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Comparison of PDE-5 inhibitors used in erectile dysfunction with some candidate molecules: A study involving molecular docking, ADMET, DFT, biological target, and activity

Süleyman Sağır[1], Velid Unsal[2*], Erkan Oner[3], Reşit Yıldız[2] and Başak Doğru Mert[4]

Abstract

Erectile dysfunction (ED) is a urological condition defined as the inability of a man to achieve or maintain an erection. This condition negatively affects his sexual performance and the performance of his partner. Phosphodiesterase type 5 (PDE5) inhibitors are commonly used to treat ED. Arginase II plays an important role in regulating L-arginine to NO synthase in the smooth muscle of the human corpus cavernosum of the penis. NO is a molecule essential for regulating a variety of functions, including arterial blood pressure, penile erection, and energy balance. Substances such as vardenafil, alprostadil, papaverine, and resveratrol increase NO production, thereby supporting sexual function and vascular health. Additionally, NO donors such as L-arginine, L-citrulline, and α-lipoic acid provide effective alternatives when used in combination with PDE5 inhibitors. Medications used in the treatment of ED include vardenafil, alprostadil, and papaverine. In addition, although molecules such as L-arginine, citrulline, resveratrol, alpha-lipoic acid, and rutin are thought to play a role in ED, their pharmacological and molecular effects have not been sufficiently elucidated. The aim of this study was to investigate the effects of these molecules in the treatment of ED by computer-based calculations, to obtain new information about them and to inspire new treatment strategies for ED. The physicochemical, molecular and pharmacokinetic properties of the compounds were determined by SwissADME software, and ADMET (absorption, distribution, metabolism, excretion and toxicity) data were determined by ADMETlab 3.0 software. Biological target and activity data were obtained by MolPredictX and PASS Online software. While the Gaussian 09 program was used for DFT calculations, PyMOL, AutodockTools 4.2.6, AutoDock Vina, and Biovia Discovery programs were used for molecular docking studies. It was found that L-arginine, citrulline, resveratrol and α-lipoic acid were well absorbed from the intestine, while rutin showed limited absorption. When their metabolic risks were evaluated, L-arginine and citrulline were found to have lower toxicity. Molecular docking results of rutin and resveratrol were remarkable. The electronic properties of the compounds were explained by DFT calculations. L-arginine and citrulline were found to have low toxicity and positive therapeutic effects. L-arginine and citrulline stand out as promising candidates for future research. Although resveratrol data are promising, unfortunately their potential toxicity and metabolic interactions require further investigation. It is important to learn more about these compounds or conduct research to improve their therapeutic efficacy. Although computer-based calculations play an important role in toxicity predictions, drug interactions, pharmacokinetics and toxicity properties should be carefully evaluated.

Keywords Erectile dysfunction, PDE-5 inhibitors, NOS, Arginase II, ADMET, Molecular docking

*Correspondence:
Velid Unsal
velidunsal@artuklu.edu.tr
Full list of author information is available at the end of the article

Introduction

Erectile dysfunction (ED), which has detrimental effects on a man's quality of life, is one of the important urological problems, defined as the inability to achieve and maintain penile erection for satisfactory sexual performance [1–3]. ED increases with age; from 5% in men aged 20-39 to 70% in men over 70. The aetiology of ED includes organic, psychogenic and mixed factors that are intertwined with vascular, neurological and endocrinological disorders, and is therefore not limited to older men. Risk factors such as hypertension, diabetes, high cholesterol, and smoking have been shown to have a strong correlation with ED. If left untreated, it can lead to anxiety, depression, low self-esteem, and strained interpersonal relationships. Therefore, comprehensive assessment and management of EDs are of great importance [4, 5].

Phosphodiesterase Type 5 (PDE5) is the most well-known phosphodiesterase that targets nitric oxide-mediated cGMP and regulates cGMP signaling in cardiovascular and other tissues. Due to cGMP's role in many physiological processes, PDE5 inhibitors are used in treatments such as erectile dysfunction and pulmonary hypertension [6].

Nitric oxide (NO), which is produced through the action of nitric oxide synthases (NOS), has several important functions within the body. It acts as both a neuromodulator and neurotransmitter, playing a crucial role in various processes, including the regulation of arterial blood pressure, the process of penile erection, maintaining energy balance at the central level, memory, learning, and sexual behavior [7].

PDE5 inhibitors are similar in structure to cGMP; NO synthesis is produced from L-arginine by the NOS enzyme and plays critical roles in various physiological processes through mechanisms such as stimulation of potassium-dependent calcium channels or inhibition of upregulation of the RhoA/Rho-kinase pathway. The increase/accumulation of cGMP in smooth muscle cells causes smooth muscle relaxation in the corpus cavernosum and increased blood flow to the penis, which leads to prolongation of erection [8–11].

Arginase is a well known enzyme of the urea cycle that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. The effect of arginase goes beyond the limits of hepatic urogenic function and is widespread in extrahepatic tissues. Two isoforms of arginase coexist, Arginase I (Arg1) is predominantly expressed in the liver, and Arginase II is expressed in extrahepatic tissues [12]. Given that human arginase II is involved in regulating L-arginine bioavailability to NO synthase in the smooth muscle of the human penile corpus cavernosum, inhibition of human arginase II is a potential strategy for the treatment of erectile dysfunction [13]. The increased activity of arginase can reduce the availability of L-arginine for NO synthase, thereby reducing the production of NO while increasing the formation of reactive oxygen species and ultimately leading to endothelial dysfunction [14].

Vardenafil is a selective inhibitor of PDE5 that promotes the release of NO in the corpus cavernosum during sexual arousal. The FDA approved the use of vardenafil for the treatment of erectile dysfunction in 2003 [15]. alprostadil (Prostaglandin E1) improves microcirculation, dilates blood vesselsi and inhibits platelet aggregation. It is widely used as anticoagulation therapy for limb ulcers, cardiovascular and cerebrovascular microcirculation disorders caused by chronic arterial obstruction, and organ transplantation. Supplementation is also a promising treatment option for many diseases such as hepatitis, pancreatitis, diabetes and erectile dysfunction [16, 17]. papaverine relaxes the smooth muscles of large blood vessels, including the coronary, cerebral, and pulmonary arteries, by direct action. PDE5 and PDE10A are phosphodiesterase inhibitors, mostly found in the striatum of the brain. papaverine is used to treat vasospasm and erectile dysfunction.

In addition, current pharmacological research has revealed that papaverine exerts a variety of biological activities, including antiviral, cardioprotective, anti-inflammatory, anticancer, neuroprotective, and pregnancy effects [18–20].

L-citrulline, which is produced from l-ornithine and carbamoyl phosphate in the urea cycle and then converted to L-arginine, supporting the production of NO, is a precursor of L-arginine and is involved in the production of NO [21]. Given the important role of NO in erection physiology, NO donors, especially L-arginine and L-citrulline, are attracting attention as an effective alternative to PDE-5 inhibitors [22].

Resveratrol, a natural polyphenol, is found in foods such as grape skins, blueberries, and peanuts. Thanks to its antioxidant properties, it may be effective in preventing cardiovascular diseases, cancer, and neurodegenerative disorders by reducing oxidative stress. On the other hand, its cardioprotective effects improve vascular health by promoting the production of NO, which dilates blood vessels and increases blood flow. In addition, it protects endothelial function by reducing inflammation and preventing the development of atherosclerosis [23].

Thioctic acid is an acid derivative of α-lipoic acid, a natural antioxidant. As one of the few antioxidants that is both water- and fat-soluble, it can act on the inner and outer regions of cells. α-lipoic acid works as a cofactor of mitochondrial enzymes involved in energy production and supports cellular energy metabolism α-lipoic acid is useful in the management of health problems associated with oxidative stress, such as diabetes and

neurodegenerative diseases. For treating diabetic neuropathy, it can help relieve symptoms by reducing nerve damage and improving nerve function. Moreover, it positively affects overall health by reducing inflammation and strengthening antioxidant defense mechanisms. α-lipoic acid shows positive effects on erectile dysfunction and metabolic disorders in men with type 2 diabetes. Owing to its ability to inhibit the glycosylation of free radicals and increase endothelial NOS activity, it improves vascular function. These properties may enable the use of α lipoic acid in combination with PDE-5 inhibitors and contribute to nitric oxide production in patients with severe diabetic neuropathy [24–26].

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a bioflavonoid found mainly in plants such as apples, green tea, *Betula pendula leaves, Fagopyrum esculentum* (buckwheat), *Ruta graveolens* (seposis) and *Sophora japonica* (Japanese acacia). It has a wide range of biological effects, including anti-inflammatory, antioxidant, neuroprotective, nephroprotective, and hepatoprotective activities. Rutin has been shown to increase NO production in the HUVEC cell line, i.e., to increase eNOS at both the mRNA protein level and enzyme activity [27, 28]. On the other hand, it has been reported that plant extracts containing high concentrations of rutin inhibit arginase in vitro conditions, increase the amount of arginine, and act as a direct inhibitor on PDE5 [29].

Recently, artificial intelligence (AI) and machine learning (ML) have become revolutionary technologies in pharmaceutical research and development. Advances in computational technologies and the disappearance of big data processing restrictions have accelerated this process. Aware of the high costs, the pharmaceutical industry has made the increasing complexity of drug discovery in the last 15-20 years more understandable, systematic, more efficient. AI/ML's most recent contribution has also been to clinical trial design and analysis. In addition, John Hopfield and Geoffrey Hinton were awarded the 2024 Nobel Prize in physics for having pioneering computational methods that enabled the development of neural networks [30, 31]. The discovery of oral PDE5 inhibitors 30 years ago was a breakthrough in the treatment of ED. For treating ED, local and/or centrally acting drugs are used, mainly orally administered PDE5 inhibitors and intracavernously injected phentolamine, prostaglandin E1 and even papaverine. However, these drugs have not been effective for every man and new treatment strategies are being sought for persistent forms of ED. The failure of PDE5 inhibitors for treating ED is attributed to low levels of NO in the cavernous tissue (NO synthase deficiency, L-arginine deficiency, etc.). Various pharmacological strategies are being investigated to overcome this situation [32].

ED treatments aim to achieve and maintain an erection sufficient for sexual intercourse. The safety and pharmacokinetic properties of pharmacological agents such as vardenafil, alprostadil, and papaverine, which are widely used in the treatment of ED, were included in the control group of drugs. However, available data on the efficacy, safety, and optimum dosage profiles of alternative compounds such as L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin are insufficient and controversial. Unfortunately, this poses a challenge for clinicians regarding the efficacy of these compounds. Although there are hundreds of studies on molecules such as L-arginine, citrulline, resveratrol, α-lipoic acid and rutin, comprehensive studies including pharmacological and chemoinformatic data of these compounds are lacking. In addition, experimental studies examining the role of these molecules in the pathophysiology of ED and their potential therapeutic effects are insufficient. On the other hand, studies comparing the efficacy and side effect profiles of these compounds are almost nonexistent. The aim of this study was to comprehensively reveal the pharmacological properties, efficacy and side effect profiles of molecules such as L-arginine, citrulline, resveratrol, α-lipoic acid and rutin as potential therapeutic agents in the treatment of ED using computational methods. The reason for selecting these molecules is their known effects on biological processes such as endothelial dysfunction, oxidative stress and nitric oxide (NO) synthesis, which play an important role in the pathophysiology of ED. In particular, L-arginine and citrulline support vascular relaxation as precursor molecules in NO synthesis, while resveratrol and α-lipoic acid can reduce oxidative stress with their antioxidant properties. Rutin draws attention with its flavonoid structure that supports vascular health. In addition, this study aims to pioneer the investigation of new therapeutic strategies targeting PDE5, NOS and arginase-II inhibitors in the treatment of ED. This targeting approach emphasizes the originality and innovative aspect of the study. It is thought that the findings obtained may constitute an important data source for future pharmacological research and drug development processes related to ED treatment. This study aims to contribute to the development of new and effective therapeutic options in the treatment of ED.

Materials and methods

Physicochemical and molecular descriptor analysis

The chemical structures of vardenafil, alprostadil, papaverine, arginine, citrulline, resveratrol, α-lipoic acid and rutin compounds in canonical SMILES format were downloaded from PubChem database. These structures were used as input to SwissADME online

platform (http://www.swissadme.ch/, access date, 19 August 2024) for the evaluation of drug-like properties [33]. SwissADME was used to analyze the basic physicochemical properties of these compounds such as conformity to Lipinski rule, water solubility, lipophilicity (logP), number of hydrogen bond donors and acceptors. These analyses were aimed to determine whether the compounds have drug-like properties. In addition, the same compounds were subjected to more comprehensive analysis using ADMETlab 3.0 software (https://admetlab3.scbdd.com, access date, 20 August 2024) [34]. ADMETlab 3.0 was used to calculate the molecular and pharmacokinetic properties of these molecules. These analyses aimed to evaluate the absorption, distribution, metabolism, elimination and toxicity (ADMET) profiles of the compounds. In particular, parameters such as plasma protein binding, intestinal permeability, hepatic metabolism, CYP enzyme inhibition, toxicity risk (e.g. hERG,Blockers, DILI, AMES Mutagenicity) were investigated. These comprehensive in silico analyses provided critical information to predict the pharmacological potential and possible side effects of the compounds.

Identification of biological targets by molecules

This tool predicts potential targets and interactions of specific compounds with these targets by analyzing their molecular structures and biological effects. Potential biological targets of vardenafil, alprostadil, papaverine, arginine, citrulline, resveratrol, α-lipoic acid, and rutin were predicted. These predictions were made using MolPredictX (https://www.molpredictx.ufpb.br/.) (Access date, 17 August 2024) These predictions are usually based on various computational biology methods such as molecular docking, bioinformatics analyses, and machine learning models. The calculated probabilities are presented based on whether a given compound is active or inactive against the target. The reliability and precision of these calculations can be an important issue because predictions are often limited to theoretical models that are not validated by experimental data. Potential targets, outcome, probability of activity or inactivity, and reliability are reported in the same table. K: Predicted Outcome, L:Probability, M: Probability Active, N: Probability Inactive [35].

Biological activity site of metabolism

The biological activity of the metabolism zone was determined using a prediction service called PASS Online (https://www.way2drug.com/passonline). (Access date, 22 August 2024).The user enters PASS Online with the touch of a chemical owner. This structure can typically be in SMILES format or in concise file formats (e.g. MOL, SDF). PASS analyzes this structural structure and compares data relationships to known images. PASS predicts various biological activities of a compound based on molecular structure. These activities can include enzyme inhibition, receptor binding affinities, effects on intracellular signaling pathways, and many other pharmacological effects. Predictions are presented as "Pa" (likely activity) and "Pi" (likely inactivity) values [36, 37]

Molecular Modelling Studies

Ligand System

Vardenafil, alprostadil, papaverine, Arginine, citrulline, resveratrol, α-lipoic acid and rutin chemical structures were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Details about the compounds are given in Table 1.

