions:** For instance, epoxides formed from PAHs or other xenobiotics.
- **Reactive oxygen species products:** Such as 4-hydroxynonenal (a product of lipid peroxidation) and malondialdehyde.
- **Alkylating agents:** Used in chemotherapy or present as environmental pollutants.
- **Certain pesticides and herbicides.**

Mechanism of Action of GSTs: GSTs facilitate the conjugation reaction by lowering the pKa of thiol group of glutathione, making it a more potent nucleophile. Each monomer of a GST dimer contains two distinct substrate binding sites:

1. **G-site (Glutathione-binding site):** This site binds glutathione and is highly conserved across GST isoforms. It plays a vital role in activating the thiol group of GSH.
2. **H-site (Hydrophobic or Electrophilic-binding site):** This site accommodates

the electrophilic xenobiotic. The H-site is highly variable among GST isoforms, accounting for their broad and overlapping substrate specificities.

The catalytic mechanism involves:

1. **GSH Binding:** GSH binds to the G-site, where specific residues (e.g., tyrosine, serine) interact with the glutathione molecule, polarizing its thiol group and increasing its nucleophilicity.
2. **Electrophile Binding:** The electrophilic substrate binds to the adjacent H-site.
3. **Nucleophilic Attack:** The activated thiolate anion of GSH launches a nucleophilic attack on the electrophilic centre of the xenobiotic, forming the S-conjugate.
4. **Product Release:** The glutathione S-conjugate is released from the active site, allowing for further processing and excretion.

In order for various GST isoforms to detoxify a broad range of chemically diverse electrophiles, the structural diversity of the H-site is essential. Thus, the structure of 1GTA provides a model for comprehending how various toxins may attach to and be broken down by this family of enzymes.

2.2 Molecular Docking: A Computational Technique for In-silico Study

Molecular docking is type of computational technique which use to prediction binding affinity between suitable molecules one is ligand (small molecule) to another macromolecule (Receptor) and one of the important techniques used in in-silico study [13]. Typically, one of the substances functions as a ligand attached to a macromolecule (protein). This potent method is a great tool for comprehending the pertinent physiological processes in a variety of systems and creatures. It is frequently called a "lock-and-key" dilemma and is based on molecular recognition. Generally, shape complementarity and a scoring system based on binding energy affinity yield the optimal orientation. To obtain the optimal binding complexes in protein-ligand simulations, dockings are typically used in a stochastic search approach. Molecular mechanic force fields can be used to quantify the energy.

Molecular docking calculates binding affinity score by three main types i.e., force-field, knowledge-based statistical function and empirical scoring [14]. Scoring function used to estimate binding affinity and binding affinity is directly related to the Gibbs binding energy. It is also employed in drug development and biological research with some limitation [15]. In this modern era computer-aid drug design and discovery, molecular docking is empirically known and is an efficient computational tool in discovering pharmacological activity in new drug research.

2.3 Glutathione and Ethacrynic acid as Control Compounds Used in Molecular Docking Analysis

Glutathione (GSH), a tripeptide consisting of γ-L-glutamyl-L-cysteinyl-glycine, is the most significant low molecular weight antioxidant produced by cells. Cysteine and glutamate are added one after the other, and then glycine is added to create it. The sulfhydryl group (−SH) of the cysteine is involved in the reduction and conjugation reactions that is considered to be the most important functions of GSH [16].

Ethacrynic acid, often known as Edecrin, is a loop diuretic used in treating high blood pressure that causes a rapid and significant diuresis. Ethacrynic acid's main function is to block the Na^{+}-K^{+}-$2Cl^{+}$ symporter's activity in the loop of Henle's thick ascending limb [17]. It works well for all kinds of edemas, regardless of whether there is an electrolyte imbalance, clinical acidosis, or alkalosis. Ethacrynic acid's efficacy (volume depletion) is responsible for the majority of its negative effects.
A library of 4 ligands (each for 2HI4 and 1GTA) compiled for the current experiment from multiple sources, including PubChem database, research journals, scientific database and pharmacological references. A thorough literature review and investigation was conducted for the proceedings of the experiment. This study conducts for investigation and interpretation of binding interaction and structure-activity relationship between the ligands and the proteins.

