Insilico identification of a ferroportin inhibitor for the management of iron-overload condition in Beta Thalassemia major patients.

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Research Article

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Abstract

The reason of high mortality rate in the patients of beta-thalassemia major is iron overload because it leads to many secondary complications. Condition of iron overload is known as hemochromatosis (HC). HC causes distorted formation of HFE protein that disturbs the whole pathway of HAMP protein synthesis which results in unbounded form of ferroportin and hence iron keeps absorbing in the body, leading to iron accumulation. The current study was conducted to identify a potential phytochemical that could bind to ferroportin and inhibits its iron absorbing activity within the body. The 3D structure of Ferroportin was unavailable in protein data bank PDB, therefore, it was developed by using different bioinformatics tools and best structure was identified through SAVES and RAMPAGE analysis. This best structure was docked with a library of 1010 bioactive phytochemicals by using MOE-2009 software. The top-ten ranked potential inhibitors were then evaluated for drug-like properties through molsoft and Molinspiration server followed by ADMET analysis. Our study demonstrated that “Taxifolin’ showed the maximum binding affinity with Ferroportin and also demonstrates maximum drug-like properties. Thus this compound could be used as a potential inhibitor of ferroportin. However, in-vitro and in-vivo studies must be conducted to validate the therapeutic potential of taxifolin against hemochromatosis.

Introduction

Hemochromatosis (HC) is a multifactorial (Burke W *et al.*, 1998) and genetically heterogeneous (Porto G *et al.*, 2001) disease which results in excess of iron accumulation in the body. There are two different types of HC first one is primary Hemochromatosis that is due to genetic mutations and the other is secondary Hemochromatosis which occurs due to some other disease or group of disease (Hussain B *et al.*, 2011). In primary Hemochromatosis, mutations in five different genes are responsible for iron accumulation in the body. Genes that are involved in HHC are HFE, HJV, HAMP, TfR2 and SLC40A1 coding for HFE protein, hemojuvelin, hepcidin, transferrin receptor 2, and ferroportin respectively (Lok C Y *et al.*, 2009, Santos P C J d L *et al.*, 2012, Yasir S *et al.*, 2019). All these five proteins are involved in proper regulation of HFE-TFR2-Hepcidin pathway. Thus mutation in any one of these genes results in the disruption of iron regulation mechanism, leading to iron overload in the body (Kawabata H, 2018).

In Beta-Thalassemia major, synthesis of the beta chain of hemoglobin is adversely affected; consequently, the hemoglobin is defected in nature. This defective hemoglobin results in imperfect RBCs that cause a severe form of Anemia (Fairweather D *et al.*, 1978). Regular blood transfusion is the commonly employed options to cope up with anemic condition; however, this repeated transfusion results in iron overload in the body. Transfused blood encompass 200mg iron while the body is only capable of removing 1-2mg iron in a single day, the excess iron starts accumulating in vital body organs and ultimately prompts death of the individual (Ginzburg Y and S Rivella, 2011, Oerter K E *et al.*, 1993). Besides that blood transfusion also enhances the chances of transfusion transmitted infections, among which hepatitis is the most prevalent one (Shah N *et al.*, 2010).

At present, no efficacious anemia modifying medication for patients with Beta-Thalassemia major is known which can be delivered orally, therefore, the current study was aimed to identify a bioactive agent, from different classes of phytochemicals available in PubChem that could be used as a substituent of Hepcidin and

show good binding affinity with ferroportin, thereby prohibiting further iron absorption by the body cells. Active biological agents that are extracted straightforwardly from the plants are termed as Phytochemicals, They show remarkable acceptance in both sustenance and pharmaceutical because of their prominent natural qualities (Georgiev M I, 2014, Xiao J et al., 2014). There are many different types of phytochemicals such as carbohydrates, steroids, flavonoids, stilbenoids, alkaloids, lignins, polysaccharides, saponins and lignans, which due to their notable biological characteristics are highly acceptable in both food and pharmaceutical (Georgiev M I, 2014, Xiao J et al., 2014). These phytochemicals have been reported to show remarkable effects against different illnesses including cancer, diabetes, and arthritis.(Zhang Y *et al.*, 2020, Zainab B *et al.*, 2020, BHATTACHARYA S *et al.*, 2020). In the present study, phytochemicals from every class were selected and docked with 3D modeled structure of ferroportin to identify its inhibitor. This will ultimately led the foundation of the identification of a novel phytochemical that could be used as a life-saving drug with minimum side-effects.

