In Silico Study of the 5-Hydroxytryptamine-2C Receptor Antagonist Activity of Anthocyanins as Antidepressant Therapy

Nia Kurnianingsih\textsuperscript{a,c,*}, Novita Titis Harbianti\textsuperscript{a}, Ardani Galih Prakosa\textsuperscript{a}, Retty Ratnawati\textsuperscript{a}, Fatchiyah Fatchiyah\textsuperscript{b,c}

\textsuperscript{a} Department of Physiology, Faculty of Medicine, Universitas Brawijaya, 65145 Malang, East Java, Indonesia
\textsuperscript{b} Department of Biology, Faculty of Mathematic and Natural Science, Universitas Brawijaya, 65145 Malang, East Java, Indonesia
\textsuperscript{c} Research Center of Smart Molecule of Natural Genetic Resources, Universitas Brawijaya, 65145 Malang, East Java, Indonesia

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\*Corresponding author
nia_fkub@ub.ac.id

\textbf{Abstract}
This study aimed to evaluate the drug-likeness, pharmacokinetic and safety prediction of six types of anthocyanins (ANC) as well as virtual molecular interaction between ANC and 5-hydroxytryptamine-2C (5HT-2C) receptor for antagonist target of antidepressant drug development. The Lipinski rule of five was used to predict the oral drug-likeness of ANC. The pharmacokinetic and safety prediction was analyzed with a free accessible web server. The ligands of ANC were retrieved from PubChem National Centre for Biotechnology Information (NCBI) database. The protein of the 5HT-2C receptor was obtained from Protein Data Bank. Molecular docking was performed by PyRx software and visualized using Discovery Studio Software. The results showed ANC is proposed as an oral drug candidate. The pharmacokinetic prediction of ANC was demonstrated to have high absorption in the intestinal route, solubility in the aqueous phase, capability to evade hepatic first-pass metabolism and high total clearance from the kidney. Virtual toxicity prediction showed a higher threshold of chronic lethal dose than control with no toxicity on the salmonella typhimurium reverses mutation assay (AMES) test, liver, and skin. Molecular prediction found ANC type of delphinidin has the most similar interaction site with the control antagonist ligand on the 5HT-2C receptor which is facilitated with hydrogen bonds and hydrophobic bonds at amino residues of Trp324, Phe328, Ala222 and Val135. We concluded ANC particularly delphinidin is proposed as an oral drug candidate potentially used as a 5HT-2C receptor antagonist and thus, further in vitro and in vivo studies are necessary to confirm the effect on antidepressant activity.

\textbf{Keywords} Anthocyanin, antidepressant, in-silico, serotonin receptor

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1.0 INTRODUCTION
Anthocyanin (ANC) is an interesting polyphenol derivative which is abundant in various colours of exotic plants. ANC provides red to blue colour among plants such as red cabbage, purple corn, elderberry, purple sweet potatoes, red berries, purple berries, grapes, and apples [1]. ANC has six basic structures specifically cyanidin, pelargonidin, petunidin, delphinidin, and malvidin. The structures differ in the position of glycosidic bond binding, number of hydroxyl groups, type of sugar, number of sugar, and type of aromatic acid [2]. Health benefits of ANC include antioxidant, anti-cancer, anti-angiogenesis, cardiovascular health,
visual health, anti-diabetes, anti-obesity, anti-microbial and neuroprotective effect [3]. An initial study reported that the ANC works in brain tissues and resists the effect of psychological stress [4]. Anthocyanins reached brain regions following oral administration and give an improvement effect on neurotransmitter status as well as behavioural changes [5,6]. This correlates with a previous study which reveals the influence of ANC from purple sweet potatoes on the depressive behaviour of prenatally stressed offspring mice. However, the behavioural effect's molecular pathway remains uncertain and needs to be evaluated [7].

The serotonergic neurotransmission pathway is strongly correlated with depression disorder. The inhibition of the serotonin re-uptake process is well established as a molecular target of antidepressants. Serotonin has several subtypes of receptors including the 5-hydroxytryptamine-2C (5HT-2C) subtype. This receptor differs from other subtypes according to its preferential location in gamma-aminobutyric acid (GABA) interneurons instead of in serotonergic neurons [8]. The inhibition of the 5HT-2C receptor provides an antidepressant effect as well as an anxiolytic. The administration of psychoactive drugs that acts as selective antagonist to the 5HT-2C receptor was reported to possess several behavioural benefits [9,10]. However, circular challenges limit the therapeutic efficacy of antidepressants, for instance, long onset of action, low remission rate, drug dropout rate and drug tolerance. The limitations are a point of attention which should be considered [11].