CHAPTER 3

Computational tools and database used in molecular docking

For Current study and investigation, we used computational software which is free available such as PyRx, Biovia Discovery Studio, SwissDock ADME and biological Database including PubMed, PubChem, and PDB (Protein Data Bank).

1. **PyRx**- Computational software with search engine is Autodock-vina used to studies molecular docking and binding affinities between ligand and target protein based on protein-ligand interaction.
2. **Biovia Discovery Studio**- It is a computational toolkit programme used to modification of ligand and target proteins, visualization in 2D and 3D and analysis of interaction.
3.**PubMed**- PubMed is database which contains large number of literature review and research articles, most use of PubMed for citations and review of publication.
4. **PubChem**- PubChem is world largest database in the field of chemicals sciences because it contains a vast number of chemicals information like physical properties, structure, biological activity, safety, toxicity, patient and literature citations.
5. **PDB (Protein Data Bank)**- It is a globally recognized, freely accessible database that archives large number of experimentally design 3D structure of biological macromolecules like proteins, nucleic acids and complex tertiary assemblies.

CHAPTER 4

METHODOLOGY

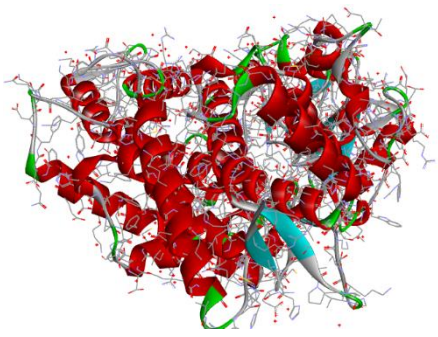
4.1 Data Collection

This project work contains In-silico investigation of potential ligands for. For completing of this project, collection of data-set from different database: 2 Target protein (enzymes) from Protein Data Bank and2 controls from PubChem, which is one of the most famous databases for retrieving chemical compounds and ligands.

4.2 Target Protein Selection and Modification

Selection of target protein on the basis of complete structure available on Protein Data Bank. The 3D structure of Human Microsomal P450 1A2 (2HI4) and Glutathione S-transferase(1GTA) were downloaded from RCSB PDB with resolutions of 1.95Å and 2.40 Å respectively. The 2HI4 protein classified as oxidoreductase, found in *Homo sapiens* which expression shows in E. coli. Similarly, the 1GTA protein classified as glutathione-s-transferase, found in *Schistosoma japonicum*. Downloaded protein contains hetatm (Hetero-atom), protein group and ligand group (see figure 1a & 2a). Hetero-atoms makes unfavourable for molecular docking and gives unreal results. Thus, need to modified target proteins by removing hetatm and add polar hydrogen atoms (see figure 1b.), these process makes suitable for molecular docking with enhance electrostatic interaction. Now protein ready for docking and protein-ligand interaction.

a



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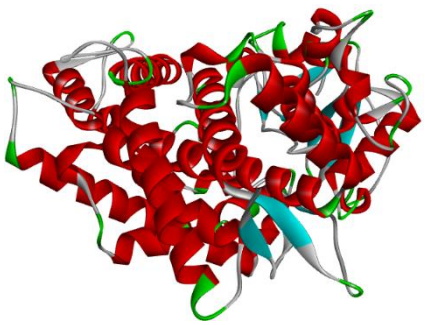

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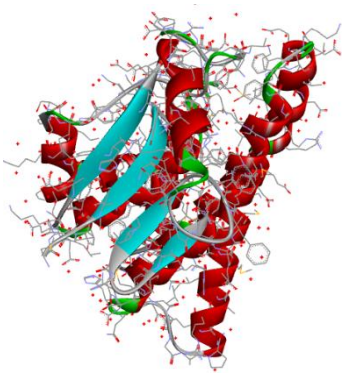


d

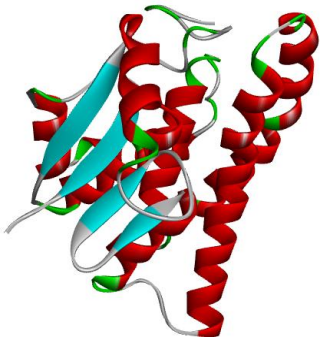


Figure 1. (a) PDB downloaded 3D structure of Human Microsomal P450 1A2.
(b) Modified PDB 3D structure of Human Microsomal P450 1A2.