Materials And Methods

3D Structure modeling

NCBI Database (Jenuth J P, 2000) was used to obtain the primary structure of “Ferroportin” which was then operated to construct its secondary structure by using CFSSP (Chou & Fasman Secondary Structure Prediction Server) tool (Kumar T A, 2013). Tertiary structure of Ferroportin was predicted by using all three approaches of 3D modeling i.e. Homology Modeling, Threading and Ab-initio. Swiss Model (Benkert P *et al.*, 2010) and IntFOLD (McGuffin L J *et al.*, 2015) were employed for Homology modeling, Phyre 2 (Kelley L A *et al.*, 2015) and MUSTER (Wu S and Y Zhang, 2008) were consumed for Threading and i-TASSER (Zhang Y, 2008) and FALCON (Wang C *et al.*, 2015) were operated as a method of ab-initio modeling. In order to estimate the best structure among all of the predicted ones, SAVES and RAMPAGE server was manipulated, where SAVES assessed them on the basis of Whatcheck (Hooft R W, 1996), PROVE (Pontius J *et al.*, 1996), ERRAT (Colovos C and T O Yeates, 1993), Verify 3D (Luthy R *et al.*, 1992) and PROCHECK software (Laskowski R A *et al.*, 1996) and RAMPAGE evaluated them on the basis of Ramachandran plot (Wang W *et al.*, 2016). Docking is an in-silico prediction of most appropriate spatial configuration of ligand-target complex and also anticipate the free energy of corresponding complex (Bortolato A *et al.*, 2013). The best structure, investigated with SAVES and RAMPAGE server, was then selected for molecular docking analysis.

Preparation of ligand molecules and Target Proteins

For the purpose of docking, 2D conformation of 1010 bioactive phytochemicals, belonging to seven different classes of phytochemicals i.e. alkaloids, aromatic, carbohydrates, flavonoids, lignans, tannins and polycyclic aromatic ligands, were retrieved from different databases including; PubChem (Bolton E E *et al.*, 2008), MPD3 (Mumtaz A *et al.*, 2017) and Zinc database (Irwin J J and B K Shoichet, 2005) in sdf file format. This step was followed by the preparation of ligands by adding partial charges via Protonate3D module and energy minimization by selecting MMFF94x force-field. Afterwards each of the selected ligands was added individually to the MOE ligand database for docking purpose. The protein structure preparation included protonation and energy minimization via Protonate3D algorithm and AMBER99 force-field (Labute P, 2007).

The idea of conducting docking analysis was to pick compounds with minimum docking score, RMSD value and interacting residues involved.

Evaluation of Drug-like properties

The top ranked compounds was then evaluated for Lipinski rule of five for the assessment of drug-like characteristics by using Molinspiration and molsoft software (Molsoft L, Cheminformatics M). Furthermore, the qualitative assessment of absorption, distribution, metabolism, excretion and toxicity profile of these hits were predicted virtually by using ADMETsar server (Cheng F *et al*., 2012). The schematic diagram of the entire methodology is shown in figure 1.

Results

Prediction of 3D Structure:

Tertiary structure of ferroportin was not found in Protein Data Bank, therefore, it was predicted through different bioinformatics tools. Amino acid sequence of ferroportin was retrieved in Fasta format from Uniprot (accession no: CAG38595.1). This sequence was then practiced to examine the secondary structure of ferroportin through CFSSP (Chou & Fasman Secondary Structure Prediction Server) tool, according to which 79% (451) amino acid residues were shown to have extended strands and 77.9% (445) residues were of helix nature, while only 9.8% (56) amino acids were undergoing beta turn. Tertiary organization of protein was anticipated by utilizing all three tactics of constructing protein structure; Homology modeling, Threading and ab-intio modeling. Intfold and Swiss modeling were employed for homology modeling, MUSTER and Phyre 2 were used for threading and Falcon and i-TASSER were used for the 3D construction of ferroportin protein on the basis of Ab-initio analysis. Top 2 models from each software was taken and SAVES server was manipulated for picking up the best established structure. SAVES server evaluated the constructed models by visualizing through, ERRAT, PROCHECK, WHATCHECK, Prove and Verify 3D. Results of SAVES analyses are shown in table 1, from where it can be seen that model 5, developed by MUSTER, was found to be the best constructed model of Ferroportin, its 3D structure and Ramachandran plot is given in figure 2.

