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Research Article

MOLECULAR DOCKING STUDIES OF NEW ANTIHYPERTENSIVE DRUG BASED ON DELPHINIDIN-3-SAMBUBIOSIDE ANALOGS USING ANGIOTENSIN I–CONVERTING ENZYME AS THE TARGET

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ABSTRACT

Objective: Hypertension is a major health concern which is responsible for various cardiovascular and renal disorders. Although being one of the most commonly prescribed medicines, antihypertensive drugs have been shown to have negative side effects for some patient groups, posing a need for the development of novel therapies. This study aimed to evaluate the potential of Delphinidin-3-sambubioside (D3S), an *hibiscus* anthocyanin, and its stereochemically altered analogs as angiotensin I–converting enzyme (ACE) inhibitors.

Methods: The ACE inhibitory activity of D3S and its stereochemically altered analogs was evaluated using an *in silico* molecular docking methods. To determine the important interaction stabilizing the ligand-protein complexes, binding affinities were computed and molecular interaction studies were performed. The binding pocket's druggability was also investigated.

Results: The highest binding affinity was recorded for one of the analogs with a binding energy of −10.4 kcal/mol, which is greater than that obtained for D3S at −8.2 kcal/mol. Molecular interaction analysis of this analog demonstrated key hydrogen bond interactions with ACE residues along with other hydrophobic interactions and salt bridges at the active site stabilizing the protein-ligand complex. The possible druggable pocket was also predicted to explore any overlapping with binding regions and a drug score of 0.81 was obtained.

Conclusion: The increased binding affinity and favorable interaction profile of the D3S analog, combined with reported pharmacokinetic properties, predict D3S analogs as a potential alternative to be further investigated *in vitro* and *in vivo* as antihypertensive agents.

Keywords: Angiotensin-converting enzyme, Delphinidin-3-sambubioside, Antihypertensive drug, Molecular docking.

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INTRODUCTION

Arterial hypertension is a chronic condition where the pressure of blood against the walls of the arteries is consistently high and has been deemed as one of the leading causes of premature death worldwide [1]. It is often termed a silent killer because high blood pressure in itself is asymptomatic but can lead to serious health problems such as heart disease, stroke, and kidney failure when left untreated. Almost 7% of the world is affected by hypertension [2-4]. As per a recent report by the World Health Organization (WHO), around 1.28 billion adults from low and middle-income families have hypertension. Although in most situations, hypertension is left undiagnosed and untreated, the WHO aims to reduce the widespread presence of hypertension by 33% between 2010 and 2030 as one of the global targets for non-communicable diseases. India has also contributed to this initiative by adopting the India Hypertension Control Initiative to enhance hypertension management, offer a consistent supply of antihypertensive drugs, and promote patient-centered care [5]. Despite the presence of established therapies for treating hypertension, there are still persistent shortcomings in understanding the pathogenesis of hypertension. Therefore, novel therapeutic routes to treat the increasing rate of cardiovascular diseases have been a priority for researchers worldwide [6]. Several pharmacological classes of antihypertensive drugs such as beta blocker, diuretic, angiotensin-converting enzyme (ACE) inhibitor, renin inhibitors, and angiotensin II receptor antagonist have been identified [7]. Amongst these, modulation of the renin-angiotensin-aldosterone system (RAAS) has been used as a promising approach to develop novel antihypertensive therapy. ACE is one of the components of the RAAS which plays a vital role in the control of blood pressure by converting angiotensin I to angiotensin II [8,9]. ACE inhibitors have been promoted as one of the most effective therapies for hypertension, myocardial infarction, congestive heart failure, left ventricular systolic dysfunction, etc. [10]. Although several ACE inhibitors have been developed, some side effects have been reported for these drugs such as low blood pressure, dry cough, angioedema, hives, arrhythmia, headache, nausea, and decline in renal function. [11,12]. Newer strategies that are with more therapeutic efficacy and less side effects must therefore be developed. Since antihypertensive therapies are widely related to various categories such as age, race, and history of other disorders such as diabetes and cardiovascular issues, studying hypertension in clinical settings has proven difficult [13,14]. Moreover, drug discovery is a costly and prolonged procedure with a high rate of failure [15]. *In silico* studies have proved to be an effective alternative in this respect which have integrated the advancements in the arena of bioinformatics and computational biology to enable significant progress in the field of drug discovery [16,17]. *In silico* studies make use of large datasets of potential compounds, which speed up drug development by reducing laboratory synthesis providing few potential leads [18-20].