Therefore, the exploration of new antidepressant drug therapy dramatically increases the utilization of plant bioactive sources through several pathways such as monoamine neurotransmitter upregulation, binding to serotonergic receptors, reducing inflammation, increasing antioxidant, and relieving the oxidative stress state [12]. Recently, the drug development process involved computer-aid drug design which is crucial and cost-effective to predict the rational design of animal research. In silico approaches have been established as promising methods to predict the pharmacokinetics, metabolism, and toxicity of drug candidates [13]. Thus, this study performed in silico analysis of quantitative structure-activity relationship prediction of ANC as an antidepressant drug based on drug-likeness, pharmacokinetics, safety, as well as molecular docking interaction prediction between ANC and 5HT-2C receptor. This study would support the prediction pathway of ANC in behavioural advantages, particularly depressive behaviour.

2.0 EXPERIMENTAL

2.1 The drug-likeness prediction of anthocyanins

Since human gastric has low pH, this study evaluated all six aglycones of basic ANC structure which are stable at lower pH [14]. The physicochemical scores of ANC were performed using Molinspiration, a web-based software, (https://www.molinspiration.com) [15]. The Rule of Five by Lipinski et al. [16] was used for determining the potential oral absorption of cyanidin as a drug candidate, based on molecular weight, number of hydrogen bond donors, hydrogen bond acceptors and logP. The drug-likeness prediction of ANC was then compared to the 5HT-2C antagonist control ligand, SB-242084 [9]. The administration of selective antagonist 5HT-2C receptor, i.e., SB-242084, possessed several behavioural benefits on animal models [9,10].

2.2 The pharmacokinetic properties and safety analysis of Anthocyanins

The profiling of absorption, distribution, metabolism, and excretion of anthocyanins was carried out using a free accessible web server, pkCSM (http://biosig.unimelb.edu.au/pkcsms) [17]. The absorption was predicted by the percentage of human intestinal absorption. The percentage of <30% was considered to be poorly absorbed. The volume distribution was classified into low distribution if below 0.71 L/kg (log VDss < 0.15) and high if above 2.81 L/kg (log VDss > 0.45. The hepatic metabolism was classified according to inhibitory properties against enzymes CYP2D6 and CYP3A4. The clearance of anthocyanins was predicted as total clearance. The AMES toxicity, hepatic toxicity, skin sensitization, lethal dose 50 (LD50), and log lowest observed adverse effect (LOAEL) were used for predicting the safety of anthocyanins [18]. The profile of pharmacokinetics and toxicity was compared to SB-242084 as a 5HT-2C antagonist [9].

2.3 In silico molecular interaction

The ligand of cyanidin (CID: 128861), delphinidin (CID: 128853), malvidin (CID: 159287), pelargonidin (CID: 440832), peonidin (CID: 441773), petunidin (CID: 441774) and native ligand SB-242084 (CID: 3644637) were elected from PubChem National Centre for Biotechnology Information (NCBI) database [19], [20]. The ligand is converted into pdb using PyRx 0.8 software [7]. The protein of the 5HT-2C receptor (PDB: 6bqh) was retrieved from Protein Data Bank (PDB) (http://rcsb.org). The associated molecule on protein structure was removed using Discovery Studio Visualizer v19.1.0.18287 program [7]. Molecular interaction prediction was performed as flexible docking using PyRx 0.8 software and then visualized using Discovery Studio Visualizer v19.1.0.18287 program [21]. The energy binding, type of binding interaction and distance interaction was recorded for docking parameters [22]. Docking validation was carried out using redocking between the 5HT-2C receptor and the control ligand of SB-242084. The root means square deviation (RMSD) was then explored for further validation. The point of RMSD ≤ 2 Å was set as validation criteria for the test docking compound [23].
3.0 RESULTS AND DISCUSSION

The rule of five (RO5) was used to predict the absorption and permeability of ANC as an oral drug candidate. Better absorption and permeability were more likely if the compound has molecular weight < 500, hydrogen bond acceptors < 10, hydrogen bond donors < 5 and the logP < 5. Table 1 shows that most of the ANC meet the rule’s requirement of Lipinski’s rule of five. Therefore, ANC is predicted to have a good permeability to perform biological activities via oral route administration. Anthocyanins have a lower molecular weight than SB-242084 as 5HT-2C receptor antagonists. Anthocyanins have the logP negative value instead of SB-242084, which means that the ANC has a higher affinity at the aqueous phase compared to the lipid phase.

