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DESIGN OF SOME 2-PHENYL CHROMANE DERIVATIVES AS NEW POTENTIAL TYROSINASE INHIBITORS BY IN SILICO APPORACH

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ABSTRACT

One of the most glaringly varied phenotypes in humans is hyperpigmentation disorders, which are frequent in the Indian population. It is well recognised that pigmentation disorders can be exacerbated or brought on by UV light exposure. Tyrosinase inhibitors can be useful tools in the cosmetics and pharmaceutical sectors since they are involved in the manufacture and control of melanin. In the last five years, chalcones, hydroxystilbenes, thiosemicarbazones, and lignans have been identified in the literature as the most effective tyrosinase inhibitors among the most often used scaffold, flavonoids. According to a number of published inhibition mechanisms, the inhibitors discussed here must contain copper chelating and/or hydrophobic moieties in order to have effective inhibition capabilities. In the current study, however, 18 derivatives of the 2-Phenyl chromane scaffold were subjected to in silico studies using Pass online, Mol Inspiration, Pro Tox II, and IGEMDOCK to investigate their *in vitro* inhibitory effect against mushroom tyrosinase. *In silico* docking studies with mushroom tyrosinase (PDB ID 2Y9X) predicted that these compounds would interact with the active site of mushroom tyrosinase. The findings highlighted the significance of the flavonoid core with a hydroxyl group at C-7 as a significant contributor to tyrosinase inhibitory activity. These compounds could form metal-ligand interactions with the Cu^{2+} ions in the active site, according to the docked conformations. As a result, the *in silico* results served as a template for subsequent synthesis, *in vitro*, and *in vivo* studies to obtain promising mushroom tyrosinase inhibitors.

Keywords: *In silico* approach, Tyrosinase, 2-Phenyl chromane

1. INTRODUCTION

Tyrosinase, a multifunctional copper-containing oxidase enzyme, is important in biology because it is essential for the manufacture of the pigment melanin in living things. It causes polyphenolic substrates to be o-hydroxylated, resulting in diphenol (catechol) derivatives that oxidize to produce o-quinone products. As a rate-limiting enzyme, tyrosinase is known to catalyse the hydroxylation of L-tyrosine to L-DOPA and 3,4-dihydroxyphenylalanine to dopaquinones, which can result in an unusual buildup of melanin pigment in the epidermis (1). The standard tyrosinase inhibitors (TIs) in this situation, such as kojic acid, tropolone, hydroquinone, and mercury, are clinically effective and may serve as medications for treating facial aesthetic conditions and other more severe dermatological conditions linked to melanin hyperpigmentation in human skin. Moreover, they are utilised in agriculture as bio-insecticides and in cosmetics as skin-whitening agents (2). Tyrosinase is also in charge of a number of biological procedures in arthropods, such as cuticle sclerotization, wound healing, and protective encapsulation.

In order to identify potentially effective chemicals, we looked at the tyrosinase inhibitory activity of 8 flavonoids, including kojic acid, 4, 6, 4-trihydroxyaurone, isoliquiritigenin, Baicalein, Dihydromyricetin, 7, 3, 4-trihydroxyisoflavone, Steppogenin, and Quercetin. In order to identify the types of inhibition for the compounds with the highest activity, tyrosinase inhibition and enzyme kinetics were first examined in an in vitro model. Second, docking investigations were conducted in an in silico model to define SARs, which were also evaluated using a statistical model. Consequently, the functional mechanisms of the investigated substances are described using a mix of bioinformatics simulation and biological in vitro studies (3).

1.1 The role of tyrosinase enzyme in the biosynthesis of melanin pigment

Melanin is an essential pigment produced by dermal cells in the deepest layer of epidermis, known as the basal layer. It is produced by specialised cells called melanocytes via a multi-step chemical process that begins with the oxidation of the amino acid tyrosine and ends with polymerization. Melanogenesis occurs in the skin after UV radiation exposure and is capable of dissipating nearly all of the absorbed UV radiation. Thus, melanin plays an important role in protecting the skin from the harmful effects of the sun's ultraviolet rays. Even though melanin's primary function in human skin is photoprotection, melanin accumulation in various parts of the skin results in more pigmented spots and patches, which may become an aesthetic issue (4),(5).

In the worst-case scenario, frequent exposure to UV radiation may be linked to an increased risk of malignant melanoma, a cancer of melanocytes 3, 4. Melanocytes, as previously stated, are cells produced in the dermal basal layer and play an important role in melanogenesis, the process of biosynthesizing melanin. Melanocytes are melanoblasts formed-dendritic cells that are unpigmented cells derived from embryonic neural crest cells. These are primarily involved in the synthesis of melanin pigments. Each melanocyte is surrounded by approximately 36 keratinocytes, the primary type of cell found in the skin's outermost layer. After being synthesised, melanin is stored inside melanosomes and then transferred to the outermost layer of suprabasal keratinocytes via extended dendrites (6).