Table 1: Comparative Analysis of manufactured 3D structures by using different software, the best model is highlighted yellow

Model	Software used	Amino Acid residues in final structure	Ramachandran Plot Residues in favored: allow :outer region	ERRAT (Quality Factor A)	WhatCheck Error: Warning: Pass	PROVE % error	Pro-check Error: Warning:Pass
Model 1	Swiss-Model	523	90.3: 7.3: 2.7	80.1636	9: 15: 23	6.0	6: 1: 2
Model 2	Falcon	571	88.0: 7.2: 4.7	41.0811	6: 11: 30	10	4: 1: 3
Model 2a	Falcon	571	83.5: 10.4: 6.2	24.7191	6: 13: 28	15.0	5: 1: 2
Model 3	I-Tasser	571	81.2: 10.7: 8.1	80.1066	10: 19: 19	6.7	7: 2: 0
Model 3a	I-Tasser	571	78.6: 13.0: 8.4	77.7975	9: 18: 22	7.9	7: 2: 0
Model 4	PHYRE	395	81.2: 10.7: 8.1	84.6154	6: 13: 30	6.8	3: 2: 4
Model 4a	PHYRE	402	97.2: 1.8: 1.0	92.3077	6: 15: 28	7.8	2: 4: 3
Model 5	MUSTER	571	94.4: 4: 1.6	64.4068	6: 13: 28	6.5	5: 1: 3
Model 5a	MUSTER	571	92.4: 4.4: 3.2	49.5396	7: 12: 28	9.7	6: 1: 2
Model 6	Int-fold	571	94: 2.6: 3.3	63.1365	6: 12: 29	6.4	5: 1: 3
Model 6a	Int-fold	571	93.7: 3.9: 2.5	57.3146	6: 12: 29	7.4	5: 0: 3

Molecular Docking Analysis

Constructed 3D structure of ferroportin was further used for molecular docking analysis. For the docking purpose, a library of 1010 phytochemicals was made and docked against the selected structure of ferroportin. All of these ligands were docked with 3D modeled structure of Ferroportin by using MOE 2009 software. Top ten compounds were selected on the basis of lower binding energies and more interactions with target's residues. Table 2 represents the IUPAC names of these compounds, there docking scores, RMSD values and interacting residues

Table.2 Moe results of docking analysis of top ten compounds, with interacting residues of ferroportin protein

Sr no	IUPAC Name of Compound	Docking score	RMSD-refine	Interacting residues
439533	Taxifolin	-25.4428	0.5962	Arg40 & Arg466
10393772	XVFLUEXTEKGHHD-LQHJLSERSA-N	-25.1922	2.2630	Asn 185, Ser349 & Tyr501
72284	Chebulinic Acid	-24.9710	2.9446	Arg466 and Asp504
10575105	FAUBWJZFUIHBLR-QOCNXNKDSA-N	-22.6099	0.9936	Gln496 & Tyr501
10088963	Taxiresinol	-22.1488	1.8077	Ala151, Arg 156 and Asn 497
6683	Purpurin	-22.0526	1.4723	Tyr 501
442431	Narirutin	-21.3499	1.5958	Arg 40, Tyr 318, Gln 496 & Asn 500
10436972	Ferrugunin C	-20.1111	1.1287	Arg40 & Asn 185
5281702	Tricin	-19.2699	1.5273	Ile389, Phe 392, Met 393, & Ser401

Assessment of Drug-like Properties

Selected top ten compounds were then subjected to the prediction of drug-like properties via molsoft and Molinspiration online server. The molsoft software was practiced to compare the drug like and non-drug like properties and Molinspiration was adopted for the assessment of Lipinski rule of five. Ligands in table 2 were examined from minimum docking score to maximum until a particular ligand was found that could show all of the scrutinized parameters within the normal range. Table 2 shows that Taxifolin (PubChem_id “439533”) displays lowest binding energy and maximum interaction with ferroportin in comparison to all other phytochemicals, its 2 dimensional and 3 dimensional interactions within the binding pocket of ferroportin are displayed in figure 3.