The pharmacokinetic prediction was carried out to evaluate the absorption, metabolism, distribution, and excretion of anthocyanins. The absorption in the human intestine is considered relatively low when the percentage was reported to be less than 30%. Anthocyanins as well as SB-242084 were suggested to have good absorption in the human intestine because the percentage of intestinal absorption was more than 70% (Table 2). The intestinal absorption of ANC was lower than SB-242084. In line with previous studies, ANC was transported through the monolayer human colon Caco-2 cell line as intact aglycone form [24]. The absorption of ANC in Caco-2 cells was enhanced by adding phospholipids that form the ANC-phospholipid complex [25]. Another study was performed concerning intragastric cannulation of ANC in anaesthetized rats. Anthocyanin was rapidly absorbed into the gastric wall. It might correlate to the stability of ANC at low pH [3,26]. However, the ANC aglycone remained efficiently absorbed in the intestine. It probably was due to their water solubility [27]. As flavonoids derivate, the mechanism of ANC absorption has remained debatable. ANC is possibly absorbed using an active transport mechanism through the intestinal epithelial cells, such as glucose transporter-2 (GLUT-2) and Na+/glucose cotransporter 1 (SGLT1) [27]. Among the drug deliveries, an oral pathway was considered as having many potential benefits, including easier administration and patient compliance [28]. The current study provides a promising prospect for ANC to be developed into oral drug candidates.

Table 1. Comparison of physicochemical prediction of ANC with SB-242084 (5HT-2C receptor antagonist) analyzed using Molinspiration (www.molinspiration.com) [29]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Weight</th>
<th>Hydrogen Bond Acceptor (nON)</th>
<th>Hydrogen Bond Donors (nONH)</th>
<th>logP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvidin</td>
<td>331.30</td>
<td>7</td>
<td>4</td>
<td>-0.42</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>303.24</td>
<td>7</td>
<td>6</td>
<td>-1.04</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>271.24</td>
<td>5</td>
<td>4</td>
<td>-0.26</td>
</tr>
<tr>
<td>Petunidin</td>
<td>317.27</td>
<td>7</td>
<td>5</td>
<td>-0.72</td>
</tr>
<tr>
<td>Peonidin</td>
<td>301.27</td>
<td>6</td>
<td>4</td>
<td>-0.44</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>287.24</td>
<td>6</td>
<td>5</td>
<td>-0.75</td>
</tr>
<tr>
<td>SB-242084</td>
<td>394.86</td>
<td>6</td>
<td>1</td>
<td>4.59</td>
</tr>
</tbody>
</table>

We have also compared ANC with SB-242084 as 5HT-2C antagonist drugs. ANC was more soluble in an aqueous solution than in SB-242084. The positive value of logP of SB-242084 indicated the solubility in the lipid phase. This study is in line with previous work reported that exhibits the effectiveness of SB-242084 both in intraperitoneal and intramuscular administration in rats and squirrel monkey, respectively [10,25]. Despite ANC being more soluble in the water phase, it can penetrate the brain, including the cortex and cerebellum [5], and provide antioxidant effects to several brain areas of psychological stress model mice [4].

