Toxicity prediction and analysis of flavonoid apigenin as a histone deacetylase inhibitor: An in silico approach

Divya Rajaselvi N
Kerala Veterinary and Animal Sciences University
Jida M.D
Kerala Veterinary and Animal Sciences University
Devu B Nair
Kerala Veterinary and Animal Sciences University
Varsha B
Kerala Veterinary and Animal Sciences University
Nisha A. R (nisha@kvasu.ac.in)
Kerala Veterinary and Animal Sciences University

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Abstract

Occurrence of cancer is driving up on a global scale that exerts greater implications on the physical, psychological and economic stability of the human population. In the present context, numerous research studies are being conducted to explore and discover the drug molecule as an anticancer agent. Diverse scales of flavonoids entail the human diet, and they displayed prospective curative effects against an array of ailments. Among various categories of flavonoids, apigenin a trihydroxy flavone has been proven to have various pharmacological effects. Molecular docking is a key tool in structural molecular biology and computer assisted drug design. In this study, HDAC inhibitory action of apigenin and its probable toxicity was assessed by docking study using Auto dock platform. Toxicity predictions were evaluated by using tools such as CarcinoPred for carcinogenicity study, pkCSM for ADMET analysis, ProTox-II for rodent oral toxicity, lazar for estimating mutagenicity, BOILED Egg plot analysis for examining the gastrointestinal absorption and blood brain permeability, PASS prediction to identify the various biological functions and DruLiTo program to compute the drug likeness property.

Introduction

Cancer, a highly life-threatening disease is manifested by its proficient proliferation rather than normal cells which arises because of multiple factors as faulty cell division, damage to the DNA structure, genetic alterations. Histone, the basic component of chromatin is regulated by two antagonistic enzymes like histone deacetylase (HDAC) and histone acetyl transferase (HAT). Different classes of HDACs have been evolved as an appealing target for therapeutic candidates in several human cancers due to its ubiquitous mutation and unusual expression in various human diseases, specifically cancer (Barneda zahonero and Parra, 2012). One of the ultimate breakthroughs in the pharmaceutical sectors is the quest and exploration of molecules as histone deacetylase inhibitors (HDACi) to function as effective anticancer drugs. Recently plant derived compounds showed promising HDAC inhibitory activity due to its antiproliferative effect and the potential to induce apoptosis (Senawong et al., 2013).

Plants in general possess a class of approximately 4000 kinds of naturally existing polyphenolic substances known as flavonoids. Vegetables, fruits, seeds, nuts, spices, flowers, stems and red wine are the rich sources of flavonoids (Nayaka *et al.*, 2014). The metabolism of acetate and shikimic acid pathways yield a C6-C3-C6 chemical skeleton composed of heterocyclic benzopyran with oxygen and two rings of aromatic molecules commonly known as flavonoids. It is widely acknowledged that flavonoids have plenty of implications in several physiological pathways. (Filho *et al.*, 2015). One of the most ubiquitously distributed and extensively studied flavonoids is apigenin. The primary sources of this substance are Asteraceae plants, specifically those in the genera Achillea, Matricaria, Artemisia and Tanacetum. Chamomile, made from the withered flowers of *Matricaria chamomilla*, serves as one of the foremost prominent sources of apigenin (Salehi et al., 2019).

Based on the research findings, it is speculated that only eight percent of drug compounds which undergo clinical trials have the likelihood to be commercialised, whereas 20% of the drugs breakdown in the later phases of drug discovery owing to their toxicity as the core factor (Drwal et al., 2014). The most dreadful

toxicological implications posing threat to human health are the carcinogenicity and mutagenicity. Testing on animals are an essential milestone for assessing the probable lethality of newly developed drugs and cosmetics. *In silico* prediction approaches provide an alternate option to elucidate the preclinical drug discovery process and also it has an advantage as minimizing the time, expenditure, and testing on animals that are involved (Arulanandam et al., 2022). Toxicological risk assessment is progressively being executed by computer-based (*in silico*) forecasting, yet there remains a bit of scepticism about these approaches. *In silico* techniques now afford valuable incentives to both legislative standards and criteria for risk evaluations, facilitating the pharmaceutical sector to review the risk and safety picture of a compound. This has been made achievable by breakthroughs in the discipline of algorithmic computing. Hence in this study, apigenin as HDAC inhibitor and its toxicity predictions are carried out using various computational i*n silico* models.