Being on the top of the list, Taxifolin was firstly screened for molsoft and molinspiration analysis. Fortunately, this very first compound showed all of the parameters within the acceptable range, comparison of its drug and no-drug like properties can be seen in figure 4 while results of Molinspiration are shown in table 3. Finally ADMET properties, evaluated from ADMETsar online tool also confirmed its acceptable ADMET properties (table 4).

Table.3 Results of Molinspiration server for PubChem compound “Taxifolin”

Analyzed Parameter	Analyzed value	Comments
miLogP	4.39	Acceptable
TPSA	114.69	Acceptable
Natoms	35	Acceptable
nON	8	Acceptable
nOHNH	3	Acceptable
Nviolations	0	Acceptable
volume	422.79	Acceptable

Table 4: ADMET profiling enlisting absorption, metabolism and toxicity related drug like parameters of Taxifolin

Phytochemical	Taxifolin
A. Absorption	
Blood-Brain Barrier permeant	No
Gastro- Intestinal Absorption	High
Caco-2 Permeability	Yes
P-glycoprotein substrate	No
B. Metabolism	
CYP450 1A2 Inhibitor	No
CYP450 2C19 Inhibitor	No
CYP450 2C9 Inhibitor	No
CYP450 2D6 Inhibitor	Yes
CYP450 3A4 Inhibitor	No
C. Toxicity	
Human either-a-go-go inhibition	No
Carcinogens	No
AMES mutagenesis	No

Discussion

This study was designed to identify an inhibitor of ferroportin protein that is overexpressed in Hemochromatosis patients, the most common reason of deaths in Beta Thalassemia patients (Ando J *et al.*, 2017). In the patients of Hemochromatosis, mutation in HAMP gene produces deformed Hepcidin, which

cannot bind to ferroportin protein, therefore, ferroportin continue to absorb iron which ultimately prompts iron-overload. To develop a drug against a protein its structure should be known but ferroportin structure was not present in Protein Data Bank (PDB), henceforward Ferroportin structure was forecasted through different software based on different modes of protein modeling i.e. homology modeling, threading and ab-initio modeling and to choose the best structure ERRAT, WhatCheck, PROVE and PROCHECK software were used, same methodology is also adopted by many of the previous studies (Kumar *et al.*, 2017). In order to develop a drug, docking was done with the best-designed structure of ferroportin. 1010 phytochemicals were selected from seven different classes of phytochemicals available in PubChem. Top ten compounds were selected on the basis of lower binding energies and more interactions with target's residues these compounds were then subjected to prediction of drug like properties via molsoft and Molinspiration server. Docking results showed that PubChem compound "439533" (Taxifolin) showed the best interaction with ferroportin. Taxifolin is a flavonoid with IUPAC name of (2R, 3R)-2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-2,3-dihydrochromen-4-one. In the structure of ferroportin two basic amino acids; Arginine 40 and Arginine 466 acts as a side-chain donor (denoted as the dotted arrow, with arrowhead towards the compound which acts as a side-chain acceptor). Both of these basic amino acids interact with the hydroxyl group of aromatic rings at the one side of Taxifolin structure. Arg 466 interacts with the hydroxyl group at one corner of Taxifolin whilst Arg 40 interrelate with two hydroxyl groups of another aromatic ring, present at the other corner of Taxifolin. This interaction ultimately prohibits ferroportin to perform its usual task i.e. iron absorption in the body. If a compound is supposed to be considered as a drug it should follow the Lipinski rule of Five (LRo5), according to which a compound shows inefficient absorption if it has more than 10 H-bond acceptors (HBA), 5 H-bond donors (HBD), Log P (CLogP) more than 5 and Molecular Weight (M.W) is more than 500 (Lipinski C A *et al.*, 2001). Lipinski rule for Taxifolin was evaluated by molsoft and Molinspiration where Molinspiration also determines TPSA (Topological Polar Surface Area) and Molecular Volume, where former one forecasts the transportation ability of the drug within the body and latter one represents features of crossing blood-brain barrier and absorption in intestinal tract (Cheminformatics M, 2011). Both Molinspiration and molsoft have already been used for identification of drug-like properties in many previous studies (Raj V *et al.*, 2015, Raj V *et al.*, 2014, Lalitha P and S Sivakamasundari, 2010) In short "Taxifolin" (PubChem_id 439533) came out to be the best Phytochemical that could bind with ferroportin, its binding lessens the flexibility protein, the same docking procedure was adopted to analyze the binding capability of chronic acid two sites that can decrease the YopH bacterial virulence activity (Kuban-Jankowska A *et al.*, 2016) and to report the phytochemicals action against HCV NS3 protease (Ashfaq U A *et al.*, 2016).

