In-silico drug-likeness analysis, ADME properties, and molecular docking studies of cyanidin-3-arabinoside, pelargonidin-3-glucoside, and peonidin-3-arabinoside as natural anticancer compounds against acting receptor-like kinase 5 receptor

Hasan Kurter[a], Nazli Mert-Ozupek[b], Hulya Ellidokuz[c] and Gizem Calibasi-Kocal[d]

Background **The aim of the study was in-silico drug-likeness analysis, absorption, distribution, metabolism, and excretion (ADME) properties, and molecular docking studies of anthocyanins as natural anticancer compounds against acting receptor-like kinase 5 (ALK5) receptor. Transforming growth factor-β (TGF-β) plays an essential role in various cellular processes. Increased expression of TGF-β and its receptor TGFβR-I (i.e. ALK5) have been associated with poor prognosis in cancer patients.**

Methods **The drug-likeness activity of anthocyanins was performed using SwissADME tool. Molecular docking studies were carried out by using the Autodock Vina 1.5.6 tool.**

Results **The results revealed that cyanidin-3-arabinoside (C3A), pelargonidin-3-glucoside (P3G), and peonidin-3-arabinoside (P3A) were able to use both Lipinski's rule of five and Ghose variations. The binding energies of C3A, P3G, and P3A against ALK5 were found as −8.0, −8.3, and −8.4 kcal mol^{-1}, respectively.**

Conclusion **These selected anthocyanins have shown higher binding energies than known inhibitors to the ALK5 receptor. Further in-vitro and in-vivo studies were strongly recommended to clarify the whole mechanism.**
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[a]Department of Translational Oncology, Institute of Health Sciences, Dokuz Eylul University, [b]Department of Basic Oncology, Institute of Health Sciences, Dokuz Eylul University, [c]Department of Preventive Oncology, Institute of Oncology, Dokuz Eylul University and [d]Department of Translational Oncology, Institute of Oncology, Dokuz Eylul University, İzmir, Turkey

Correspondence to Gizem Calibasi-Kocal, PhD, Department of Translational Oncology, Institute of Oncology, Dokuz Eylul University, İzmir, Turkey
Tel: +90 232 28233; e-mail: gizem.calibasi@deu.edu.tr; gizemcalibasi@gmail.com

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Introduction

The transforming growth factor-β (TGF-β) is secreted from cells as a cytokine [1]. TGF-β plays key roles in numerous biological and cellular processes such as immune response [2], inflammation [3], extracellular matrix design and remodeling [4], proliferation [5], migration, invasion, and epithelial-mesenchymal transition (EMT) [6]. TGF-β is related to many diseases, including cancer. Although TGF-β plays a tumor-suppressor role by inducing apoptosis at the early stages of carcinogenesis, cancer cells lose tumor-suppressor gene function at the later stages of carcinogenesis [7]. Therefore, TGF-β acquires a tumor-promoting feature [8]. Besides carcinogenesis, TGF-β, well-known EMT-related cytokine, promotes EMT, and it leads to drug resistance in cancer treatment [9]. For instance, TGF-β plays a crucial role in triple negative breast cancer epirubicin resistance by adjusting EMT, stemness, and apoptosis [10]. Furthermore, TGF-β–mediated EMT has been demonstrated to cause in colorectal cancer oxaliplatin resistance [11].

TGF-β cytokines signal via transmembrane receptors including TGFβR-II and TGFβR-I [also known as acting receptor-like kinase 5 (ALK5), EC number: 2.7.11.30; receptor protein serine/threonine kinase] [12]. ALK5 exists in inactive form without ligand [12]. However, TGFβR-II is structurally active in the presence or absence of the ligand. When the binding of TGF-β to TGFβR-II, they form a ligand-receptor complex. After that, ALK5 is activated by TGFβR-II-mediated phosphorylation in the glycine-serine domain [13]. SMAD2 and SMAD3 (R-SMADs) are phosphorylated through C-terminal by activated ALK5 receptors [14]. R-SMADs connect with SMAD4, and these complexes (SMAD4-R-SMADs) translocate to the nucleus where it stimulates the TGF-β target genes [15]. All this pathway is called SMAD-dependent TGF-β signaling pathway. In addition to SMAD-dependent TGF-β signaling pathway, TGF-β receptors may also activate other intracellular pathways via phosphorylation or with critical signaling intermediates [16]. This pathway is also called 'SMAD-independent TGF-β signaling pathway' [17].

