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

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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

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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 (Casiiraghi 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 (Ciocan et al., 2019; Protopogerou 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 errectogenic 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](image-url)
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**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Docking score (kcal/mol)</th>
<th>Hydrogen bond</th>
<th>Hydrophobic interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbralisib</td>
<td><img src="image1" alt="Structure" /></td>
<td>-13.53</td>
<td>Ser663, Phe820</td>
<td>Val660, Arg667, Ile665, Ile824, Phe820, Leu804, Phe786, Leu725</td>
</tr>
<tr>
<td>Amentoflavone</td>
<td><img src="image2" alt="Structure" /></td>
<td>-13.49</td>
<td>Phe820</td>
<td>Met816, Ile824, Gly819, Ala823, Phe820, Val782, Leu725</td>
</tr>
<tr>
<td>Nicotiflorin</td>
<td><img src="image3" alt="Structure" /></td>
<td>-11.97</td>
<td></td>
<td>Ala767, Val782, Phe820, Tyr612</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Docking score (kcal/mol)</td>
<td>Hydrogen bond</td>
<td>Hydrophobic interaction</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------</td>
<td>--------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Eriocitrin</td>
<td></td>
<td>-11.38</td>
<td>Glu682, Arg667, Asp724, His613, His657, Asp654, Asp764, Leu765, Ile768, Gln817, Thr723</td>
<td>Leu725, Ile768, Gln775, Val782</td>
</tr>
<tr>
<td>Narirutin</td>
<td></td>
<td>-11.35</td>
<td>Asp724, Asp654, Thr723</td>
<td>Leu725, Ile768, Phe820, Tyr612, Gln775, Ala767, Val782</td>
</tr>
<tr>
<td>Rutin</td>
<td></td>
<td>-11.25</td>
<td>Gln775, Leu765, Thr723, Asn661</td>
<td>Ala767, Tyr612, Val782, Phe820, Phe786, Leu804</td>
</tr>
<tr>
<td>Kaempferitrin</td>
<td></td>
<td>-11.21</td>
<td>Asn662, His657</td>
<td>Arg667</td>
</tr>
<tr>
<td>Compound</td>
<td>Structure</td>
<td>Docking score (kcal/mol)</td>
<td>Hydrogen bond</td>
<td>Hydrophobic interaction</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
<td>--------------------------</td>
<td>--------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Rhoifolin</td>
<td><img src="image1" alt="Structure" /></td>
<td>-11.13</td>
<td>Tyr612, Asp764, His617, Leu725, Glu682, Thr723</td>
<td>Val782, Phe820, Phe786, Leu765</td>
</tr>
<tr>
<td>Rutin hydrate</td>
<td><img src="image2" alt="Structure" /></td>
<td>-11.12</td>
<td>Gln775, Tyr612, Asp654, His685, Asn661</td>
<td>Ala767, Val782, Phe820, Phe786, Leu804</td>
</tr>
<tr>
<td>Ligustroflavone</td>
<td><img src="image3" alt="Structure" /></td>
<td>-11.06</td>
<td>Met816, Asn662, Leu725, Thr723, Asp654</td>
<td>Val782, Ala783, Phe820, Ile813, Phe786, Ile665, Ile824, Met816</td>
</tr>
<tr>
<td>Naringin</td>
<td><img src="image4" alt="Structure" /></td>
<td>-11.00</td>
<td>Tyr612, Asp654, Thr723</td>
<td>Leu765, Val782, Phe820, Phe786, Leu725</td>
</tr>
</tbody>
</table>

