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In silico binding affinity of multi-therapeutic agent Pinostrobin on various mammalian albumins: Computational evaluation of animal models

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Abstract

Pinostrobin as a famous member of flavonoid family has been investigated in terms of its therapeutic effect on a variety of diseases, and positive effect has been reported in many in vitro and in vivo studies. As one of the essential elements of blood plasma in human body, serum albumin functions a carrier protein for fatty acids, hormones, and drugs because of its abundance and strength in blood. For that, serum albumin plays an important role on the understanding of pharmacological effect of the promising therapeutic agent, pinostrobin. For providing insight into the preclinical studies of albumin targeted therapeutics, we, in this study, investigated the binding characteristics of human serum albumin – pinostrobin complex in terms of binding energy, bounded residues, and association constants, and compared them with various mammalian albumins such as goat, bovine, porcine, rabbit, sheep, and dog albumins. We used molecular modeling and molecular docking methods with the softwares PyRX and PyMol. We found that pinostrobin-human serum albumin had an association constant in between $(10.26\text{-}20.16)10^5$ M^{-1} with the interaction energy in a range of (-8.2-(-8.6)) kcal/mol. Among animal proteins, porcine (5IIU) and sheep (4LUF) showing the interaction energy of -8.4 kcal/mol and -8.1 kcal/mol, respectively, were found to be the most appropriate animal models to be used in albumin based preclinical investigations.

Keywords: Serum albumin, molecular docking, pinostrobin, animal model, binding affinity, in silico, PyRX, PyMol

Introduction

Flavonoids form an important class of polyphenol secondary metabolites. Their structure is basically formed by carbon rings of C_6-C_3-C_6 [1]. With different substitution of C_6 rings, approximate 9000 flavonoids have been reported so far [2]. Pinostrobin or 5-hydroxy-7-methoxyflavanone is a member of broadly presented flavonoids in nature. It is a dietary mostly found in plants such as *Pinus strobus, K. pandurata*, and *Cajanus cajan* [3]. Previous studies have shown that pinostrobin has a variety of important biological activities such as antiviral, anti-inflammatory, anticancer, anti-Alzheimer [3-5]. This natural pharmaceutical agent was studied on different cell cultures such as human mammary carcinoma, human breast cancer cell line MCF-7, HeLa, and HepC2 cell lines been evaluated based on an animal study of wistar rats with the calculated LD50 value of more than 500 mg / ml [9]. Therefore, for its cytotoxicity [6-8]. Moreover, its nontoxic property has also pinostrobin gathers a significant interest as a natural bioactive compound and as a candidate of herbal drug investigations in recent studies.

Interaction of bioactive therapeutic compounds with blood plasma is of important issue. Some important therapeutic parameters such as circulatory half-time of a drug, rapid renal clearance, degradation, or toxicity is closely related to how strong a drug interacts with plasma protein [10]. Significant part of plasma protein is formed by serum albumin with more than half (50%-60%) of the total. It is the most abundant protein in human blood plasma. Serum albumin or simply referred as albumin plays significant roles on variety of biological functions such as regulating plasma oncotic pressure, transforming nutrients or ligands to cells, or balancing plasma pH. Human serum albumin is a globular protein with the molecular weight of 66.5 kDa as shown in Figure 2(a) with accompanying water molecules and ligands [11]. It is a robust molecule with stability in a wide range of pH from 4 to 9. According to the literature, heating for up to 10 h even at 60^0C has not altered its structure [11]. Half-life of human serum albumin in blood circulation was reported as 19 days. Its high solubility and stability make albumin as an appropriate drug delivery platform. Heart

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shaped structure of albumin has multiple binding sites which are used as carrier for both endogenous compounds like ligands, fatty acids, and exogenous drug molecules [11, 12]. Therefore, we, in this study, investigated in the binding characteristics of therapeutic flavonoid, pinostrobin with plasma protein serum albumin taken from the various mammalians including human to give insight into the preclinical studies.

Animal studies play important roles in drug discovery and development for characterization of disease pathophysiology, drug mechanism of action, and/or assessment of toxicity and safety profile. For that, we investigated pinostrobin-albumin interaction characteristics for different mammalian such as goat, bovine, human, porcine, rabbit, sheep, and dog to get insight regarding which animal model is the best for albumin-targeted therapeutics for future studies, using computational molecular modeling and molecular docking procedure performed by PyRX and PyMol softwares [13].

