Molecular Docking Approach to Identify Flavonoids as Potential Phosphodiesterase 5 Inhibitors for Erectile Dysfunction Treatment

Nguyen Tan Khanh[a], Pham Thi Khanh Huyen[b], Nguyen Thi Huynh Van[c], Le Ba Nam[d], Nguyen Ngoc Linh[e*]

Abstract:

An erection that is not strong enough for sexual activity might be caused by erectile dysfunction. Periodically experiencing difficulties achieving an erection is not always a reason for alarm. On the other hand, if erectile dysfunction persists, it may lead to stress, undermine confidence, and exacerbate interpersonal issues. In addition to being a risk factor for heart disease, difficulties achieving or maintaining an erection may also indicate an underlying medical issue that needs to be treated. Many studies have also steadily examined flavonoids' capacity to inhibit the phosphodiesterase 5 enzyme in several recent studies. This is an enzyme expressed in erectile tissue that is the most important substance that degrades cGMP in the body's smooth muscle cells. This study identified two prominent flavonoids that had inhibitory effects on the phosphodiesterase 5 enzyme: Amenoflavone and Umbralisib. Our research suggests new molecular pathways by which these substances might influence biological processes. One compound that shows promise for treating erectile dysfunction and is safe for healthy cells is Amentoflavone.

Keywords: *Erectile dysfunction, Phosphodiesterase 5, virtual screening, molecular docking*

Received: 13.01.2024; Accepted: 15.6.2024; Published: 30.6.2024

DOI: 10.59907/daujs.3.2.2024.283

[a] Dong A University; 33 Xo Viet Nghe Tinh Street, Hoa Cuong Nam Ward, Hai Chau District, Da Nang City, Vietnam. e-mail: khanhnt@donga.edu.vn

[b] School of Medicine and Pharmacy, The University of Da Nang; Hoa Quy Ward, Ngu Hanh Son District, Da Nang City, Vietnam. e-mail: phamthikhanhhuyen22299@gmail.com

[c] Dong A University; 33 Xo Viet Nghe Tinh Street, Hoa Cuong Nam Ward, Hai Chau District, Da Nang City, Vietnam. e-mail: vannth.nckh@donga.edu.vn

[d] College of Pharmacy, Thanh Do University; Kim Chung Ward, Hoai Duc District, Hanoi City, Vietnam. e-mail: banamdkh@gmail.com

[e] College of Pharmacy, Thanh Do University; Kim Chung Ward, Hoai Duc District, Hanoi City, Vietnam. e-mail: nnlinh@thanhdouni.edu.vn

[*] Corresponding Author.

ISSN: 2815 - 5807

Introduction

Erectile dysfunction (ED) is the sexual ailment that is most frequently researched (Porst et al., 2013). A frequent medical problem in males is ED, which is defined as a man's continuous or recurring inability to achieve and/or sustain a penile erection strong enough for sexual engagement (Lue et al., 2004; McCabe et al., 2016). It has been demonstrated that ED has a seriously detrimental effect on a person's life and happiness (Christensen et al., 2011). Millions of men worldwide are impacted by ED, a dysfunction that worsens with age and is prevalent in 40 % of men between the ages of 40 and 70 (Lewis, 2004). According to future forecasts, 322 million men will have ED by 2025, which is more than 100 % more than similar rates in 1994 (about 154 million men with ED) (Aytaç et al., 1999). In addition to psychological variables, identified risk factors for ED include age, obesity, smoking, depression, hypertension, previous pelvic surgery, and spinal cord damage (McCabe et al., 2016). Current therapies for ED include stem cell therapy (Albersen et al., 2010; Lin et al., 2009; Mangner et al., 2013; Sun et al., 2012), stem cell delivery (Casiraghi et al., 2013; Lin et al., 2010; Prockop, 2007; Ryu et al., 2016; Ryu et al., 2014; Uccelli et al., 2008; You, Jang, Lee, Jeong, et al., 2013; You, Jang, Lee, Suh, et al., 2013), sex therapy (Bilal & Abbasi, 2020), oral medications (Boulton et al., 2001; Hatzimouratidis & Hatzichristou, 2004, 2005; Hatzimouratidis et al., 2016; Kalsi & Kell, 2004; Padma-Nathan & Giuliano, 2001; Peng et al., 2017; Wrishko et al., 2009), intracavernosal injections (Elena et al., 2023; Giuliano et al., 2023), urethral alprostadil suppositories (Karakus & Burnett, 2020; Rezaee et al., 2020), vacuum erection devices (Beaudreau et al., 2021; Jones et al., 2021; Khayyamfar et al., 2021; Sultana et al., 2022), and penile implants (Ciocanel et al., 2019; Protogerou et al., 2021). Since they are the least intrusive option, phosphodiesterase type 5 inhibitors (PDE5i) are frequently used as the first-line therapy for ED (Jarow et al., 1996; Shamloul & Ghanem, 2013). Because of its safety and efficacy in treating ED, it is regarded as the most widely used therapy globally (Langarizadeh et al., 2023; Singh et al., 2005).

Phosphodiesterases (PDEs) have eleven subtypes, and they are expressed in many different bodily tissues. They work as a class to modulate signaling cascades' second messengers, and they have a wide range of downstream cellular and physiological effects. Although almost all PDEs are expressed in erectile tissue, Phosphodiesterase 5 (PDE5) is the most crucial one for cGMP degradation in the cells of the body's smooth muscle. The complex interactions that lead to an erection include psychological sexual excitement, sensory input, peripheral neurotransmitter release, relaxation of smooth muscle cells, and vascular engorgement of the corporal penile tissue. The most significant erectogenic chemical released by postsynaptic neurons and endothelial cells in the penis during sexual stimulation is nitric oxide (NO). This gaseous molecule has an extremely short half-life, yet it may diffuse across smooth muscle cell membranes very fast, initiating a signaling

cascade that leads to vascular engorgement, erection, and relaxation of the smooth muscle in the arteriolar region. Soluble guanylyl cyclase is activated by NO and converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). The second messenger that initiates vascular smooth muscle relaxation is cyclic GMP. PDE5 is an enzyme that converts cGMP to GMP enzymatically, which reduces erectogenic signaling downstream. Therefore, by boosting the NO/cGMP-dependent signaling cascade and stabilizing cGMP, phosphodiesterase type 5 inhibitor (PDE5i) aids in erections. PDE5i do not induce erections in the absence of sexual stimulation, though, since there is no signal to amplify in the absence of sexual stimulation and the creation of NO (Ferguson III & Carson III, 2013; Ghofrani et al., 2006).