Materials and methods

Structure of apigenin was inputted as simplified molecular input line entry system (SMILES) format to estimate toxicity predictions. To carry out molecular docking apigenin structure was downloaded from pubchem database and HDAC enzymes were selected using RCSB protein data bank.

Molecular docking

Molecular docking is a computerised simulation study to sought out the binding and interactions exist between a ligand and receptor.Receptor structure for HDAC (PDB ID: 4a69, 4bkx) was downloaded from RCSB PDB website (https://www.rcsb.org/). Docking was performed for apigenin and HDACs in the Autodock tools 1.5.6 platform. Receptor-ligand interactions were further visualized using Discovery studio visualizer 3.5.

Mutagenicity prediction

The bacterial reverse mutation assay (AMES test) is the most routinely used assay to figure out the mutagenic characteristic of any chemical substance. Lazar, a web-based tool helps in the forecasting of complicated toxicological outcomes such as reproductive toxicity, carcinogenicity, blood brain barrier permeation and long-term toxic effects (Arulanandam et al., 2022). To generate predictions of unknown chemical compounds, lazar inputs data from experimental findings by using data mining algorithms. Mutagenicity, the most critical outcome of a toxic compound is due to the persistent heritable genetic alterations in the DNA sequence of an organism. Lazar prediction tool was downloaded from https://nano-lazar.in-silico.ch/predict (Maunz et al., 2013).

Carcinogenicity prediction

The probable likelihood of substance to trigger carcinogenicity in humans and animals can be estimated by the Carcino Pred- EL (Carcinogenicity Prediction using Ensembled Learning Methods) computational tool which can be accessed with the link as http://112.126.70.33/toxicity/CarcinoPred-EL/index.html. This

model is assembled with conglomeration of three programs such as Ensemble RF, SVM and XGBoost. Ensemble XGBoost has been demonstrated to be the most effective carcinogenicity predictor in CarcinoPred-EL as it has an increased rate of sensitivity, accuracy and specificity compared to other two models (Zhang et al., 2017).

PASS prediction

The PASS online software tool enables users to predict the expected profile of biological function of an organic compound that resembles a drug. Prediction can be obtained online by inserting the chemical structure of an organic compound to be predicted either in the Molfile format or SMILES code or direct adding of structure using Marvin applet as a file. The PASS tool applies the category labels of "active" (Pa) or "inactive" (Pi) as biological activity (Filimonov et al., 2014).

Prediction of ADMET

Pharmacokinetic parameters like absorption, distribution, metabolism and excretion (ADME) properties of apigenin was predicted by inputting SMILES format in the pkCSM website http://biosig.unimelb.edu.au/pkcsm/ (Syahputra et al., 2020). The capability of the chemical molecule to function as a drug can be assessed based on its ADME features.

Drug likeness prediction

Drug-likeness prediction of apigenin was predicted using the DruLiTo web application. Chemical structure of apigenin was inputted in the software in the form of sdf file format. To investigate the prediction, three filters were applied which includes Lipinski's rule, Veber filter and Ghose filter (Jadhav et al., 2015; Andhiarto *et al*., 2022).

Rodent oral toxicity prediction

ProTox-II, an online computerised programme that has been designed to anticipate the toxicity of drugs before the process of drug discovery. Toxicity can be predicted from the website https://tox-new.charite.de/protox_II/. ProTox-II is an innovative approach in toxicity prediction, and it runs based on the juxtaposition of unknown molecule with the median lethal dose (LD50) of known compounds and incorporates the identified hazardous components. The only prerequisite to access the ProTox web server is having the two-dimensional structure of the chemical to be estimated for toxicity. Within seconds, toxicity prediction report of a compound along with LD50 value will be constructed as an output. Furthermore, Pro Tox webserver analyse the toxicity class of a chemical value and it will be grouped into the class from I to VI based on the obtained LD50 value as per the guidelines of globally harmonized system of classification of labelling of chemicals (Drwal et al., 2014).

Fish acute toxicity prediction

The inclusion of fish in acute toxicity investigations is incredibly important for the assessment of possible ecological threats of a unknown substance in the aquatic environment according to the guidelines issued

by national legislation. The Virtual models for property Evaluation of chemicals within a Global Architecture VEGA version 1.1.4 platform, using the Sarpy/IRFMN v.1.0.2 acute fish lethality model was used to predict the acute fish toxicity (Zhou et al., 2021).