Anthocyanins are a class of flavonoids that have a large range of pharmacological actions such as protecting of heart and brain, reducing inflammation, and preventing cancer and muscular atrophy. [21]. Delphinidin which is an anthocyanin has well-established health benefits and can be isolated from beans, berries, fruits, and flowers. Delphinidin-3-sambubioside (D3S) is one of the bioactive compounds which has been isolated from *Hibiscus sabdariffa*

and has been shown to have anti-inflammatory, hypotensive, and hypolipidemic properties [22]. One of the most common ingredients found in herbal tea blends is the dry flower powder of *H. sabdariffa* L. The consumption of this herbal tea has been associated with the lowering of blood pressure in adults with hypertension [23-25]. The D3S present in its flower has been linked to lowered blood pressure through a number of pathways [26]. D3S causes blood vessel relaxation and enlargement which improves blood flow by lowering resistance which in turn contributes to reduced blood pressure. D3S also shows strong antioxidant properties like other anthocyanins. Antioxidants assist in lowering inflammation and oxidative stress both of which contribute to lowering blood pressure [27]. *Hibiscus* flower extracts have also exhibited a slight diuretic effect and anti-inflammatory properties which are considered to be due to D3S [28]. This helps in lowering the blood volume and consequently the blood pressure by increasing the generation of urine. From previous studies, it has also been found that D3S has the ability to reduce cardiac hypertrophy, oxidative stress, and fibrosis with MAPR pathway modulation making it a potential candidate for antihypertensive and cardioprotective drug development [29,30]. It can inhibit ACE to a comparable extent to captopril, a clinically used ACE inhibitor [31-33]. Modifications to its structure have also been reported to improve its stability and bioavailability [34]. In addition, natural ACE inhibitors such as flavonoids, peptides, and polyphenols have shown fewer side effects for hypertension and provide cardiovascular protection through their antioxidant and vasodilatory properties and therefore have been considered as a more viable alternative for hypertension treatment [35,36].

Based on these attributes, we have chosen D3S and its four analogs (ZINC000100826162 [L162], ZINC000100826163 [L163], ZINC000100826165 [L165], and ZINC000100826166 [L166]) to evaluate their potential as antihypertensive drugs. These analogs were obtained by stereo-chemically altering the backbone to evaluate the resultant impacts on pharmacological activity [37]. We have employed molecular docking to evaluate the most stable ligand-ACE complex according to their binding free energy values and obtain the best possible drug compound which can produce reduced side effects along with other additional cardiovascular benefits. Although the investigation of analogs of commercial ACE inhibitors has been reported, D3S and its analogs as ACE inhibitors have not been thoroughly examined for its antihypertensive activity by molecular docking studies.

METHODS

Computational analysis

The software used in this study is PyMOL for molecular visualization and analysis, AutoDock Vina 4.0 as the molecular docking software, and MGL Tools for preparing and analyzing molecular structures [38]. AutoDock Vina performs different types of docking, using a scoring function for predicting binding energies and identifying the best poses which can accommodate the conformational flexibility of ligands in the active site of proteins. It allows the screening of both large and small libraries of molecules [39-43]. PyMOL is a powerful tool coded in Python for high-quality visualization of the docked complex providing details such as orientation, bond lengths, and proximity of specific residues, which helps in interpreting the docking results. Protein-Ligand Interaction Profiler (PLIP) can be used for the identification of non-covalent interactions such as hydrogen bonds, hydrophobic contacts, π-stacking, and salt bridges involved in binding [44]. Protein-Plus Web Server is used for obtaining the druggability of the protein-ligand complex and predicting the most flexible pocket for the drug-like molecule.

Selection and preparation of ligand and protein

The ligand SDF structure format was downloaded from NCBI PubChem compound database for D3S and the 3D structure of the protein ACE with the protein data bank (PBD) ID (1O8A) (Fig. 1) was downloaded from RCSB PBD.