PDE5Is are a family of medications that work by inhibiting cGMP degradation to extend the physiological effects of NO/cGMP signaling in tissues. Examples of these medications are Vardenafil, Tadalafil, and Sildenafil. Even though these drugs were once created to treat angina and hypertension, unforeseen side effects allowed for advancements in the management of pulmonary arterial hypertension and erectile dysfunction. Evidence has been mounting over the last ten years suggesting PDE5Is may offer more therapeutic advantages than previously thought for the treatment of ED (Tzoumas et al., 2020). The ability of flavonoids to inhibit the PDE5 enzyme has also been gradually investigated in several recent research (Adefegha et al., 2018; Gu et al., 2021). This gives pharmacognosy researchers a new avenue to pursue in their hunt for naturally occurring flavonoid compounds to treat ED. Given the significance of the problem, we have also looked for flavonoid compounds that influence PDE5 in this study to identify safe, efficient, and low-side-effect medications for the treatment of ED.

Materials and methods

Protein and Ligand Preparation

PDE5's crystal structure (PDB ID: 2H42) was downloaded from the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics. After that, ubiquitin, water molecules, and crystal ligands were eliminated. Using Auto Dock tools (version 1.5.6), polar hydrogen and Kollman charges were introduced to the protein (Cosconati et al., 2010). Finally, the macromolecule was exported into a dockable pdbqt format for molecular docking. 3D structures of flavonoid library were downloaded from Selleckchem database. After that, hydrogen atoms were added to all ligands, which were converted to dockable pdbqt format utilizing Open Babel 3.1.1 (O'Boyle et al., 2011).

Molecular Docking

AutoDock Vina was used to perform molecular docking procedures. Using the CASTp server, the grid boxes covering proteins' active sites were predicted (Cosconati et al., 2010). Based on the sildenafil binding site and PDE5 active site locations in the crystal structure (PDB ID: 2H42), the grid box's center was established. The grid box was created with the following parameters when the center site was determined: size_x = 22.5, size_y = 18.75, size_z = 22.5, center_x = 30, center_y = 120, and center_z = 9. Compound docking scores were used to categorize the data, with docking scores expressed in kcal/mol. Finally, a visualization of the molecular interactions between proteins and ligands was achieved using Discovery Studio Visualizer.

In-silico Drug-Likeness and Toxicity

The Lipinski rule was used to forecast a compound's drug-likeness based on an existing idea (Benet et al., 2016). SwissADME server was used to calculate the ADME properties of ligands which provided information about the absorption, distribution, metabolism, and excretion (ADME) properties of the ligands (Daina et al., 2017). The DL-AOT prediction serve was utilized to forecast acute oral toxicity (Mukherjee et al., 2021).

Results and discussion

Interaction and Binding Affinity Between Ligands and PDE5

Sildenafil was shown activity to inhibit PDE5 (**Figure 1**) (Francis & Corbin, 2005). Therefore, we decided to choose Sildenafil as a reference inhibitor.

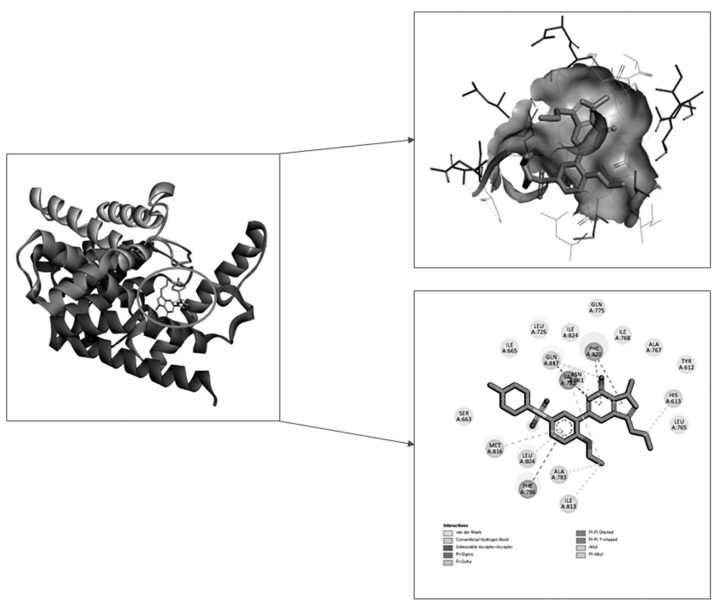
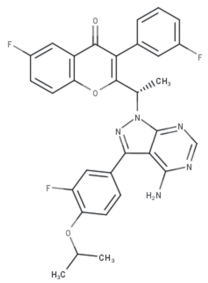
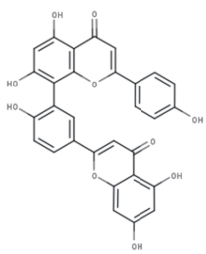
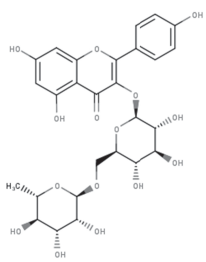
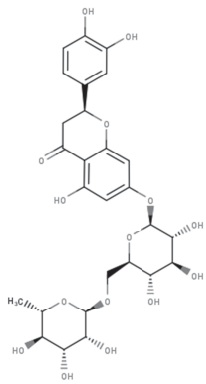
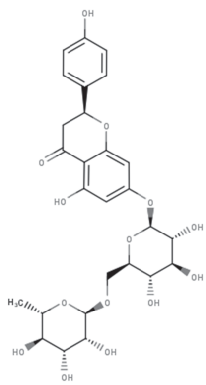
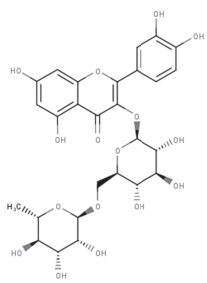
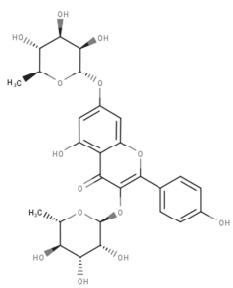