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 Å, 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 Umbraisib 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight (g/mol)</th>
<th>Log P</th>
<th>nHBD</th>
<th>nHBA</th>
<th>TPSA (Å²)</th>
<th>MR</th>
<th>Lipinski Violation</th>
<th>Log Kp (cm/s)</th>
<th>Log S</th>
<th>nRotB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbraisib</td>
<td>571.55</td>
<td>5.80</td>
<td>1</td>
<td>9</td>
<td>109.06</td>
<td>153.18</td>
<td>1</td>
<td>-5.65</td>
<td>-7.21</td>
<td>6</td>
</tr>
<tr>
<td>Amentoflavone</td>
<td>538.46</td>
<td>3.62</td>
<td>6</td>
<td>10</td>
<td>181.80</td>
<td>146.97</td>
<td>2</td>
<td>-6.01</td>
<td>-6.75</td>
<td>3</td>
</tr>
<tr>
<td>Nicotiflorin</td>
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<td>-0.73</td>
<td>9</td>
<td>15</td>
<td>249.20</td>
<td>139.36</td>
<td>3</td>
<td>-9.91</td>
<td>-3.42</td>
<td>6</td>
</tr>
<tr>
<td>Eriocitrin</td>
<td>596.53</td>
<td>-1.30</td>
<td>9</td>
<td>15</td>
<td>245.29</td>
<td>136.94</td>
<td>3</td>
<td>-10.90</td>
<td>-2.50</td>
<td>6</td>
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<tr>
<td>Narirutin</td>
<td>580.53</td>
<td>-0.95</td>
<td>8</td>
<td>14</td>
<td>225.06</td>
<td>134.91</td>
<td>3</td>
<td>-10.54</td>
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<tr>
<td>Rutin</td>
<td>610.52</td>
<td>-1.29</td>
<td>10</td>
<td>16</td>
<td>269.43</td>
<td>141.38</td>
<td>3</td>
<td>-10.26</td>
<td>-3.30</td>
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<tr>
<td>Kaempferitin</td>
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<td>-0.53</td>
<td>8</td>
<td>14</td>
<td>228.97</td>
<td>137.93</td>
<td>3</td>
<td>-9.89</td>
<td>-3.33</td>
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<tr>
<td>Rheofolin</td>
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<td>-0.66</td>
<td>8</td>
<td>14</td>
<td>228.97</td>
<td>137.33</td>
<td>3</td>
<td>-9.94</td>
<td>-3.22</td>
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<tr>
<td>Rutin hydrate</td>
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<td>-1.52</td>
<td>11</td>
<td>17</td>
<td>278.66</td>
<td>144.43</td>
<td>3</td>
<td>-10.71</td>
<td>-3.10</td>
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<tr>
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<td>-2.01</td>
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<td>-3.45</td>
<td>8</td>
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<tr>
<td>Naringin</td>
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<td>8</td>
<td>14</td>
<td>225.06</td>
<td>134.91</td>
<td>3</td>
<td>-10.15</td>
<td>-2.98</td>
<td>6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Log Kp (cm/s)</th>
<th>GI Abs</th>
<th>BBB Per</th>
<th>P-gp Substrate</th>
<th>CYP1A2 Inhibitor</th>
<th>CYP2C19 Inhibitor</th>
<th>CYP2C9 Inhibitor</th>
<th>CYP2D6 Inhibitor</th>
<th>CYP3A4 Inhibitor</th>
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</thead>
<tbody>
<tr>
<td>Umbilisib</td>
<td>-5.65</td>
<td>Low</td>
<td>No</td>
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<td>No</td>
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<td>No</td>
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<td>Nicotiflorin</td>
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<td>No</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Eriocitrin</td>
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<td>No</td>
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<td>No</td>
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<td>No</td>
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<td>No</td>
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<tr>
<td>Rutin hydrate</td>
<td>-10.71</td>
<td>Low</td>
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<td>No</td>
<td>No</td>
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<td>No</td>
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<td>Ligustroflavone</td>
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<td>Yes</td>
<td>No</td>
<td>No</td>
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</tbody>
</table>

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, Umbilisib, Eriocitrin, and Narirutin are all classified as “warning”, indicating they are predicted to be toxic to normal cells. The remaining compounds, including Nicotiflorin, Rutin, Kaempferitin, 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

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

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


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-