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Anthocyanins, secondary herbal metabolites, are water-soluble pigments that belong to the flavonoids subgroup, a subclass of polyphenols [18]. The red, blue, and purple-colored anthocyanins have some clinical and biotechnological properties such as antioxidant [19] and anti-inflammatory [20] properties, and show antitumorigenic effects during carcinogenesis as regulate autophagy [21]. Park *et al*. [22] demonstrated that soybean anthocyanins inhibited radiation-induced skin fibrosis by inhibiting TGF-β and SMAD3 expression. Thus, in this study, the in-silico inhibitory effect of anthocyanins on ALK5 that is a receptor within the TGF-β signaling pathway was mainly aimed. The computational tools (absorption, distribution, metabolism, and excretion [ADME], virtual screening, and molecular docking approach) were used to determine the inhibitory activity of natural compounds (anthocyanins) against ALK5 protein.

Methods

Receptor preparation

Protein receptor structure [ALK5, Protein Data Bank (PDB) ID: 1VJY, resolution: 2.00 Å] was retrieved from Research Collaboratory for Structural Bioinformatics PDB (https://www.rcsb.org/) as a 3D structure for molecular docking studies. Before docking analysis, all water molecules were removed. Polar hydrogen atoms and Gasteiger charges were added by Autodock Vina 1.5.6 tool (The Scripps Research Institute, La Jolla, California, USA: ADT 1.5.6). Then, the ALK5 structure was saved in PDBQT files for using molecular docking analysis. ALK5 inhibitors such as LY364947, SB505124, and SD208 were used as a control for the study.

Ligand preparation

The 24 most abundant anthocyanins were found in the literature. The structure of anthocyanins was retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as 3D SDF file format. The inhibitors of ALK5 (SD208, LY364947, and SB505124) also were retrieved from the PubChem database. After energy minimizing of all ligands, they were converted to PDBQT file format for molecular docking analysis by the Autodock Vina 1.5.6 tool.

Drug-likeness prediction and ADME analysis

Pharmacokinetic and pharmacodynamic features of the anthocyanins were predicted by the SwissADME (The Scripps Research Institute, La Jolla, California, USA: ADT 1.5.6) (http://www.swissadme.ch/) tool (Molecular Modelling Group of the Swiss Institute of Bioinformatics) [23]. The chemical structure (canonical SMILES format) of anthocyanins was obtained from the PubChem database. The drug likeness and ADME properties of anthocyanins were calculated by the SwissADME tool.

Molecular docking analysis

Molecular docking experiments were carried out with AutoDock Tools 1.5.2. The grid box resolution was set at 12.0034, 70.3969, and 18.1330 along x, y, and z points, respectively. The grid dimensions of all receptors were adjusted to $10 \times 13 \times 17$ for all molecular docking studies. The results of graphical representations were prepared via Maestro Schrödinger.

Results

Evaluation of drug-likeness properties

The drug-likeness feature of anthocyanins was evaluated by five different filters (Lipinski, Ghose, Veber, Egan, and Muegge). Only three anthocyanins, C3A, P3G, and P3A, were found suitable for Lipinski's rule of five (RO5) (Table 1). The molecular weights of C3A, P3G, and P3A are 419.36, 433.39, and 433.39 g mol^{-1}, respectively. The number of hydrogen donors (-OH and -NH atoms) of C3A, P3G, and P3A were found as 7, 7, and 6, respectively. The number of hydrogen acceptors (O and N atoms) of C3A, P3G, and P3A were found as 10. According to Lipinski's RO5, the number of hydrogen donors and acceptors must be 5 and 10, respectively. However, these anthocyanins violated the hydrogen donor limit in Lipinski's rule. Lipophilicities [*n*-octanol and water coefficient (MLogP)] of C3A, P3G, and P3A were found as –1.22, –1.27, and –1.00, respectively. According to Lipinski's rule, this value should be less than 4.15.