Figure 2. (a) PDB downloaded 3D structure of Glutathione S-transferase.
(b) Modified PDB 3D structure of Glutathione S-transferase.

4.3 Selection and Preparation of LIGANDS

After an extensive review of scientific literature and database like PubChem, certain ligands were downloaded in three-dimensional configuration. All ligands were downloaded in SDF format (see table)

COMPOUND NAME	PUBCHEM ID
Glutathione	124886
Bisphenol A	6623
Methyloxindole	6096
Chlorpyrifos	2730
Benzo[a]pyrene	2336

Table 2. list of selected ligands for library preparation
for 2HI4 with PubChem IDs

COMPOUND NAME	PUBCHEM ID
Ethacrynic acid	3278
Atrazine	2256
Malathion	4004
1,2,3-tribromopropane	7279
Quercetin	5280343

Table 3. list of selected ligands for library preparation for 1GTA with PubChem IDs

4.4 Molecular Docking Studies

Docking calculations were performed on PyRx software which run on the basis of AutoDock-Vina module available at (https://pyrx.sourceforge.io/).

In PyRx programme, target proteins (enzymes) in PDB format were uploaded and make macro-molecule. Then we upload ligand in which download in 3D SDF, after uploading file minimize ligands after that convert all to Autodock ligand in pdbqt format by Biovia discovery studio.

Two target proteins (enzymes) were docked- one, Human Microsomal P450 1A2 was docked with Glutathione as a control and second, Glutathione S-transferase was docked with ethacrynic acid as control. PyRx was operated and binding energy was calculated. After few minutes, the process was completed and the best docking result was saved in PDB at zero. Further, 4 ligands of Human Microsomal P450 1A2 (2HI4) and Glutathione S-transferase(1GTA), corresponding to them were also docked. Docking results were evaluated based on binding affinity, interaction energy, and key molecular interactions (hydrogen bonding and hydrophobic contacts).

In covalent docking, there are two methods- one is grid-based approach which calculates a blueprint for the site of attachment of covalent ligand and second one is the modification of the flexible side chain method. The results were loaded into Biovia discovery studio- both target protein and ligand and these makes complex structure for visualization and analysis of receptor ligand interactions in 3D and 2D spaces.

4.5 Docking Analysis

Different ligands binding energy corresponding to 2HI4 and 1GTA were compared with Glutathione and Ethacrynic acid depicting the protein-ligand interactions were studied.

The study evaluated the interaction, inhibitory potential, and detoxification efficiency of environmental pollutants by molecularly docking them with the detoxifying enzymes CYP1A2 (2HI4) and GST (1GTA). Swiss-ADME's profiling and visual analysis revealed that the majority of ligands had appropriate pharmacokinetics. Future experimental validation for environmental risk assessment and drug discovery is supported by the results, which demonstrate the ability of in-silico docking in predicting enzyme-toxin dynamics.

CHAPTER 5

RESULT AND DISCUSSION

5.1 Binding affinity and scoring analysis of Protein-Ligand interactions

5.1.1 Molecular Docking

The comparison of the docking result between the Human Microsomal P450 1A2 (2HI4) enzyme with Glutathione and Glutathione S-transferase (1GTA) enzyme with Ethacrynic acid were performed. For 2HI4, glutathione was used as a control model which is a detoxifying enzyme that catalyses the conjugation of glutathione to electrophilic compounds had a binding affinity of -6.1 Kcal/mol. The binding affinities with the other ligands are lower as seen in Table. Similarly, for 1GTA, ethacrynic acid was used as a control model which is a known loop diuretic used for treating high blood pressure and also being a glutathione S-transferase inhibitor, had a binding affinity of -6.1 Kcal/mol. The binding affinities with the other ligands are lower as seen in Table.