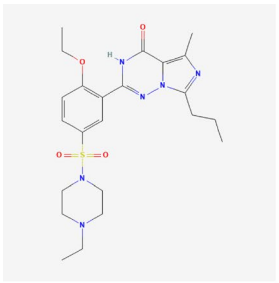
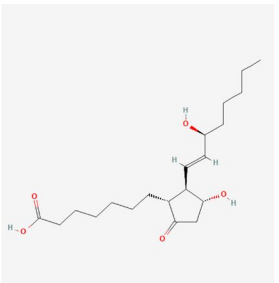
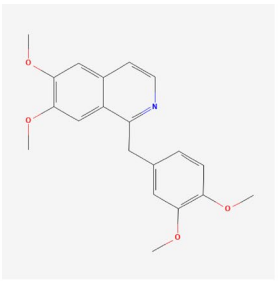
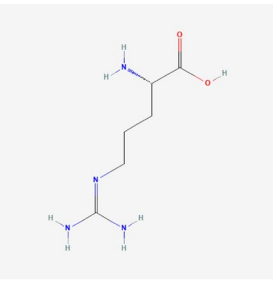
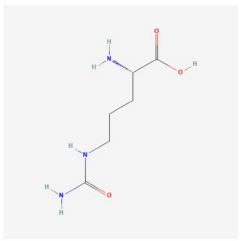
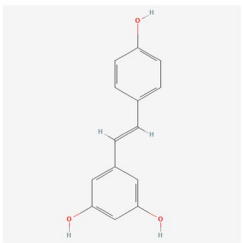
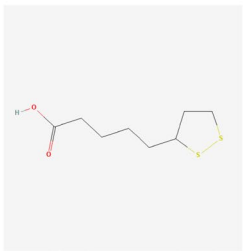
Protein system

Catalytic Domain Of Human Phosphodiesterase 5A (PDB ID: 1T9R), Nitric Oxide Synthase (PDB ID: 2FLQ) and Crystal structure of Human Arginase-II (PDB ID: 4IXU) protein structures were obtained from the protein database (https://www.rcsb.org/). The information of the proteins is given in Table 2.

Molecular docking method

Molecular docking studies were performed according to our previous studies [38–40]. The 2D structures of the compounds vardenafil, alprostadil, papaverine, arginine, citrulline, resveratrol, α-lipoic acid and rutin molecules, which we selected from the PubChem database, were obtained in SDF file format from the protein database. The energy minimisations required to prepare the obtained compounds for the docking environment were performed in ChemDraw and converted to pdb format. PyMOL (Schrödinger, Inc., New York, NY, USA) v. 2.5 software was used to check for the presence of missing atoms in the PDE5, Arg II and NOS proteins, the presence of other ligands and bound ions, and to remove the ligand from the protein. The pdb file of the protein was transferred from Autodock Tools (The Scripps Research Institute, San Diego, CA, USA) v. 1.5.7 to MGL Tools. Here, water molecules were removed from the protein and polar bonds were added. The final version of the protein was saved in pdb file format. The saved PDB file was transferred to Swiss-Pdb Viewer v. 4.1 (Geneva, Switzerland) where energy minimisation of the protein was performed. The pdb file of the protein was then analysed in Autodock Tools v. 1.5.7 software. Autodock Vina v. 2.5 software was used to dock the protein and each ligand separately. The affinity results of the ligands were saved in txt file format. As software output, the out.pdbqt file of each ligand and the out.pdbqt file of

Table 1 General information about compounds (https://pubchem.ncbi.nlm.nih.gov/compound)

1. Vardenafil Compound ID: 135400189	2. Alprostadil Compound ID: 5280723	3. Papaverine Compound ID: 4680	4. Arginine Compound ID: 6322
			
5. Citrulline CompoundID: 9750	6. Resveratrol Compound ID: 445154	7. alpha-Lipoic acid Compound ID: 864	8.Rutin CompoundID: 5280805
			

the protein were opened in PyMOL v. 2.5 software and the 1st conformation complex states were saved in pdb file format. Biovia Discovery Studio Visualizer software was used for visualisations PDE5 coordinates XYZ: (48.110538, -0.370462, -6.293923), Arg II coordinates XYZ: (33.923936, 89.071106, 69.113553), NOS coordinates XYZ: (76.314945, 30.151691, 12.487127) [41–43].

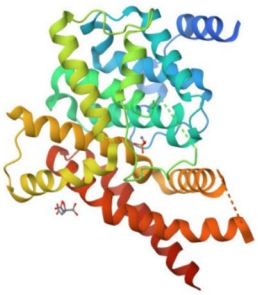
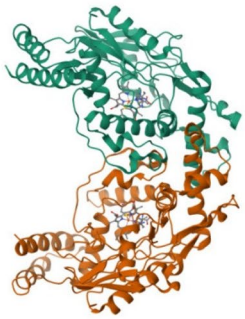
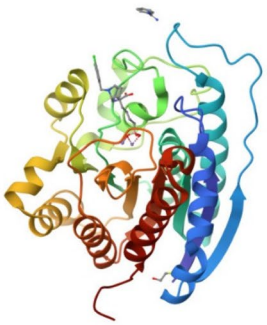
Autodock Protocols Semi-flexible docking allows ligands to sample various conformations during a semi-flexible docking step while the protein receptor remains rigid. By default, all three software utilise the semi-flexible docking algorithm. The receptor docking site was within the binding site of the co-crystallised ligand. Polar hydrogen atoms and Kollman charges were added and assigned to the receptor respectively [44].

Autodock Vina Protocols The centre of the crystalline proteins was set as the centre of the docking box. In this study, the Vina force field was used instead of the AutoGrid4.2 force field. Affinity grid maps were not calculated. The maximum number of binding modes was 9. The maximum energy difference between modes was 2.5 kcal/mol. The scoring function weights and terms were set to default [44].

DFT calculations

The Gaussian 09 software package and GaussView 5.0 software were used to carry out geometry optimization techniques using density functional theory-based quantum chemistry computations (B3LYP with a 6-311++G(d,p) basis set). The energy gap (ΔE) between the lowest unoccupied molecular orbital (*LUMO*) and the highest occupied molecular orbital (*HOMO*) was calculated as part of the computations. In addition, evaluations were conducted for factors including nucleophilicity (*Nu*), softness (s), electrophilicity (ω), electronegativity (χ) and hardness (η). These quantum chemical techniques

Table 2 3D structures of Phosphodiesterase 5A, Nitric Oxide Synthase, Arginase-II

No.	Target proteins	PDB ID	Structure
1	PDE 5A	1T9R	
2	NOS	2FLQ	
3	Arginase-II	4IXU	

were also used to compute the dipole moment (μ) and Mulliken charges on the backbone atoms [45–54].

The following formula was used to get the parameters

$$\Delta E = E_{LUMO} - E_{HOMO} \quad (1)$$

$$A = -E_{LUMO} \quad (2)$$

$$I = -E_{HOMO} \quad (3)$$

$$\eta = \frac{1}{2}(I - A) \quad (4)$$

$$s = 1/\eta \quad (5)$$

$$\chi = 1/2(I + A) \tag{6}$$

$$\omega = \frac{\chi^2}{2\eta} \tag{7}$$

$$Nu = 1/\omega \tag{8}$$

Predicting the chemical reactivity and stability of the molecules under study requires a thorough understanding of their electrical characteristics, which is provided by these computations. The data can provide important insights into molecular behavior and possible chemical and biological applications by clarifying these features. This knowledge helps with the design and synthesis of novel compounds with desired stability and reactivity profiles in chemical applications. In terms of biology, the data is crucial for forecasting how these compounds will interact with biological targets, maximizing their effectiveness as medications, and evaluating their safety and metabolic stability.

Results and Discussion

In the process of drug discovery, which chemicals to synthesize and test is determined by considering various parameters, if possible, it is to select molecules that are effective and have low toxicity. In addition, the reach and concentration of the molecules to the therapeutic target is also important. Parameters affecting target access are examined separately to assess pharmacokinetics [33]. The SwissADME Web tool, which allows the calculation of pharmacokinetic, basic physicochemical, drug-like and related parameters simultaneously for a compound or sometimes for more than one compound, is a program designed to support experts and non-experts in their discovery efforts [33]. A radar map was created using six physicochemical characteristics such as size, polarity, lipophilicity, solubility, elasticity and saturation to evaluate and compare the drug similarity of Verdenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, rutin. The region shown in the diagram corresponded to the optimal range of values for each parameter, while according to the diagrams obtained, vardenafil, papaverine, L-arginine, resveratrol, α-lipoic acid exhibited an optimal range (shown in the pink area) for all criteria. In addition, according to these diagrams, alprostadil exhibited an optimal range for all criteria except Flexibility; citrulline and resveratrol showed optimal range in all criteria except saturation; and rutin, on the other hand, has the optimal range in all criteria except Polarity (Figure 1). The rotatable bond numbers of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid and rutin compounds were determined as 8, 13, 6, 5, 6, 2, 5 and 6, respectively, while the hydrogen bond acceptor values were measured as 8, 5, 5, 4, 4, 3, 2 and 16, respectively. The Csp3 hybridization rates of the same compounds were found to be 0.52, 0.80, 0.25, 0.67, 0.67, 0.67, 0.00 and 0.88, respectively. Vardenafil and alprostadil had high rotatable bonds. High rotatable bond values may increase the conformational diversity of these molecules, but this may adversely affect bioavailability. Rutin had the highest hydrogen bond acceptor value. Because of this feature, rutin can often be associated with better water solubility and membrane permeability. Low Csp3 values generally indicate more planar and aromatic structures, while high Csp3 values indicate more complex and three-dimensional structures [33].

The Total Polar Surface Area (TPSA) values of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid and rutin compounds were 121.28, 94.83, 49.81, 127.72, 118.44, 60.69, 87.90 and 269.43 $Å^2$, respectively. When these values were examined, it was observed that the TPSA values of all compounds except rutin were in accordance with the range of 20-130 $Å^2$, which is accepted as the ideal range. Rutin showed that it has a TPSA value above this range, which may have potential limitations in terms of pharmacokinetic properties.

Vardenafil, alprostadil, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin fully met the Lipinski and Egan rules. However, vardenafil violated the Ghose rules twice (Violed parameters: Molecular weight >480 and molar refractive index >130). Alprostadil violated the Veber rule once (Violed parameters: Number of rotatable bonds >10). L-arginine violated the Ghose rule once (Violed parameters: WLOGP < -0.4) and twice the Muegge rules (Violed parameters: Molecular weight <200 and XLOGP3 < -2). Citrulline violated the Ghose rule once (Violed parameters: WLOGP < -0.4) and twice the Muegge rules. Rutin, on the other hand, did not meet any of the Lipinski, Ghose, Veber, Egan and Muegge rules, which limits the acceptance of rutin as an ideal drug candidate in terms of pharmacokinetic properties (Table 3).

When the bioavailability scores are examined; vardenafil: 0.55, alprostadil: 0.56, papaverine: 0.55, L-arginine: 0.55, citrulline: 0.55, resveratrol: 0.56 α-lipoic acid: 0.55, rutin: 0.17 compounds have been observed to exhibit moderate bioavailability. In terms of lipophilicity values (XLOGP3), vardenafil: 3.80, alprostadil: 3.21, papaverine: 2.95, resveratrol: 3.13 and α-lipoic acid: 1.68 compounds conformed to the ideal lipophilicity range of −0.7 to +5.0. However, L-arginine: -4.19, citrulline: -3.19 and rutin: -0.33 compounds remained below the lower limit of

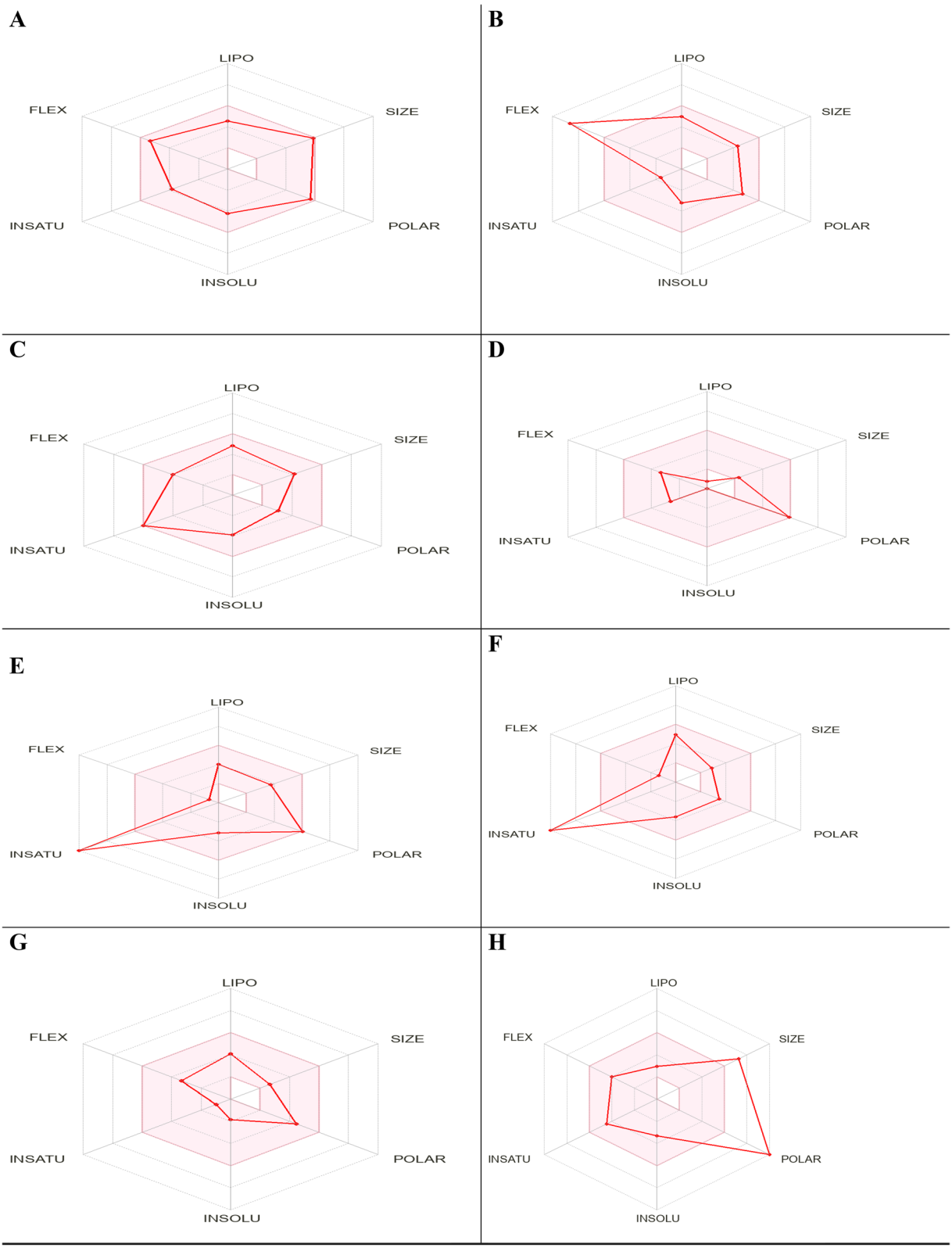


Fig 1 Radar map of Vardenafil (1A), Alprostadil (1B), Papaverine (1C), Arjinine (1D), Citrulline (1E), Resveratrol (1F), α-Lipoic acid (1G), Rutin (1H) molecule from Swissadme database.