Materials and Methods

Pinostrobin input file preparation

Pinostrobin smiles code and its sdf file were obtained from PubChem database. The sdf file was converted to pdb using Open Babel tab embedded in PyRX virtual screening toolbox. Energy minimization of pinostrobin was performed with Open Babel using the force field of uff (unrestricted force field), and the optimization algorithm of conjugate gradients, and minimization stopped, if energy difference is less than 0.1. The 2D structure drawn with Molinspiration, the optimized 3D structure produced by Avogadro softwares, and the smiles code of pinostrobin were shown in Figure 1.

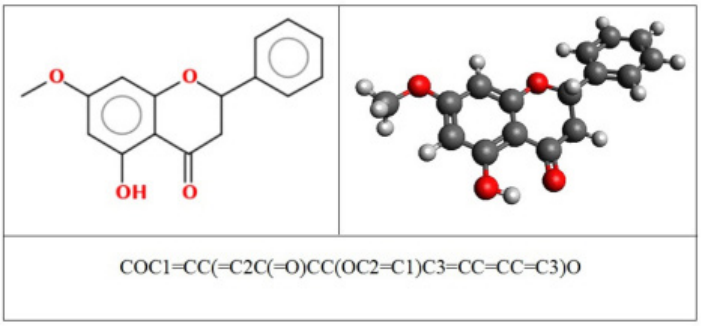

Figure 1. The 2D (top left), the optimized 3D (top right) structure, and canonical SMILES code of pinostrobin (bottom)

Molecular pharmaceutical properties of pinostrobin

Molecular structural and physico-chemical properties of a molecule provide information regarding whether the molecule could be considered as a candidate of biologically active therapeutic agent or not. Therefore, we explored some molecular physico-chemical properties of pinostrobin such as partition coefficient (Log P), topological polar surface area (TPSA), number of hydrogen bond donor and number of hydrogen bond acceptors using the SMILES code given in Figure 1. The calculations were performed via Molinspiration cheminformatics software by inserting the SMILES obtained from PubChem into the software. The calculated results of these molecular physico-chemical properties were provided in Table 1.

Table 1. Physico-chemical properties of pinostrobin were given. [l]Logarithm of partition coefficient between n-octanol and water (miLog P), [b]topological polar surface area (TPSA), [c]molecular weight (MW), [d]number of hydrogen bond donors (NHD), and [e]number of hydrogen bond acceptors (NHA). The SMILES code of pinostrobin was provided in Figure 1

PubChem ID	MiLog P[a]	TPSA[b]	MW[c]	NHA[d]	NHD[e]
73201	3.13	55.77	270.28	4	1

Protein input file preparation

Protein source files of albumins for different organisms were obtained from the Protein Data Bank, https://www.rcsb.org/ in a complex form of natural ligands, het atoms, and substructures. For human serum albumin structures, we restricted the web search to `Homo sapiens only'. For computation, the following proteins of PDB ID codes were used. The resolutions were given in parenthesis: 5ORI (1.94 Å) for goat serum albumin, 4F5S (2.47 Å) for bovine serum albumin, 5Z0B (2.17 Å) and 1N5U (1.90 Å) for human serum albumin, 5IIU (2.30 Å) for porcine serum albumin, 4F5V (2.27 Å) for rabbit serum albumin, 4LUF (2.30 Å) for sheep serum albumin, and 5GHK (3.20 Å) for dog serum albumin were downloaded as pdb files. All of the macromolecules were cleaned by removing het atoms, miscellaneous groups, and water molecules using ArgusLab, molecular modeling package. The cleaned file of protein molecules was saved in pdb format and used for docking procedure.

Molecular docking

Molecular docking was performed using PyRX, an open source virtual screening software used for computational drug discovery with an exhaustiveness value of 8 [14]. The association constant of albumin-pinostrobin complex was calculated using the highest binding energy of the docking results using the following Gibbs free energy formula [15]:

$$\Delta G = -RT \ln(K_i)$$

$$K_i = e^{-\Delta G/RT}$$

where ΔG is the lowest binding energy of serum albumin docked with pin ostrobin in the unit of Joule (J), R represents the regression constant which equals 8.314 J/(K.mol), and T is temperature in the unit of Kelvin as 298.15. Visualization and analysis of the docking results of pinostrobin - albumin complex were performed by PyMol, an open-source molecular graphics system [13].