According to Fig. 1, keratinocyte cornification, which comprises mature melanosomes that are biosynthesized, employed to store melanin, and transported from the melanocytes to the keratinocytes, is what causes skin colour. Eventually, keratinocytes

go through a process called cornification or programmed cell death and move to the skin's surface with the encapsulated melanin. The cornified layer, formed by cornification often known as the outermost skin barrier (7).

Tyrosinase inhibitors are important inhibitory drugs to fight the overproduction of melanin since tyrosinase is the primary enzyme that speeds up the process of melanin creation. On the other hand, as free radicals can also cause the manufacturing of melanin, several natural and artificial antioxidant systems that can scavenge such radicals may be able to regulate excessive melanin production (8).

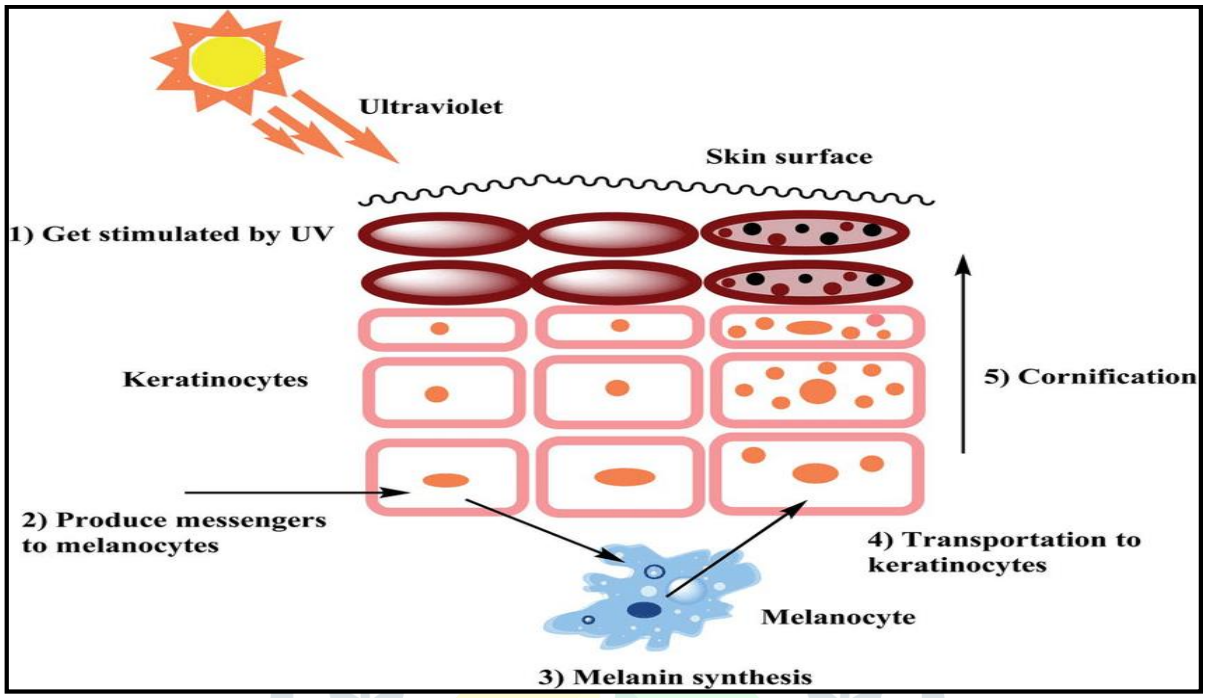

Fig. 1. Mechanism of pigmentation

1.2. Tyrosinase inhibition mechanism

The two divalent copper ions that are surrounded by three histidine residues are structurally responsible for tyrosinase's catalytic activity. An in silico docking study revealed that the various TIs expressed their inhibitory potential via hydrophobic p-alkyl, hydrophobic p-s, hydrophobic p-p T-shaped, and hydrophobic p-p stacked type interactions with His 263, Phe264, Ala 286, Val 283, and His 85 amino acid residues within the activation loop of the targeted tyrosinase biological molecule (9).

According to thorough structure-activity relationship investigations, the flavonol class stands out among all flavonoid derivatives and provides the most effective inhibitory activity because of its structural likeness to the common inhibitor kojic acid. The phenyl-g-benzopyran core structure in particular is crucial for the tyrosinase inhibitory action. It should be emphasised, however, that variations in the locations and types of substituents on the aryl ring (B) as well as the characteristics of ring B are responsible for variations in the action of these inhibitors. Ring A of 2-phenylchromones gains in electron density and hydrophobicity when halogen atoms are added, which promotes strong bonding contacts. As a result, the structure of such inhibitors is customizable and can be tuned to achieve maximum potency. According to the findings, 3-hydroxyflavone molecules can be intriguing candidates for the

treatment of tyrosinase-related diseases and as lead compounds for the development of potent new tyrosinase inhibitors (8).