Table 3 Physicochemical properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
Formula	$C_{19}H_{19}ClN_2$	$C_{20}H_{34}O_5$	$C_{20}H_{21}NO_4$	$C_6H_{14}N_4O_2$	$C_6H_{13}N_3O_3$	$C_{14}H_{12}O_3$	$C_8H_{14}O_2S_2$	$C_{27}H_{30}O_{16}$
Molecular weight g/mol	488.60	354.48	339.39	174.20	175.19	228.24	206.33	610.52
Num. heavy atoms	34	25	25	12	12	17	12	43
Num. arom. heavy atoms	15	0	16	0	0	12	0	16
Fraction Csp3	0.52	0.80	0.25	0.67	0.67	0.00	0.88	0.44
Num. rotatable bonds	8	13	6	5	6	2	5	6
Num. H-bond acceptors	8	5	5	4	4	3	2	16
Molar Refractivity	138.53	99.96	97.16	44.54	41.53	67.88	55.41	141.38
TPSA (Å^2)	121.28	94.83	49.81	127.72	118.44	60.69	87.90	269.43
Log $P_{o/w}$ (XLOGP3)	3.80	3.21	2.95	-4.19	-3.19	3.13	1.68	-0.33
Log S (ESOL)	-4.22	-3.20	-3.88	2.05	1.48	-3.62	-1.85	-3.30
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	No; 3 violations: MW>500, NorO>10, NHorOH>5
Ghose	No; 2 violations: MW>480, MR>130	Yes	Yes	No; 1 violation: WLOGP<-0.4	No; 1 violation: WLOGP<-0.4	Yes	Yes	No; 4 violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70
Veber	Yes	No; 1 violation: Rotors>10	Yes	Yes	Yes	Yes	Yes	No; 1 violation: TPSA>140
Egan	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No; 1 violation: TPSA>131.6
Muegge	Yes	Yes	Yes	No; 2 violations: MW<200, XLOGP3<-2	No; 2 violations: MW<200, XLOGP3<-2	Yes	Yes	No; 4 violations: MW>600, TPSA>150, H-acc>10, H-don>5
Bioavailability Score	0.55	0.56	0.55	0.55	0.55	0.56	0.55	0.17
Synthetic accessibility	4.26	4.91	2.62	2.47	1.85	2.02	2.87	6.52

this range and exhibited low lipophilicity values. A positive Log P value indicates that the molecule is lipophilic. Compounds such as vardenafil, alprostadil, papaverine, resveratrol, and α-lipoic acid exhibit significant lipophilic properties due to their high Log P values, which allows them to easily pass through cell membranes. However, while high lipophilicity provides advantages in terms of cell membrane permeability, it may cause potential limitations in terms of pharmacokinetic properties such as water solubility. In contrast, rutin and L-arginine are hydrophilic due to their negative Log P values, which may make it difficult for them to pass through cell membranes. Low membrane permeability of hydrophilic compounds is generally associated with low bioavailability. Therefore, solubility and permeability properties of compounds such as rutin and L-arginine need to be optimized in order to be used in pharmaceutical applications (Table 3) [33, 55, 56]. ADMETlab 3.0 is the most comprehensive ADMET online prediction platform known to date, by studying the ADMET properties of molecules in vitro or in vivo in depth [34].

Absorption properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin

When Caco-2 permeability values were examined, Vardenafil: -4.801, alprostadil: -5.273, papaverine: -4.487, L-arginine: -6.029, citrulline: -5.98, resveratrol: -4.942,

α-lipoic acid: -5.017, rutin: -6.307 were found. Optimal Caco-2 permeability indicates the ability of the compound, which should usually be above -5.15 Log units, to pass through intestinal cells and enter the blood. Vardenafil, papaverine, resveratrol, and α-lipoic Acid can cross the intestinal barrier well because their log value is greater than -5.15 and have good absorption potential when taken orally. However, alprostadil, L-arginine, citrulline and rutin have low intestinal permeability because their log values are less than -5.15 and their absorption may be limited when taken orally (Table 4).

While Madin-Darby Canine Kidney (MDCK) cell culture systems are used to assess intestinal permeability, the Parallel Artificial Membrane Permeability Test (PAMPA) is a low-cost and high-throughput method for early drug discovery (Table 4).

When MDCK and PAMPA values were examined, Vardenafil: -4.572; 0.008, alprostadil: -4.74; 0.998, papaverine: -4.48; 0.007, L-arginine: -4.572; 1.0, citrulline: -4.672; 0.995, resveratrol: -4.769; 0.405, α-lipoic acid: -4.844; 0.789, rutin: -4.996; 1.0 has been found. All compounds are in the low permeability category because their MDCK logPeff values are less than 2.0. This indicates that the passive passage of these compounds through kidney cells is limited. Alprostadil, L-arginine, citrulline, α-lipoic acid and rutin show high permeability, while vardenafil, papaverine and resveratrol show low permeability. This reflects the potential for these compounds to cross biological barriers by passive diffusion.

P-glycoprotein (P-gp) is a molecule that is important in the active transport of various substrates with various structures out of cells, resulting in poor intestinal permeability and limited bioavailability after oral administration. P-gp inhibitors, including small molecule drugs, natural ingredients, and pharmaceutically inert excipients, have been used to overcome P-gp outflow and increase the oral absorption and bioavailability of many P-gp substrates [57]. When Pgp-inhibitor and Pgp-substrate values were examined, Vardenafil: 0.985; 1.0, alprostadil: 0.0; 0.016, papaverine: 0.531; 0.753, L-arginine: 0.0; 0.99, citrulline: 0.0; 0.455, resveratrol: 0.001; 0.011, α-lipoic acid: 0.006; 0.005, rutin: 0.0; 0.959 were found, respectively (Table 4). Vardenafil and papaverine, which are likely to be both inhibitors and substrates, have a high potential to interact with Pgp. L-arginine and rutin compounds, on the other hand, are compounds that are likely to be transported by P-gp but are not inhibitory. Alprostadil, citrulline, resveratrol, and α-lipoic acid, on the other hand, are compounds that are unlikely to interact with Pgp.

When HIA values were examined, Vardenafil: 0.009, alprostadil: 0.882, papaverine: 0.008, L-arginine: 0.001, citrulline: 0.004, resveratrol: 0.00, α-lipoic acid: 0.002, rutin: 0.974 were found. Vardenafil, papaverine, L-arginine, citrulline, resveratrol, and α-lipoic acid are compounds that are well absorbed from the intestine, and these compounds may have high bioavailability when used orally. However, since Alprostadil and rutin are compounds with low intestinal absorption, their bioavailability may be low when used orally, so different formulations or routes of administration may be required (Table 4).

When F20%, F30%, F50% values were examined, vardenafil: 0.959; 0.973; 0.997, alprostadil: 0.058; 0.74;

Table 4 Absorbsion properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
Caco-2 Permeability	-4.801	-5.273	-4.487	-6.029	-5.98	-4.942	-5.017	-6.307
MDCK Permeability	-4.572	-4.74	-4.48	-4.572	-4.672	-4.769	-4.844	-4.996
PAMPA	0.008	0.998	0.007	1.0	0.995	0.405	0.789	1.0
Pgp-inhibitor	0.985	0.0	0.531	0.0	0.0	0.001	0.006	0.0
Pgp-Substrate	1.0	0.016	0.753	0.99	0.455	0.011	0.005	0.959
HIA	0.009	0.882	0.008	0.001	0.004	0.0	0.002	0.974
F20%	0.959	0.058	0.03	0.0	0.005	0.329	0.002	0.939
F30%	0.973	0.741	0.006	0.0	0.007	0.24	0.861	1.0
F50%	0.997	0.358	0.166	0.05	0.014	0.982	0.985	1.0

Caco-2: Optimal: higher than -5.15 Log unit

MDCK: low permeability: $< 2 \times 10^{-6}$ cm/s, medium permeability: 2-20 $\times 10^{-6}$ cm/s, high passive permeability: $> 20 \times 10^{-6}$ cm/s

PAMP: The experimental data for Peff was logarithmicall transformed (logPeff). Molecules with log Peff values below 2.0 were classified as low-permeability (Category 0), while those with log Peff values exceeding 2.5 were classified as high-permeability (Category 1).

Pgp-inhibitor and Pgp-Substrate : Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being Pgp-inhibitor and Pgp-substrate

HIA: Human Intestinal Absorption, Category 1: HIA+(HIA < 30%); Category 0: HIA-(HIA >= 30%);The output value is the probability of being HIA+

F20%: Category 1: F 20% + (bioavailability < 20%);Category 0: F 20% - (bioavailability ≥ 20%);The output value is the probability of being F 20%+

F 30% :Category 1: F 30% + (bioavailability < 30%); Category 0: F 30% - (bioavailability ≥ 30%);The output value is the probability of being F 30%+

F50%: Category 1: F 50% + (bioavailability < 50%); Category 0: F 50% - (bioavailability ≥ 50%);The output value is the probability of being F 50%+

0.358, papaverine: 0.03; 0.006; 0.166, L-arginine: 0.0; 0.0; 0.05, citrulline: 0.005; 0.007; 0.014, resveratrol: 0.329; 0.24; 0.982, α-lipoic acid: 0.002; 0.861; 0.985, rutin 0.939; 1.0; 1, respectively. F20%, F30% and F50% can be considered as percentages showing the absorption or bioavailability levels of different compounds [34]. These data are usually used to evaluate the bioavailability of the compound over a given time period (e.g. 20%, 30%, and 50% time-corresponding data). Compounds such as vardenafil, rutin, and α-lipoic acid have the highest bioavailability, so it can be interpreted that the therapeutic efficacy of the compounds is high. However, for L-arginine and citrulline, its absorption is quite low, unlike other molecules, it can be interpreted that their therapeutic effectiveness is low. These findings suggest investigating different formulations or transport mechanisms to increase oral bioavailability of L-arginine and citrulline (Table 4).

Distribution properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin

PPB affects the distribution and biological activity of the drug, while VDss measures the degree of concentration of a drug in body tissue compared to its concentration in the blood. [58, 59]. When PPB and VDss values were examined, Vardenafil: 95.525; 0.345, alprostadil: 88.455; 0.285, papaverine: 89.065; 0.261, L-arginine: 10.637; 10.637, citrulline: 8.74; -0.522, resveratrol: 88.141; 0.16, α-lipoic acid: 85.37; -0.961, rutin: 81.741; -0.151. Vardenafil, alprostadil, and papaverine have high PPB and low VDss values. This suggests that they stay longer in the blood and bind tightly to proteins. The therapeutic effects of such compounds may be limited and dose adjustments should be made with caution. L-arginine and Citrulline have low PPB and high VDss (for L-arginine) values. This suggests that their free form is high and they can be widely distributed in tissues. Compounds with negative VDss values could not be commented on [34] (Table 5). When BBB and Fu values were examined, they were found to be Vardenafil: 0.001; 3.912, alprostadil: 0.001; 8.119, papaverine: 0.042; 8.165, L-arginine: 0.021; 86.634, citrulline: 0.218; 93.095, resveratrol: 0.007; 14.409, α-lipoic acid: 0.946; 11.107, rutin: 0.0; 19.664, respectively.Since all compounds except α-lipoic Acid are unlikely to cross the BBB, their direct effects on the brain are limited. L-arginine and citrulline have very high free fractions in plasma, while vardenafil, alprostadil, papaverine, resveratrol and α-lipoic Acid have medium free fractions. L-arginine and citrulline have high bioavailability and high potency due to their high free fractions. Rutin has a high free fraction, but its effect on the brain is limited because it does not cross the BBB (Table 5). Organic anion transporter polypeptides OATP1B1 and OATP1B3 membrane proteins that facilitate the uptake of various xenobiotics, including many cancer drugs, into the liver before metabolism and enable subsequent conjugation and excretion of bile; This is an important step in the elimination of drugs from the human body [60, 61]. When OATP1B1 and OATP1B3 inhibitor values were examined, respectively; vardenafil: 0.012; 0.019, alprostadil: 0.009; 0.981, papaverine: 0.995; 0.999, L-arginine:

Table 5 Distribution properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
PPB	95.525	88.455	89.065	10.637	8.74	88.141	85.37	81.741
VDss	0.345	0.285	0.261	10.637	-0.522	0.16	-0.961	-0.151
BBB	0.001	0.001	0.042	0.021	0.218	0.007	0.946	0.0
Fu	3.912	8.119	8.165	86.634	93.095	14.409	11.107	19.664
OATP1B1 inhibitor	0.012	0.009	0.995	0.926	0.976	0.998	0.916	0.001
OATP1B3 inhibitor	0.019	0.981	0.999	0.999	0.999	0.999	0.968	0.941
BCRP inhibitor	0.038	0.012	0.0	0.0	0.995	0.381	0.0	0.793
MRP1 inhibitor	0.999	0.85	0.412	0.423	0.838	0.452	0.676	0.83

PPB: Plasma Protein Binding, Optimal < 90%, Drugs with high protein-bound may have a low therapeutic index

Volume Distribution: Optimal: 0.04-20L/kg

Blood-Brain Barrier Penetration: Category 1: BBB+; Category 0: BBB-; The output value is the probability of being BBB+

Fu: Low: <5%; Middle: 5~20%; High: > 20% The fraction unbound in plasms

OATP1B1, OATP1B3 inhibitor, BCRPinhibitor, and MRP1 inhibitor: Category 0: Non-inhibitor; Category 1: inhibitor.The output value is the probability of being inhibitor, within the range of 0 to 1

0.926; 0.999, citrulline: 0.976; 0.999, resveratrol: 0.998; 0.999, α-lipoic acid: 0.916; 0.968, rutin: 0.001; 0.941 found (Table 5). Papaverine, L-arginine, citrulline, resveratrol, and α-lipoic acid exert strong inhibitory action on both OATP1B1 and OATP1B3 transporters. These compounds can lead to significant drug interactions through carrier proteins in the liver. While alprostadil does not produce any inhibitory effect on OATP1B1, it exerts a strong inhibitory effect on OATP1B3. Similarly, rutin does not have an inhibitory effect on OATP1B1, but it has a strong inhibitory effect on OATP1B3. When the inhibitory values of BCRP inhibitor and MRP1 inhibitor were examined, they were found to be; vardenafil: 0.038: 0.999, alprostadil: 0.012; 0.85, papaverine: 0.0; 0.412, L-arginine: 0.0; 0.423, citrulline: 0.995; 0.838, resveratrol: 0.381; 0.452, α-lipoic acid: 0.0; 0.676, rutin: 0.793 0.83, respectively.