Although the current study proposes that the "Taxifolin" shows very good binding affinity with the binding sites of ferroportin, yet for how much time this interaction will sustain within the real body conditions is still required to be explored. For this purpose, Molecular Dynamic (MD) Simulations can be employed, which has been used previously in many studies to evaluate the durability of bonds established between ligands and receptors (Dos Santos E G et al., 2020, Gao Y et al., 2020). If the stable bonding of Taxifolin with ferroportin is confirmed, it can be considered as a bioactive phytochemical with a strong potential to be used for the appropriate management of iron regulation within the patients of Beta-Thalassemia.

Conclusion

The present study reinforces the importance of usage of phytochemicals for the treatment of this life-threatening disorder. In the current study, phytochemical “Taxifolin” is identified to be a potential drug for the management of iron regulation in hemochromatosis condition of the body. This phytochemical shows very good binding affinity with ferroportin thus inhibits its action of iron absorption. However, further in-vitro and in-vivo experimentation is needed to validate our result.

Declarations

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Author contributions

Sharif Y performed research, data acquisition and manuscript writing; Tariq MH performed research and data acquisition; Irshad S did supervision, conceptualization, interpretation of data, and manuscript writing; Asghar MN did conceptualization, interpretation of data, manuscript writing and editing.

Conflict of Interest

Authors have no conflict of interest to disclose.

Data availability

We will share the original data whenever needed.