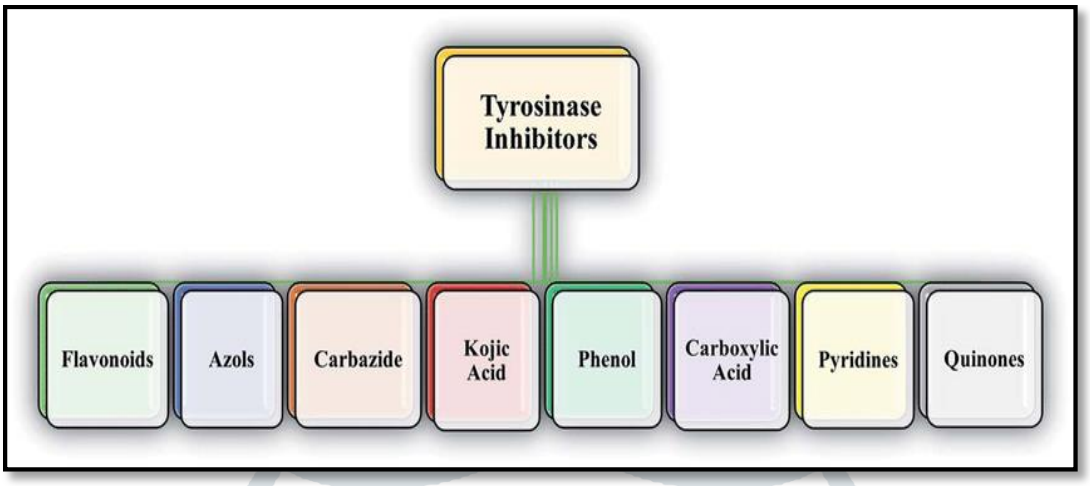

Fig. 2 Representative anti-tyrosinase scaffolds

1.3 Natural phenolic tyrosinase inhibitors and their sources

To protect themselves from the harmful effects of UV radiation, living organisms have evolved shielding mechanisms. As a result, nature provides a plethora of tyrosinase inhibitor sources. Several researchers have described the isolation of tyrosinase inhibitors from a variety of sources, including mycological metabolites, plants, and aquatic algae. Tyrosinase inhibitors derived from natural resources are more commonly sought after than synthetic ones in order to meet cosmetic demand and requirements. Many researchers have since used the most well-known TI, such as kojic acid, as a standard to adjust and normalise the reported inhibitory activity values (10).

A structure-based virtual screening method called docking predicts, with respect to experiment, the preferred binding orientation of the ligand to the chosen receptor in a stable compound. This method looks through a wide range of potential interactions to find a collection of ligand poses that correspond to the ligand's local minimum-energy locations. In order to precisely rank-order various ligands in relation to empirically determined binding affinities, a binding energy is calculated (11).

In the current investigation, Mol inspiration (Absorption, Distribution, Metabolism and Excretion: ADME) and ProTox-II were used to predict toxicity and conduct activity spectrum predictions for 18 leads of the 2-phenylchromane scaffold (basic flavonoid skeleton) (12). Eight of these leads that were found using these filters were put through molecular docking tests. These substances are very near mimics of kojic acid and possess structural components that enable them to chelate copper ions and establish contacts with important residues in the active site. Some of these compounds displayed tyrosinase inhibitory activity and were taken into consideration when designing the study compounds based on the in vitro assessments.

2. MATERIALS AND METHODS:

In silico design was carried out using the online or web server free softwares

- 18 known flavonoids based on review of literature and some of them authenticated by PUB-CHEM database (13-15).