Evaluation of ADME properties

ADME properties [i.e. topological surface area (TPSA), rotatable bonds, water solubility (Log S), human intestinal absorption (HIA), blood–brain barrier (BBB) access P-gp substrates, and cytochrome enzymes (CYP)] were evaluated (Tables 2 and 3). The TPSA value should be between 20 and 130 $Å^2$ according to a colored zone of bioavailability radar (Fig. 1). The TPSA values of C3A, P3G, and P3A were determined as 173.21, 173.21, and 162.21 $Å^2$, respectively. Rotatable bonds of C3A, P3G, and P3A were determined as 3, 4, and 4, respectively. Log S (SILICOS-IT) values of C3A, P3G, and P3A were found as –1.36, –1.52, and –2.05, respectively. The boiled-egg chart is categorized into three colored areas such as yellow, white, and gray (Fig. 2). The yellow area in the boiled-egg graph represents the BBB access, the white area represents HIA, and the gray area represents compounds not showing both these properties. According to boiled-egg chart, it was found that anthocyanins do not have BBB access properties. Intestinal absorption properties were also found low. According to this figure, C3A, P3G, and P3A were not P-gp substrates. Thus, it is not excreted out of the cell via the P-gp carrier protein. However, all known ALK5 inhibitors were found as P-gp substrates. In addition, C3A, P3G, and P3A were not found as inhibitors of CYP enzymes. However, known inhibitors show inhibitory properties for most CYP enzymes.

Molecular docking analysis

The molecular docking analyses were performed between ALK5 and the P3A, C3A, P3G, and known

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Table 1 Drug-likeness properties of anthocyanins and known inhibitors by SwissADME

Compound name	Formula	MW (g/mol)	NHA	NHD	MLOGP	Lipinski's rule of five violation
C3A	$C_{20}H_{19}O_{10}$	419.36	10	7	−1.22	1
P3G	$C_{21}H_{21}O_{10}$	433.39	10	7	−1.27	1
P3A	$C_{21}H_{21}O_{10}$	433.39	10	6	−1.00	1
SD208	$C_{17}H_{10}ClFN_6$	352.75	6	1	2.02	0
LY364947	$C_{17}H_{12}N_4$	272.30	3	1	1.74	0
SB505124	$C_{20}H_{21}N_3O_2$	335.40	4	1	2.06	0

ADME, absorption, distribution, metabolism, and excretion; MW, molecular weight; NHD, number of hydrogen donors; NHA, number of hydrogen acceptors.

Table 2 ADME properties of C3A, P3G, and P3A and known inhibitors (SD208, LY364947, and SB505124)

Compound name	Formula	TPSA (Å^2)	NRB	Log S	HIA	BBB	P-gp substrate
C3A	$C_{20}H_{19}O_{10}$	173.21	3	−2.18	Low	No	No
P3G	$C_{21}H_{21}O_{10}$	173.21	4	−3.70	Low	No	No
P3A	$C_{21}H_{21}O_{10}$	162.21	4	−3.51	Low	No	No
SD208	$C_{17}H_{10}ClFN_6$	76.48	3	−4.29	High	No	Yes
LY364947	$C_{17}H_{12}N_4$	54.46	2	−3.54	High	Yes	Yes
SB505124	$C_{20}H_{21}N_3O_2$	60.03	3	−5.24	High	Yes	Yes

ADME, absorption, distribution, metabolism, and excretion; BBB, blood-brain barrier; HIA, human intestinal absorption; NRB, number of rotatable bonds; TPSA, topological surface area.

Table 3 ADME properties (cytochrome enzymes inhibitory effect) of C3A, P3G, and P3A and known inhibitors (SD208, LY364947, and SB505124)

Compound name	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
C3A	No	No	No	No	No
P3G	No	No	No	No	No
P3A	No	No	No	No	No
SD208	Yes	No	Yes	Yes	Yes
LY364947	Yes	Yes	No	Yes	Yes
SB505124	Yes	Yes	Yes	Yes	Yes

inhibitors, LY3649471, SB50524, and SD208. According to the results, the highest and lowest binding energies of ALK5 were found as –8.4 and –7.0 kcal/mol for P3A and LY364947, respectively. Inside the cavity of ALK5, the hydroxy group on the B ring of the aglycone part of anthocyanin makes a hydrogen bond with Gly374 and Lys376 (distances were 1.89 and 2.18, respectively). Moreover, the hydroxy group of arabinose, which is bound to anthocyanidin, makes a hydrogen bond with Lys335 (distance 2.04) (Fig. 3).