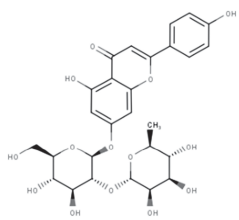
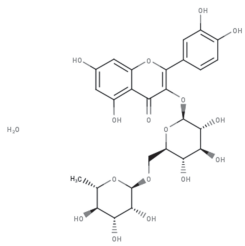
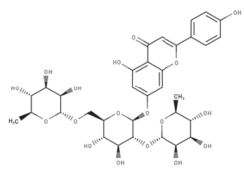
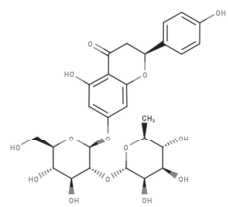
Figure 1. Docking pose of Sildenafil at the crystal structures of PDE5

After docking, the results revealed 33 compounds that have better docking scores than -10.01 Kcal/mol, a docking score of Sildenafil. For selecting bioactive compounds that have a good binding affinity with PDE5, a cut-off of -11 Kcal/mol was set. Eleven compounds surpassed this threshold, and the results of these top-ranked compounds are represented in **Table 1**.

Table 1. Docking results of eleven compounds towards PDE5

Compound	Structure	Docking score (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Umbralisib		-13.53	Ser663, Phe820	Val660, Arg667, Ile665, Ile824, Phe820, Leu804, Phe786, Leu725
Amentoflavone		-13.49	Phe820	Met816, Ile824, Gly819, Ala823, Phe820, Val782, Leu725
Nicotiflorin		-11.97	Tyr612, Gln775, Met816, Asp654, Thr723, Asn662	Ala767, Val782, Phe820, Tyr612

Compound	Structure	Docking score (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Eriocitrin		-11.38	Glu682, Arg667, Asp724, His613, His657, Asp654, Asp764, Leu765, Ile768, Gln817, Thr723	Leu725, Ile768, Gln775, Val782
Narirutin		-11.35	Asp724, Asp654, Thr723	Leu725, Ile768, Phe820, Tyr612, Gln775, Ala767, Val782
Rutin		-11.25	Gln775, Leu765, Thr723, Asn661	Ala767, Tyr612, Val782, Phe820, Phe786, Leu804
Kaempferitrin		-11.21	Asn662, His657	Arg667

Compound	Structure	Docking score (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Rhoifolin		-11.13	Tyr612, Asp764, His617, Leu725, Glu682, Thr723	Val782, Phe820, Phe786, Leu765
Rutin hydrate		-11.12	Gln775, Tyr612, Asp654, His685, Asn661	Ala767, Val782, Phe820, Phe786, Leu804
Ligustroflavone		-11.06	Met816, Asn662, Leu725, Thr723, Asp654	Val782, Ala783, Phe820, Ile813, Phe786, Ile665, Ile824, Met816
Naringin		-11.00	Tyr612, Asp654, Thr723,	Leu765, Val782, Phe820, Phe786, Leu725

Among these compounds, Umbralisib and Amentoflavone have demonstrated the highest binding affinity to the PDE5 enzyme with docking scores of -13.53 and -13.49 Kcal/mol, respectively. Interaction analysis has revealed that Umbralisib and Amentoflavone form several significant interactions with the PDE5 enzyme at the points Phe820, Ile824, and Leu725 within the catalytic domain (residues 537–860) of human PDE5. As shown in Figure 2, Umbralisib forms two hydrogen bonds with Ser663, and Phe820 and eight hydrophobic interactions with Val660, Arg667, Ile665, Ile824, Phe820, Leu804, Phe786, and Leu725. Similarly, Amentoflavone forms one hydrogen bond with Phe820 and seven hydrophobic interactions with Met816, Ile824, Gly819, Ala823, Phe820, Val782, Leu725 **(Figure 2).** These interactions are quite similar to the interactions between Sildenafil and

PDE5. Sildenafil was also found to interact with PDE5 at Phe820, Gln817, Val782, Met816, Leu804, Phe786, Ala783, Ile813, and His613 with one hydrogen bond and eight hydrophobic interactions **(Figure 1).**