Predicting gastrointestinal absorption and brain penetration

The ability of the bioactive substance to be absorbed in the gastrointestinal tract and its permeability into the brain are the two major pharmacokinetic parameters and can be estimated by the Brain Or Intestinal EstimateD permeation (BOILED- Egg) computational *in silico* prediction model. Tight junctions between the endothelial cells and the presence of efflux pumps form a physical and biochemical barrier in the brain thereby protects the tissues of central nervous system. Prior evaluation of oral bioavailability and the proportion of the drug that enters into circulation after oral administration can be detected in advance before doing animal experimentation. Both GI absorption and brain penetration are ascertained by evaluating the lipophilicity and polarity of the inputted molecule and the results are created with clear comprehensible graphical illustrations (Dain and Zoete, 2016). The SwissADME web platform with the accession link http://www.swissadme.ch/ can be employed to carry out the BOILED-Egg plot assessment.

Results and Discussion

Molecular docking

Results are validated based on the energy values in the RMSD table obtained after docking. The bonding is calculated based on the Gibbs free energy (ΔG) the more negative the energy, which is suggestive of stronger the affinity and binding. From this study, apigenin exhibited stronger HDAC inhibitory activity with a value of -8.9 kcal/mol and -7.98 kcal/mol.

Table 1
ADMET prediction

Property	Model name	Predicted value	Unit
Absorption	Water solubility	-3.329	Log mol/L
Absorption	Intestinal absorption	93.25	% absorbed
Absorption	Skin permeability	-2.735	log Kp
Distribution	BBB permeability	-0.734	Log BB
Distribution	VDss	0.822	Log L/kg
Metabolism	CYP3A4 substrate	No	Yes/ No
Metabolism	CYP2C19 inhibitor	Yes	Yes/ No
Excretion	Total clearance	0.566	log ml/min/kg
Excretion	Renal OCT2 substrate	No	Yes/ No
Toxicity	Ames toxicity	No	Yes/ No
Toxicity	Hepatotoxicity	No	Yes/ No
Toxicity	Oral Rat acute toxicity (LD50)	2.45	mol/kg

Table 2
PASS prediction

Sl.NO	Activity	Pa	Pi
1.	Chlordecone reductase inhibitor	0,973	0,001
2.	Membrane integrity agonist	0,967	0,002
3	HIF1A expression inhibitor	0,911	0,005
4	Kinase inhibitor	0,941	0,002
5	Anaphylatoxin receptor antagonist	0,931	0,003
6	2-Dehydropantoate 2-reductase inhibitor	0,942	0,002
7	Aldehyde oxidase inhibitor	0,937	0,003
8	Antimutagenic	0,921	0,002
9	Aryl-alcohol dehydrogenase (NADP+) inhibitor	0,936	0,001
10	CYP1A inducer	0,907	0,002
11	CYP2C12 substrate	0,946	0,004
12	Histidine kinase inhibitor	0,918	0,002
13	Membrane permeability inhibitor	0,946	0,002
14	NADPH-ferrihemoprotein reductase inhibitor	0,914	0,002
15	P-benzoquinone reductase (NADPH) inhibitor	0,931	0,001
16	Peroxidase inhibitor	0,924	0,002
17	Ubiquinol-cytochrome-c reductase inhibitor	0,902	0,005
18	Antihemorrhagic	0,837	0,002
19	Apoptosis agonist	0,847	0,005

Table 3
Carcinogenicity prediction

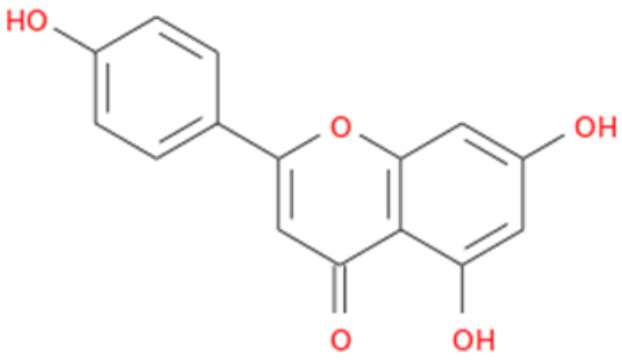
Compound Name	CarcinoPred -EL method	Average	Predicted result
Apigenin 	RF	0.43	Non-Carcinogen
	SVM	0.42	Non-Carcinogen
	XGBoost	0.44	Non-Carcinogen