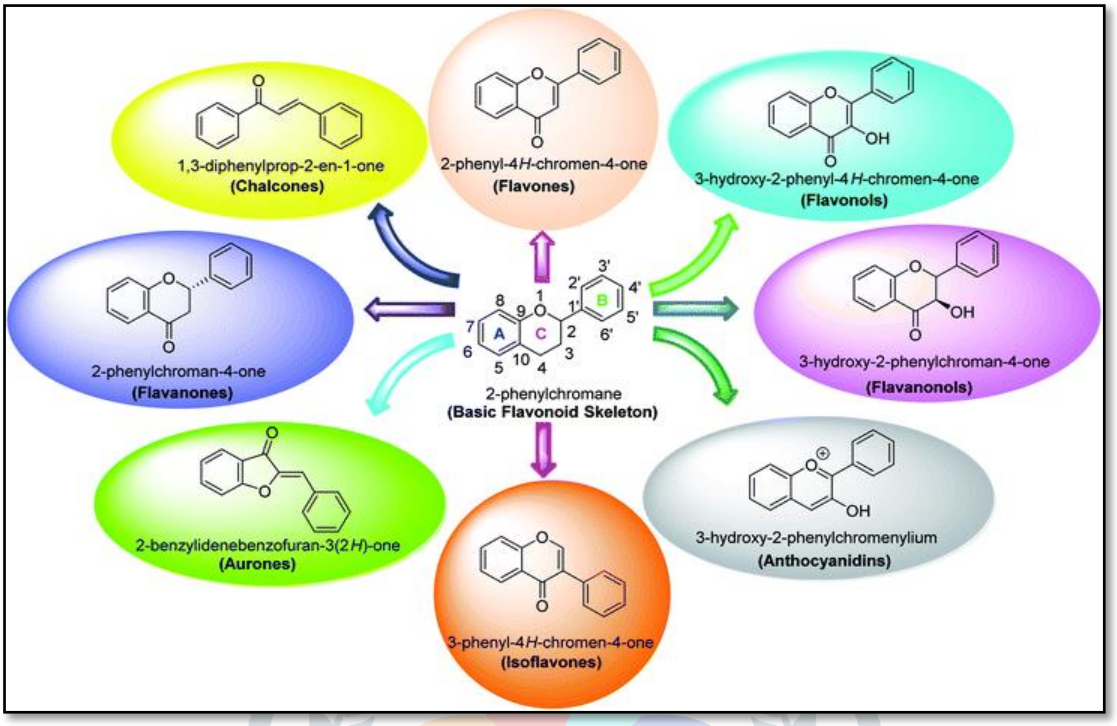

Fig. 3 Selected leads from sub classes of flavonoids

2.1 ACD/ Chemsketch Version (15)

2.2 PASS Prediction (16)

2.3 Mol Inspiration (17)

2.4 Pro Tox II (18)

2.5 iGEMDOCK (19)

- Target protein 2Y9X (20),(21),(22)

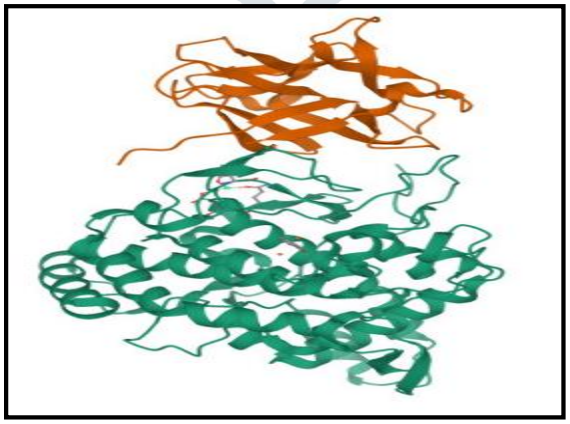

Fig 4. 3D structure of protein 2Y9X

2.1 PASS Prediction

2.1.1 How to draw a structure of a molecule:

The PASS tool will analyse the 2D molecular structures to decipher the biologically active spectra (23). ACD/ChemSketch version 12 (http://www.acdlabs.com/home/) can be used to depict the structure of a molecule. To determine the molecule's predicted biological activity spectrum, the structure of the molecule can be saved as a ChemSketch 2.0 document (*.SK2) or MDL Mol file (*.mol). A JAVA applet that employs a 2D chemical sketch-drawing application can also be used to directly design the structure on the PASS prediction website.

2.1.2 Pass prediction approach:

By "comparing" the structure of the novel chemical with that of a well-known biological active substrate that is already present in the database, activity of the molecule is anticipated. The activity spectrum estimate algorithm is based on the Bayesian method. For prediction thresholds of Pa > 30%, Pa > 50% and Pa > 70%, the PASS prediction tool will predict the Pa: Pi (active, inactive ratio). Leave-one-out cross validation (LOO CV) estimation indicates that the average prediction accuracy is approximately 95%. The estimation of biological activity is more accurate since the accuracy of PASS prediction depends on detailed information about the biological activity spectrum for each molecule provided in PASS training set.

2.2 Mol Inspiration:

Mol inspiration visualises a set of molecules encoded as SMILES or SDfiles. Our depiction engine automatically converts SMILES into molecule 2D representations (24). Displaying associated data, selecting molecules, performing built-in substructure searches, and exporting selected molecules are all supported. Because the Viewer is written in Java, it is platform independent and can be used on any computer that has the Java runtime installed. Mol inspiration helps the internet chemistry community by calculating important molecular properties (logP, polar surface area, number of hydrogen bond donors and acceptors, and so on), as well as predicting bioactivity scores for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors).