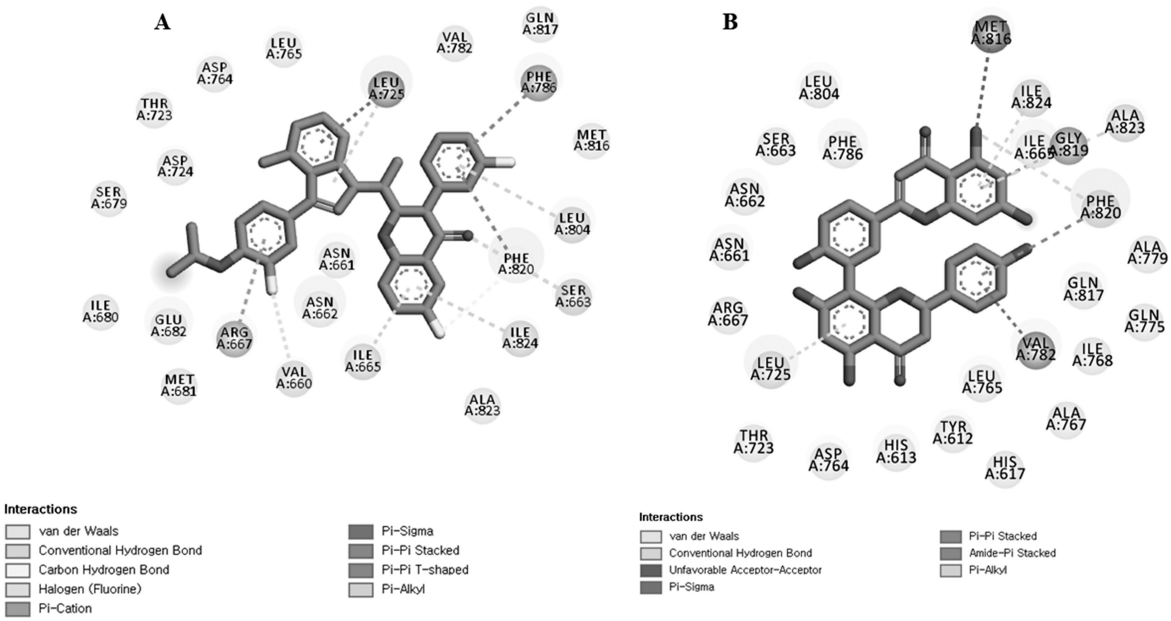


Figure 2. Interaction between compounds and PDE5. (A) Umbralisib; (B) Amentoflavone

ADMET Properties

A method for determining a compound's drug-likeness and potential oral activity is Lipinski's rule of five. The hydrophobicity and oral bioavailability of drugs with excellent membrane permeability are both reflected in the logP, TPSA, MW, HBA, and HBD values. Drugs' oral bioavailability and intestinal absorption are predicted using nRotB and MR values.

In silico predictions regarding the absorption, distribution, metabolism, and excretion of 11 potential compounds are presented in **Table 2** and **Table 3**. To predict whether a drug is suitable for oral administration, certain criteria must be met, including a molecular weight between 150 and 500 g/mol, polarity (TPSA) between 20 and 130 , solubility (log S) not higher than 6, and flexibility with no more than 9 rotatable bonds (Daina et al., 2017). **Table 2** shows that all 11 compounds are predicted not to be orally available. However, these are only predictions, so further research or alternative oral formulation designs may be necessary to address this issue.

These compounds are predicted to have poor absorption through the digestive tract and are not expected to cross the blood-brain barrier. Furthermore, through metabolic biotransformation, the superfamily of isoenzymes is essential for drug clearance (Testa & Kraemer, 2007). According to some research, P-glycoprotein (P-gp) and cytochrome P450 (CYP) may complement one another to improve tissue and organism protection (van Waterschoot & Schinkel, 2011). It is expected that five key isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) are substrates of between 50 and 90 percent of medicinal compounds (Di, 2014; Wolf et al., 2000). Drug-drug interactions linked to pharmacokinetics are greatly influenced by the inhibition of these isoenzymes, which can have harmful or undesirable consequences because of decreased drug clearance and increased buildup of the drug or its metabolites (Hollenberg, 2002; Huang et al., 2008; Kirchmair et al., 2015). The results in **Table 3** indicate that all 11 compounds are predicted not to interact with CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4, which is crucial for the drug's biotransformation process (Zanger & Schwab, 2013). Furthermore, the prediction tool SwissADME suggests that Umbralisib and Amentoflavone do not inhibit the P-glycoprotein background, but the remaining nine compounds are predicted to have the potential to inhibit the P-glycoprotein background.

Table 2. Drug Properties of Compounds with SwissADME

Compound	Molecular weight (g/mol)	Log P	nHBD	nHBA	TPSA (Å²)	MR	Lipinski Violation	Log Kp (cm/s)	Log S	nRotB
Umbralisib	571.55	5.80	1	9	109.06	153.18	1	-5.65	-7.21	6
Amentoflavone	538.46	3.62	6	10	181.80	146.97	2	-6.01	-6.75	3
Nicotiflorin	594.52	-0.73	9	15	249.20	139.36	3	-9.91	-3.42	6
Eriocitrin	596.53	-1.30	9	15	245.29	136.94	3	-10.90	-2.50	6
Narirutin	580.53	-0.95	8	14	225.06	134.91	3	-10.54	-2.64	6
Rutin	610.52	-1.29	10	16	269.43	141.38	3	-10.26	-3.30	6
Kaempferitrin	578.52	-0.53	8	14	228.97	137.93	3	-9.89	-3.33	5
Rhoifolin	578.52	-0.66	8	14	228.97	137.33	3	-9.94	-3.22	6
Rutin hydrate	628.53	-1.52	11	17	278.66	144.43	3	-10.71	-3.10	6
Ligustroflavone	724.66	-2.01	10	18	287.89	168.55	3	-11.38	-3.45	8
Naringin	580.53	-0.79	8	14	225.06	134.91	3	-10.15	-2.98	6

MW, Molecular weight; LogP, Log of octanol/water partition coefficient; nHBD, Number of hydrogen bond donor(s); nHBA, Number of hydrogen bond acceptor(s); TPSA, Total polar surface area; MR, Molar refractivity; Log Kp, Log of skin permeation; Log S, log of solubility; nRotB, Number of rotatable bonds.