Table 4
Druglikeness assessment with DruLiTo program

Name	MW	logP	HBA	nRB	HBD	TPSA	nAtom	Lipinski's rule	Ghose filter	Verber filter
Apigenin	270.05	1.138	5	1	3	86.99	30	1	1	1

MW- Molecular weight, LogP: Partition coefficient, HBA: H-Bond Acceptor, HBD: H-Bond Donor, TPSA- Total Polar Surface Area, nRB- Number of rotatable bonds, nAtom- Number of Atoms. 1- compound follows the rule.

Human intestinal absorption (HIA), the white region of the plot is the total of the rate of intestinal absorption plus the bioavailability of the unchanged portion of the chemical substance that ascends to the systemic circulation. The endothelium of cerebral arteries contains P-glycoprotein (Pgp) which is having role as a defense barrier in the brain by restricting the entry of xenobiotics from the systemic circulation which is represented by the yellow region of the plot (Cezario *et al.*, 2022).Based on the BOILED-Egg plot analysis, compounds seen in the yellow region of the plot were considered to have higher blood–brain barrier permeability, whereas compounds found in the white region of the plot were considered to have higher gastrointestinal absorption properties (Arulanandam et al., 2022). Results from the Fig. 2 portrayed that apigenin was perceived in the white region and hence it is suspected to have higher absorption in the GI tract.

Drug-likeness prediction for apigenin showed that it follows the Lipinski, Veber and Ghosh rule which is mentioned in the Table 4. The analysis of the possibilities of flavonoid apigenin activity revealed that the molecule was drug-like with antifungal activity (Pa:0.524 and Pi:0.027), antiviral activity (Hepatitis B)

(Pa:0.469 and Pi:0.007), antineoplastic (lung cancer) (Pa: 0.403 e Pi: 0.023) and antioxidant activity (Pa:0.553 and Pi: 0,005) through PASS online tool and other activities with more than 70% are listed in the Table 4.

PROTOX-II web tool predicted that apigenin was predicted as class 5 toxicant with a LD50 of 2500mg/kg and accuracy as 70.97%(Fig. 1). In accordance with the results of Sarpy/IRFMN v.1.0.2 acute fish toxicity model, apigenin was categorised as toxic class 3 with dose range from 10 to 100mg/ml. Mutagenicity prediction of apigenin was done in the lazar web server against Salmonella typhimurium model showed that mutagenic probability was 0.556 and non-mutagenic probability as 0.319 with estimated prediction as mutagenic whereas results of CarcinoPred EL showed that it is a non-carcinogen.

Conclusion

The screening, discovery and development of novel potential anti-neoplastic drugs with maximum therapeutic efficacy and minimal adverse effects are an instant necessity in the present milieu. Other than *in vitro* models, computer assisted forecasting algorithms or prediction tools are the highly utilisable models in the available repository of alternate modalities. The toxicological features of a compound can be promptly and efficiently examined by computerised approaches. From this study based on different *in silico* prediction methods, it was evidenced that apigenin displayed an acceptable drug-like property without any carcinogenicity and mutagenicity. This study evaluates the anticancer activity of flavonoid apigenin targeting HDAC. Further validation should be done in wet lab to investigate its safety profile and interactions with other drugs.