Ligand	Binding Affinity (kcal/mol)
Benzo[a]pyrene	-14.4
Chlorpyrifos	-7.8
Methyloxindole	-7.6
Glutathione (Ctrl)	-6.1
Bisphenol A	-6.1

Table 4. list of all analysed ligands with control for 2HI4 with their respective name and binding affinities (RMSD value=0)

Ligand	Binding Affinity (kcal/mol)
Quercetin	-6.5
Atrazine	-5.4
Ethacrynic Acid (Ctrl)	-4.6
Malathion	-4.4
1,2,3-Tribromopropane	-2.5

Table 5. list of all analysed ligands with control for 1GTA with their respective name and binding affinities (RMSD value=0)

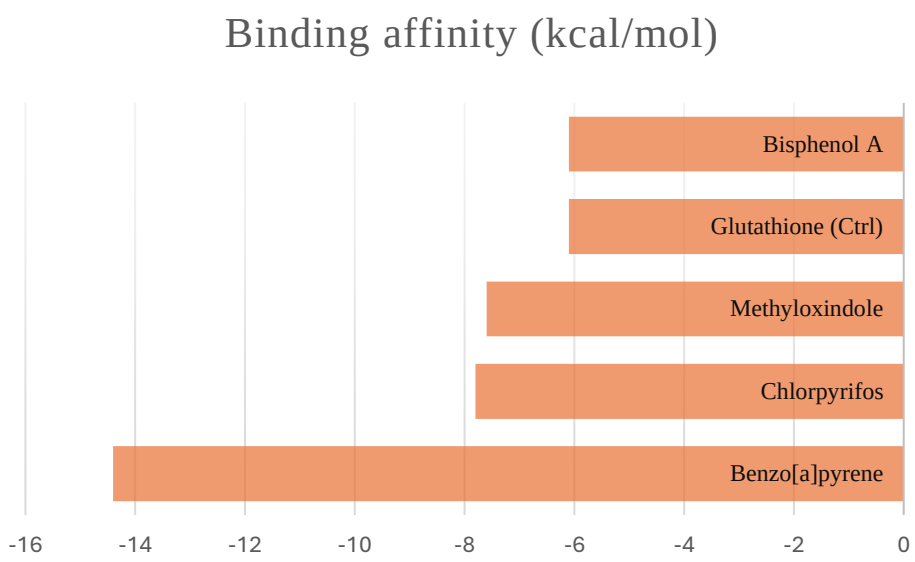


Figure 3. Graphical represent of binding affinity of ligands w.r.t 2HI4

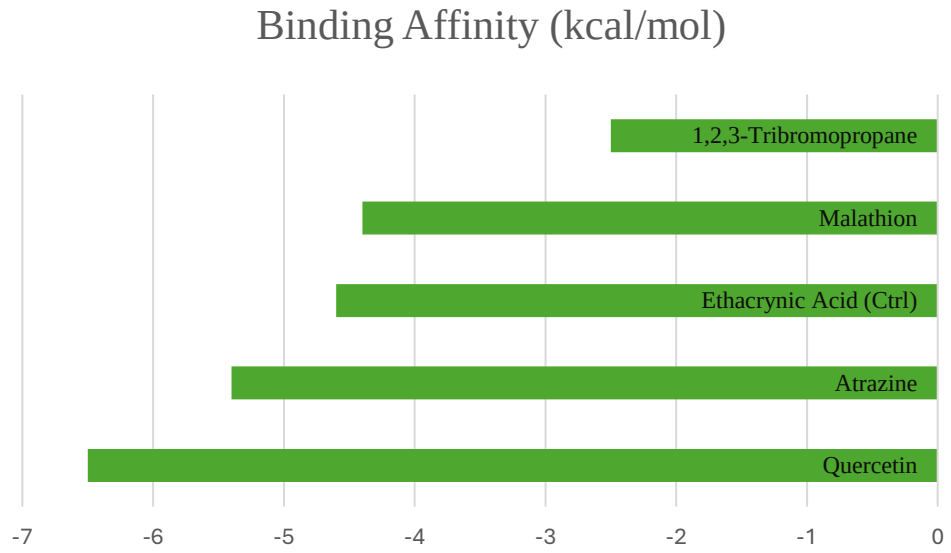


Figure 4. Graphical represent of binding affinity of ligands w.r.t 1GTA

5.2 ADME ANALYSIS

The SWISS-ADME is a free web-based tool which helps in predicting and estimate the value of certain physiochemical properties such as lipophilicity, water solubility, pharmacokinetics, drug-likeness, etc.

Pictorial representation of BOILED-Egg and Radar plot of ligands of 2HI4 and 1 GTA.