2.3 Pro Tox II:

Prediction of compound toxicity is an important step in the drug development process. Computational toxicity estimations are not only faster than animal toxic dose determinations, but they can also help to reduce the number of animal experiments. To learn more about reducing animal testing, click here. ProTox-II uses molecular similarity, fragment propensities, most frequent features, and (fragment similarity based CLUSTER cross-validation) machine-learning to predict toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox 21), pathways, and toxicity targets (25).

Toxicity Targets:

Protein targets known to cause toxic effects and unfavourable medication reactions are known as toxicity targets. Here, we use a set of pharmacophores based on protein ligands to forecast potential binding to hazardous targets to gain greater knowledge on models based on paharmacophore.

Toxicity Pathway:

The Tox 21 (Toxicology in the 21st Century) platform evaluated the effects of substances using a variety of tests that focus on cellular functions. The programme aims to discover mechanisms of action for additional research, such as disease-associated pathways, and to prioritise chemicals for detailed toxicological screening for more information about the Tox21 Program to discover more about prediction models that are based on machine learning.

2.4 iGEMDOCK:

A graphical environment for pharmacological interaction recognition and virtual screening. Pharmacological interactions aid in the identification of lead compounds and the understanding of ligand binding mechanisms for a therapeutic target. Currently, these interactions are frequently inferred from a collection of active compounds obtained experimentally. Furthermore, most docking programmes only loosely linked the stages of structure-based virtual screening (VS) from preparation to post-screening analysis. An integrated VS environment is useful for drug discovery because it provides a friendly interface for seamlessly combining different-stage programmes for VS and identifying pharmacological interactions from screening compounds. We created iGEMDOCK, an easy-to-use graphic environment for docking, virtual screening, and post-screening analysis (26).

Method:

iGEMDOCK is a nearly automatic virtual screening tool. GEMDOCK can be used sequentially in four computational phases: target and database preparation, molecular docking, and post-docking analysis.

iGEMDOCK employs an empirical scoring function as well as an evolutionary strategy. The electrostatic, steric, and hydrogen-bonding potentials comprise the iGEMDOCK energy function. The latter two terms employ a simple linear model that recognises potential complexes quickly. The central idea of this evolutionary approach is to create multiple operators who collaborate using a family competition paradigm similar to a local search procedure.

• Algorithm generic

• Scoring function

3. RESULT AND DISCUSSION

3.1 PASS prediction

For a molecule to be a powerful drug molecule, it must meet a number of requirements. The evaluation of PASS, which provides thorough details regarding the biological activity spectrum for each molecule present in the PASS training set, is a step in the drug development process.

The findings from a library of 18 flavonoids showed in Table 1. Out of that 12 of the leads had passed the first filter's PASS prediction and had melanin-inhibitory action with a Pa between 0.300 and 0.700.

Table 1: Pass prediction

Lead	Pub Chem ID	Pa	Pi
4,6,4-Trihydroxyaurone	42607763	0.354	0.007
Taxifolin	439533	0.752	0.001
Isoliquiritigenin	638278	0.393	0.005
Flavone	10680	0.427	0.004
Baicalein	5281605	0.462	0.004
Flavanol	7309334	0.415	0.005
Dihydromyricetin	161557	0.708	0.001
Isoflavones	70267806	0.032	0.023
7,3,4,trihydroxy isoflovones	5284648	0.384	0.005
Flavanone	10251	0.511	0.003
Steppogenin	21596130	0.703	0.001
Flavonols	11349	0.415	0.005
Quercitin	5280343	0.538	0.003
Kuraridin	9954815	0.690	0.001
Kojic acid	3840	0.433	0.004
Pelargonidin	440832	0.383	0.005
Xanthohumol	639665	0.605	0.002
Alpinetin	154279	0.645	0.002

3.2 Drug -likeness and in silico ADME prediction analysis

Furthermore, the in silico drug design process includes evaluating a molecule's ADME properties, which stand for absorption, distribution, metabolism, and excretion. The pharmacokinetic profile of a small molecule is critical in the drug discovery process. The bioavailability radar gives the first indication of a molecule's drug-likeness. Table 2, revealed that 12 leads passed the Lipinski's five criteria (MW500 Da, LogP 5, nHBD 5, NHBA 10, and TPSA). Enzyme inhibition availability is one of the parameters assessed by the Mol Inspiration web server. All chemicals are capable of inhibiting enzymes to varying degrees (-0.12 to 0.32). The results for the ADME parameters (Molecular weight, Log P, no hydrogen bond donor, no hydrogen bond acceptor, no rotatable bond, TPSA, no Lipinski rule violation, Log Kp, enzyme inhibition capability, and synthetic accessibility) were all favourable for the compounds included in this study. All have good oral bioavailability or permeability as a result.