Table 3. ADME Predictions of Compounds Computed by SwissADME

Compound	Log Kp (cm/s)	GI Abs	BBB Per	Inhibitor Interaction					
				P-gp Substrate	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
Umbralisib	-5.65	Low	No	No	No	No	No	No	No
Amentoflavone	-6.01	Low	No	No	No	No	No	No	No
Nicotiflorin	-9.91	Low	No	Yes	No	No	No	No	No
Eriocitrin	-10.90	Low	No	Yes	No	No	No	No	No
Narirutin	-10.54	Low	No	Yes	No	No	No	No	No
Rutin	-10.26	Low	No	Yes	No	No	No	No	No
Kaempferitrin	-9.89	Low	No	Yes	No	No	No	No	No
Rhoifolin	-9.94	Low	No	Yes	No	No	No	No	No
Rutin hydrate	-10.71	Low	No	Yes	No	No	No	No	No
Ligustroflavone	-11.38	Low	No	Yes	No	No	No	No	No
Naringin	-10.15	Low	No	Yes	No	No	No	No	No

GI Abs: Gastro-intestinal absorption; BBB Per: Blood brain barrier permeability; P-gp, P-glycoprotein; CYP, cytochrome-P

Predict toxicity

Eleven compounds were found to have the potential to inhibit the PDE5 enzyme in the treatment of ED. However, the activity of these compounds on normal cells needs to be assessed to ensure selectivity in disease treatment and the development of treatment methods without posing risks to the patient's health. In this study, their toxicity was predicted by the DL-AOT prediction server.

DL-AOT classifies compounds into four groups: "danger/poison", "warning", "caution", and "none required". Amentoflavone is predicted as "none required", with an LD50 value of 3.60 mg/kg, meaning it is not toxic to normal cells. However, Umbralisib, Eriocitrin, and Narirutin are all classified as "warning", indicating they are predicted to be toxic to normal cells. The remaining compounds, including Nicotiflorin, Rutin, Kaempferitrin, Rhoifolin, Rutin hydrate, Ligustroflavone, and Naringin, are all classified as "caution", meaning caution is needed when using these compounds in treatment. Our *in silico* data suggests that these eleven compounds have the potential to inhibit the PDE5 enzyme in the treatment of ED. However, they may have the potential to be toxic to cells, excluding Amentoflavone. Therefore, experimental studies on the biological activity of these compounds are needed **(Table 4).**

Table 4. Toxicity of Compounds Predicted by DL-AOT Prediction Server

Compound	LD50 (mg/kg)	Label (probability)	Toxicity
Umbralisib	3.18	2 (0.98)	warning
Amentoflavone	3.60	4 (1.00)	none required
Nicotiflorin	3.08	3 (1.00)	caution
Eriocitrin	2.93	2 (0.93)	warning
Narirutin	2.92	2 (0.72)	warning
Rutin	3.08	3 (0.99)	caution
Kaempferitrin	2.82	3 (1.00)	caution
Rhoifolin	3.31	3 (1.00)	caution
Rutin hydrate	3.10	3 (1.00)	caution
Ligustroflavone	3.36	3 (1.00)	caution
Naringin	2.95	3 (0.99)	caution

Among potential candidates, Umbralisib exhibited the strongest binding affinity to PDE5 at catalytic site. Umbralisib is a kinase inhibitor, targeting for cancer treatment. The toxicity of umbralisib was predicted “warning”, and the Gastro-intestinal absorption was predicted “low”. Therefore, we need further studies related structure to decrease the toxicity, and increase the absorption to develop Umbralisib derivatives as specific PDE5 inhibitor.

On the other hand, the second candidate, Amentoflavone, a flavonoid constituent of a number of plants. At in vitro studies, Amentoflavone showed antimalarial activity, anticancer activity, as well as neurological dysfunction suppression. This compound was predicted “no” toxicity in in-silico studies, however; it also has low gastro-intestinal absorption. Therefore, further investigation is needed to improve absorption to develop compound as oral drug.

Conclusion

Amentoflavone and Umbralisib are two notable flavonoids with inhibitory properties against the PDE5 enzyme. Our study proposes novel biological mechanisms through which these compounds may exert their biological effects. Amentoflavone emerges as a promising candidate for ED therapy that is not toxic to normal cells. The *in-silico* data

in our research provides suggestive information for further investigations into designing pharmaceutical formulations from the eleven compounds with potential PDE5 inhibitory capabilities. Subsequent experiments are needed to demonstrate the biological activity of these compounds.

ABBREVIATIONS

BBB Per: Blood brain barrier permeability

CYP: Cytochrome P450

ED: Erectile dysfunction

GI Abs: Gastro-intestinal absorption

cGMP: Cyclic guanosine monophosphate

GTP: Guanosine triphosphate

Log Kp: Log of skin permeation

LogP: Log of octanol/water partition coefficient

Log S: Log of solubility

MR: Molar refractivity

MW: Molecular weight

nHBA: Number of hydrogen bond acceptor(s)

nHBD: Number of hydrogen bond donor

NO: Nitric oxide

nRotB: Number of rotatable bonds

PDE5: Phosphodiesterase 5

PDE5i: Phosphodiesterase type 5 inhibitor

PDEs: Phosphodiesterases

P-gp: P-glycoprotein

TPSA: Total polar surface area

CONFLICTS OF INTEREST

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.