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Figures

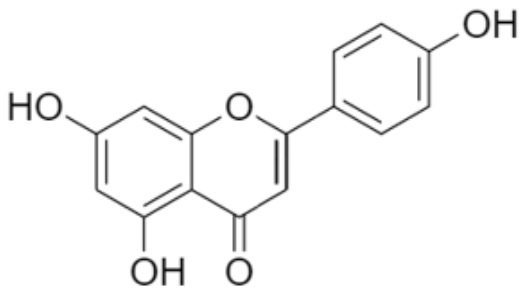

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Figure 1

Structure and SMILES format of apigenin

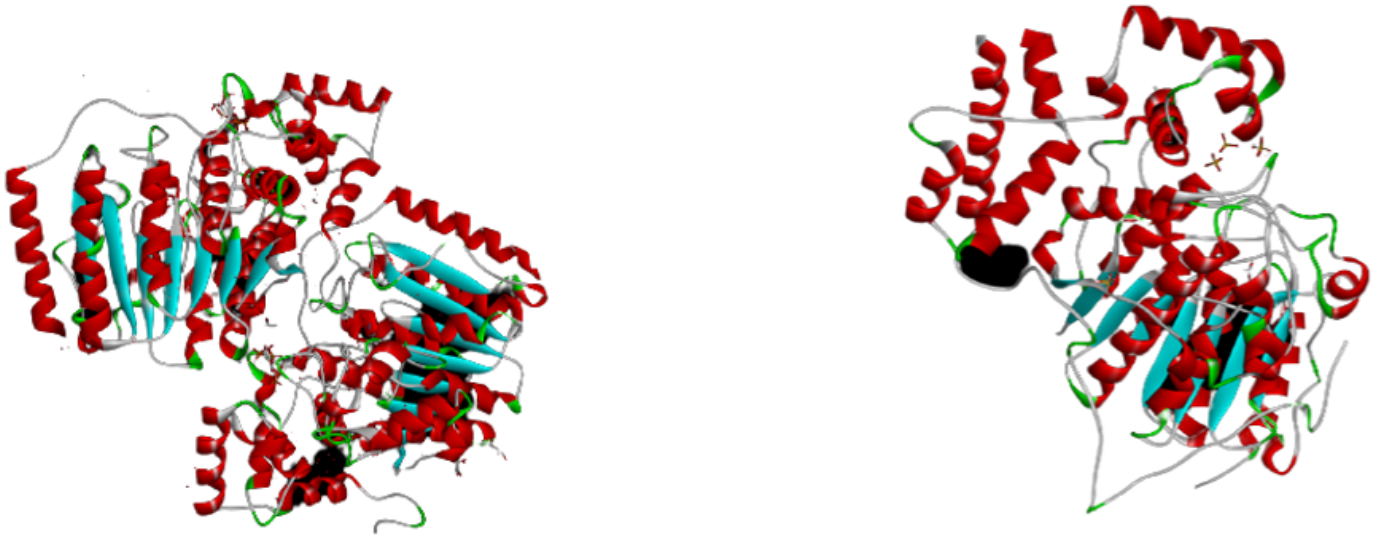

Figure 2

Structure of histone deacetylase HDAC 3 and HDAC 1 (PDB id: 4a69 , 4bkx)