Swiss-ADME tool is used to study and predict adsorption, distribution, metabolism and excretion in early stage of drugs development [18].
BOILED-Egg represent that number of molecules which cross the Blood-Brain Barrier under the yellow zone and not cross Blood Brain Barrier beyond the yellow. Another molecular prediction to be PGP+ and PGP-marked with red, Pgp+ marked with blue dot indication that they may be effluxed from brain or gut and less effective. Whereas PGP- not pumped out by P-gp and higher chance to absorbed in Gastrointestine.

The analysis focused on molecular weight, consensus log P, Water solubility Class, Gastrointestinal. (GI) absorption, blood-brain barrier (BBB.) permeability, P-glycoprotein (Pgp) substrate activity, skin permeability (log Kp), Lipinski rule of five's violations, bioavailability score, Leadlikeness violations, and synthetic accessibility and manifest that most of the under range which is given in Table 6 & 7.

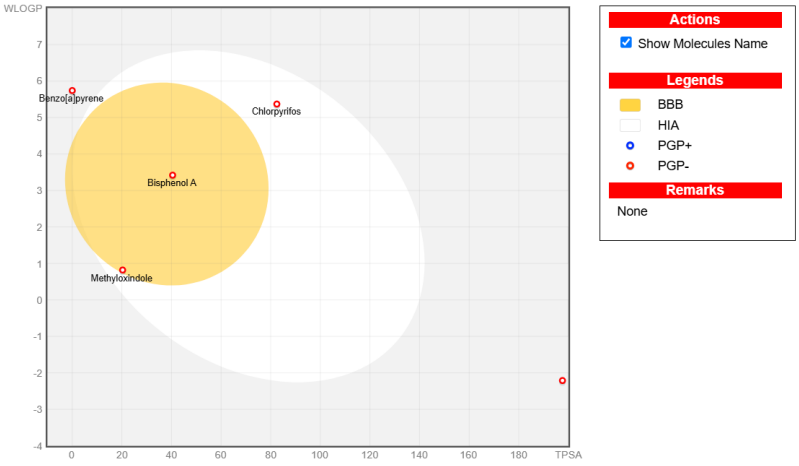


Figure 5. BOILED-Egg diagram of ligands with control (2HI4)

All ligands bioavailability under limit that is 0.55 excepted Glutathione (0.11), violate Lipinski rule of five (Table 6). whereas most of molecules shows high GI absorption and approx. 40% molecule are BBB permeability.

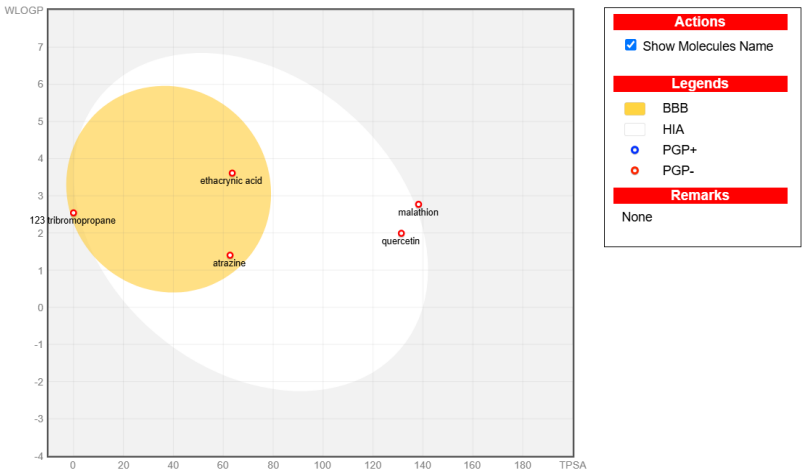


Figure 6. BOILED-Egg diagram of ligands with control (1GTA)

Similarly, all ligands bioavailability under limit that is 0.55 excepted Ethacrynic acid (0.84), violate Lipinski rule of five (Table 7). whereas most of molecules shows high GI absorption and approx. 60% molecule are BBB permeability.

Table 6 & 7 shows SWISS-ADME value of 4 ligands and its controls (Glutathione & Ethacrynic acid) with their respective molecular weight, consensus log P, gastrointestinal. (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (Pgp.) substrate activity, skin permeability (log Kp.), Lipinski rule of five's violations, bioavailability score, and synthetic accessibility.

