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Molecular Docking and in Silico Drug-Likeness Evaluation of Higenamine for its Potential Anticancer Effect

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Abstract

The current research explores the anti-breast cancer effect of higenamine through in silico studies. Higenamine was docked with key protein targets like PTEN, SMO, RTK, CHK2, and TYK2, which are involved in various biological processes. The results revealed strong binding affinities with the targets suggesting effective modulation of these protein functions. Additionally, drug-likeness analysis via Swiss ADME confirmed that higenamine meets Lipinski, Ghose, Veber, Egan, and Muegge criteria, indicating favorable pharmacokinetic properties and potential for oral bioavailability. These findings underscore higenamine as a promising bioactive

compound with a favorable pharmacological profile. Future research should focus on preclinical and clinical tests to assess its safety and efficacy as a therapeutic candidate.

Keywords: Higenamine, Breast Cancer, Molecular Docking, Pharmacokinetics, Drug-Likeness, ADMET Analysis

Introduction

Breast cancer, the second leading cause of death among women accounts for approximately 10.4% of all cancer cases in women (Waks and Winer, 2019). Breast cancer classically originates in the breast tissue either within the milk-producing lobules or the inner lining of the ducts that transport milk to the nipple (Lester et al., 2016) Various lifestyle, environmental, and genetic factors contribute to the of risk breast cancer. These include lack of exercise, family history, aging, chemical exposure, use of birth control pills, DNA alterations, and hormonal influences. Primary prevention involves minimizing exposure to risk factors, while secondary prevention focuses on early detection (Sun et al., 2017).

In recent years, natural compounds have gained significant attention due to their wide biological activities and anti-cancer effect is one of them. One such compound is higenamine which is a naturally occurring alkaloid derived from plant sources. This has shown promising pharmacological activities like anti-inflammatory, antioxidant, and cardioprotective effects. Emerging research suggests this compound could be an anti-cancer lead, particularly in the context of breast cancer (Zhang et al., 2017).

Progression of breast cancer is found to be regulated by several molecular pathways like smoothened homolog (SMO) (Jeng et al., 2020), receptor tyrosine kinases (RTK) (Butti et al., 2018), phosphatase and tensin homolog (PTEN) (Lu et al., 2016), tyrosine kinase 2 (TYK2) (Hynes, 2000), and checkpoint kinase 2 (CHK2) (Tsoi et al., 2022). These proteins play vital role in cell growth, apoptosis, and DNA repair, making them key targets in the development of new therapeutics. Therefore, the current study is designed to assess the anti-breast cancer effect of higenamine by targeting these proteins via molecular docking and pharmacokinetic studies. Along with this in silico toxicity prediction would be done for assessing the safety profile of higenamine.

Materials and Methods

Ligand Preparation

The protein structures of the target receptor molecules Phosphate and Tensin homolog Gene (PTEN) [PDB ID: 7JTX], Smoothened receptor (SMO) [PDB ID: 6XBJ], Receptor tyrosine kinase (RTK) [PDB ID: 4BGK], Checkpoint kinase 2 (CHK2) [PDB ID: 2CN5], and Tyrosine kinase 2 (TYK2) [PDB ID: 4OLI] were retrieved from the RCSB Protein Data Bank. Each protein was prepared by adding missing hydrogen atoms and charges, followed by saving them in PDBQT format within PyRx workspace folders. The ligand, higenamine, was sourced from the PubChem database, imported, and converted into PDBQT format for compatibility with AutoDock Ligand (Kirubhanand et al., 2023).

Molecular Docking Studies

Molecular docking studies were conducted to investigate the interactions between higenamine and the selected target proteins. The

three-dimensional structures of the proteins were obtained from the Protein Data Bank. Binding affinities between higenamine and the target receptors (7JTX, 6XBJ, 4BGK, 2CN5, and 4OLI) were evaluated using AutoDock4, which allowed for blind docking to determine optimal binding box sizes and grid dimensions for each ligand's atoms. Charges, including Gasteiger charges and polar hydrogen atoms, were calculated with AutoDock4. The binding affinity score (kcal/mol) for each ligand-receptor complex was used to determine the optimal orientation of higenamine with each receptor. The interactions were visualized and analyzed using BIOVIA Discovery Studio v21.1. Density Functional Theory (DFT) calculations and molecular dynamics simulations were performed on the compound with the highest docking score (Nachammai et al., 2023).