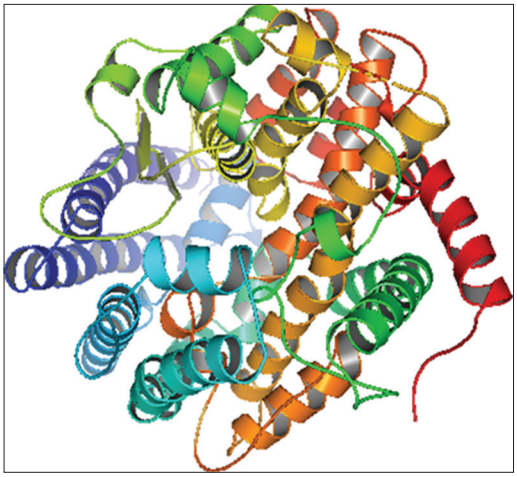

Fig. 1: 3D structure of the protein angiotensin-converting enzyme with Protein Data Bank ID (1O8A)

D3S obtained from PubChem database serves as a reference for comparing the analogs selected from ZINC20 database with similarity in their core structure and substituents but having variation in stereochemistry at the backbone (Fig. 2). These differences in the chiral centers affect how they bind with protein targets. The SDF structure format of the four analogs, L162, L163, L165, and L166, from the ZINC20 database is given below:

- L162 (https://zinc20.docking.org/substances/ZINC000100826162/)
  L163 (https://zinc20.docking.org/substances/ZINC000100826163/)
- L165 (https://zinc20.docking.org/substances/ZINC000100826165/) and
  L166 (https://zinc20.docking.org/substances/ZINC000100826166/)

Molecular docking

The protein target and ligands (D3S and analogs) were prepared for molecular docking using AutoDock Tools 1.5. Water molecules were removed from the protein molecule as they can interfere with the docking process. Gastieger charges and polar hydrogens were added to the protein to account for the electrostatic interactions. The molecular docking parameters included a center grid box set up around the active site of size 40×40×40 (x, y, z) points, with a spacing of 0.375 Å and the center dimensions ofx=40.064, y=37.371, z=43.69 Å, respectively. The exhaustiveness parameter was set to 8 to allow a search of possible binding poses with minimum Gibbs free energy. All the prepared ligands and protein files were uploaded to AutoDock Vina as PDBQT files for docking and energy calculations. After docking, close interactions of the binding of the ACE with the D3S and its analogs were analyzed and visualized using PyMOL and all the polar hydrogens were displayed. The results obtained were then analyzed by two Web Servers, namely PLIP and the Protein Plus web server to generate 2D and 3D figures for the detection of cavities. The PDB files of protein-ligand complex (Fig. 3) were provided as input to the DoGSiteScorer server to calculate the ligand/protein overlap and to determine the druggability for the pockets.

RESULTS

The binding interactions between the ACE macromolecule and D3S and its analogs were studied using molecular docking and the log files generated as a result of docking (Supplementary Table 1a) represent the net predicted binding free energy of the best nine pose. Out of these, the binding energy of the pose with root mean square deviation value <1 was chosen and the subsequent results are summarized in Table 1.

The binding site amino acid residues and the polar contacts were visualized for all the five ligands by PyMOL visualization and are represented in Table 1 and Fig. 4 [45,46]. L162 and ACE complex showed substantial binding affinity of −10.4 kcal/mol, indicating stable and strong interaction leading to the inhibition of ACE activity. The docking