Molecule	MW	Consensus Log P	GI absorption.	BBB permeant.	Pgp substrate	log Kp. (cm/s)	Lipinski #violations	Bioavailability Score	Synthetic Accessibility
Glutathione	307.32	-2.36	Low	No	No	-11.37	0	0.11	3.06
Bisphenol A	228.29	3.06	High	Yes	No	-5.34	0	0.55	1.43
Methyloxindole	147.17	1.37	High	Yes	No	-6.52	0	0.55	1.33
Chlorpyrifos	350.59	4.12	High	No	No	-4.92	0	0.55	3.31
Benzo[a]pyrene	252.31	5.33	Low	No	No	-3.6	1	0.55	1

Table6. Pharmacokinetics of ligands(2HI4) by SWISSADME

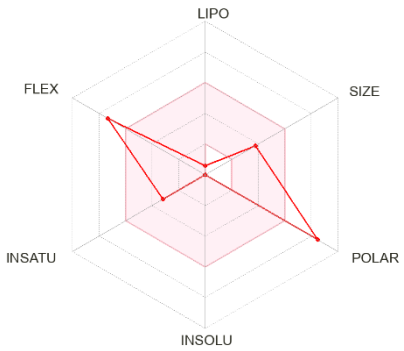
Molecule	MW	Consensus Log P	GI absorption	BBB permeant	Pgp substrate	log Kp (cm/s)	Lipinski #violations	Bioavailability Score	Synthetic Accessibility
Ethacrynic acid	303.14	3.14	High	Yes	No	-5.44	0	0.85	2.43
Atrazine	215.68	1.65	High	Yes	No	-5.76	0	0.55	2.42
Malathion	330.36	2.14	Low	No	No	-6.64	0	0.55	4.35
Tribromo-propane	280.78	2.58	Low	Yes	No	-6.19	0	0.55	3.07
Quercetin	302.24	1.23	High	No	No	-7.05	0	0.55	3.23

Table7. Pharmacokinetics of ligands(1GTA) by SWISSADME

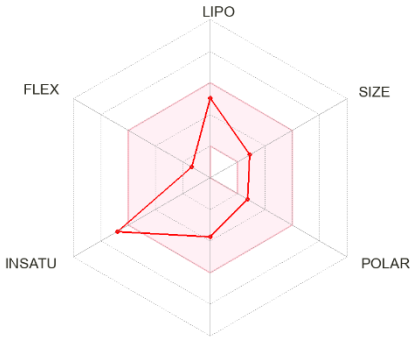
The RADAR plot integrated into SWISSADME web tool which represent novel graph of molecule to facilitate the rapid assessment of multiple physiochemical properties like lipophilicity, polarity, size, solubility, and flexibility onto a single radial graph. Each axis represents to one property and shape give overview of compound' ADME profile. Controls Glutathione and Ethacrynic acid and their

corresponding ligands on the basis of their binding affinity.

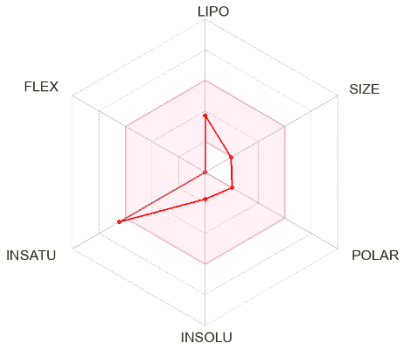
RADAR plots of these compounds are represented in figure. The pink region represents the standard range for every characteristic.



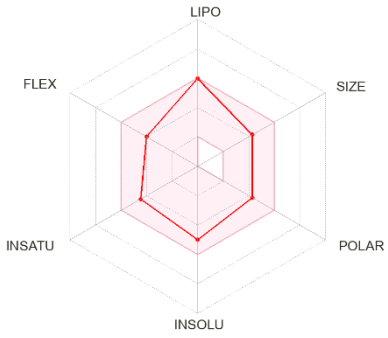
Glutathione (control)