Table 2. Comparison of computed pharmacokinetics anthocyanins with SB-242084 (5HT-2C receptor antagonist) analyzed using ADMET (http://biosig.unimelb.edu.au/pksc/prediction)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Intestinal absorption (human) (% absorbed)</th>
<th>VDss (human) (log L/kg)</th>
<th>CYP2D6 inhibitor</th>
<th>CYP3A4 inhibitor</th>
<th>Total clearance (log ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvidin</td>
<td>88.79</td>
<td>0.76</td>
<td>No</td>
<td>No</td>
<td>0.69</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>77.39</td>
<td>0.86</td>
<td>No</td>
<td>No</td>
<td>0.58</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>87.29</td>
<td>0.65</td>
<td>No</td>
<td>No</td>
<td>0.58</td>
</tr>
<tr>
<td>Petunidin</td>
<td>84.43</td>
<td>0.84</td>
<td>No</td>
<td>No</td>
<td>0.65</td>
</tr>
<tr>
<td>Peonidin</td>
<td>89.20</td>
<td>0.56</td>
<td>No</td>
<td>No</td>
<td>0.63</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>87.30</td>
<td>0.95</td>
<td>No</td>
<td>No</td>
<td>0.53</td>
</tr>
<tr>
<td>SB-242084</td>
<td>93.17</td>
<td>-0.015</td>
<td>No</td>
<td>No</td>
<td>-0.024</td>
</tr>
</tbody>
</table>
The distribution of ANC in various tissues was characterized by the volume of distribution (VDss). The distribution is considered relatively low when the VDss is lower than 0.71 L kg\(^{-1}\) (log VDss < -0.15), otherwise higher when the VDss is more than 2.81 L kg\(^{-1}\) (log VDss > 0.45). The results showed that ANC has a moderate volume of distribution which is larger than SB-242084. The ADMET prediction revealed that ANC was not assigned as CYP2D6 and CYP3A4 inhibitors. The CYP2D6 and CYP3A4 were the subtypes of cytochrome 450, which are enzymes for drug metabolism in the liver [30]. This suggested that ANC might be metabolized in the liver, as well as in SB-242084. The total clearance of ANC was higher than SB-242084. Among six ANC, malvidin was the highest total clearance, followed by petunidin, peonidin, delphinidin, pelargonidin and cyanidin. The total clearance was a combination of hepatic clearance and kidney clearance (excretion via kidney). It is important for determining the half-life of the drug candidate for achieving bioavailability [31].

Table 3 indicates ANC has no AMES toxicity, hepatotoxicity as well as skin sensitization. Meanwhile, SB-242084 as a 5HT-2C antagonist has positive hepatotoxicity. The concentration of acute toxicity was determined using lethal dose 50 (LD50). The lethal dose that caused the death of 50% of the group animal ranged between 2.35 and 2.55 log mg/kgBW/day. Anthocyanins have lower LD50 than SB-242084. The oral chronic toxicity was determined using Log Lowest Observed Adverse Effect (LOAEL). The observable adverse effects dose at chronic administration of ANC were predicted larger than marketed SB-242084.

Table 3. Comparison of computed safety ANC with SB-242084 (5HT-2C receptor antagonist) analyzed using ADMET (http://biosig.unimelb.edu.au/pksc/prediction) [21]

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Ames toxicity</th>
<th>LD50 (mol/kg)</th>
<th>LOAEL (log mg/kg_BW/day)</th>
<th>Hepatotoxicity</th>
<th>Skin sensitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malvidine</td>
<td>No</td>
<td>2.35</td>
<td>2.41</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>No</td>
<td>2.55</td>
<td>2.93</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Pelargonidine</td>
<td>No</td>
<td>2.43</td>
<td>2.46</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Petunidin</td>
<td>No</td>
<td>2.46</td>
<td>2.45</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Peonidin</td>
<td>No</td>
<td>2.41</td>
<td>2.43</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>No</td>
<td>2.68</td>
<td>1.14</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>SB-242084</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition to the pharmacokinetic profile, drug safety is essential to be evaluated [32]. The safety prediction of ANC is shown in that the ANC was safer than SB-242084 based on the hepatotoxicity effect. The AMES toxicity prediction was a bacterial-based mutagenicity test. This test detects the capability of the substrate as a carcinogenic agent [33]. On the other hand, Yoshimoto et al. [34] reported that ANC particularly from purple-fleshed potatoes has an antimutagenic effect. As for the beneficial value of ANC, it is necessary to perform further investigations to predict the effect of ANC on the target molecule.

We performed a computerized analysis to evaluate the interaction between ANC and the 5HT-2C receptor. Cyanidin has the most negative binding affinity followed by pelargonidin, delphinidin, petunidin, peonidin and malvidin (Table 4). A more negative score on energy binding affinity can predict stronger binding interaction on the target molecule [20]. However, delphinidin has the most similar interaction site with SB-242084 on the 5HT-2C receptor. Those interactions were facilitated with 4 hydrogens bonds and 8 hydrophobic bonds. The distance of the hydrogen bond between delphinidin and the 5HT-2C receptor was closer than that between SB-242084 and 5HT-2C receptor. Regarding the SB-242084, only two hydrogen bonds on the 5HT-2C receptor were found. Therefore, delphinidin is suggested to have a stronger interaction with the 5HT-2C receptor. Seven amino residues were found as similar interaction sites in both delphinidin and SB-242084 on the 5HT-2C receptor that are Ala222, Trp324 (3 bonds), Phe328 (2 bonds), Phe328 and Val135 on the 5HT-2C receptor (Figure 1). Even though cyanidin has the highest affinity on the 5HT-2C receptor, the interaction is only supported by 1 hydrogen bond and 5 hydrophobic bonds. The interaction between protein and ligand is performed by neighbouring interaction at amino residues. The greater number of amino residue bonds would stabilise the interaction [35].