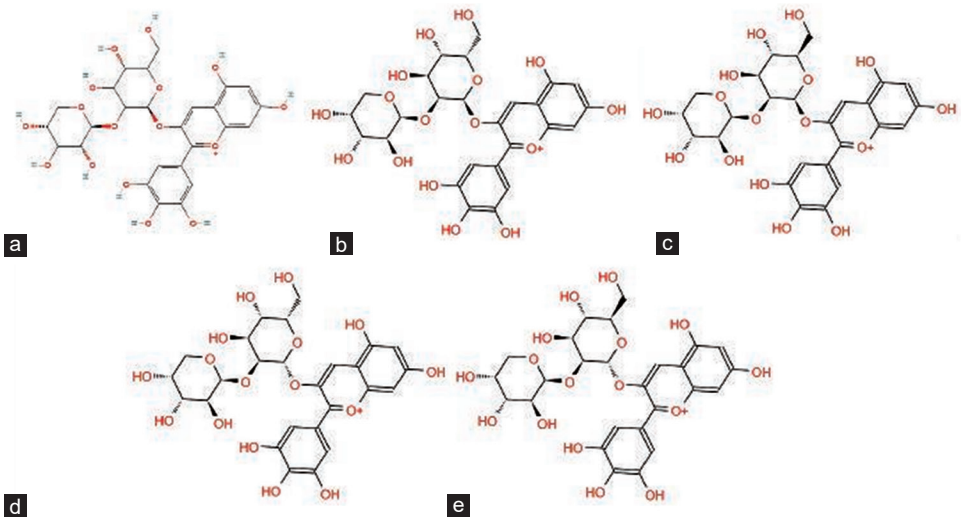

Fig. 2: (a) Delphinidin-3-sambubioside and its structural analogs with different stereochemistry, (b) L162, (c) L163, (d) L165, (e) L166

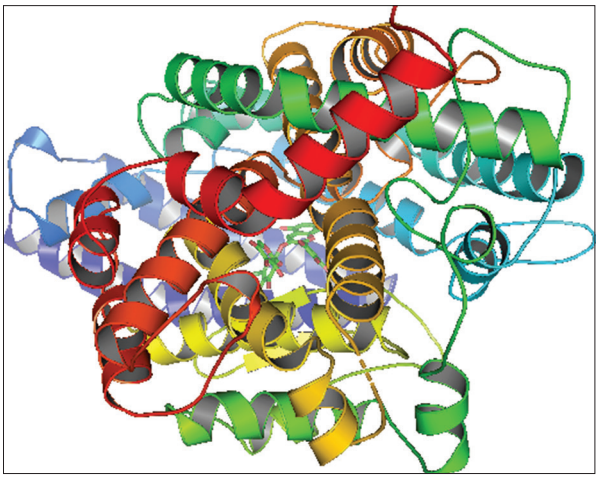

Fig. 3: 3D interaction pose of L162 and angiotensin-converting enzyme after docking

of commercial ACE inhibitors such as enalapril, captopril, and lisinopril with ACE has shown a binding affinity of −6.38, −6.54, and −12.47 kcal/mol, respectively [47,48]. When these values are compared, the binding energies of the analogs of D3S are similar to known ACE inhibitors. A total of 5 polar bond formations with active sites were observed with multiple amino acid residues including LYS-368, ARG-124, SER-35, GLU-411, and ALA-356.

For further investigation of the molecular interaction, L162 was selected for analysis using the PLIP web server at the active site of ACE's chain A. The results showed a total of 8 chemical features including five hydrogen bonds with residues, ASN 66, SER 355, ALA 356, and LYS 368 (Table 2). In addition, a hydrophobic contact with PHE 512 and two salt bridges with ARG 126 and ARG 522 were identified (Table 3) which add stability to the ligand-protein complex contributing toward good inhibitory activity [49]. These findings not only aligned with the molecular docking results but also indicated a detailed map of the specific interactions found in the ligand-protein binding. The hydrogen bonds increased the degree of affinity in the ligand-protein binding thereby stabilizing the L162-ACE complex. Therefore, a concordance is observed in the results of molecular docking, PLIP analysis (Fig. 5) and PyMOL visualizations (Fig. 4).