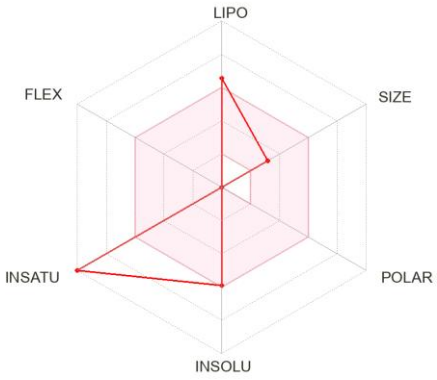
Bisphenol A



Methyloxindole

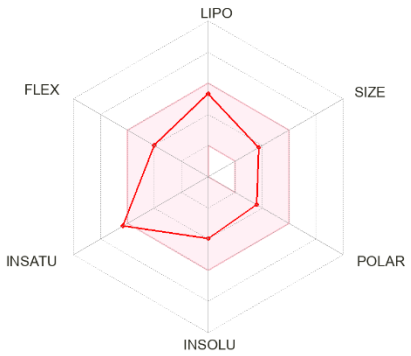


Chlorpyrifos

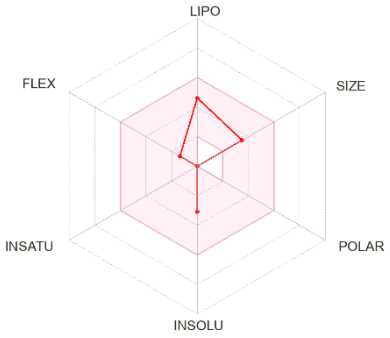


Benzo[a]pyrene

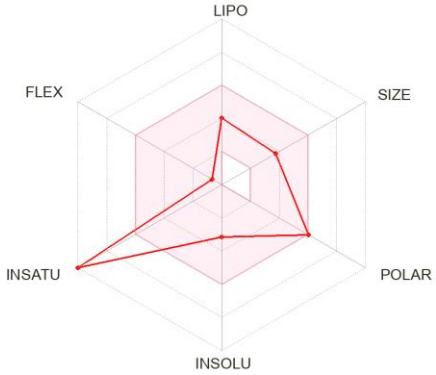
Figure 7. Radar plots of one control and 4 ligands for Human Microsomal P450 1A2 (2HI4)



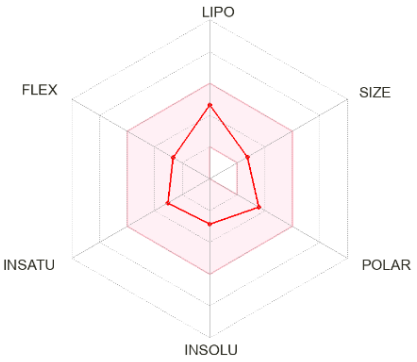
Ethacrynic acid (Control)



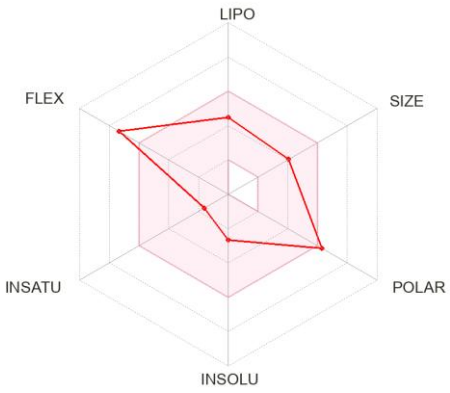
1,2,3-tribromopropane



Quercetin



Atrazine



Malathion

Figure 8. Radar plots of one control and 4 ligands for Glutathione S-transferase (1GTA)

CHAPTER 6

CONCLUSION

Using molecular docking as a predictive in-silico technique, this study provides molecular insights into the responses of two key detoxification enzymes, glutathione S-transferase and cytochrome P450 1A2, to environmental contaminants. By means of methodical docking simulations and binding affinity investigations, we discovered important ligand-enzyme interactions that may indicate metabolic activation or inhibitory pathways. The strongest binding affinity was shown by benzo[a]pyrene with CYP1A2, suggesting that it has a high potential for enzymatic bioactivation or disruption of regular detoxification processes. Similarly, quercetin and GST exhibited positive interactions, indicating effective detoxifying capacity.

Furthermore, ADME profiling offered crucial pharmacokinetic information such as the bioavailability and drug-likeness of the majority of ligands but also emphasizing the limitations imposed by particular compounds such as ethacrynic acid and glutathione. The visual comprehension of each compound's pharmacological appropriateness was improved by the combination of BOILED-Egg models with RADAR plots.