Table 2: mol inspiration prediction data

Lead	MW ≤500 Da	LogP <5	nHBD ≤5	NHBA ≤10	TPSA	No of violation
Baicalein	270.24	2.68	3	5	90.89	0
Pelargonidin	271.25	5.12	4	5	92.08	1
Isoliquiritigenin	258.27	1.48	3	4	77.75	0
7,3,4-Trihydroxyisoflaones	270.24	2.07	3	5	90.89	0
Steppogenin	288.25	2.03	4	6	107.22	0
Kojic acid	142.11	-0.89	2	4	70.67	0
4,6,4-Trihydroxyaurone	270.24	1.96	3	5	90.89	0
Quercitin	302.24	1.68	5	7	130.35	0
Flavone	222.24	3.74	0	2	30.21	0
Kuraridine	438.52	6.37	4	6	107.22	1
Flavanonols	240.36	6.10	1	3	46.53	1
Flavanone	224.26	3.18	0	2	26.30	0
Flavonols	238.24	3.45	1	3	50.44	0
Alpinetin	270.28	2.66	1	4	55.77	0
Xanthohumol	354.40	4.80	3	5	86.99	0

3.3 ProTox II Predication Analyses

Pro Tox II generates prediction findings for acute toxicity and toxicity targets in real time. To forecast the toxicity parameters, we used the web-server ProTox-II. Table 3 show that chemicals (IIb) and (IIc) have no effect on (hepatotoxicity) and (carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity). Compound (IIa) on the other hand is active on (hepatotoxicity) and (carcinogenicity and mutagenicity).

Table 3. Predicted toxicity data for leads

Lead	Hepato-toxicity	Carcino-toxicity	Immuno-toxicity	Muta-genicity	Cyrto-toxicity
Baicalein	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Isoliquiritigenin	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
7,3,4-Trihydroxyisoflaones	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Steppogenin	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Kojic acid	Y(0.69)	N(0.62)	Y(0.92)	N(0.97)	N(0.93)
4,6,4-Trihydroxyaurone	Y(0.69)	N(0.62)	Y(0.92)	N(0.97)	N(0.93)

Quercitin	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Flavone	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Xanthohumol	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Alpinetin	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Flavonol	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)
Flavanone	Y(0.69)	N(0.62)	Y(0.96)	N(0.97)	N(0.93)

(Probability): Y (Yes, active), N (No, inactive)

Activity color key	Color
Active	
Inactive	

4. Molecular docking

Molecular docking studies were performed on eight natural flavonoids derivatives, and the docking scores (Binding energy) of these leads varied from -137.2 to -200.5 kcal/mol. (Table 4). IGEMDOCK software predicted the possible hydrophobic interaction between the docked ligand (Fig. 5: Tropolone). According to the findings, the lead compounds Baicalein, 7, 3, 4-Trihydroxyisoflaones, Steppogenin, and 4, 6, 4-Trihydroxyaurone create four bonds with H-S-HIS-61, H-S-HIS-85, H-S-CU-400, and H-M-CU-401. (Fig 6).

Table 4 .The protein-ligand interactions of native ligand of tyrosinase 0TR with active site 2Y9X

Lead	**Binding energy kcal/mole)**	**Amino acids involved in the interaction**	**Length of hydrogen bonds in A^0**
Native ligand of Tyrosinase 0TR	-69.9	H-S-HIS-61 H-S-HIS-85 H-S-HIS-259 H-S- CU-400 H-M- CU-401	3.49 3.50 5.98 2.50 4.03
Baicalein	-156.0	H-S-HIS-61 H-S-HIS-85 H-S- CU-400 H-M- CU-401	4.77 2.87 7.46 7.41
Isoliquiritigenin	-146.7	H-S-HIS-263 H-M- CU-401	5.90 6.00

7,3,4- Trihydroxyisoflaones	-156.1	H-S-HIS-61 H-S-HIS-85 H-S- CU-400 H-M- CU-401	4.80 2.90 7.50 7.50
Steppogenin	-165.4	H-S-HIS-61 H-S-HIS-85 H-S- CU-400 H-M- CU-401	5.80 3.50 9.00 9.00
4,6,4- Trihydroxyaurone	-156.1	H-S-HIS-61 H-S-HIS-85 H-S- CU-400 H-M- CU-401	4.80 2.50 7.50 7.40
Xanthohumol	-200.5	H-S-HIS-263 H-M- CU-401	7.10 7.50
Alpinetin	-154.3	H-S-HIS-263 H-M- CU-401	5.80 6.00

Derivatives Alpinetin, Xanthohumol, and Isoliquiritigenin each form two bonds with H-S-HIS-263 and H-M-CU-401 (Fig. 7). The hydrophobic interactions with residues are the same for the derivatives Baicalein, 7, 3, 4-Trihydroxyisoflaones, Steppogenin, 4, 6, 4-Trihydroxyaurone, and tropolone, according to a comparison of their binding mechanisms.