Discussion

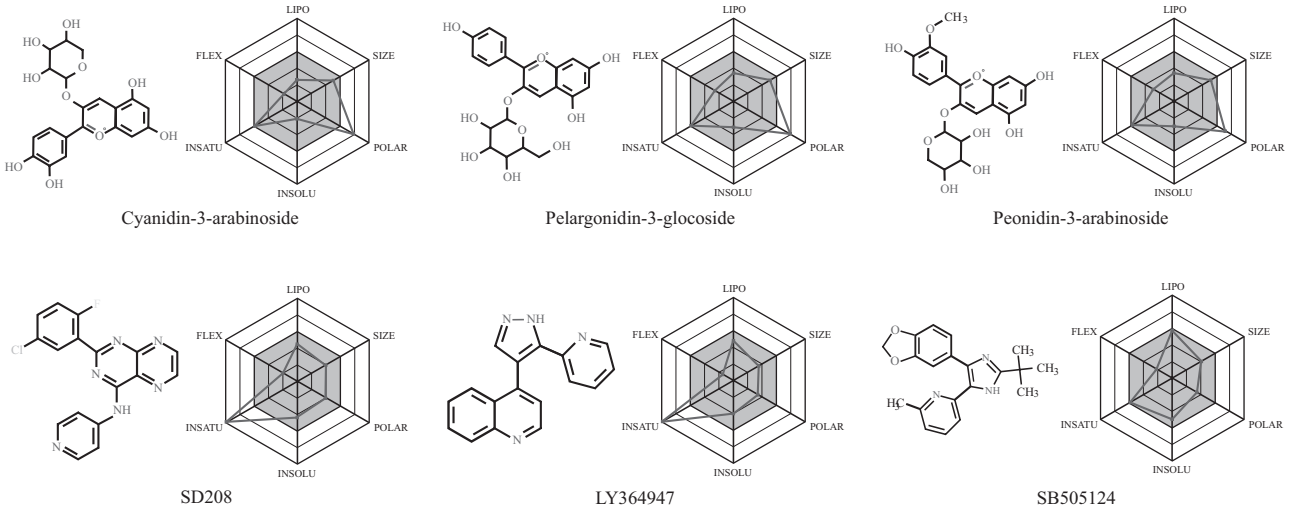
In this study, in-silico drug-likeness prediction and ADME efficiency of 24 anthocyanins were tested, and the effectiveness of the most suitable compounds for the Lipinsiki's RO5 on ALK5 were tested by in-silico molecular docking method. ADME results revealed that C3A, P3G, and P3A were fit for Lipinski's RO5 (Table 4). The molecular weights of C3A, P3G, and P3A are 419.36, 433.39, and 433.39 g mol^{-1}, respectively, which are the acceptable range for the Lipinski filter (MW ≤ 500). The TPSA values of C3A, P3G, and P3A were found as 173.21, 173.21, and 162.21 Å^2, respectively. The number of rotatable bonds refers to the flexibility of bioactive molecules, and this number should be between 0 and 9 [23]. Rotatable bonds of C3A, P3G, and P3A were determined as 3, 4, and 4, respectively. Log S (SILICOS-IT) values of C3A, P3G, and P3A were found as –1.36, –1.52, and –2.05, respectively, that is means all of them show feature water solubility. WLOGP and TPSA values give information about HIA and BBB access of compounds. The boiled-egg chart is created with the help of these values. This chart allows us to predict the HIA and BBB properties of the compounds.

Examination of bioactive compounds as a drug candidate increases the drug discovery. It is important to estimate the drug likeness and ADME properties of a new drug candidate with bioinformatic tools. These tools allow us to learn the physicochemical properties of natural bioactive compounds in a short time and in a cheaper way by avoiding time and cost [24]. The SwissADME web tool that is one of these ways has been used to estimate the drug likeness of anthocyanins. Drug-likeness properties of anthocyanins were predicted by Lipinski, Veber, Ghose, Egan, and Muegge rules. Lipinski's RO5 are one of the important rules for characterizing the bioactive compounds [25]. The drug-likeness properties of the three anthocyanins were found to obey Lipinski rules, except for a violation. At the same time, anthocyanins also were found to comply with Ghose rules, and they have no violation of Ghose rules. Other rules also have been examined for determining the drug likeness of three anthocyanins. However, they did not fit with other drug-likeness guidelines.

ADME is defined as a 'new chemical space' (in-silico ADME behavior analysis) for drugs and drug-like natural product (NP)–based molecules [26]. ADME analyses were performed to estimate the water solubility,