The Pose View tool of the Protein Plus web server was highly useful in generating a two-dimensional diagram showing the interaction of

Table 1: Binding energies and amino acid residues at the binding site of ACE and D3S analogs

No	Protein	Ligand	Binding affinity kcal/mol	Amino acid residues at binding sites
1	ACE	D3S	−8.2	LYS-368, TYR-360, ARG-402, ARG-522, GLU-411
2	ACE	L162	−10.4	LYS-368, TYR-360, ARG-124, SER-355, GLU-411, ALA-356
3	ACE	L163	−10.3	LYS-368, ASN-70, SER-355, ALA-356, GLU-411, ARG-522, ARG-124
4	ACE	L165	−10.2	LYS-368, ASN-70, SER-355, ARG-124, ARG-522, GLU-411, ALA-356
5	ACE	L166	−10.1	LYS-368, ALA-356, GLU-411, ARG-124, ASN-70, SER-355

ACE: Angiotensin-converting enzyme, D3S: Delphinidin-3-sambubioside

Table 2: Hydrogen bond distance from amino acid

Index	Residue	Amino acid	Distance H-A (Å)	Distance D-A (Å)	Donor angle
1	66A	ASN	3.45	3.82	103.72
2	355A	SER	2.52	3.27	135.81
3	356A	ALA	2.71	3.61	146.63
4	356A	ALA	2.83	3.42	120.28
5	368A	LYA	2.74	3.46	128.24

Table 3: Hydrophobic interaction and Salt bridge between amino acid and L162

Type of interaction	Residue	Amino acid	Distance (Å)
Hydrophobic interaction	512A	PHE	3.88
Salt bridge	126A	ARG	4.53
Salt bridge	522A	ARG	4.79

structural elements in atomic details and the overall binding of L162 in the ACE receptor structure (Fig. 6). This analysis showed five hydrogen bonds, involving three donor hydrogen atoms, indicating strong connections with amino acid residues GLU 411, TYR 360, TYR 360, TYR 62, and SER 355 that are present in the binding pocket. The hydrogen bonds are shown as dashed lines indicating the specificity and strength of molecular interactions of the three hydroxyl groups of the B ring system of L162.