Metabolism properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin

When the inhibition values of Vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, rutin molecules of CYP 3A4, 2D6, 2C19, 2C9 and 1A2 enzymes were examined, respectively: CYP1A2 inhibitors: 0.0; 0.0; 0.012; 0.0; 0.0; 1.0; 0.0; 0.0; 0.0, CYP2C19 inhibitors: 0.001; 0.0; 0.126; 0.0; 0.0; 0.001; 0.0; 0.0, CYP2C9 inhibitor 0.0; 0.13; 0.952; 0.034; 0.0; 0.003; 0.02; 0.0, CYP2D6 inhibitor: 0.0; 0.012; 0.002; 1; 0.0; 0.0; 0.01; 0.0; 0.0, CYP3A4 inhibitor: 0.806; 0.0; 0.999; 0.01; 0.937; 1; 0.0; 0.01, were found. (Table 6). Cytochrome p450 is a superfamily of membrane-bound hemoprotein isozymes with different classifications, these enzymes predominantly occupy the liver, intestines and kidneys, with the highest concentrations in the liver. Of the total 57 isozymes discovered to date, identified with the function of carrying out oxidative metabolism of xenobiotics and endogenous compounds, 6 are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 among the six isozymes, and these enzymes are responsible for 90% of drug metabolism [62, 63]. Enzyme inhibition occurs when two co-administered drugs share the same biotransformation mechanism and compete for metabolism at the same enzyme receptor site [64]. The findings that clearly reveal the inhibition potential of each molecule on different CYP enzymes are as follows: papaverine and alprostadil on CYP2C9, resveratrol on CYP1A2, papaverine on CYP2C19, alprostadil on CYP2D6, α-lipoic acid, papaverine and resveratrol on CYP3A4 show a significant inhibition value. Many molecules do not appear to have an effect on CYP1A2 (Vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin, alprostadil, L-arginine, citrulline, α- lipoic acid, rutin) and CYP2C19 (alprostadil, L-arginine, citrulline, α- lipoic acid, rutin), CYP2D6 (Vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin, citrulline, α- lipoic acid, rutin). The fact that some molecules, such as papaverine and alprostadil, exert a strong inhibitory effect on certain CYP enzymes can be interpreted as the need to be careful in the interactions of these drugs with other drugs (Table 6).

Excretion properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin

Clearance (CL), volume of distribution and elimination half-life, which are used to estimate the first dose in humans, are the most important pharmacokinetic parameter that is most frequently extrapolated [65]. T1/2 is defined as the time it takes for the plasma concentration of a drug to decrease by 50% [65]. When CLplasma and T1/2 values were examined, they were found to be vardenafil: 13.387; 0.824, alprostadil: 3.428; 1.082, papaverine: 11.327; 1.962, L-arginine: 2.314; 2.115, citrulline: 6.69; 1.296, resveratrol: 9.967; 1.536, α-lipoic acid: 7.284; 1.65, rutin: 2.05; 3.878, respectively. While not all compounds had high clearance rates, compounds such as vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin, papaverine, citrulline, resveratrol, and α-lipoic acid had moderate clearance rates, indicating that these compounds have a reasonable elimination time in the body. Additionally, alprostadil, L-arginine, and rutin had lower clearance rates, meaning that these compounds tend to stay in the body longer (Table 7).

Table 6 Metabolism properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
CYP1A2 inhibitor	0.0	0.0	0.012	0.0	0.0	1.0	0.0	0.0
CYP2C19 inhibitor	0.001	0.0	0.126	0.0	0.0	0.001	0.0	0.0
CYP2C9 inhibitor	0.0	0.13	0.952	0.034	0.0	0.003	0.02	0.0
CYP2D6 inhibitor	0.0	0.012	0.002	1	0.0	0.001	0.0	0.0
CYP3A4 inhibitor	0.806	0.0	0.999	0.01	0.937	1	0.0	0.001

CYPs: Category 1: Inhibitor; Category 0: Non-inhibitor; The output value is the probability of being inhibitor.

Toxicity properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin

Human ether-a-go-go-related (hERG) has been used in silico approaches to predict cardiotoxicity, while drug-induced liver injury (DILI) represents a variety of responses after exposure to any produced or naturally occurring chemical compound [66, 67]. When hERG Blockers and DILI values were examined, they were found to be vardenafil: 0.763; 0.999, alprostadil: 0.019; 0.005, papaverine: 0.281; 0.579, L-arginine: 0.196; 0.013, citrulline: 0.055; 0.062, resveratrol: 0.487; 0.011, α-lipoic acid: 0.026; 0.994, rutin: 0.029; 0.812, respectively. (Table 8). Vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α- lipoic acid, and rutin may pose serious safety concerns with its therapeutic use as it carries a high risk of both hERG blockade and DILI. Alprostadil, L-arginine, and Citrulline carry a low risk of both hERG blockade and DILI. In other words, it can be interpreted that both the cardiovascular side effects of these compounds are lower and the effects on the liver are generally less worrisome. Papaverine carries moderate risks of both hERG blockade and DILI. Papaverine should be used with caution, especially in patients with preexisting cardiac or hepatic disease.

Due to the high cost and inconvenience in experimental testing, it is more practical to resort to robust in silico assays to estimate Mutagenicity, which is one of the important points of toxicity [68].Acute rat oral toxicity plays an important role in hazard identification and drug risk management by measuring with a 50% lethal dose (LD50), but alternative methods have been developed to replace these costly and time-consuming studies [69]. When the AMES Mutagenicity and Rat Oral Acute Toxicity values were examined, Vardenafil: 0.45; 0.746, alprostadil: 0.147; 0.248, papaverine: 0.475; 0.387, L-arginine: 0.488; 0.507, citrulline: 0.552; 0.227, resveratrol: 0.814; 0.316, α-lipoic acid: 0.038; 0.036, rutin: 0.753; 0.415 were found to be one of the safest compounds due to its low AMES mutagenicity and rat oral acute toxicity values. Similarly, Alprostadil is also a compound that is considered safe with low mutagenicity and toxicity risk. On the other hand, Resveratrol and Citrulline can be classified as Ames positive due to their high AMES mutagenicity potential; however, their low overall toxicity risk makes these compounds relatively safe to use. In contrast, Vardenafil and L-arginine are compounds that should be used with caution due to their high toxicity potential. The safety profiles of these compounds have been evaluated based on AMES and acute toxicity data. However, more extensive toxicological studies are required for definitive conclusions (Table 8). The FDA-recommended maximum daily dose (FDAMDD) provides an estimate of the toxic dose threshold of chemicals in humans, while carcinogenicity testing of pharmaceutical drugs aims to assess the risk of use in humans by determining the potential for tumor-forming in 2-year studies in rodents or 6-month studies in transgenic mice [70]. When FDAMDD and Carcinogenicity values were examined, vardenafil: 0.864; 0.898, alprostadil: 0.802; 0.283, papaverine: 0.447; 0.682, L-arginine: 0.184; 0.194, citrulline: 0.074; 0.225, resveratrol: 0.688; 0.263, α-lipoic acid: 0.065; 0.24, rutin: 0.636; 0.111 were found, respectively (Table 8). Vardenafil and alprostadil are close to the maximum recommended daily dose, indicating that these drugs require careful dose management, while citrulline and α-lipoic acid can be considered safe with a very low probability of FDAMDD. Vardenafil is the most likely to be carcinogenicity, and long-term use of this drug may carry a risk of cancer. Therefore, vardenafil is a drug that requires careful use with high FDAMDD and high probability of carcinogenicity. It can be interpreted as carrying potential risks in terms of both dose and long-term use. However, alprostadil carries a low risk of carcinogenicity, although there is a high probability of FDAMDD, and this may support its safe use with proper dose management. Both Arginine and citrulline are noted for their low probability of FDAMDD and low carcinogenicity.

Hematoxycity is serious toxicity in drug discovery [71]. Immunotoxicity, the negative effect of xenobiotics on the immune system, is evaluated based on cytotoxicity data from the B cell line RPMI-8226 in the public database of the US National Cancer Institute (NCI), with a model in which compounds with a growth inhibition (GI50) value of less than 10 μM are identified as toxic [72]. When hematotoxicity and immunotoxicity data of RPMI-8226 were evaluated, vardenafil: 0.598;

Table 7 Excretion properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
CLplasma	13.387	3.428	11.327	2.314	6.69	9.967	7.284	2.05
T1/2	0.824	1.082	1.962	2.115	1.296	1.536	1.65	3.878

CLplasma: The unit of predicted CLplasma penetration is ml/min/kg. >15 ml/min/kg: high clearance; 5-15 ml/min/kg: moderate clearance; < 5 ml/min/kg: low clearance

T1/2: The unit of predicted T1/2 is hours. ultra-short half-life drugs: 1/2 < 1 hour; short half-life drugs: T1/2 between 1-4 hours; intermediate short half-life drugs: T1/2 between 4-8 hours; long half-life drugs: T1/2 > 8 hours

Table 8 Toxicity properties of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
hERG Blockers	0.763	0.019	0.281	0.196	0.055	0.487	0.026	0.029
DILI	0.999	0.005	0.579	0.013	0.062	0.011	0.994	0.812
AMES Mutagenicity	0.45	0.147	0.475	0.488	0.552	0.814	0.038	0.753
Rat Oral Acute Toxicity	0.746	0.248	0.387	0.507	0.227	0.316	0.036	0.415
FDAMDD	0.864	0.802	0.447	0.184	0.074	0.688	0.065	0.636
Carcinogenicity	0.898	0.283	0.682	0.194	0.225	0.263	0.24	0.111
Hematotoxicity	0.598	0.01	0.428	0.126	0.339	0.014	0.192	0.005
RPMI-8226 Immunitoxicity	0.067	0.021	0.115	0.018	0.026	0.091	0.001	0.034
A549 Cytotoxicity	0.033	0.0	0.009	0.477	0.012	0.78	0.001	0.424
Hek293 Cytotoxicity	0.571	0.109	0.036	0.473	0.023	0.809	0.025	0.865
Drug-induced Neurotoxicity	0.967	0.015	0.184	0.204	0.529	0.908	0.036	0.024

hERG Blockers: Molecules with IC50 ≤10mM or ≥50% inhibition at 10 mM were classified as hERG+ (Category 1), while molecules with IC50 >10mM or < 50% inhibition at 10mM were classified as hERG - (Category 0). The output value is the probability of being hERG+, within the range of 0 to 1

Drug Induced Liver Injury: Category 1: drugs with a high risk of DILI; Category 0: drugs with no risk of DILI. The output value is the probability of being toxic

AMES Toxicity: Category 1: Ames positive(+); Category 0: Ames negative(-); The output value is the probability of being toxic

Rat Oral Acute Toxicity. Category 0: low-toxicity, > 500 mg/kg; Category 1: high-toxicity; < 500 mg/kg. The output value is the probability of being toxic, within the range of 0 to 1

FDA Maximum (Recommended) Daily Dose.:Category 1: FDAMDD (+); Category 0: FDAMDD (-); The output value is the probability of being positive

Carcinogenicity: Category 1: carcinogens; Category 0: non-carcinogens; The output value is the probability of being toxic

Hematotoxicity: Category 0: non-hematotoxicity (-); Category 1: hematotoxicity (+). The output value is the probability of being hematotoxicity (+), within the range of 0 to 1

RPMI-8226 Immunitoxicity: Category 0: non-cytotoxicity (-); Category 1: cytotoxicity (+). The output value is the probability of being ototoxicity (+), within the range of 0 to 1

A549 Cytotoxicity and Hek293 Cytotoxicity: Category 0: non-cytotoxicity (-); Category 1: cytotoxicity (+). The output value is the probability of being cytotoxicity (+), within the range of 0 to 1

Drug-induced Neurotoxicity: Category 0: non-neurotoxic (-); Category 1: neurotoxic (+). The output value is the probability of being neurotoxic (+), within the range of 0 to 1

0.067, alprostadil: 0.01; 0.021, papaverine: 0.428; 0.115, L-arginine: 0.126; 0.018, citrulline: 0.339; 0.026, resveratrol: 0.014; 0.091, α-lipoic acid: 0.192; 0.001, rutin: 0.005; 0.034 were found, respectively (Table 8). Compounds such as alprostadil, L-arginine, resveratrol, α-lipoic acid and rutin appear to be safer in terms of both hematotoxicity and immunotoxicity, while vardenafil and papaverine carry a higher risk of hematotoxicity [71, 72]. When A549 Cytotoxicity, Hek293 Cytotoxicity, Drug-induced Neurotoxicity values were examined vardenafil, : 0.033; 0.571; 0.967, alprostadil: 0.0; 0.109; 0.015, papaverine: 0.009; 0.036; 0.184, L-arginine: 0.477; 0.473; 0.204, citrulline: 0.012; 0.023; 0.529, resveratrol: 0.78; 0.809; 0.908, α-lipoic acid: 0.001; 0.025; 0.036, rutin: 0.424; 0.865; 0.024 were found, respectively. While Alprostadil and α-lipoic Acid are among the safest compounds in terms of both cytotoxicity and neurotoxicity, Resveratrol is the compound with the highest risk in both respects. Vardenafil poses a serious risk, especially in terms of neurotoxicity. Rutin is a safe compound in terms of neurotoxicity, although it carries a high risk of toxic effects against Hek293 cells. (Table 8).