Results

Molecular properties of pinostrobin

To understand the experimentally observed therapeutic effects of pinostrobin, we investigated its molecular structural and physico-chemical properties. The molecular physico-chemical properties are used to evaluate the molecule in term of pharmaceutical aspect,

whether the molecule would be a good drug candidate. Some of these properties are listed as log P, molecular weight, number of rotatable bonds, number of hydrogen bond donor and hydrogen bond acceptor. Based on the properties of the FDA (The Federal Drug and Disease Association of USA) approved drug molecules, certain ranges were defined for them called drug-likeness criteria. According to the commonly used drug-likeness criteria, Lipinski's rule of five which was given in the previous studies [16-18], molecular weight of a drug candidate should be less than 500 gr/mol for having high possibility of effective ADME properties. From the similar manner, the number of hydrogen bond donor, the number of hydrogen bond acceptor, and log P value are considered to be less than 5, 10, and 5, respectively. Pinostrobin shows 4 hydrogen bond acceptors and 1 hydrogen bond donor according to our calculations (Table 1). Log P value of pinostrobin is also in the range with a value of 3.13. The molecular weight of the molecule was calculated as 279.28 gr/mol. According to the drug likeness criteria 'Lipinki's rule of five', pinostrobin obeys all of five criteria with the properties summarized in Table 1.

Molecular docking

Binding affinity of a ligand with a receptor molecule is often studied with in silico docking procedure before performing costly experimental and animal studies [19]. Docking actually is a simulation procedure of the interaction profile of a drug or bioactive molecule with a drug target, mostly proteins. We, in this study, aimed to explore the binding characteristics of pinostrobin - albumin complex with the binding energy, the bonded residues, the number of interactions, and the association constant of the final complex structure for different animal albumins.

Human serum albumin crystal structure 1N5U docked with pinostrobin and the binding cavity of the complex structure was shown in Figure 2b and Figure 2c, respectively. The docking results of AutoDock Vina implemented in PyRX virtual screening tool were successfully recorded eight most favorable binding poses of the albumin - pinostrobin complex. The pose showing the lowest energy was accepted as a favorable binding position of the complex structure, although it is not guaranteed. Our computation gave the binding pose of 1N5U shown in Figure 2b and Figure 2c with a binding energy of -8.2 kcal/mol and with three polar bonds as listed in Table 2. Another human albumin structure of 5Z0B resulted in two polar interactions given in Table 2 and those interactions produced the total binding affinity of -8.6 kcal/mol, which is a quite favorable energy for a drug-target interaction.

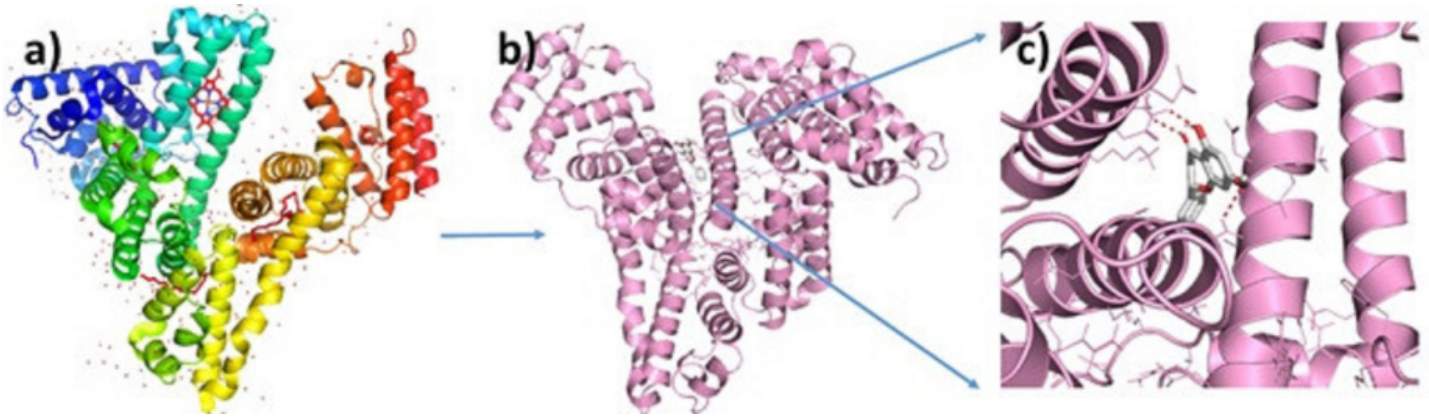

Figure 2. a) Human serum albumin (1N5U) structure with accompanying water molecules (the red dots), natural ligands, and metals were given. b) 1N5U 3D structure docking with our ligand molecule, pinostrobin shown in grey and red, c) Close view of active binding site with all interaction shown in red color