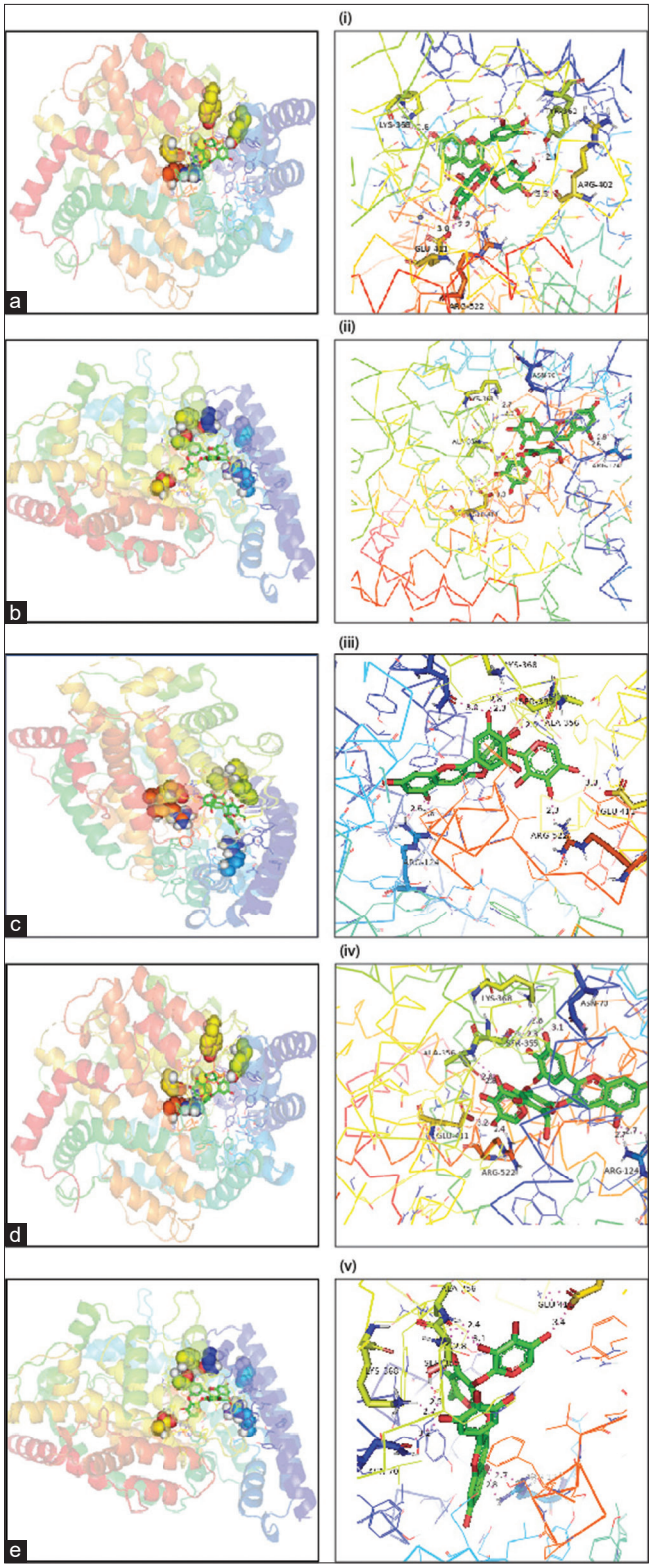

Fig. 4: (a-e) PyMOL visualization representing the Binding mode of delphinidin-3-sambubioside, L162, L163, L165, L166 and angiotensin-converting enzyme with amino acid residues

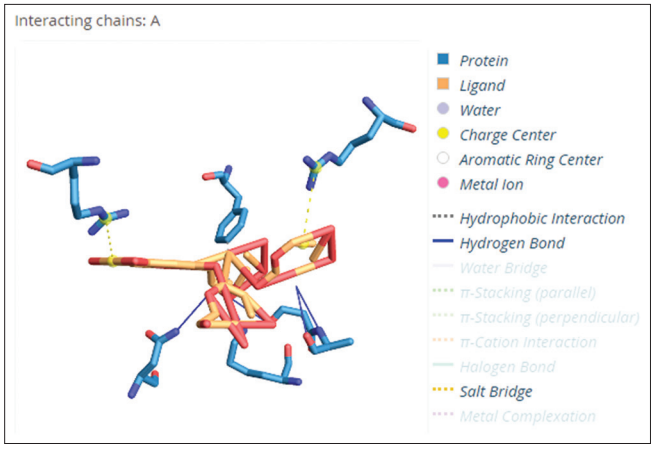

Fig. 5: Protein-ligand interaction profiler analysis depicting the interactions between amino acid residues and L162

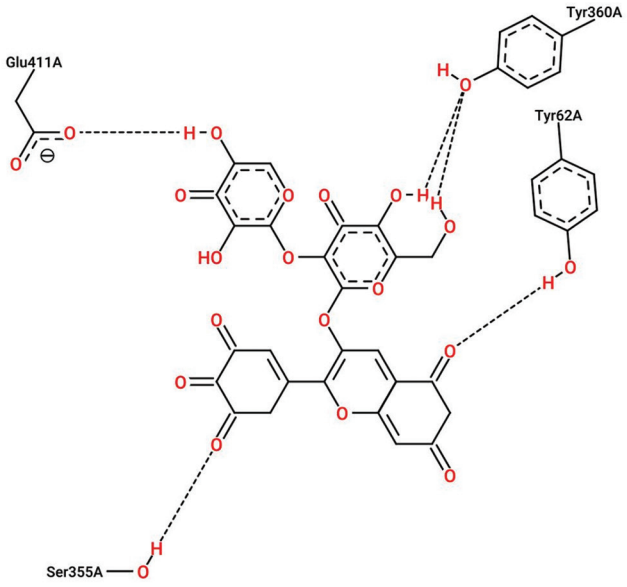

Fig. 6: 2D diagram of amino acid residues forming hydrogen bonds with L162

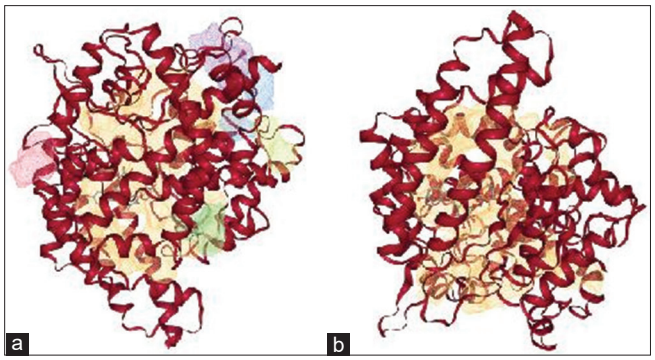

Fig. 7: Analysis of the pockets of the protein structure (a) with angiotensin-converting enzyme chain A and (b) with the highest drug score