References

Adefegha, S. A., Oyeleye, S. I., Dada, F. A., Olasehinde, T. A., & Oboh, G. (2018). Modulatory effect of quercetin and its glycosylated form on key enzymes and antioxidant status in rats penile tissue of paroxetine-induced erectile dysfunction. *Biomedicine & Pharmacotherapy*, *107*, 1473-1479. https://doi.org/https://doi.org/10.1016/j.biopha.2018.08.128

Albersen, M., Fandel, T. M., Lin, G., Wang, G., Banie, L., Lin, C.-S., & Lue, T. F. (2010). Injections of adipose tissue-derived stem cells and stem cell lysate improve recovery of erectile function in a rat model of cavernous nerve injury. *The journal of sexual medicine*, *7*(10), 3331-3340. https://doi.org/https://doi.org/10.1111/j.1743-6109.2010.01875.x

Aytaç, Mckinlay, & Krane. (1999). The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. *BJU international*, *84*(1), 50-56. https://doi.org/https://doi.org/10.1046/j.1464-410x.1999.00142.x

Beaudreau, S. A., Van Moorleghem, K., Dodd, S. M., Liou-Johnson, V., Suresh, M., & Gould, C. E. (2021). Satisfaction with a vacuum constriction device for erectile dysfunction among middle-aged and older veterans. *Clinical Gerontologist*, *44*(3), 307-315. https://doi.org/https://doi.org/10.1080/07317115.2020.1823922

Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). BDDCS, the Rule of 5 and drugability. *Advanced drug delivery reviews*, *101*, 89-98. https://doi.org/https://doi.org/10.1016/j.addr.2016.05.007

Bilal, A., & Abbasi, N. U. H. (2020). Cognitive behavioral sex therapy: An emerging treatment option for nonorganic erectile dysfunction in young men: A feasibility pilot study. *Sexual medicine*, *8*(3), 396-407. https://doi.org/https://doi.org/10.1016/j.esxm.2020.05.005

Boulton, A., Selam, J.-L., Sweeney, M., & Ziegler, D. (2001). Sildenafil citrate for the treatment of erectile dysfunction in men with type II diabetes mellitus. *Diabetologia*, *44*, 1296-1301. https://doi.org/https://doi.org/10.1007/s001250100656

Casiraghi, F., Remuzzi, G., Abbate, M., & Perico, N. (2013). Multipotent mesenchymal stromal cell therapy and risk of malignancies. *Stem cell reviews and reports*, *9*(1), 65-79. https://doi.org/https://doi.org/10.1007/s12015-011-9345-4

Ciocanel, O., Power, K., & Eriksen, A. (2019). Interventions to treat erectile dysfunction and premature ejaculation: an overview of systematic reviews. *Sexual medicine*, *7*(3), 251-269. https://doi.org/https://doi.org/10.1016/j.esxm.2019.06.001

Cosconati, S., Forli, S., Perryman, A. L., Harris, R., Goodsell, D. S., & Olson, A. J. (2010). Virtual screening with AutoDock: theory and practice. *Expert opinion on drug discovery*, *5*(6), 597-607. https://doi.org/https://doi.org/10.1517/17460441.2010.484460

Christensen, B. S., Grønbæk, M., Osler, M., Pedersen, B. V., Graugaard, C., & Frisch, M. (2011). Associations between physical and mental health problems and sexual dysfunctions in sex-

ually active Danes. *The journal of sexual medicine*, *8*(7), 1890-1902. https://doi.org/https://doi.org/10.1111/j.1743-6109.2010.02145.x

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, *7*(1), 42717. https://doi.org/https://doi.org/10.1038/srep42717

Di, L. (2014). The role of drug metabolizing enzymes in clearance. *Expert opinion on drug metabolism & toxicology*, *10*(3), 379-393. https://doi.org/https://doi.org/10.1517/17425255.2014.876006

Elena, B. W., Zachary, M., Haritha, P., Graham, B. A., & Wayne, H. J. (2023). Current status of intracavernosal injection therapy in erectile dysfunction. *Expert Opinion on Pharmacotherapy*, *24*(8), 925-933. https://doi.org/https://doi.org/10.1080/14656566.2023.2204189

Ferguson III, J. E., & Carson III, C. C. (2013). Phosphodiesterase type 5 inhibitors as a treatment for erectile dysfunction: Current information and new horizons. *Arab Journal of Urology*, *11*(3), 222-229. https://doi.org/https://doi.org/10.1016/j.aju.2013.07.009

Francis, S. H., & Corbin, J. D. (2005). Sildenafil: efficacy, safety, tolerability and mechanism of action in treating erectile dysfunction. *Expert opinion on drug metabolism & toxicology*, *1*(2), 283-293. https://doi.org/https://doi.org/10.1517/17425255.1.2.283

Ghofrani, H. A., Osterloh, I. H., & Grimminger, F. (2006). Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nature reviews Drug discovery*, *5*(8), 689-702. https://doi.org/https://doi.org/10.1038/nrd2030

Gu, S. j., Li, M., Yuan, Y. m., Xin, Z. c., & Guan, R. l. (2021). A novel flavonoid derivative of icariside II improves erectile dysfunction in a rat model of cavernous nerve injury. *Andrology*, *9*(6), 1893-1901. https://doi.org/https://doi.org/10.1111/andr.13065

Giuliano, F., Denys, P., & Joussain, C. (2023). Safety and Effectiveness of Repeated Botulinum Toxin A Intracavernosal Injections in Men with Erectile Dysfunction Unresponsive to Approved Pharmacological Treatments: Real-World Observational Data. *Toxins*, *15*(6), 382. https://doi.org/https://doi.org/10.3390/toxins15060382

Hatzimouratidis, K., & Hatzichristou, D. G. (2004). Treatment options for erectile dysfunction in patients failing oral drug therapy. *EAU Update Series*, *2*(2), 75-83. https://doi.org/https://doi.org/10.1016/j.euus.2004.03.001

Hatzimouratidis, K., & Hatzichristou, D. G. (2005). A comparative review of the options for treatment of erectile dysfunction: which treatment for which patient? *Drugs*, *65*, 1621-1650. https://doi.org/https://doi.org/10.2165/00003495-200565120-00003

Hatzimouratidis, K., Salonia, A., Adaikan, G., Buvat, J., Carrier, S., El-Meliegy, A., McCullough, A., Torres, L. O., & Khera, M. (2016). Pharmacotherapy for erectile dysfunction: recommendations from the Fourth International Consultation for Sexual Medicine (ICSM 2015).