W hen the biological target of molecules is examined, for *Sars-Cov* (alprostadil, resveratrol, α-lipoic acid), for *E.coli* (alprostadil), *C.albicans* (alprostadil, papaverine, citrulline), *Leishmania infantum - Promastigota* (vardenafil, resveratrol, α-lipoic acid, rutin), *Dengue larvicida* (vardenafil, rutin), *Hepatitis C - Type1* (vardenafil), *Leishmania amazonensis - Promastigota* (vardenafil, papaverine, arginine, rutin*), Leishmania braziliensis* (alprostadil, papaverine), *Leishmania major* (papaverine, arginine, rutin), *Epimastigote Chagas* (vardenafil, papaverine) Alprostadil may be effective against a variety of pathogens including *SARS-CoV, E. coli, C. albicans,* and *Leishmania braziliensis* and has antibacterial, antifungal,

and antiparasitic potential. Resveratrol has antiviral and antiparasitic properties against *SARS-CoV and Leishmania infantum*; α-lipoic acid may also show antiviral and antiparasitic effects against *SARS-CoV* and *Leishmania infantum*. Vardenafil is effective against *Leishmania infantum*, Hepatitis C, *Leishmania amazonensis, epimastigote chagas, and dengue larvicida,* and has the potential to be used in antiparasitic, antiviral, and vector control. Papaverine has antifungal and antiparasitic properties against *C. albicans, Leishmania amazonensis, Leishmania braziliensis, Leishmania major*, and *Epimastigote chagas*. Arginine provides antiparasitic activity against *Leishmania amazonensis and Leishmania major*. Finally, rutin is a compound with potential for antiparasitic and vector control against *Leishmania infantum, dengue larvicida*, *Leishmania amazonensis and Leishmania major* [35] (Table 9).

Vardenafil (Phosphodiesterase I inhibitor, Phosphodiesterase 1B inhibitor, Erectile dysfunction treatment, Phosphodiesterase inhibitor, Phosphodiesterase 5A inhibitor), alprostadil (Prostaglandin-E2 9-reductase inhibitor, Antisecretoric, GST A substrate, Mucomembranous protector, Vasodilator, peripheral), papaverine (Cyclic AMP) phosphodiesterase inhibitor, Aspulvinone dimethylallyltransferase inhibitor, Nucleotide metabolism regulator, Gluconate 2-dehydrogenase (acceptor) inhibitor, Preneoplastic conditions treatment), Arginine (Arginine 2-monooxygenase inhibitor, Dimethylargininase inhibitor, Omptin inhibitor, Mucositis treatment, Glutamine-phenylpyruvate transaminase inhibitor) citrulline (Protein-disulfide reductase (glutathione) inhibitor, Arginine 2-monooxygenase inhibitor, Glutamine-tRNA ligase inhibitor, Amino-acid N-acetyltransferase inhibitor, Omptin inhibitor), resveratrol (Membrane integrity agonist, Aspulvinone dimethylallyltransferase inhibitor, Feruloyl esterase inhibitor, APOA1 expression enhancer, JAK2 expression inhibitor 0(S)-2 -hydroxy-acid oxidase inhibitor), α-lipoic acid ((S)-2-hydroxy-acid oxidase inhibitor, Reductant, Dopamine precursors, Antiischemic, cerebral, Lipotropic), rutin (Hemostatic, Membrane permeability inhibitor, Free radical scavenger, Cardioprotectant, Lipid peroxidase inhibitor) shows biological activity [36] (Table 10).

Molecular docking methods in modern drug design evaluate the interactions of macromolecular targets by investigating ligand conformations and ligand-receptor binding free energy. Understanding the advantages and limitations of different nesting algorithms is important for the development of effective strategies [73].

The binding energies (Gibbs free energies, ΔG) of phosphodiesterase 5A and compounds such as vardenafil, alprostadil, papaverine, Arginine, citrulline, resveratrol, α-lipoic acid and rutin as a result of ligand-protein interaction, H bond and hydrophobic interactions are shown in Table 11. The negative value of the ΔG results indicates that the reaction occurred spontaneously. The tested compounds showed a binding energy in the range of – 8.6 to -5.2 kcal/mol. The H bond Interaction, Hydrofobic Interaction, Electrostatic Interation and binding energies of phosphodiesterase 5A with the compounds are as follows: rutin (-8.6 kcal/mol, H bond Interaction:ASP568, ALA611, ASN614, TRP615, ARG616, Hydrophobic Interaction: ALA611, Electrostatic Interation: No), papaverine (-8.5 kcal/mol, H bond Interaction: ASP563, ALA611, ASN614, HIS617, ASN620, Hydrophobic Interaction: LEU781, VAL782, ILE778, Electrostatic Interation: No), resveratrol (-8.2 kcal/mol, H bond Interaction: LEU785, Hydrophobic Interaction: HIS617, HIS678, ALA767, ILE778, Electrostatic Interation: CYS677) , vardenafil (-8.0 kcal/mol, H bond Interaction: ASP568, ALA611, ASN614, TRP615, ARG616 , Hydrophobic Interaction: ILE778 , Electrostatic Interation: No), alprostadil (-5.8 kcal/mol H bond Interaction: ALA611, TYR612 , Hydrophobic Interaction: PHE564, ARG616 , Electrostatic Interation: No), α-lipoic acid (-5.7 kcal/mol, H bond Interaction: HIS617, ASN620, THR621, CYS763, Hydrophobic Interaction: ILE778 , Electrostatic Interation: No), citrulline (-5.4 kcal/mol, H bond Interaction: ALA611, TYR612, ASN614, CYS763, ASP764 , Hydrophobic Interaction: No, Electrostatic Interaction: No), Arginine (-5.2 kcal/mol, H bond Interaction: HIS617, ASN620, GLU785, Hydrophobic Interaction: ARG616, CYS677, HIS678, LEU781, VAL782, ALA767, Electrostatic Interaction: ASP56) (Figure 2, Figure 3 and Table 11). In the current study, vardenafil, alprostadil, and, papaverine were selected as targets for phosphodiesterase type 5 (PDE5), which was cited as a target for ED, and Arginine, citrulline, resveratrol, α-lipoic acid, and rutin control as potential targets. Molecular docking showed a good binding effect for the control compounds vardenafil and papaverine PDE5. The resveratrol and rutin compounds we compared with the control compounds also showed promising binding properties (Figure 2, Figure 3 and Table 11).

The binding energies (Gibbs free energies, ΔG) formed as a result of ligand-protein interaction, H bond and hydrophobic interactions of Nitric Oxide Synthase (NOS) and compounds such as vardenafil, alprostadil, papaverine, Arginine, citrulline, resveratrol, α-lipoic acid and rutin are shown in Table 12. The negative value of the ΔG results indicates that the reaction occurred spontaneously. The tested compounds showed a binding energy in the range of – 10.5 to -5.2 kcal/mol. The H bond Interaction, Hydrophobic Interaction, Electrostatic Interaction and binding energies of NOS with compounds

Table 9 Biological target of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

Target	Vardenafil	Alprostadil	Papaverine	Arginine	Citrulline	Resveratrol	α-Lipoic acid	Rutin
Sars-Cov	K:Inactive L:0.2 M:0.8 N:0.8 Y:unreliable	K:Active* **L:0.8** M:0.8 N:0.2 Y.reliable	K:Inactive L: 0.8 M: 0.2 N: 0.8 Y: reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y.reliable	K: Inactive L: 1.0 M: 0.0 N: 1.0 Y: reliable	K:Active* **L:0.6** M:0.6 N:0.4 Y:reliable	K:Active* **L:0.6** M:0.6 N:0.4 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable
E.coli	K:Inactive L:0.2 M:0.8 N:0.8 Y:unreliable	K:Active* **L:0.6** M:0.6 N:0.4 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y:reliable	K: Inactive L: 1.0 M: 0.0 N: 1.0 Y: reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:0.6 M:0.4 N:0.6 Y:reliable
C.albicans	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Active** **L:1.0** N.0.0 Y:reliable	K:Active** L:1.0 M:0.0 N.0.0 Y:reliable	K:Inactive L:0.6 M:0.4 N:0.6 Y:reliable	K:Active* **L:0.8** M:0.8 N:0.2 Y.reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Active **L:1.0** M:1.0 N.0.0 Y.reliable
Dengue larvicida	K:Active** **L:1.0** N.0.0 Y:reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y.reliable	K:Inactive L: 0.8 M: 0.2 N: 0.8 Y: reliable	K:Inactive L: 0.8 M: 0.2 N: 0.8 Y: reliable	K:Inactive L: 0.8 M: 0.2 N: 0.8 Y: reliable	K.Active** L:1.0 M:1.0 N:0.0 Y:reliable
Salmonella	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:unreliabe	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:0.6 M:0.4 N:0.6 Y:reliable	K:Inactive L:0.6 M:0.4 N:0.6 Y:reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y.reliable
Hepatite C - Type1	K:Active* **L:0.8** M:0.8 N:0.2 Y.reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N.1.0 Y.reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y.reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y.reliable
Leishmania infantum - Promastigota	K:Active** **L:1.0** N.0.0 Y:reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N:1.0 Y:reliable	K:Active* **L:0.6** M.0.6 N.0.4 Y.reliable	K:Active* **L:0.6** M.0.6 N.0.4 Y.reliable	K:Inactive L:1.0 M:0.0 N.1.0 Y:reliable
Leishmania amazonensis - Promastigota	K:Active* **L:0.6** M:0.6 N:0.4 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Active* **L:0.8** M:0.8 N:0.2 Y.reliable	K:Active* **L:0.6** M:0.6 N:0.4 Y:reliable	K:Inactive L:0.6 M.0.4 N.0.6 Y:reliable	K.Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K.Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Active* **L:0.6** M:0.6 N:0.4 Y:reliable
Drosophila melanogaster	K:Inactive L:1.0 M:0.0 N:1.0 Y: reliable	K:Inactive L:0.6 M:0.4 N.0.6 Y:reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y.reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable
Leishmania *braziliensis*	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Active** **L:1.0** M:1.0 N:0.0 Y:reliable	K:Active** L:1.0 M:0.0 N.0.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y.reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y.reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y:reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y:reliable	K:Inactive L:0.6 M:0.4 N:0.6 Y:reliable
Leishmania major	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:0.8 M:0.2 N:0.8 Y.reliable	K:Active* **L:0.8** M:0.8 N:0.2 Y.reliable	K:Active ***L:0.8** M:0.8 N:0.2 Y.reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N.1.0 Y:reliable	K:Inactive L.1.0 M:0.0 N.1.0 Y.reliable	K:Active* **L:0.8** M:0.8 N:0.2 Y:reliable
Epimastigote Chagas	K:Active* **L:0.8** M:0.8 N:0.2 Y.reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Active** L:1.0 M:0.0 N.0.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N:1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N.1.0 Y:reliable	K:Inactive L:1.0 M.0.0 N.1.0 Y:reliable	K:Inactive L:1.0 M:0.0 N.1. Y:reliable

Table 10 Biological activity of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin

Drugs	Pa	Pi	Activity
Vardenafil	**0,985**	0,001	Phosphodiesterase I inhibitor
Vardenafil	0,967	0,000	Phosphodiesterase 1B inhibitor
Vardenafil	0,800	0,003	Erectile dysfunction treatment
Vardenafil	0,754	0,004	Phosphodiesterase inhibitor
Vardenafil	0,742	0,002	Phosphodiesterase 5A inhibitor
Alprostadil	**0,979**	0,001	Prostaglandin-E2 9-reductase inhibitor
Alprostadil	0,977	0,002	Antisecretoric
Alprostadil	0,975	0,001	GST A substrate
Alprostadil	0,972	0,002	Mucomembranous protector
Alprostadil	0,971	0,002	Vasodilator, peripheral
Papaverine	0,772	0,004	Cyclic AMP phosphodiesterase inhibitor
Papaverine	0,788	0,037	Aspulvinone dimethylallyltransferase inhibitor
Papaverine	0,716	0,010	Nucleotide metabolism regulator
Papaverine	0,735	0,038	Gluconate 2-dehydrogenase (acceptor) inhibitor
Papaverine	0,704	0,008	Preneoplastic conditions treatment
Arginine.	0,953	0,002	Arginine 2-monooxygenase inhibitor
Arginine	0,943	0,002	Dimethylargininase inhibitor
Arginine	0,935	0,002	Omptin inhibitor
Arginine	0,929	0,005	Mucositis treatment
Arginine	0,919	0,002	Glutamine-phenylpyruvate transaminase inhibitor
Citrulline	0,949	0,002	Protein-disulfide reductase (glutathione) inhibitor
Citrulline	0,942	0,002	Arginine 2-monooxygenase inhibitor
Citrulline	0,926	0,001	Glutamine-tRNA ligase inhibitor
Citrulline	0,921	0,001	Amino-acid N-acetyltransferase inhibitor
Citrulline	0,922	0,002	Omptin inhibitor
Resveratrol	0,941	0,004	Membrane integrity agonist
Resveratrol	0,930	0,004	Aspulvinone dimethylallyltransferase inhibitor
Resveratrol	0,928	0,003	Feruloyl esterase inhibitor
Resveratrol	0,923	0,002	APOA1 expression enhancer
Resveratrol	0,912	0,003	JAK2 expression inhibitor
α-Lipoic acid	0,977	0,001	(S)-2-hydroxy-acid oxidase inhibitor
α-Lipoic acid	0,961	0,002	Reductant
α-Lipoic acid	0,932	0,001	Dopamine precursors
α-Lipoic acid	0,927	0,005	Antiischemic, cerebral
α-Lipoic acid	0,911	0,001	Lipotropic
Rutin	0,993	0,001	Hemostatic
Rutin	0,990	0,000	Membrane permeability inhibitor
Rutin	0,988	0,001	Free radical scavenger
Rutin	0,988	0,001	Cardioprotectant
Rutin	0,987	0,001	Lipid peroxidase inhibitor

are as follows, respectively. rutin (-10.5 kcal/mol, H bond Interaction: TRP70, SER73, ARG75, GLY242, TRP243, Hydrophobic Interaction: GLY78, MET226, CAL305, Electrostatic Interation: CYS76, GLU248), vardenafil (-9.5 kcal/mol, H bond Interaction: TRP70, SER73, ARG75, TRP2 43, Hydrophobic Interaction: HIS134, ILE223, MET226, VAL305, ALA310, TYR362, Electrostatic Interation: CYS76), resveratrol (-9.1 kcal/mol, H bond Interaction: No, Hydrophobic Interaction: TRP70, ARG75, PHE240, Electrostatic Interation: CYS76), papaverine (-8. 7 kcal/mol, H bond Interaction: ARG75, GLY78 , Hydrophobic Interaction: TRP70, ILE77, MET226, PHE240, MET245, VAL305, PHE360 Electrostatic Interation: CYS76), alprostadil (-7.8 kcal/

Table 11 Molecular docking targets of vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin in the phosphodiesterase 5A structure

Compounds	ΔG (kcal/mol)	H bond Interaction	Hydrofobic Interaction	Electrostatic Interaction
Vardenafil	-8.0	ASP568, ALA611, ASN614, TRP615, ARG616	ILE778	-
Alprostadil	-5.8	ALA611, TYR612	PHE564, ARG616	-
Papaverine	-8.5	ASP563, ALA611, ASN614, HIS617, ASN620	LEU781, VAL782, ILE778	-
Arginine	-5.2	HIS617, ASN620, GLU785	ARG616, CYS677, HIS678, LEU781, VAL782, ALA767	ASP563
Citrulline	-5.4	ALA611, TYR612, ASN614, CYS763, ASP764	-	-
Resveratrol	-8.2	LEU785	HIS617, HIS678, ALA767, ILE778	CYS677
α-Lipoic acid	-5.7	HIS617, ASN620, THR621, CYS763	ILE778	-
Rutin	-8.6	ASP568, TRP615, ARG616, GLU785	ALA611	-