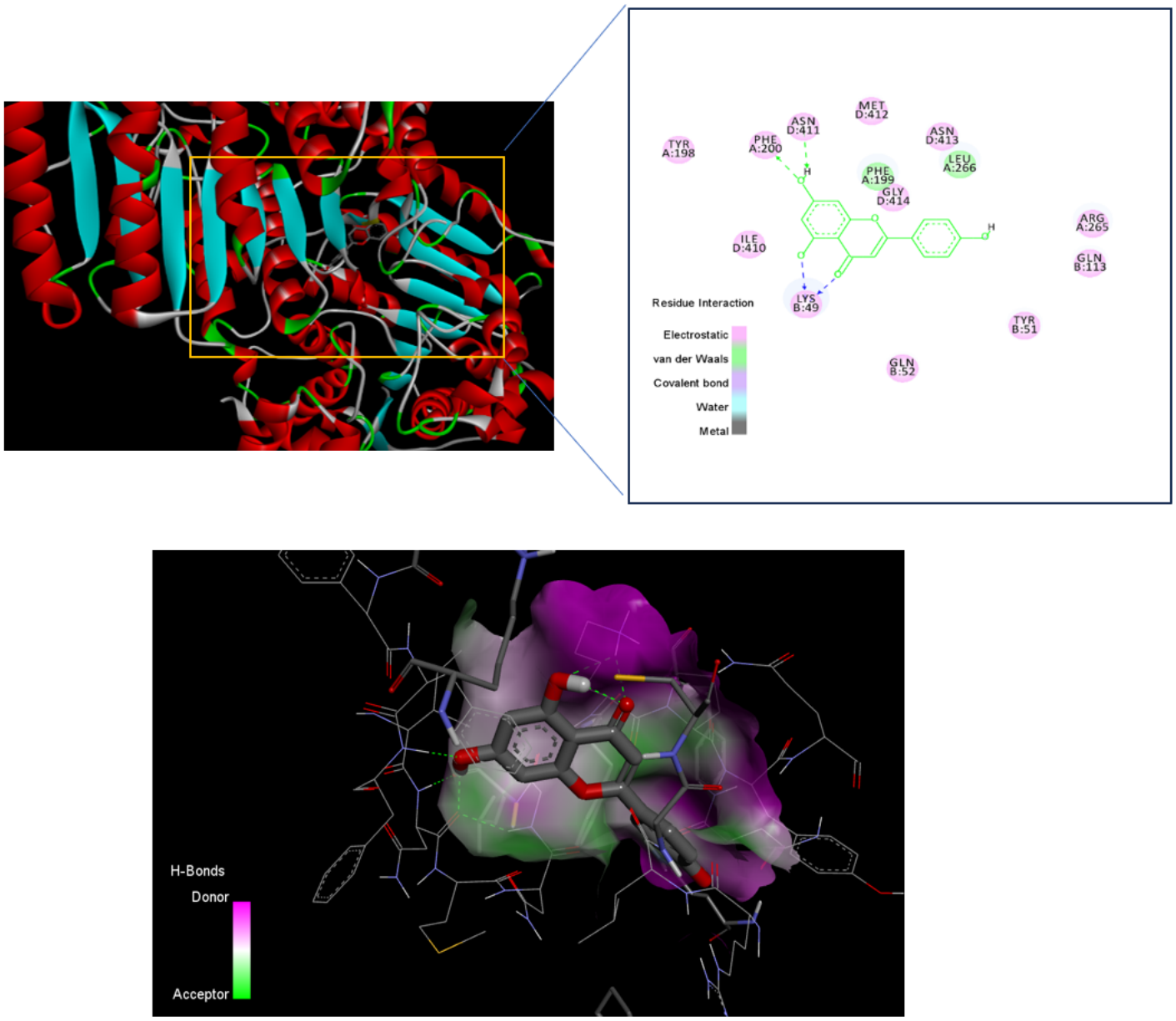

Figure 3

Docking pose of HDAC 3 and apigenin

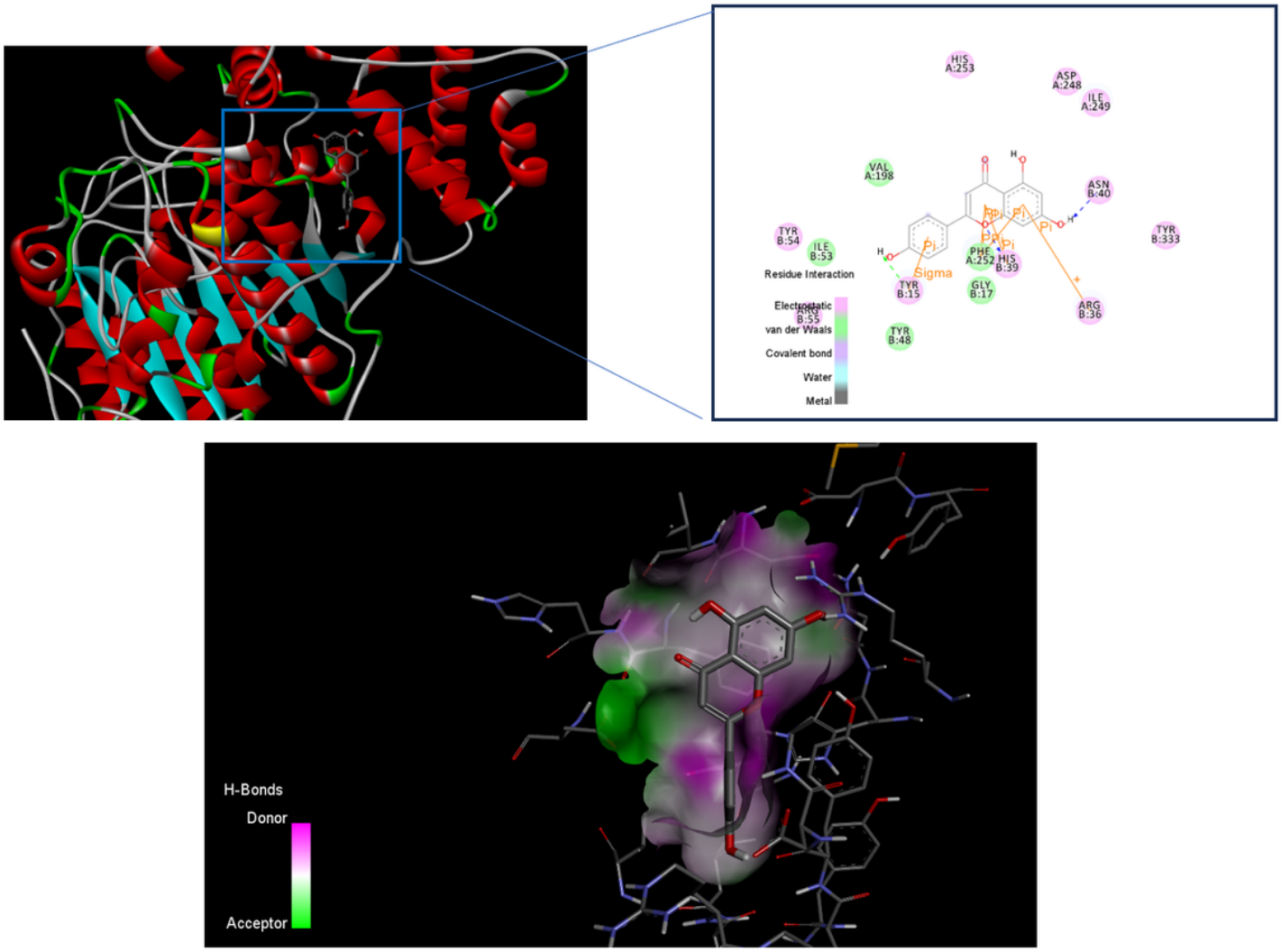

Figure 4