The journal of sexual medicine, *13*(4), 465-488. https://doi.org/https://doi.org/10.1016/j.jsxm.2016.01.016

Hollenberg, P. F. (2002). Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug metabolism reviews*, *34*(1-2), 17-35. https://doi.org/https://doi.org/10.1081/DMR-120001387

Huang, S. M., Strong, J. M., Zhang, L., Reynolds, K. S., Nallani, S., Temple, R., Abraham, S., Habet, S. A., Baweja, R. K., & Burckart, G. J. (2008). New era in drug interaction evaluation: US Food and Drug Administration update on CYP enzymes, transporters, and the guidance process. *The Journal of clinical pharmacology*, *48*(6), 662-670. https://doi.org/https://doi.org/10.1177/0091270007312153

Jarow, J. P., Nana-Sinkam, P., Sabbagh, M., & Eskew, A. (1996). Outcome analysis of goal directed therapy for impotence. *The Journal of urology*, *155*(5), 1609-1612. https://doi.org/https://doi.org/10.1016/S0022-5347(01)66142-1

Jones, P., Sandoval Barba, H., Johnson, M. I., Soomro, N., Robson, W., Ferguson, J., & Aning, J. J. (2021). Erectile dysfunction after robotic radical prostatectomy: real-life impact of vacuum erection device clinic. *Journal of Clinical Urology*, *14*(5), 325-331. https://doi.org/https://doi.org/10.1177/205141582094663

Kalsi, J., & Kell, P. (2004). Update on oral treatments for male erectile dysfunction. *Journal of the European Academy of Dermatology and Venereology*, *18*(3), 267-274. https://doi.org/https://doi.org/10.1111/j.1468-3083.2004.00885.x

Karakus, S., & Burnett, A. L. (2020). The medical and surgical treatment of erectile dysfunction: a review and update. *Can J Urol*, *27*(S3), 28-35.

Kirchmair, J., Göller, A. H., Lang, D., Kunze, J., Testa, B., Wilson, I. D., Glen, R. C., & Schneider, G. (2015). Predicting drug metabolism: experiment and/or computation? *Nature reviews Drug discovery*, *14*(6), 387-404. https://doi.org/https://doi.org/10.1038/nrd4581

Khayyamfar, F., Foroutan, S. K., Farhadi, E., & Ghasemlouei, A. (2021). The impact of vacuum constrictive device on the treatment of erectile dysfunction in spinal cord injured patients. *Translational Research in Urology*, *3*(4), 170-175. https://doi.org/https://doi.org/10.22034/TRU.2021.314753.1087

Langarizadeh, M. A., Salary, A., Tavakoli, M. R., Nejad, B. G., Fadaei, S., Jahani, Z., & Forootanfar, H. (2023). An overview of the history, current strategies, and potential future treatment approaches in erectile dysfunction: a comprehensive review. *Sexual Medicine Reviews*, qead014. https://doi.org/https://doi.org/10.1093/sxmrev/qead014

Lewis, R. (2004). Definition, classification, and epidemiology of sexual dysfunction. *Sexual dysfunction*, 3-37.

Lin, G., Banie, L., Ning, H., Bella, A. J., Lin, C. S., & Lue, T. F. (2009). Potential of adipose derived stem cells for treatment of erectile dysfunction. *The journal of sexual medicine*, *6*(Supplement_3), 320-327. https://doi.org/https://doi.org/10.1111/j.1743-6109.2008.01190.x

Lin, G., Yang, R., Banie, L., Wang, G., Ning, H., Li, L. C., Lue, T. F., & Lin, C. S. (2010). Effects of transplantation of adipose tissue derived stem cells on prostate tumor. *The Prostate*, *70*(10), 1066-1073. https://doi.org/https://doi.org/10.1002/pros.21140

Lue, T. F., Giuliano, F., Montorsi, F., Rosen, R. C., Andersson, K. E., Althof, S., Christ, G., Hatzichristou, D., Hirsch, M., & Kimoto, Y. (2004). Summary of the recommendations on sexual dysfunctions in men. *The journal of sexual medicine*, *1*(1), 6-23. https://doi.org/https://doi.org/10.1111/j.1743-6109.2004.10104.x

Mangner, N., Linke, A., Oberbach, A., Kullnick, Y., Gielen, S., Sandri, M., Hoellriegel, R., Matsumoto, Y., Schuler, G., & Adams, V. (2013). Exercise training prevents TNF- induced loss of force in the diaphragm of mice. *PLoS One*, *8*(1), e52274. https://doi.org/https://doi.org/10.1371/journal.pone.0052274

McCabe, M. P., Sharlip, I. D., Lewis, R., Atalla, E., Balon, R., Fisher, A. D., Laumann, E., Lee, S. W., & Segraves, R. T. (2016). Incidence and prevalence of sexual dysfunction in women and men: a consensus statement from the Fourth International Consultation on Sexual Medicine 2015. *The journal of sexual medicine*, *13*(2), 144-152. https://doi.org/https://doi.org/10.1016/j.jsxm.2015.12.034

Mukherjee, A., Su, A., & Rajan, K. (2021). Deep learning model for identifying critical structural motifs in potential endocrine disruptors. *Journal of chemical information and modeling*, *61*(5), 2187-2197. https://doi.org/https://doi.org/10.1021/acs.jcim.0c01409

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of cheminformatics*, *3*(1), 1-14. https://doi.org/https://doi.org/10.1186/1758-2946-3-33

Padma-Nathan, H., & Giuliano, F. (2001). Oral drug therapy for erectile dysfunction. *Urologic Clinics of North America*, *28*(2), 321-334. https://doi.org/https://doi.org/10.1016/S0094-0143(05)70141-5

Peng, Z., Yang, L., Dong, Q., Wei, Q., Liu, L., & Yang, B. (2017). Efficacy and safety of tadalafil once-a-day versus tadalafil on-demand in patients with erectile dysfunction: a systematic review and meta-analyses. *Urologia Internationalis*, *99*(3), 343-352. https://doi.org/https://doi.org/10.1159/000477496