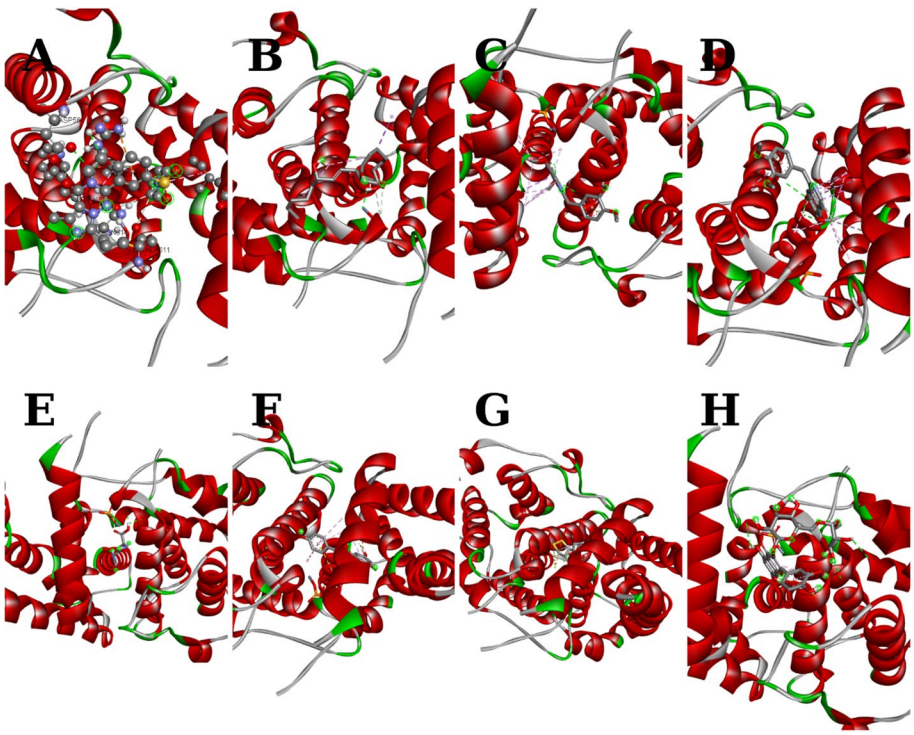


Fig 2 Best conformational regions obtained for Phosphodiesterase 5A in molecular docking analysis (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin

mol; H bond Interaction: No , Hydrophobic Interaction: TRP70, CYS76, PHE240, T RP243, PHE360 , Electrostatic Interation: No), α-lipoic acid (-5.4 kcal/mol, H bond Interaction: ASN241 , Hydrophobic Interaction: TRP70, CYS76, PRO241, PHE240 , Electrostatic Interaction: No), Arginine (-5.2 kcal/mol, H bond Interaction: HIS72, SER73, ASN74, CYS76, MET226, MET355, ASN359, PHE360, PHE361, Hydrophobic Interaction: TYR362, Electrostatic Interation: ARG75), citrulline (-5.2 kcal/mol, H bond Interaction: HIS72, ARG75, ASP331, ASN353, PHE360, Hydrophobic Interaction: No, Electrostatic Interation: No). The best binding properties were shown by rutin, vardenafil, resveratrol, papaverine, alprostadil. In light of these findings, rutin, papaverine and resveratrol stand out as interesting candidates for potential therapeutic uses due to their effects on NOS (Table 12, Figure 4 and Figure 5)

The binding energies (Gibbs free energies, ΔG) of Arginase II with compounds such as vardenafil, alprostadil, papaverine, Arginine, citrulline, resveratrol, α-lipoic

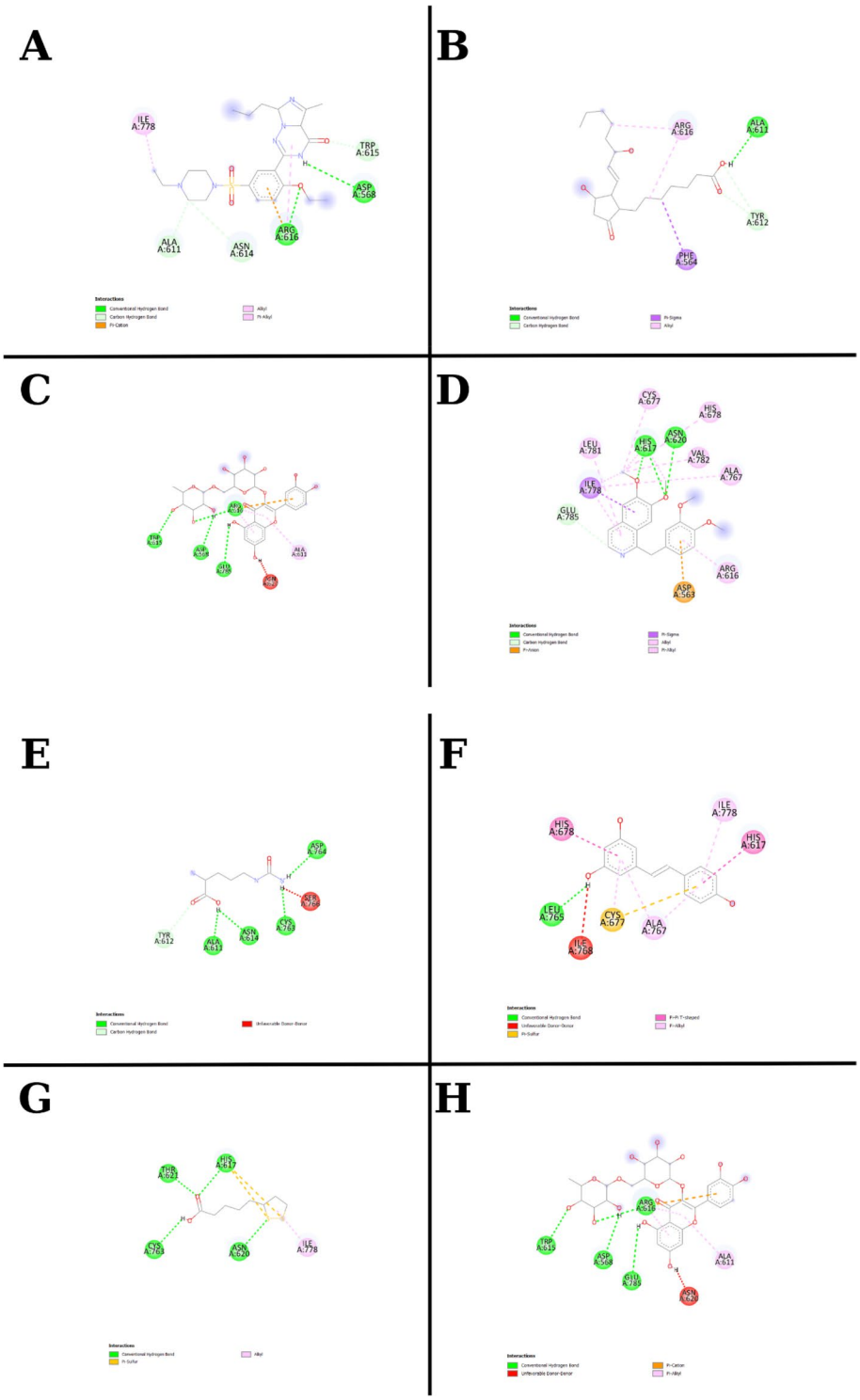


Fig 3 Ligand binding structures obtained for Phosphodiesterase 5A in molecular docking analysis: (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

Table 12 Molecular docking targets of the vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin in the NOS structure

Compounds	ΔG (kcal/mol)	H bond Interaction	Hydrofobic Interaction	Electrostatic Interaction
Vardenafil	-9.5	TRP70, SER73, ARG75, TRP243	HIS134, ILE223, MET226, VAL305, ALA310, TYR362	CYS76
Alprostadil	-7.8	-	TRP70, CYS76, PHE240, TRP243, PHE360	-
Papaverine	-8.7	ARG75, GLY78	TRP70, ILE77, MET226, PHE240, MET245, VAL305, PHE360	CYS76
Arginine	-5.2	HIS72, SER73, ASN74, CYS76, MET226, MET355, ASN359, PHE360, PHE361	TYR362	ARG75
Citrulline	-5.2	HIS72, ARG75, ASP331, ASN353, PHE360	-	-
Resveratrol	-9.1	-	TRP70, ARG75, PHE240	CYS76
α-Lipoic acid	-5.4	ASN241	TRP70, CYS76, PRO241, PHE240	-
Rutin	-10.5	TRP70, SER73, ARG75, GLY242, TRP243	GLY78, MET226, CAL305	CYS76, GLU248

acid and rutin resulting from ligand-protein interaction, H bonding and hydrophobic interactions are shown in Table 13. The negative value of the ΔG results indicates that the reaction is spontaneous. The tested compounds showed a binding energy in the range of -7.7 to -5.5 kcal/mol. Arginase II's H bond Interaction, Hydrophobic Interaction, Electrostatic Interaction and binding energies with compounds are as follows, respectively. rutin (-7.7 kcal/mol, H bond Interaction: GLN37, LYS38, ASP202, GLU205, Hydrophobic Interaction:THR265, Electrostatic Interation: No), resveratrol (-7.3 kcal/mol, H bond Interaction: SER91, Hydrophobic Interaction: PRO32, PHE73, ALA95, Electrostatic Interation: No), vardenafil (-7.2 kcal /mol, H bond Interaction: SER34, GLN35, GLN37, Hydrophobic Interaction: PRO32, PHE33, LYS38, PRO75, VAL87, VAL92, ALA95, Electrostatic Interation: No), Arginine (-7.0 kcal/mol, H bond Interaction: ASP147, ASN149, HIS160, GLY16 , Hydrophobic Interaction: HIS145, Electrostatic Interation: ASP202, GLU205), papaverine (-6.6 kcal/mol, H bond

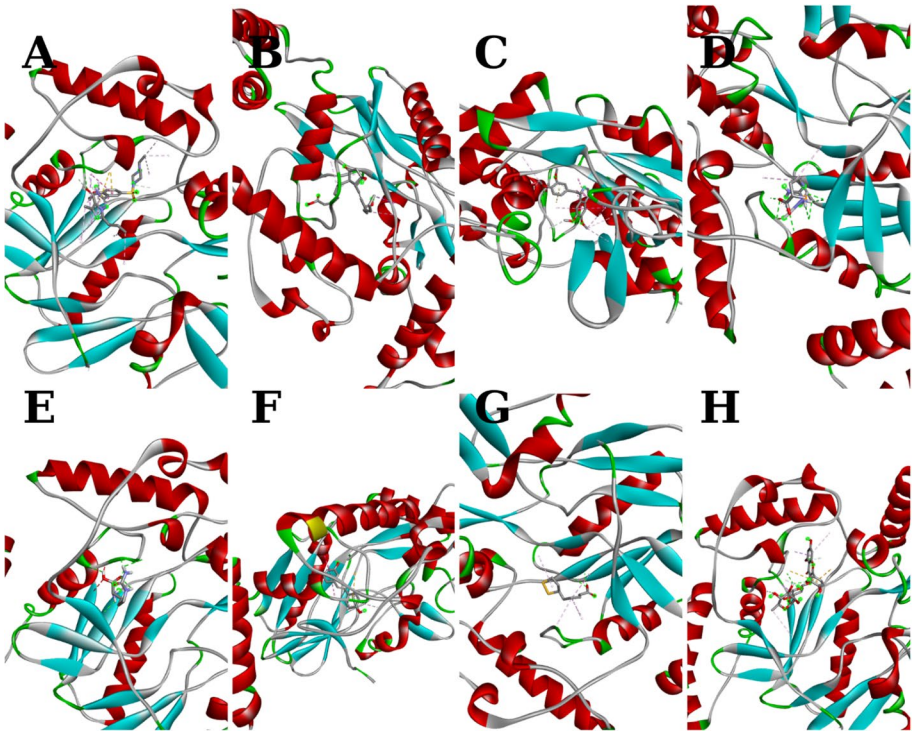


Fig 4 Best conformational regions obtained for Nitric Oxide Synthase in molecular docking analysis (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

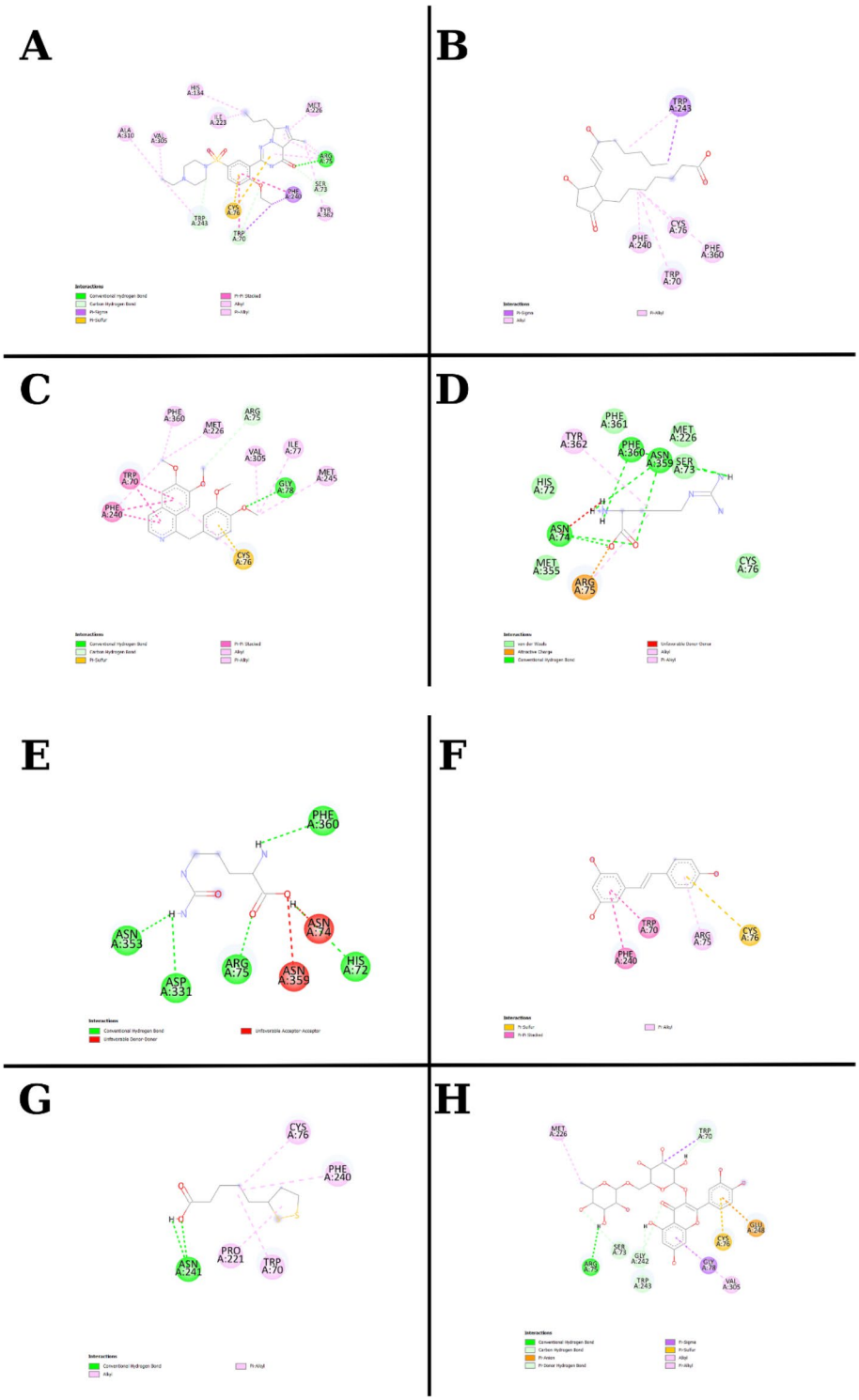


Fig 5 Ligand binding structures obtained for Nitric Oxide Synthase in molecular docking analysis: (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