The analysis of the protein structure using DoGSiteScorer showed a total of nine pockets on the surface, as shown in (Fig. 7a). Table 4 shows the main shape descriptors for all the detected pockets. Among these, the first three pockets (P_0, P_2, and P_1) were identified as potentially druggable from the drug score of 0.81, 0.6, and 0.58, respectively, obtained from the DoGSiteScorer analysis. The pocket with the highest drug score (0.81) was prioritized as the best cavity that covers the ligand L162 (Fig. 7b). The selected pocked had some distinctive properties making it suitable for drug binding. These properties included a large

Table 4: The main shape descriptors for all detected pockets

Name of pocket	Volume (Å)	Surface (Å)	Drug score
P_0	4585.56	3910.26	0.81
P_2	258.78	373.13	0.6
P_1	356.97	627.83	0.58
P_4	224.74	375.58	0.54
P_5	184.87	373.6	0.4
P_10	124.55	144.65	0.38
P_3	250.19	445.83	0.35
P_11	105.3	245.11	0.21
P_12	100.53	241.12	0.18

pocket volume of 4585.56 Å, a depth of 32.96Å, and a high apolar amino acid ratio of 0.46 %. These features together make the pocket highly druggable indicating its potential as a target site for L162 as a therapeutic lead compound. In contrast, the remaining pockets were regarded less favorable for drug binging as indicated by their smaller sizes and corresponding druggability scores below 0.5. The most promising pocket, where L162 was fully fitted was selected as the target site with optimal characteristics for potential drug development [50-55]. It also showed favorable pharmacokinetic properties essential for its potential as a drug candidate. From Fig. 6, it can be observed that there are four hydrogen bond donors, six hydrogen bond acceptors, and the log p-value, which usually indicates that a molecule's lipophilicity is −1.449. As per Lipinski's rule of 5 which evaluates the likelihood of a new molecular entity to be used as an orally active drug, log p-value should be <5. The known commercial ACE inhibitors such as enalapril and lisinopril have log p=−0.09 and −1.23, respectively [47]. A negative value of log P indicates greater hydrophilic nature of the ligand. It has been reported in previous studies that D3S exhibits an IC_{50} value of 84.55±2.2g/mL against ACE [56]. D3S and its analogs have strong antioxidant and anti-inflammatory properties which could indirectly inhibit ACE activity by reducing oxidative stress and inflammation, thus reducing blood pressure. Therefore, we believe that our studies can further the progress achieved in this field and provide exciting new directions for the development and subsequent clinical trials of D3S and its analogs as potential antihypertensive drugs.

DISCUSSION

ACE is a membrane-bound and zinc dependent peptidase. It catalyzes the transformation of angiotensin I to angiotensin II by eliminating the C-terminal amino acids. The active site of ACE contains a hydrophobic region characterized by the side chains of the C-terminal amino acid residues, a hydrophilic pocket arising from the guanidinium group of arginine, and a zinc ion. Most drugs demonstrate antihypertensive activity through two complementary modes of action, either as a diuretic which focuses on aldosterone or as an ACE inhibitor. Anthocyanins with their rigid planar structure and presence of hydroxyl groups can effectively chelate with zinc ions and also engage in hydrophilic and hydrophobic interactions through the formation of hydrogen bridges with the amino acids present in the active site [47,48]. Here, we evaluated the ACE inhibition activity of stereochemically altered analogs of D3S, which is a *Hibiscus* anthocyanin. Molecular docking results of the L162 analog of D3S exhibit an almost comparable binding affinity to the commercially available ACE inhibitors. In addition, the stability of the complex was depicted by the presence of strong hydrogen bonds as well as hydrophobic and salt bridge interactions. These hydrogen bonds of L162 bind to the active site of ACE through the hydroxyl groups creating a strong interaction which is capable of inhibiting ACE and preventing the conversion of angiotensin I to angiotensin II which in turn contributes to reduced blood pressure. The zinc ion in the active site of ACE plays a catalytic role in stabilizing the transition state of the substrate leading to the conversion of angiotensin I to angiotensin II. The L162-ACE complex in a close proximity to the zinc ion competes with the optimal function of ACE enzyme-substrate binding and its transition state stabilization thereby hindering the process. Moreover, from the log p-values, it can be deduced that L162 meets the requirement given by Lipinski's rule of five which indicates a compound's ability to form essential interactions for effective binding to biological targets while maintaining a balance that supports its absorption and distribution in the body. Therefore, L162 holds significant pharmacophore properties as a potential inhibitor of ACE and can be evaluated further experimentally as an antihypertensive drug [51,57].