Docking pose of HDAC 1 and apigenin

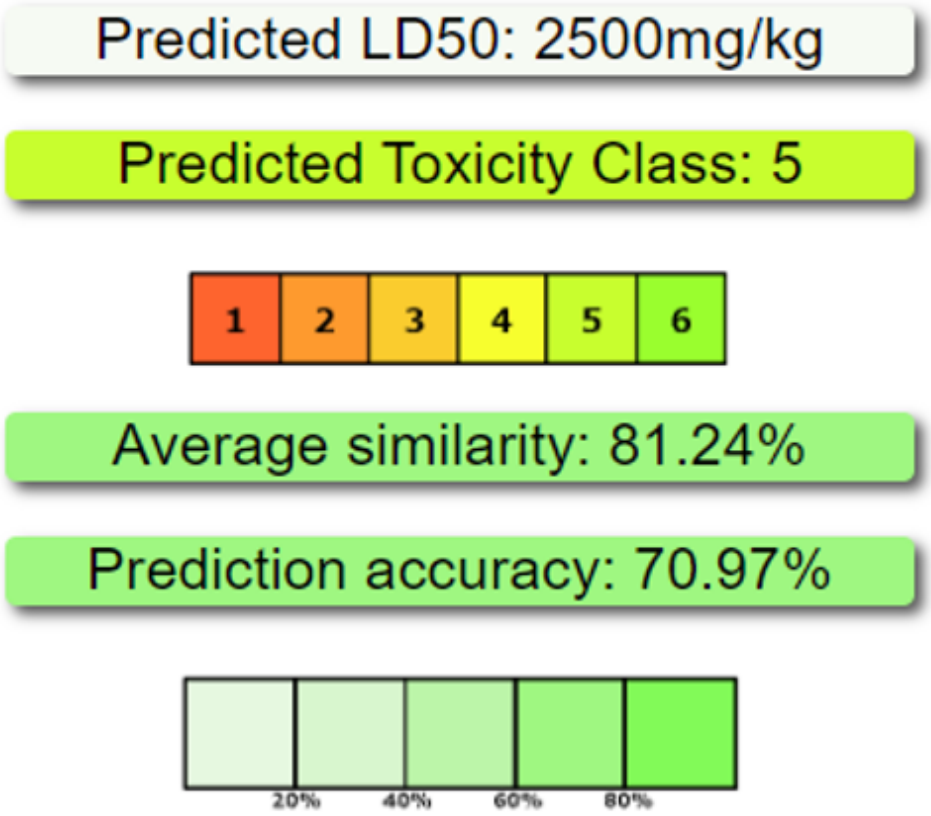

Figure 5

Rodent Oral Toxicity Prediction

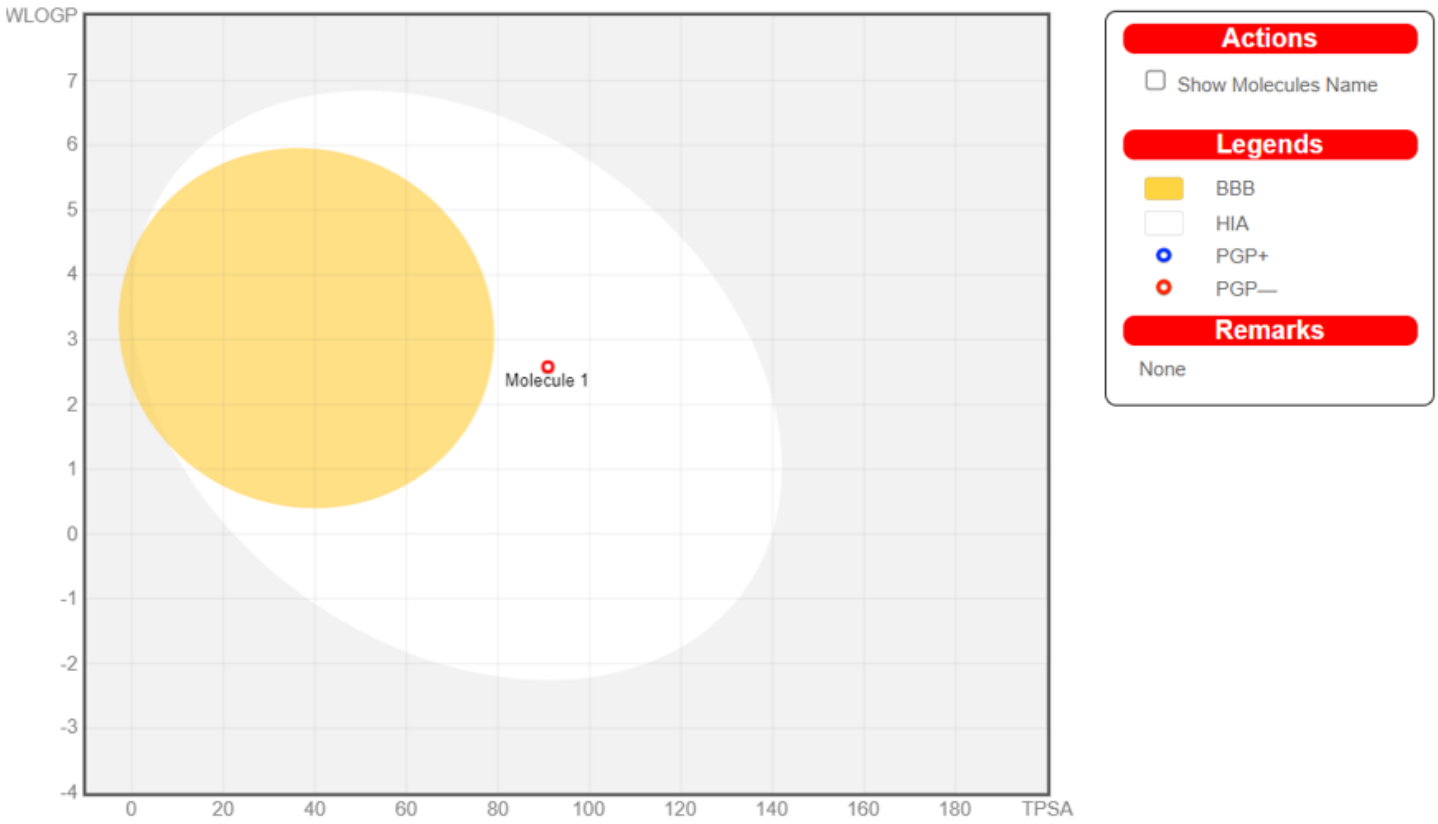

Figure 6

Prediction of gastrointestinal absorption and brain permeation of apigenin in SWISS ADME platform http://www.swissadme.ch/.