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Figures

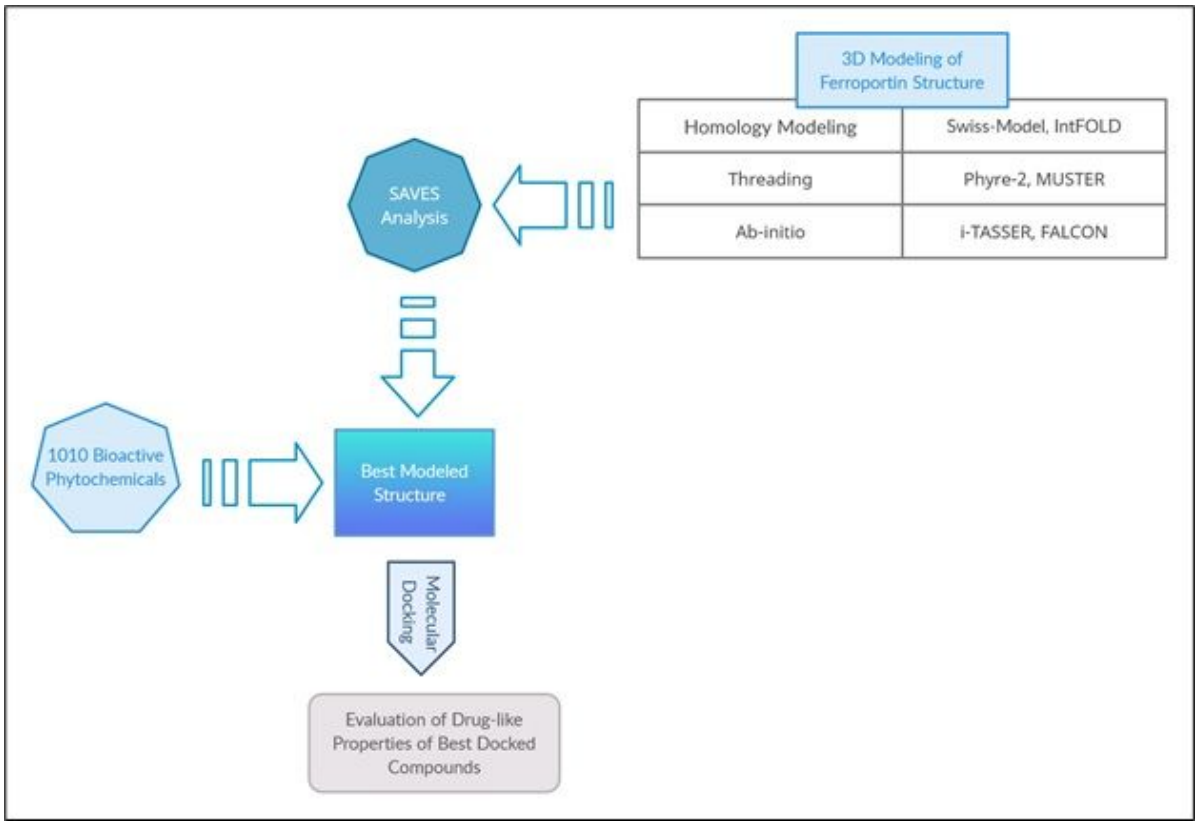

Figure 1

Schematic diagram of the methodology.