The effectiveness of molecular docking as a quick and affordable method for predicting enzyme-ligand interactions is confirmed by this in-silico method, which also provides a solid basis for environmental toxicology evaluations. Early detection of toxicological risks and possible detoxification processes is made possible by it, which helps guide further in vitro and in vivo validations. Incorporating computational toxicology into environmental risk assessments and public health policies is recommended by the findings. More varied chemical libraries, different detoxifying enzyme isoforms, and dynamic simulations should all be incorporated into this framework in future research to improve biological relevance and forecast accuracy.

REFERENCES

1. Batool M, Ahmad B, Choi S. A structure-based drug discovery paradigm. *International Journal of Molecular Sciences*. 2019;20(11). doi:10.3390/ijms20112783
2. Teodoro M, Briguglio G, Fenga C, et al. *Genetic polymorphisms as determinants of pesticide toxicity: Recent advances.* Toxicol Rep 2019;6:564-570. doi:10.1016/j.toxrep.2019.06.004
3. Seema Sing1,Swati Lal1 ,Jeena Harjit1*,Sulbha Amlathe2 and H.C.Kataria3 , Vol.3,2011,no.5,239-246 33-40
4. R.Q. Su, X.R. Yang, T.L. Zheng, Y. Tian, N.Z. Jiao, L.Z. Cai and H.S. Hong: *Harmful Algae* Vol.799-810 (2007)
5. Huang L, Zhao B, Wang S, Chang X, Klimont Z, Huang G, et al. *Global anthropogenic emissions of full-volatility organic compounds. Environ Sci Technol*. (2023) 57:16435–45. doi: 0.1021/acs.est.3c04106 16.
6. You B, Zhou W, Li J, Li Z, Sun Y. *A review of indoor gaseous organic compounds and human chemical exposure: insights from real-time measurements.* Environ Int. (2022) 170:107611. doi: 10.1016/j.envint.2022.107611
7. Sents W, Ivanova E, Lambrecht C, Haesen D, Janssens V. *The biogenesis of active protein phosphatase 2A holoenzymes: A tightly regulated process creating phosphatase specificity*. FEBS Journal. 2013;280(2):644-61. doi: 10.1111 protein 2HI4
8. Chen G. *Xenobiotic metabolism and disposition. An Introduction to Interdisciplinary Toxicology* 2020; 31–42. doi:10.1016/b978-0-12-813602-7.00003-x
9. Bhatti JS, Vijay vergiya R, Singh B, et al. *Genetic susceptibility of glutathione S-transferase genes (GSTM1/T1 and P1) to coronary artery disease in Asian Indians.* Ann Hum Genet 2018;82(6):448-456. doi:10.1111/ahg.12274
10. Lo HW, Ali-Osman F. *Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. Current Opinion in Pharmacology* 2007;7(4):367-374. DOI: 10.1016/j.coph.2007.06.009.

11. Danielson P. B. *The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Current Drug Metabolism*. 2002;3(6):561–597. doi: 10.2174/1389200023337054
12. Deng J, Yang JC, Feng Y, Xu ZJ, Kuča K, Liu M, et al. *AP-1 and SP1 transactivate the expression of hepatic CYP1A1 and CYP2A6 in the bioactivation of AFB1 in chicken.* Sci China Life Sci. 2024;67(7):1468–78. https://doi. org/10.1007/s11427-023-2512-6. GST
13. Agarwal, Shweta & Mehrotra, Ranjana. (2016). *Mini Review_ An overview of Molecular Docking*. JSM Chemistry. 2. 1024. (molecular docking)
14. Pantsar, Tatu, and Antti Poso. *"Binding Affinity via Docking: Fact and Fiction." Molecules* (Basel, Switzerland) vol. 23,8 1899. 30 Jul. 2018, doi:10.3390/molecules23081899
15. Paggi, Joseph M et al. *"The Art and Science of Molecular Docking." Annual review of biochemistry* vol. 93,1 (2024): 389-410. doi:10.1146/annurevbiochem-030222-120000.
16. Meister A. *Glutathione, metabolism and function via the gamma-glutamyl cycle.* Life Sci 1974;15(2): 177–190.
17. Janos Molnar, John C Somberg, *The clinical pharmacology of ethacrynic acid*. doi: 10.1097/MJT.0b013e318195e460.
18. Daina, Antoine et al. "*Swiss-ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules*." Scientific reports vol. 7 42717. 3 Mar. 2017, doi:10.1038/srep42717

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