In order to conduct the docking study, Agaricus bisporus mushroom tyrosinase's 3D structure was employed. Using the Pymol tool, potential H-bonding and metal interactions are evaluated. The docked ligand (Fig. 5: Tropolone; 0TR) and eight lead compounds were projected to bind hydrophobically at the residues of the catalytic site using the iGEMDOCK software. Steppogenin, 7, 3, 4-trihydroxyisoflaones, 4, 6, 4-trihydroxyaurone, and other ligand showed satisfactory docking results in accordance with binding energies.

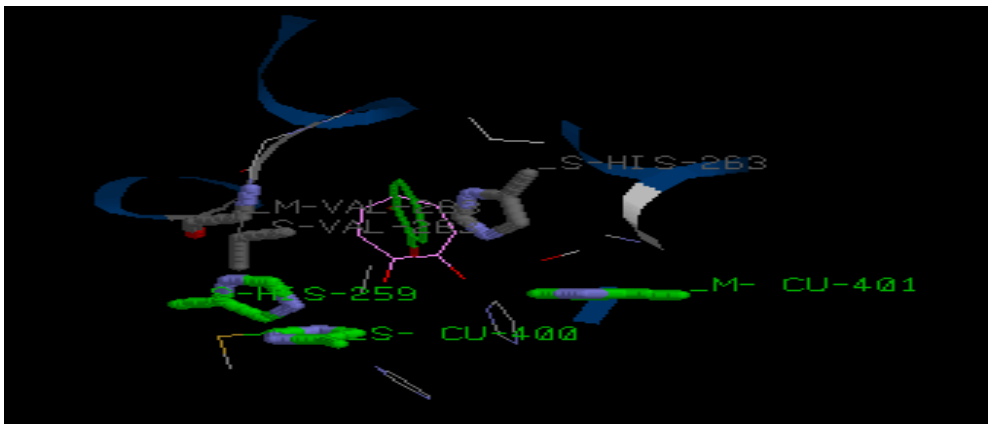

Fig 5. Docking pose of ligand 0TR at active site of tyrosinase enzyme (2y9x)

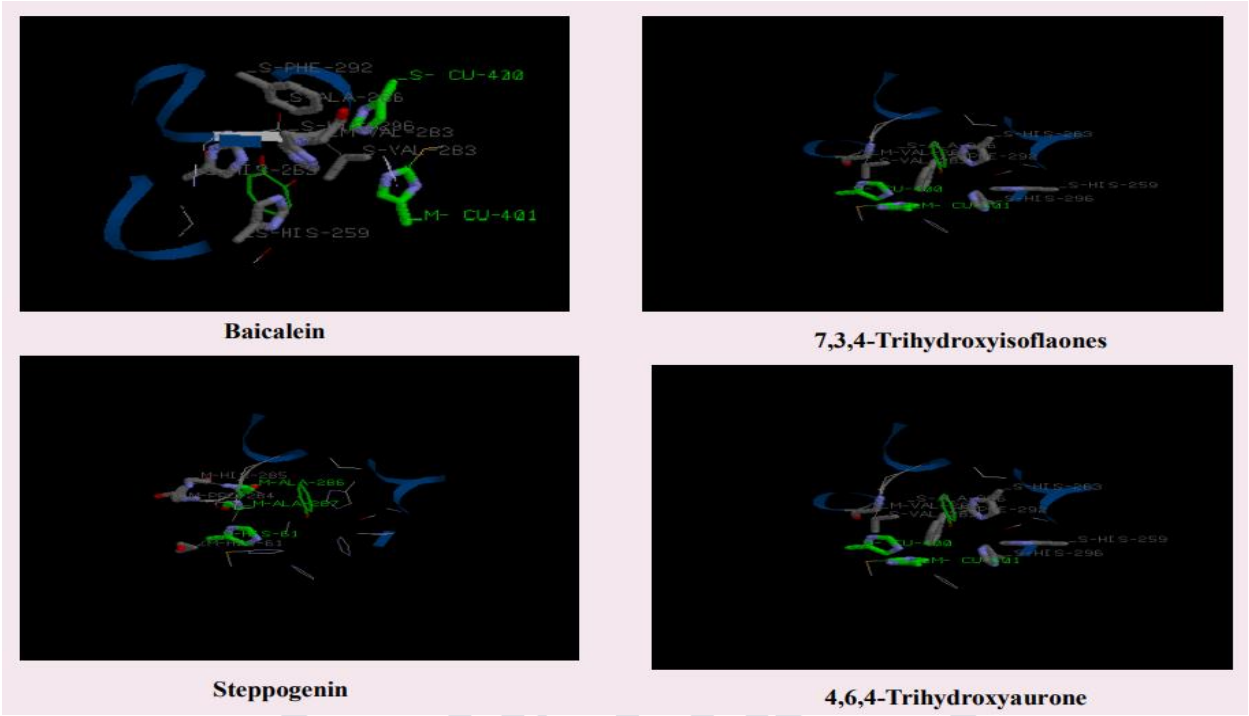

Fig. 6 Binding modes of compounds in the tyrosinase active site.