The 5HT-2C receptor showed interesting pharmacology properties, since their ability to interact with various psychoactive drugs involving antidepressant, antipsychotic, and anxiolytic [36]. The brain expression of the 5HT-2C receptor is projected on a corticolimbic pathway, hypothalamus, hippocampus, and striatum [37]. The activation of this receptor was leading to depressed mood, increase anxiety, memory interference, and reduce locomotor and appetite [38,39,40]. Those behavioural effects are related to the inhibition of dopaminergic firing and release following 5HT-2C receptor stimulation by an agonist agent [36].
**Figure 1** The molecular interaction prediction between anthocyanin and 5HT-2C receptor. The left panel shows the 3D structure interaction between ligand and receptor molecule. Ligand interaction is detailed in the box panel. The type of bond interaction is shown in the 2D structure in the right panel of each interaction. The colours green, light green, red, light purple, and dark purple visualize conventional hydrogen bonds, carbon-hydrogen bonds, unfavourable donor-donor, alkyl and Pi-Pi-shaped respectively.

**Table 4.** The molecular docking score and interaction of the 5HT-2C receptor with anthocyanin as well as SB-242084 respectively analysed using Pyrx 8.0.0 software and Discovery studio software.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Docking score (kcal/mol)</th>
<th>Hydrogen bonds interaction</th>
<th>Hydrophobic bonds interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Residues (interaction type)</td>
<td>Distance (Å)</td>
</tr>
<tr>
<td>Malvidin</td>
<td>-8.1</td>
<td>Ser219(Conventional)</td>
<td>2.27</td>
</tr>
<tr>
<td>(CID: 159287)</td>
<td></td>
<td>Asn331(Conventional)</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thr139(Conventional)</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delphinidin</td>
<td>-8.4</td>
<td>Asp134(Conventional)</td>
<td>2.11</td>
</tr>
<tr>
<td>(CID: 128853)</td>
<td></td>
<td>Ala222(Conventional)</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thr139(Conventional)</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trp324(Conventional)</td>
<td>2.68</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>-8.5</td>
<td>Ser138(Conventional)</td>
<td>2.07</td>
</tr>
<tr>
<td>(CID: 440832)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Numerous antidepressants are recognized as an inhibitor of the 5HT-2C receptor and prevent negative behaviour following the application of the 5HT-2C receptor agonist [41,42]. In the opposite finding, selective antagonist SB-242084 stimulation on 5HT-2C receptor showed a favourable effect in reducing anxiety behaviour in rat animal models [42,43]. Another study demonstrated that SB-242084 stimulates locomotor activity and prevents the hyperactivity induced by citalopram. It may suggest that the 5HT-2C antagonist acts on two distinct mechanisms involving dopamine activity enhancement and inhibition of 5HT receptors [44].
Since delphinidin and SB-242084 as 5HT-2C antagonists have similar interaction sites, it is predicted that delphinidin may have a similar antagonist role on 5HT-2C. The behavioural effect of ANC was supported by previous studies as anxiolytic and anti-depressive behaviour in animal models. The proposed mechanism of the behavioural effect of ANC was through stimulation of monoamine neurotransmitter release, neuronal growth factor upregulation, dopamine release, and GABA_B receptor activation [45,46,47]. Current results are needed for further evaluation related to the application of ANC in various models of animal behaviour. Larger studies would provide better evidence of the underlying mechanisms of ANC in restoring pathological behaviour.

4.0 CONCLUSION

All six types of ANC are proposed as oral drug candidates with effective pharmacokinetics and safety usage. Among the six types of ANC, delphinidin is predicted to act as an antagonist on the 5HT-2 receptor. Therefore, potential to be developed as an antidepressant. In the future, further studies are necessary to evaluate this prediction on larger sequential in vitro and in vivo studies.

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References