CONCLUSION

This study provides a structure-based design and discovery of D3S and its stereochemically altered analogs as a novel, natural ACE blocker. A comprehensive evaluation was carried out using molecular docking studies and molecular interaction analysis of new promising analogs of D3S. This provided a clear understanding of the potential therapeutic applications of these compounds. The compound's optimal molecular interactions such as hydrogen bonding, hydrophobic interactions, and electrostatic interference to chelating active site zinc ion indirectly, coupled with favorable pharmacokinetic attributes from previous studies, align with the established criteria for drug-likeness. The evaluation of potential drug-binding pockets highlighted a particularly promising site with a drug score of 0.81. This pocket accommodated the ligand L162 optimally reinforcing its potential to interfere in the enzymatic activity of ACE by inhibiting the catalytic site of ACE akin to traditional ACE inhibitors such as captopril and enalapril. Therefore, high binding affinity, strong hydrogen bonding, and hydrophobic and salt bridge interactions at the enzyme-substrate binding site almost completely filling cavity make this analog a good inhibitor of ACE. Collectively, the computational analysis performed in this study yielded better results for L162 than commercially used ACE inhibitors and it, therefore, paves the way for the further investigation of D3S and its analogs through *in vitro* and *in vivo* studies for a potent antihypertensive drug.

AUTHORS' CONTRIBUTIONS

All the authors have equally contributed to the article.

CONFLICTS OF INTEREST

No conflicts of interest.

AUTHORS' FUNDING

Nil.

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Supplementary Table 1a

Log file for D3S

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b
1	-8.2	0.000	0.000
2	-8.1	11.648	15.003
3	-8.1	1.911	7.199
4	-8.1	10.015	14.996
5	-8.0	9.402	14.877
6	-7.8	11.356	14.502
7	-7.8	2.551	7.153
8	-7.5	3.878	8.681
9	-7.5	13.518	18.427

Log file for L162

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b
1	-10.4	0.000	0.000
2	-10.3	3.085	7.483
3	-10.3	2.765	3.524
4	-10.2	2.655	5.874
5	-10.1	1.994	7.785
6	-10.1	3.695	8.869
7	-9.9	2.769	6.292
8	-9.9	3.242	6.289
9	-9.9	3.068	6.247

Log file for L163

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b
1	-10.3	0.000	0.000
2	-10.1	4.184	9.552
3	-10.0	0.802	2.010
4	-9.9	2.958	3.652
5	-9.9	2.687	5.658
6	-9.9	8.948	12.251
7	-9.8	3.718	8.751
8	-9.6	3.125	6.096
9	-9.6	4.735	9.970

Log file forL165

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b
1	-10.2	0.000	0.000
2	-9.9	0.568	1.495
3	-9.9	6.693	9.953
4	-9.9	2.929	3.490
5	-9.9	2.680	5.514
6	-9.6	2.420	7.739
7	-9.6	8.260	12.944
8	-9.5	2.793	8.149
9	-9.4	3.415	7.272

Log file for L166

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b
1	-10.1	0.000	0.000
2	-10.1	3.046	7.389
3	-10.0	4.674	9.784
4	-10.0	2.738	5.830
5	-9.9	2.954	3.478
6	-9.8	2.425	7.928
7	-9.6	3.053	5.742
8	-9.3	2.795	6.775
9	-9.3	1.501	2.305