Porst, H., Burnett, A., Brock, G., Ghanem, H., Giuliano, F., Glina, S., Hellstrom, W., Martin-Morales, A., Salonia, A., & Sharlip, I. (2013). SOP conservative (medical and mechanical) treatment of erectile dysfunction. *The journal of sexual medicine*, *10*(1), 130-171. https://doi.org/https://doi.org/10.1111/jsm.12023

Prockop, D. (2007). "Stemness" does not explain the repair of many tissues by mesenchymal stem/multipotent stromal cells (MSCs). *Clinical Pharmacology & Therapeutics*, *82*(3), 241-243. https://doi.org/https://doi.org/10.1038/sj.clpt.6100313

Protogerou, V., Chrysikos, D., Karampelias, V., Spanidis, Y., El Bisari, S., & Troupis, T. (2021). Erectile dysfunction treatment using stem cells: a review. *Medicines*, *8*(1), 2. https://doi.org/https://doi.org/10.3390/medicines8010002

Rezaee, M. E., Ward, C. E., Brandes, E. R., Munarriz, R. M., & Gross, M. S. (2020). A review of economic evaluations of erectile dysfunction therapies. *Sexual Medicine Reviews*, *8*(3), 497-503. https://doi.org/https://doi.org/10.1016/j.sxmr.2019.06.001

Ryu, J. K., Kim, D. H., Song, K. M., Ryu, D. S., Kim, S. N., Shin, D. H., Yi, T., Suh, J. K., & Song, S. (2016). Intracavernous delivery of clonal mesenchymal stem cells rescues erectile function in the streptozotocin induced diabetic mouse. *Andrology*, *4*(1), 172-184. https://doi.org/https://doi.org/10.1111/andr.12138

Ryu, J. K., Kim, D. H., Song, K. M., Yi, T., Suh, J. K., & Song, S. U. (2014). Intracavernous delivery of clonal mesenchymal stem cells restores erectile function in a mouse model of cavernous nerve injury. *The Journal of Sexual Medicine*, *11*(2), 411-423. https://doi.org/https://doi.org/10.1111/jsm.12380

Shamloul, R., & Ghanem, H. (2013). Erectile dysfunction. *The Lancet*, *381*(9861), 153-165. https://doi.org/https://doi.org/10.1016/S0140-6736(12)60520-0

Singh, J. C., Devasia, A., Gnanaraj, L., & Chacko, K. N. (2005). Erectile dysfunction. *National Medical Journal of India*, *18*(3), 139.

Sultana, A., Grice, P., Vukina, J., Pearce, I., & Modgil, V. (2022). Indications and characteristics of penile traction and vacuum erection devices. *Nature Reviews Urology*, *19*(2), 84-100. https://doi.org/https://doi.org/10.1038/s41585-021-00532-7

Sun, C., Lin, H., Yu, W., Li, X., Chen, Y., Qiu, X., Wang, R., & Dai, Y. (2012). Neurotrophic effect of bone marrow mesenchymal stem cells for erectile dysfunction in diabetic rats. *International journal of andrology*, *35*(4), 601-607. https://doi.org/https://doi.org/10.1111/j.1365-2605.2012.01250.x

Testa, B., & Kraemer, S. (2007). The biochemistry of drug metabolism–An introduction-Testa-2007-chemistry & biodiversity-wiley online library. *Chem. Biodivers*, *4*(3).

Tzoumas, N., Farrah, T. E., Dhaun, N., & Webb, D. J. (2020). Established and emerging therapeutic uses of PDE type 5 inhibitors in cardiovascular disease. *British Journal of Pharmacology*, *177*(24), 5467-5488. https://doi.org/https://doi.org/10.1111/bph.14920

Uccelli, A., Moretta, L., & Pistoia, V. (2008). Mesenchymal stem cells in health and disease. *Nature reviews immunology*, *8*(9), 726-736. https://doi.org/https://doi.org/10.1038/nri2395

van Waterschoot, R. A., & Schinkel, A. H. (2011). A critical analysis of the interplay between cytochrome P450 3A and P-glycoprotein: recent insights from knockout and transgenic mice. *Pharmacological reviews*, *63*(2), 390-410. https://doi.org/https://doi.org/10.1124/pr.110.002584

Wolf, C. R., Smith, G., & Smith, R. L. (2000). Science, medicine, and the future-Pharmacogenetics. *British Medical Journal*, *320*(7240), 987-990.

Wrishko, R., Sorsaburu, S., Wong, D., Strawbridge, A., & McGill, J. (2009). Safety, efficacy, and pharmacokinetic overview of low-dose daily administration of tadalafil. *The journal of sexual medicine*, *6*(7), 2039-2048. https://doi.org/https://doi.org/10.1111/j.1743-6109.2009.01301.x

You, D., Jang, M. J., Lee, J., Jeong, I. G., Kim, H. S., Moon, K. H., Suh, N., & Kim, C.-S. (2013). Periprostatic implantation of human bone marrow-derived mesenchymal stem cells potentiates recovery of erectile function by intracavernosal injection in a rat model of cavernous nerve injury. *Urology*, *81*(1), 104-110. https://doi.org/https://doi.org/10.1016/j.urology.2012.08.046

You, D., Jang, M. J., Lee, J., Suh, N., Jeong, I. G., Sohn, D. W., Kim, S. W., Ahn, T. Y., & Kim, C. S. (2013). Comparative analysis of periprostatic implantation and intracavernosal injection of human adipose tissue derived stem cells for erectile function recovery in a rat model of cavernous nerve injury. *The Prostate*, *73*(3), 278-286. https://doi.org/https://doi.org/10.1002/pros.22567

Zanger, U. M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & therapeutics*, *138*(1), 103-141. https://doi.org/https://doi.org/10.1016/j.pharmthera.2012.12.007