Table 13 Molecular docking targets of the vardenafil, alprostadil, papaverine, L-arginine, citrulline, resveratrol, α-lipoic acid, and rutin in the arginase II structure

Compounds	ΔG (kcal/mol)	H bond Interaction	Hydrofobic Interaction	Electrostatic Interaction
Vardenafil	-7.2	SER34, GLN35, GLN37	PRO32, PHE33, LYS38, PRO75, VAL87, VAL92, ALA95	-
Alprostadil	-5.5	HIS145, ASN149, ASP202, GLU205	HIS160	-
Papaverine	-6.6	ASN158, GLU205	HIS145, HIS160, THR265	-
Arginine	-7.0	ASP147, ASN149, HIS160, GLY161	HIS145	ASP202, GLU205
Citrulline	-6.3	ASP147, ASN149, THR154, HIS160	HIS145	-
Resveratrol	-7.3	SER91	PRO32, PHE73, ALA95	-
α-Lipoic acid	-6.0	HIS160, THR265	-	-
Rutin	-7.7	GLN37, LYS38, ASP202, GLU205	THR265	-

Interaction: ASN158, GLU205, Hydrophobic Interaction: HIS145, HIS160, THR265, Electrostatic Interation: No) citrulline (-6.3 kcal/mol, H bond Interaction: ASP147, ASN149, THR154, HIS160 Hydrophobic Interaction: HIS145, Electrostatic Interation: No), α-lipoic acid (-6.0 kcal/mol, H bond Interaction: HIS160, THR265 Hydrophobic Interaction: No, Electrostatic Interation: No). The best bonding properties were rutin, vardenafil, resveratrol. In the light of these findings, rutin and resveratrol stand out as intriguing candidates for potential therapeutic uses due to their effects on Arginase II (Table 13, Figs 6 and 7). Targets of compounds in the protein binding pocket region are given in Figs. 8, 9 and 10. Hydrophobic interactions occur between nonpolar side chains and help stabilize ligand binding. Hydrophobic interactions are also important in creating a hydrophobic pocket that supports the ligand by avoiding water molecules, thus enhancing drug binding to the protein. Hydrogen bonds play an important role in maintaining the specificity and strength of ligand-protein interactions [74].

DFT calculations provide valuable insights into the electronic characteristics of molecules, enabling the prediction of their chemical reactivity and stability. One of the key parameters in assessing a molecule's electronic

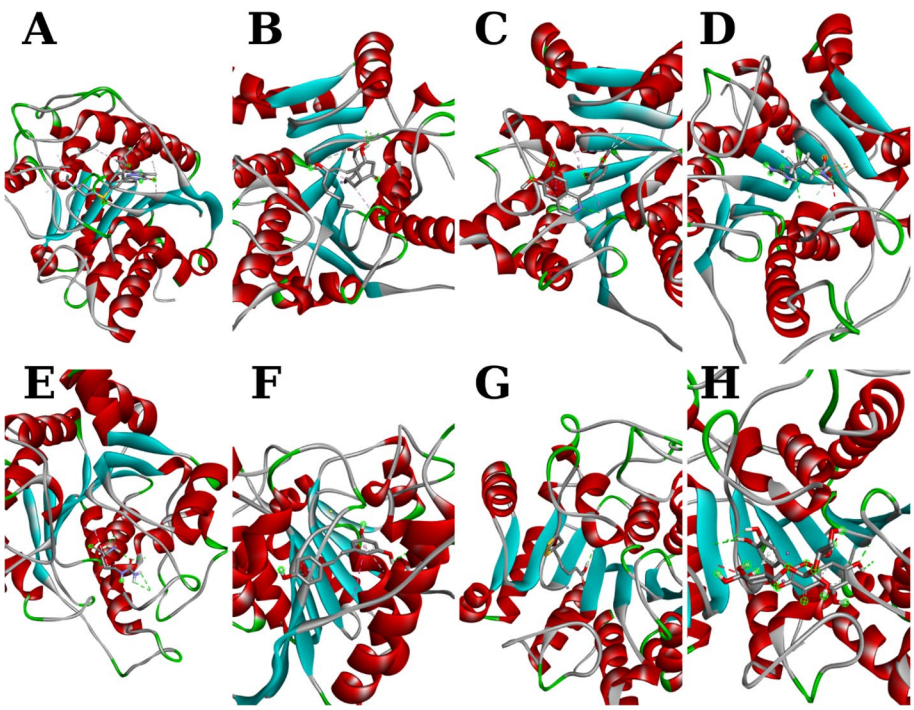


Fig 6 Best conformational regions obtained for Arginase II in molecular docking analysis (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

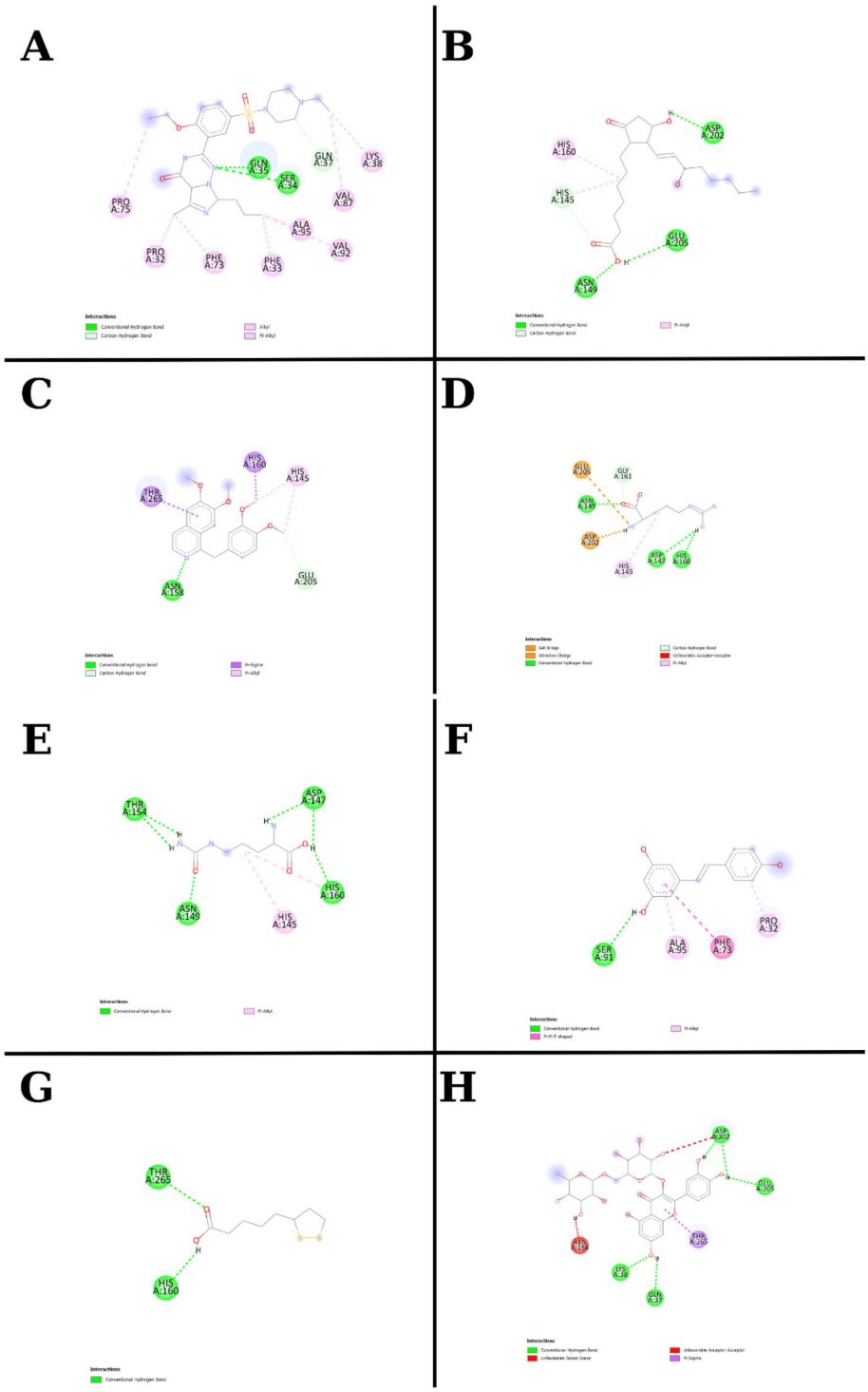


Fig 7 Ligand binding structures obtained for Arginase II in molecular docking analysis: (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

stability and reactivity is the energy gap (ΔE) between the Lowest Unoccupied Molecular Orbital (LUMO) and the Highest Occupied Molecular Orbital (HOMO). Additional parameters, including softness (s), electronegativity (χ), hardness, nucleophilicity (Nu), and electrophilicity (ω), were also evaluated. Furthermore, the

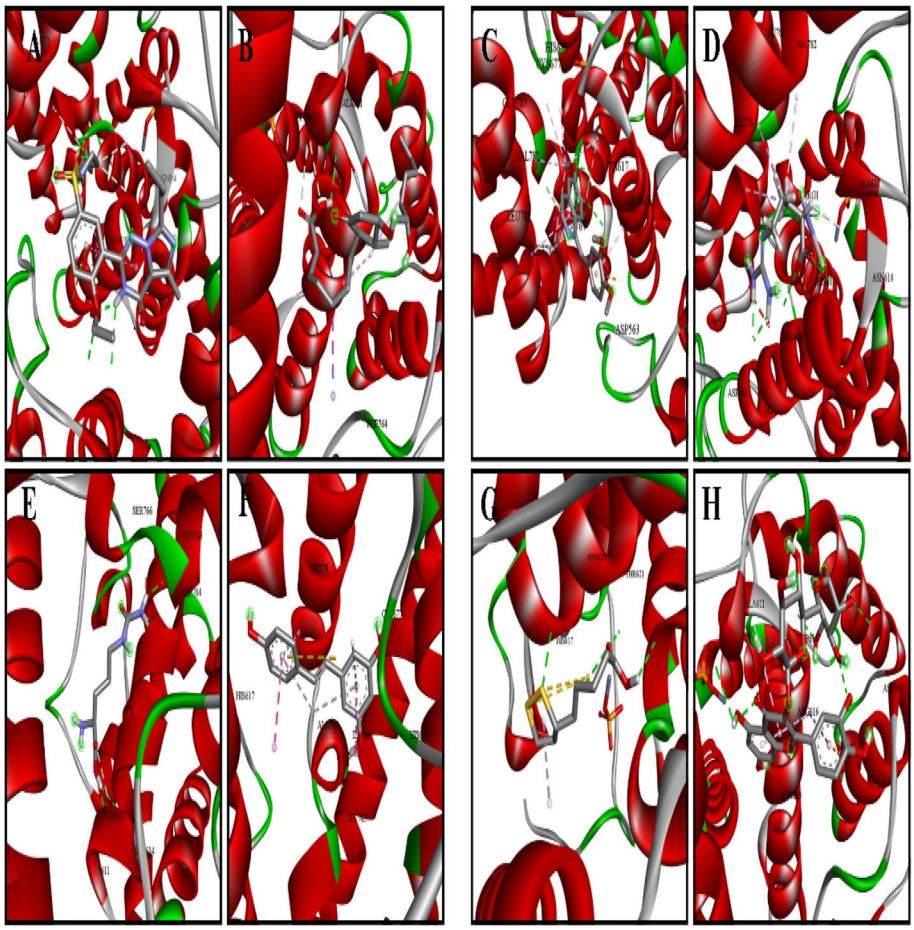


Fig 8 Targets of compounds in the Phosphodiesterase 5A protein binding site (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

dipole moment (μ) was determined to analyze charge distribution within the molecules [51]. alprostadil exhibits its significant electronic instability in Table 14 when compared to the other compounds in the investigation, with an energy gap of 2.82 eV. Higher chemical reactivity is suggested by this small ΔE. Its dipole moment of 4.9935 Debye suggests significant charge separation, which may affect its interactions with biological targets. Its high softness of 0.70 eV^{-1} and low hardness of 1.41 eV further confirm its reactive nature. In contrast, arginine is more electrically stable, with a larger energy gap of 3.52 eV. This stability is supported by its hardness (1.76 eV) and softness (0.57 eV^{-1}). Additionally, its dipole moment of 3.6923 Debye, which reflects a lower degree of charge separation, may impact its pharmacological interactions. citrulline has a moderate level of stability and reactivity with an intermediate energy gap of 3.53 eV. Its electrical properties are consistent with its hardness of 1.77 eV and softness of 0.56 eV^{-1}. Its solubility and binding affinity may be impacted by the dipole moment of 3.7357 Debye, which denotes a modest charge separation. papaverine, with an energy gap of 4.32 eV, exhibits balanced stability and reactivity. This is further corroborated by its hardness (2.16 eV) and softness (0.46 eV^{-1}). The compound's relatively low dipole moment of 3.5618 Debye suggests minimal charge separation, potentially affecting its bioavailability and interactions with target sites. With an energy gap of 3.69 eV, resveratrol has the biggest gap and could indicate limited reactivity and great electrical stability. Additional evidence for this stability comes from its high hardness (1.84 eV) and low softness (0.54 eV^{-1}). Its solubility and interaction dynamics may be impacted by the significant charge separation indicated by 2.6628 Debye dipole moment, which is noticeably high. In contrast, α-lipoic acid is characterized by lower reactivity and significant stability, with an energy gap of 2.15 eV. Its