Toxicity Prediction using Protox-II Tool

The Protox-II tool was used for in silico toxicity predictions of higenamine based on its physicochemical and structural properties. Protox-II enhances traditional toxicity prediction models by encompassing a broad range of toxicity endpoints, including oral and organ-specific toxicity, with a focus on hepatotoxicity. The tool provides insights into potential adverse outcome pathways (AOPs) through nuclear receptor signaling and stress response pathways. Notable receptors assessed include the aryl hydrocarbon receptor (AhR), androgen receptor (AR), estrogen receptor (ER), and peroxisome proliferator-activated receptor gamma (PPAR-γ), along with stress response markers like ATPase family AAA domain-containing protein 5 (ATAD5), phosphoprotein tumor suppressor (p53), and mitochondrial membrane potential (MMP) (Nachammai et al., 2023).

Drug-Likeness and Pharmacokinetics Analysis

Drug-likeness and pharmacokinetic properties of higenamine were evaluated using the SwissADME online tool. This calculates physicochemical and pharmacokinetic parameters based on established drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge). Compounds that were found to adhere to these guidelines were subjected to further in silico screening for pharmacokinetic properties. Pre-ADMET web-based applications were utilized to predict and assess various pharmacokinetic attributes, correlating the chemical and structural characteristics of higenamine with its potential for effective drug delivery and metabolism (Nachammai et al., 2023).

Result and Discussion

Molecular Docking

In this study, molecular docking of higenamine was performed various protein targets using AutoDock (Version 4), as given in Table 1. Chemical structure of higenamine is depicted in figure 1.

Table 1 Target Protein Molecules and their PDB IDs

Sl No.	Target protein	PDB ID	Structure
1	PTEN	7JTX	
2	SMO	6XBJ	

3	RTK	4BGK	
4	CHK2	2CN5	
5	TYK2	4OLI	

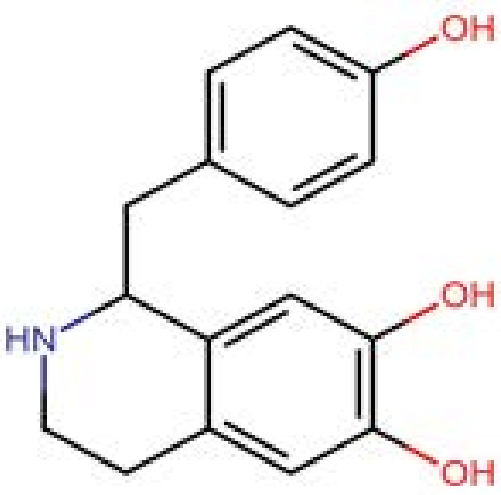

Figure 1 Chemical Structure of Higenamine

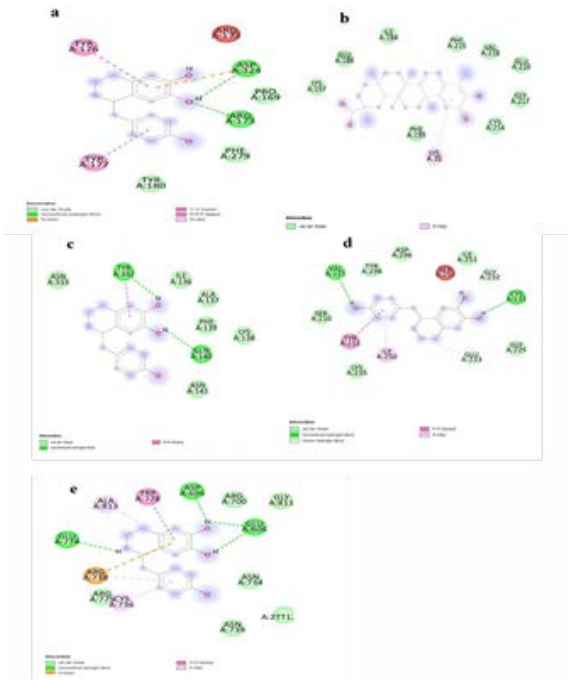

Figure 2 Molecular Docking Analysis of Marumoside A with a-Phosphatidylinositol 3,4,5-Trisphate and Dual Specific Protein Phosphate PTEN, b-Smoothened, Frizzled Class Receptor, c-Gamma-Butyrobetaine Dioxygenase, d-Serine/Threonine-Protein Kinase CHK2, e-Non-Receptor Tyrosine Protein Kinase TYK2

Figures 2a-e shows the interactions between higenamine and the protein targets. The docking interactions between higenamine and PTEN exhibited a binding affinity of 6.3 kcal/mol, with Pi-Pi and Pi-alkyl interactions observed. Specifically, tyrosine residues 176 and 177 were involved in Pi-Pi stacking with higenamine, while Asp 324 and Arg 173 formed hydroxyl bonds with the ligand. This suggests a favorable interaction profile, as Pi-Pi stacking and hydrogen bonds are key factors in ligand stabilization within the active site.