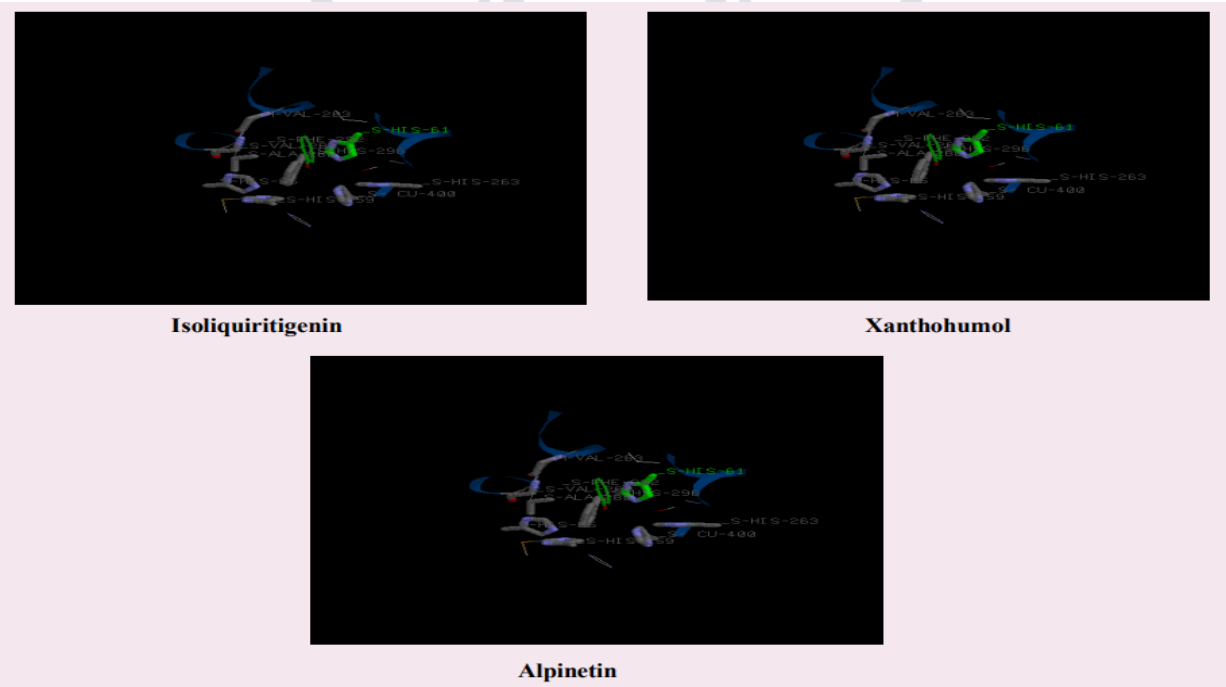

Fig. 7 Binding modes of compounds in the tyrosinase active site

5. CONCLUSION

Tropolone and its natural derivatives are recognised for their bioactive potential as tyrosinase inhibitors by the current methodologies used in the identification of *Agaricus bisporus* mushroom tyrosinase inhibitors. The potential for 2-phenyl chromane derivatives of mushroom tyrosinase to block tyrosinase has not been studied.

We used computational methods in the current investigation, such as molecular docking, to calculate the binding free energies of 18 inhibitors with tyrosinase. The molecules Steppogenin, 7, 3, 4-Trihydroxyisoflaones, and 4, 6, 4-

Trihydroxyaurone had the greatest docking results out of all those under study. All of these substances bind to the Cu^{2+} ions that are present in the active site of enzyme.

Based on their stronger binding affinity than tropolone, these three derivatives; Steppogenin, 7, 3, 4-Trihydroxyisoflaones, and 4, 6, 4-Trihydroxyaurone were recognized as powerful tyrosinase inhibitors (2y9x). The seven leads listed in Table 4 were projected as drug candidates by pass prediction, ADME properties analysis, and toxicity determination by pro Tox II because they met all requirements for absorption, distribution, metabolism, and excretion, didn't break rule 5, and appeared to be a better candidate than the frequently used kojic acid for hyper pigmentary conditions.

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