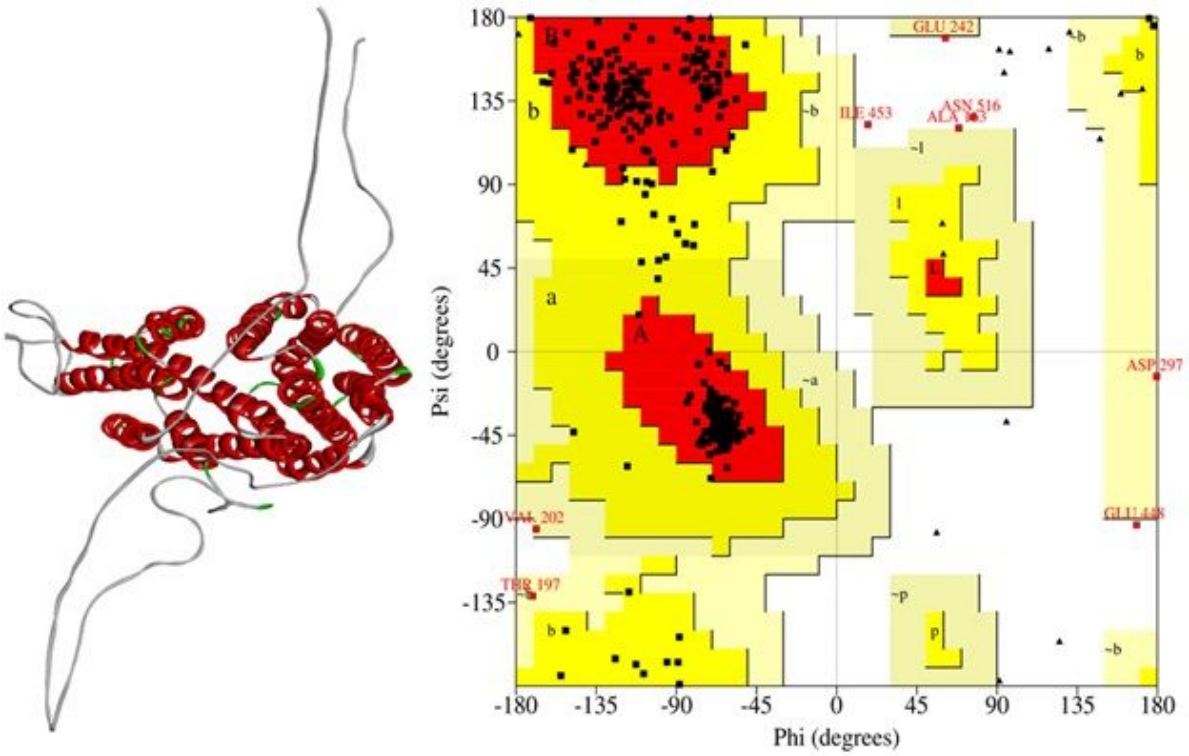

Figure 2

(A) 3D structure of Ferroportin, designed by MUSTER server (B) and its Ramachandran plot Analysis through Rampage online tool

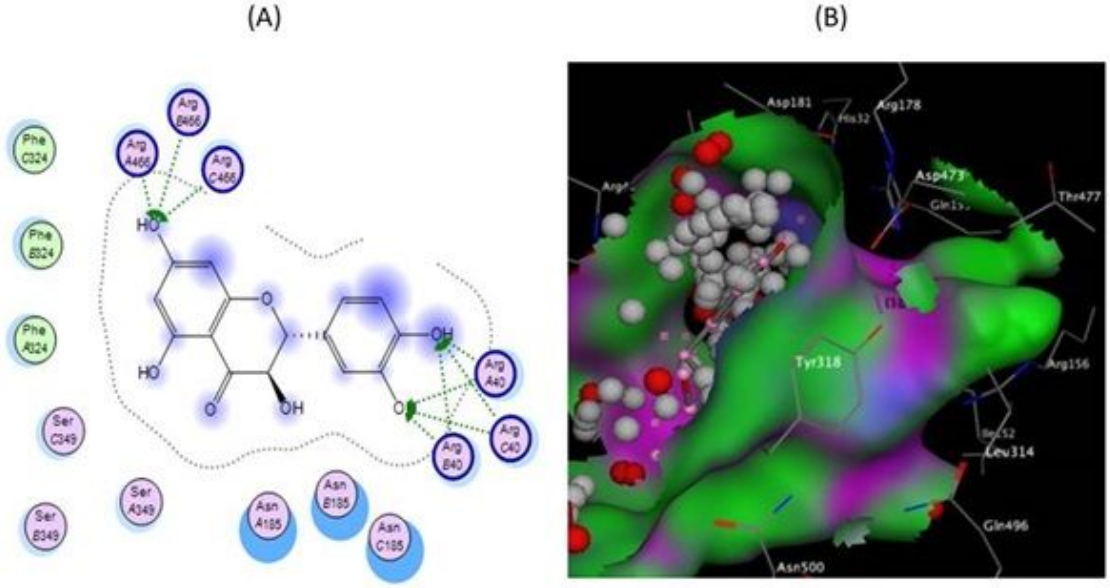

Figure 3

Binding of “Taxifolin” Ligand with the pocket of ferroportin protein; (A) represents the 2D interactions and (B) depicts the 3D interactions, where Green surface showing the binding pocket of receptor protein, while ligand is shown in the ball and stick format with balls in pink color.

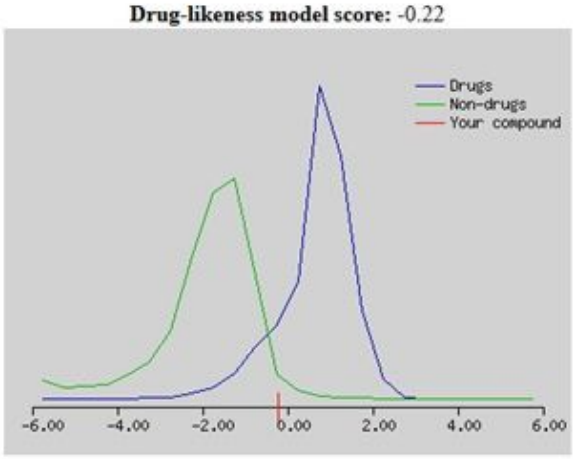

Figure 4

Graph of molsoft, showing the comparison of drug like and non-drug like properties of “Taxifolin”