Similarly, it demonstrated a binding affinity of -6.2 kcal/mol with SMO, forming Pi-Pi, Pi-alkyl, Van der Waals, and hydrogen bonds. Remarkably, Tyr 320 facilitated Pi-Pi interactions, while hydrogen bonds were formed with Gln 304 and Ile 319. Additional Van der Waals interactions with Thr 321, Phe 334, and Asp 341 further enhanced its stabilization within the binding site.

The binding affinity of higenamine with RTK was 6.5 kcal/mol. The major interactions were hydroxyl, Pi-Pi stacking and Van der Waals. Key interactions included hydrogen bonds with Tyr 332 and hydroxyl bonds with Glu 140, accompanied by Van der Waals forces contributed by Asn 141, Phe 139, and Ile 136.

In the same way, CHK2 displayed a binding affinity of -6.8 kcal/mol with higenamine, involving Pi-Pi, Pi-alkyl, and hydrogen bonding. Tyrosine 212 facilitated Pi-Pi interactions, while Ile 250 contributed to Pi-alkyl interactions. Gly 232 and Cys 231of CHK2 formed hydrogen bonds and Ser 210 and Lys 235 formed Van der Waals interactions with higenamine.

Higenamine was found to interact with TYK2 with the highest affinity of 7.7 kcal/mol. The molecular interactions included Pi-Pi stacking with Trp 778, Pi-alkyl with Ala 813, and hydrogen bonding with Asp 696 and Gln 806. Pi-cation interactions with Arg 738 and additional Van der Waals forces with Arg 700 and Gly 811 contributed significantly to ligand stability.

Table 2 Toxicity Prediction of Higenamine by Protox-II

Classification	Toxicity	Higenamine
Organ toxicity	Hepatotoxicity	Inactive
	Carcinogenicity	Inactive
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
TOX21-Nuclear receptor signalling pathway	Aryl hydrocarbon Receptor (AhR)	Inactive
	Androgen Receptor (AR)	Inactive
	Androgen Receptor Ligand Binding Domain (ER)	Inactive
	Aromatase	Inactive
	Estrogen Receptor Alpha (ER)	Inactive
	Estrogen Receptor Ligand Binding Domain (ER-LBD)	Inactive

Table 3 Toxicity Prediction of Higenamine by Stoptox

Toxicity	Score (%)	Prediction
Acute inhalation toxicity	80.0	Non-toxic
Acute oral toxicity	60.0	Non-toxic
Acute dermal toxicity	74.0	Non-toxic
Eye Irritation and Corrosion	70.0	Non-toxic
Skin Sensitization	60	Non- sensi-tizer
Skin Irritation and Corrosion	60	Negative

Toxicity and Drug-Likeness of Higenamine

Toxicity of higenamine was predicted by Protox-II and Stoptox servers and it was found to be nontoxic (Table 2 and 3). Similarly, drug-likeness predictions using the SwissADME tool assessed properties

like molecular weight, polar surface area, hydrogen bonding capacity, and lipophilicity. Higenamine adhered to the Lipinski's rule of five, indicating potential for oral administration. Similarly, it was found to be following Ghose rule which further supports its drug-likeness. Further it meets the requirements of the Veber rule, Egan rule and Muegge rule indicating favorable absorption and bioavailability (Table 4).

Overall, higenamine demonstrated adherence to all these rules, suggesting its favorable pharmacokinetic properties like oral bioavailability, intestinal absorption, and membrane permeability. These findings highlight its potential for further drug development.

Molecular docking and drug-likeness analyses collectively indicated that higenamine could be potent pharmacological agent for the targeted proteins warranting further experimental validation for clinical applications.

Table 4 Drug Likeliness Prediction of Higenamine

Sl No.	Physicochemical Properties	Higenamine
1.	Molecular weight	271.31 g/mol
2.	Rotatable bond	2
3.	Hydrogen bond acceptor	4
4.	Hydrogen bond donor	4
5.	Molar refractivity	81.15
6.	Topological polar surface area	72.72 A2
7.	Fraction Csp3	0.25
8.	Heavy atoms	20
9.	Formula	C16H17NO3

Conclusion

Molecular docking study demonstrated that higenamine interacted with multiple anti-cancer targets like PTEN, SMO, RTK, CHK2 and TYK2. These interactions were via non-covalent bonds like Pi-Pi, Pi-alkyl, hydroxyl, Van der Waals, and hydrogen bonds, with specific amino acid residues, suggesting that higenamine can effectively bind to and modulate these proteins' functions. Furthermore, Swiss ADME confirmed the drug-likeness of higenamine by meeting Lipinski, Ghose, Veber, Egan, and Muegge rules, indicating its favorable oral bioavailability and pharmacokinetic properties. This supports the potential of higenamine for oral administration and enhanced intestinal absorption. These in silico findings collectively highlight its suitability as a bioactive compound with promising pharmacological profiles, while further in vitro and in vivo studies are needed to validate these.

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