Table 2. The docking results of various albumin – pinostrobin interaction with the details of PDB ID of proteins used, the number of interactions, the binding energy, the bounded residue names, and the association constant K_a

Animal (PDB ID)	Number of interaction	Binding energy (kcal/mol)	Bound residue names	AC (K_a (M^{-1}))
Goat (5ORI)	**1 int.**	-8.0	I181	7.31 x 10^5
Bovine (4F5S)	**1 int.**	-8.1	Q220	8.67 x 10^5
Human (5ZOB)	**2 int.**	-8.6	W214 R218	20.16 x 10^5
Human (1N5U)	**3 int.**	-8.2	K432 N429 Q459	10.26 x 10^5
Porcine (5IIU)	**3 int.**	-8.4	H145 S192 E424	14.38 x 10^5
Rabbit (4F5V)	**2 int.**	-8.4	R117 R117	14.38 x 10^5
Sheep (4LUF)	**2 int.**	-8.1	K136 E118	8.67 x 10^5
Dog (5GHK)	**5 int.**	-7.6	R222 K195 K199	3.72 x 10^5

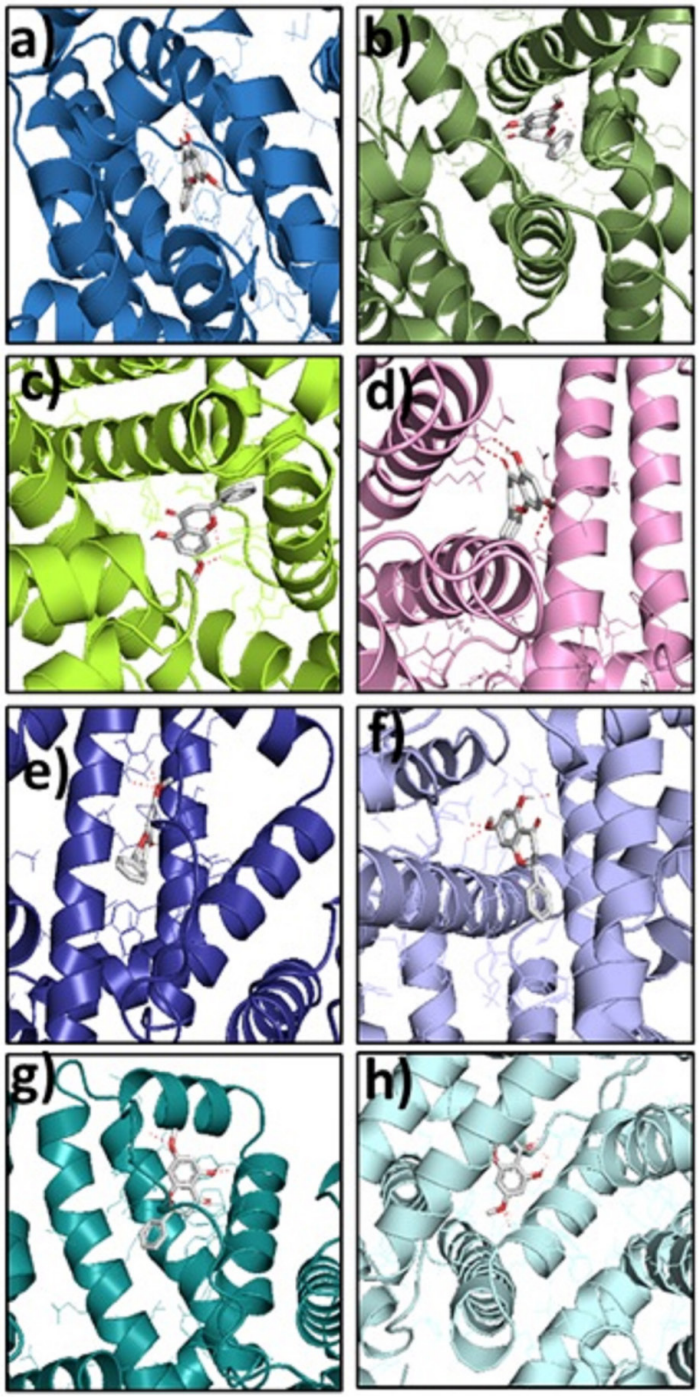

Figure 3. The binding site and the interactions between pinostrobin and some mammalian albumins were shown. The interactions were shown in red dots. a) Goat (5ORI), b) bovine (4F5S), c) human (5Z0B), d) human (1N5U), e) rabbit (4F5V), f) porcine (5IIU), g) sheep (4LUF), and h) dog (5GHK) albumin binding structure docked with pinostrobin created by PyMol

Association constant of albumin – pinostrobin complexes

The association constant of the bounded complex of albumin - pinostrobin for various mammalians was calculated using the equation provided in Section 2.4, and the results were listed in Table 2. According to the calculated K_a values, the dog albumin 5GHK gave the lowest/worst value of 3.72 x $10^5 M^{-1}$ with three polar bonds formed by the residues of R222, K195, and K199. The visualization results showed three weak interactions with only R222 and two more with K195 and K199. We count them as one interaction performed by the same residue. The Ka value of human albumins 5ZOB and 1N5U resulted in a value of 10.26 x $10^5 M^{-1}$ and 20.16 x $10^5 M^{-1}$. The closest values to human K_a were found for porcine 5IIU and rabbit 4F5V albumins. They showed a Ka value of 14.38 x $10^5 M^{-1}$. The rabbit albumin 4F5V resulted in this value of association constant with different part of the same residue, Arginine R117. The porcine 5IIU albumin, on the other hand, gave three polar bonds with different residues of H145, S192, and E424. That makes 5IIU structure more stable than 4F5V to form a strong albumin – pinostrobin complex. The association constants of goat 5ORI, bovine 4F5S, and sheep 4LUF albumins resulted in a lower value than that of human albumin.