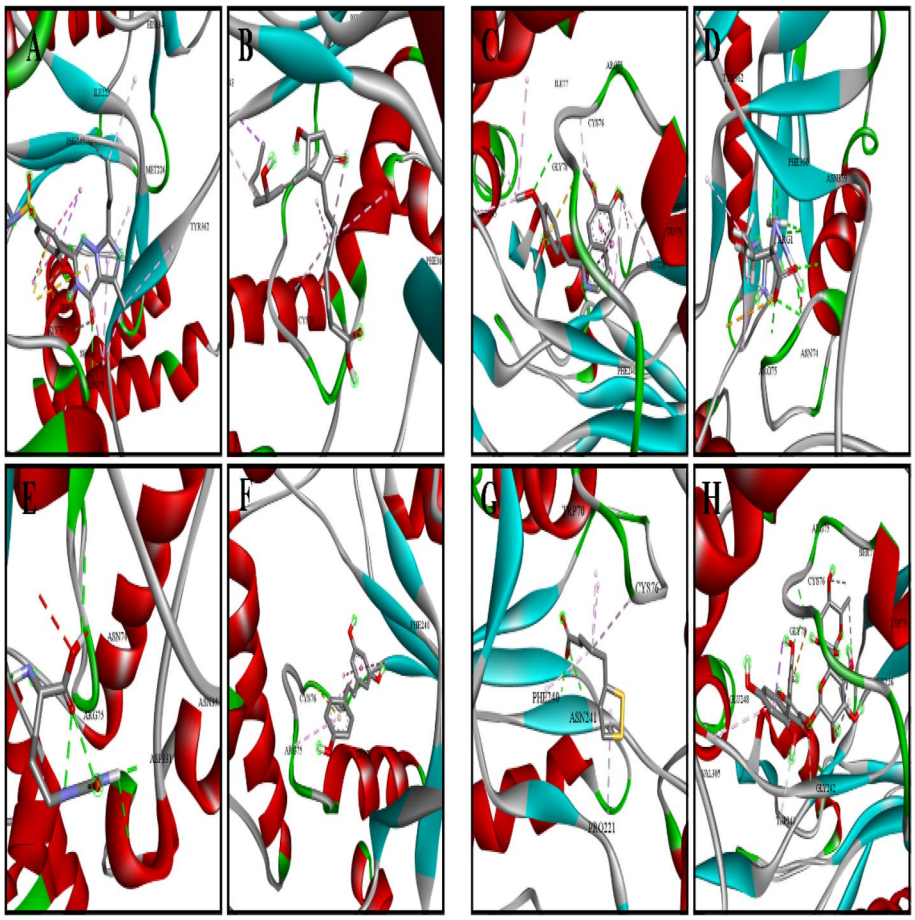


Fig 9 Targets of compounds in the Nitric Oxide Synthase binding site (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

softness (0.95 eV^{-1}) and hardness (1.05 eV) are indicative of a stable electronic nature, while its dipole moment of 5.3825 Debye suggests considerable charge separation, which may impact biological interactions. Higher reactivity is indicated by vardenafil's lower energy gap of 3.11 eV. These values, which indicate a more reactive material, are 1.55 eV for hardness and 0.64 eV^{-1} for softness. rutin is the most electronically stable and least reactive of the molecules under study, having an energy gap of 2.38 eV. Its low softness (0.83 eV^{-1}) and high hardness (1.99 eV) provide additional support for its stability (Figure 11). The dipole moment of 7.9921 Debye suggests a moderate level of charge separation, which could have an impact on how it interacts with living things. DFT simulations, in summary, show significant variations in the electronic properties and reactivity of the compounds under investigation. These differences are critical to understanding the molecules' chemical behavior and potential applications in a spectrum of fields.

Conclusion

The ADMET properties of these compounds were further analyzed using ADMETlab 3.0, which provided insights into their absorption, distribution, metabolism, excretion, and toxicity profiles. L-arginine, citrulline, resveratrol, and α-lipoic acid showed favorable intestinal absorption and permeability. L-arginine and citrulline, on the other hand, showed high tissue distribution, which may enhance their therapeutic effects. Compounds such as L-arginine, citrulline, and α-lipoic acid are safe candidates for therapeutic use due to their low toxicity profiles. On the other hand, resveratrol should be used with caution due to its high toxicity risks. Molecular docking studies highlighted the binding affinities of these compounds to key therapeutic targets such as PDE5, NOS, and arginase II. Rutin and resveratrol showed strong binding interactions with these targets, indicating their potential as therapeutic agents for erectile dysfunction. DFT calculations further elucidated the electronic properties of

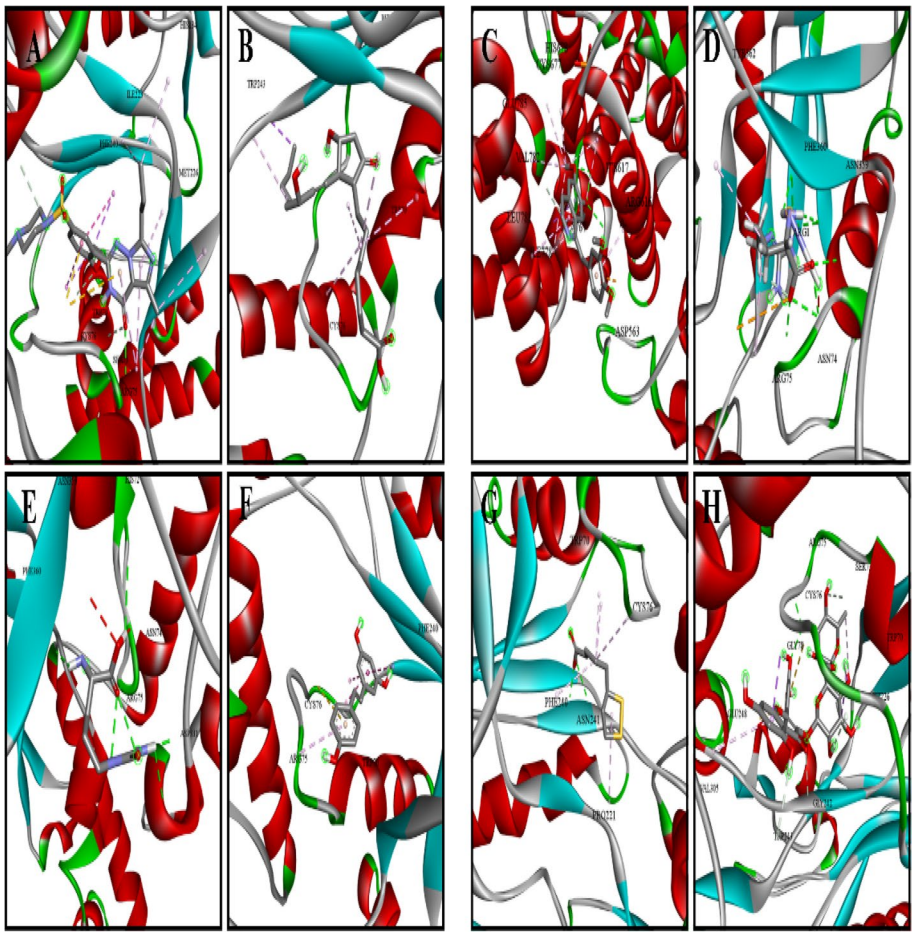


Fig 10 Targets of compounds in the Arginase II binding site (A: Vardenafil, B: Alprostadil, C: Papaverine, D: Arginine, E: Citrulline, F: Resveratrol, G: α-Lipoic acid and H: Rutin)

these compounds; alprostadil showed high reactivity due to its small energy gap, while rutin showed high electronic stability. In conclusion, this comprehensive analysis provides valuable information about the drug-likeness, pharmacokinetic properties, and therapeutic potential of the investigated compounds. Although resveratrol holds promise as a therapeutic agent, its potential toxicities and metabolic interactions require further investigation. L-arginine and citrulline emerge as promising candidates for further development with their favorable safety profiles and therapeutic effects. However, it may be recommended to conduct the necessary research studies regarding dosage and frequency.

Table 14 The obtained parameters by DFT/B3LYP/6-311G (++ d,p) for all compounds

	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	χ (eV)	(eV)	s (eV^{-1})	ω(eV)	*Nu*(eV^{-1})	μ (Debye)
Vardenafil	-5.94	-2.83	3.11	4.39	1.55	0.64	6.19	0.16	3.8362
Alprostadil	-7.05	-4.22	2.82	5.63	1.41	0.70	11.25	0.08	4.9935
Papaverine	-6.15	-1.83	4.32	3.99	2.16	0.46	3.69	0.27	3.5618
Arginine	-6.32	-2.80	3.52	4.55	1.76	0.57	5.90	0.17	3.6923
Citrulline	-6.56	-3.02	3.53	4.79	1.77	0.56	6.48	0.15	3.7357
Resveratrol	-6.14	-2.44	3.69	4.29	1.84	0.54	4.97	0.20	2.6628
α-Lipoic acid	-3.45	-1.33	2.15	2.38	1.05	0.95	2.69	0.37	5.3825
Rutin	-4.55	-2.16	2.38	3.36	1.19	0.83	4.74	0.21	7.9921

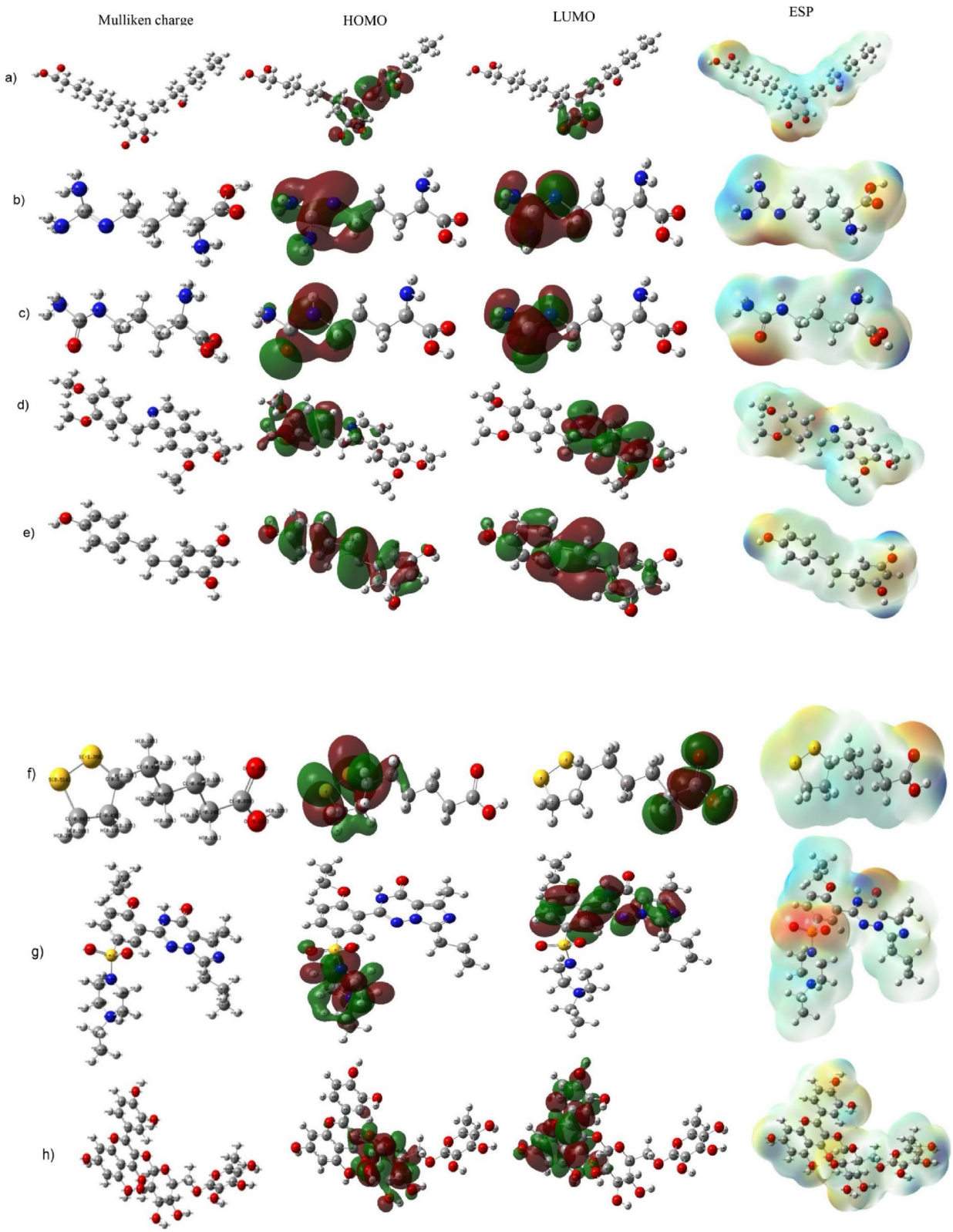


Fig 11 Compounds structures of (**a**) Alprostadil, (**b**) Arginine, (**c**) Citrulline, (**d**) Papaverine, (**e**) Resveratrol, (**f**) α-Lipoic acid, (**g**) Vardenafil and, (**h**) Rutin.

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Authors' contributions
Süleyman Sağır: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. Velid Unsal: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Project administration, Visualization, Writing – original draft, Writing – review & editing. Reşit Yıldız: Investigation, Methodology, Writing – original draft Başak Doğru Mert: Investigation, Methodology, Visualization, Data curation, Writing – review & editing Erkan Oner: Investigation, Visualization, Methodology, Writing – review & editing.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Declarations
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to particpate
This research does not require ethical approval. The study did not include any human or animal participants that required consent to participate and/or publish findings.

Consent for publication
All authors have approved the article and declared that it is an original contribution and that no material in this article has been considered for publication elsewhere.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Urology, Faculty of Medicine, Mardin Artuklu University, Mardin 47200, Türkiye. [2]Department of Nutrition and Dietetics, Faculty of Health Sciences, Mardin Artuklu University, Mardin 47200, Türkiye. [3]Department of Biochemistry, Faculty of Pharmacy, Adıyaman University, Adıyaman 02000, Türkiye. [4]Energy Systems Engineering Department, Engineering Faculty, Adana Alparslan Türkeş Science and Technology University, Adana 01250, Türkiye.

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