The experimental spectroscopic values of K_a for pinostrobin – albumin interactions of the listed mammalians were reported by Feroz and coworkers [20, 21]. Their K_a values were ranged from 1x $10^4 M^{-1}$ to 7 x $10^4 M^{-1}$ and were reported in the presence of increasing pinostrobin concentrations from 1.5 to 24 µM with a fixed albumin concentration of 3 µM [20]. And yet, the main contributors of spectroscopic measurements of a protein are the aromatic residues such as Trp and Tyr whose locations are well conserved among the mammalians of our interest. Our calculations, on the other hand, are the results of molecular based interactions and independent of the parameter concentration. Therefore, as a computational calculation, our docking results are in a plausible vicinity of the experimental reports. The binding energy were reported to lie in the range of (-5.7) – (-6.5) kcal/mol (-23.8 to -27.3 kJ/mol). The lowest binding energy of our calculation was found for dog albumin as 7.6 kcal/mol and our calculations were resulted in a range between (-7.6) – (-8.6) kcal/mol. Our calculation was seen to be in accordance with the experimental measurement of the pinostrobin-albumin systems.

Conclusion

In this study, we investigated the binding characteristics of human serum albumin with a therapeutic flavonoid pinostrobin in a comparative manner with the other six mammalian albumins such as goat, bovine, porcine, rabbit, sheep, and dog using molecular modeling and docking method to predict the most appropriate animal model. The results showed that porcine and sheep albumin exhibited the closest profile to human serum albumin with the binding energy between -8.2 and -8.6 kcal/mol. Porcine albumin resulted in three polar bonds formed by histidine (H145), serine (S192), and lysine (E424) amino acids with accompanying binding energy of -8.4 kcal/mol, while sheep albumin showed two polar bonds formed by lysine (K136) and glutamic acid (E118) with accompanying binding energy of -8.1 kcal/mol. Although bovine and rabbit showed a quite high binding energy, they were not resulted in stable binding details. Therefore, porcine (5IIU) and sheep (4LUF) albumins appear to be the best alternatives to be used in preclinical studies for developing albumin based therapeutics.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
No human or animal test to require Ethics committe approval.

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