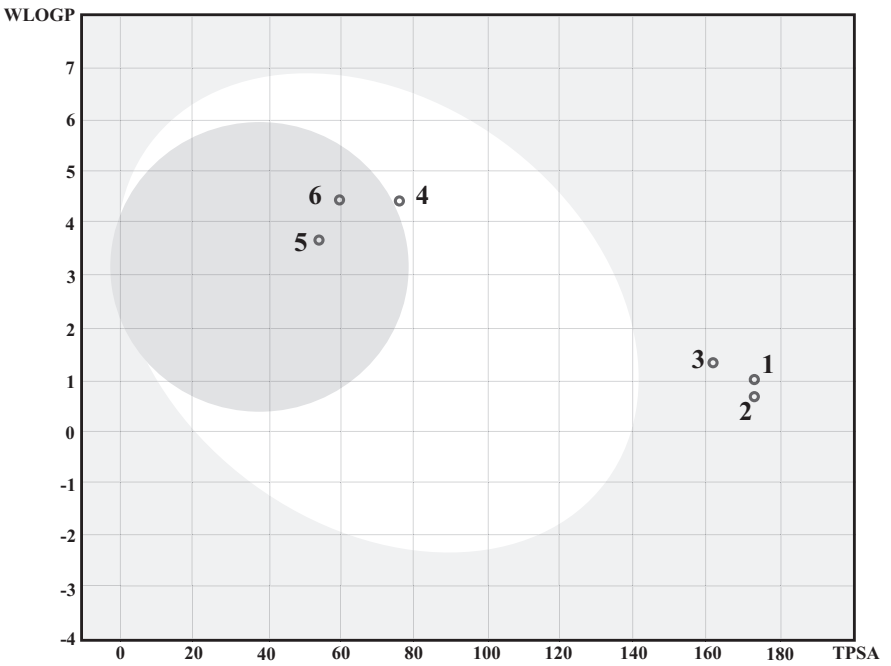
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Fig. 1



Bioavaibility radar of three anthocyanins and known inhibitors where colored zone represents of lipophilicity, molecular size, flexibility, and solubility.

Fig. 2

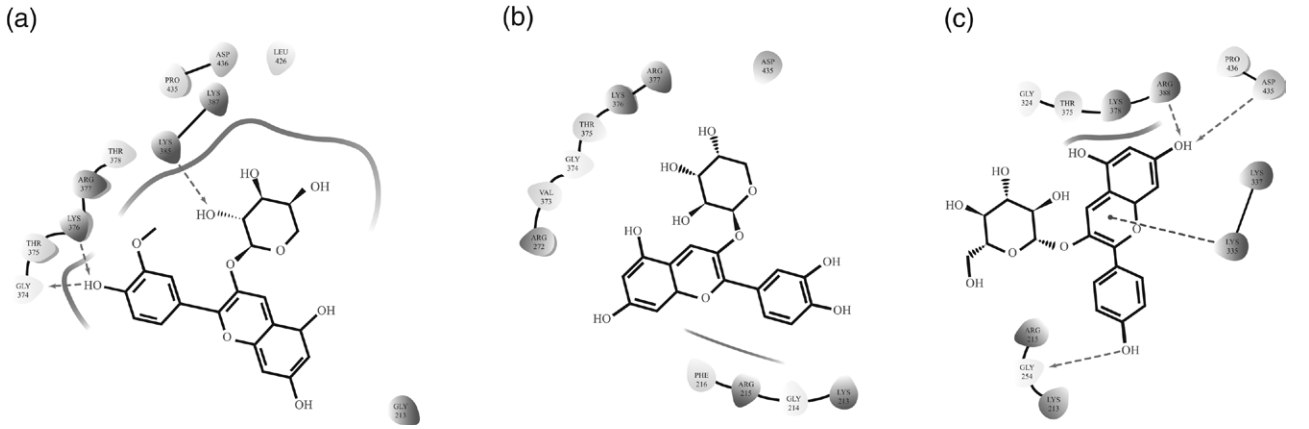


The boiled-egg plot of anthocyanins (1: C3A, 2: P3G, and 3: P3A) and known inhibitors (4: SD208, 5: LY364947, and 6: SB505124). The plot is divided into yellow, white, and gray areas. These areas represent the BBB, HIA, and not showing both, respectively. BBB, blood-brain barrier; HIA, human intestinal absorption.

lipophilic, and pharmacokinetic character of the three anthocyanins by the SwissADME web tool. The lipophilic character of bioactive compounds is defined according to *n*-octanol/water coefficient. Five models, iLOGP, XLOGP3, WLOGP, MLOGP, and SILICOS-IT, are used for predicting the lipophilic character of compounds. In this study, we used the multimodel system (SILICOS-IT). The main aim of this model is to reduce the error between the predicted and calculated values of logPo/w [27]. Lipophilicity is one of the important physicochemical properties to estimate the oral bioavailability of compounds. The lipophilic values of

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Fig. 3



Molecular docking result of (a) peonidin-3-arabinoside, (b) cyanidin-3-arabinoside, and (c) pelargonidin-3-glucoside on ALK5 receptor. ALK5, acting receptor-like kinase 5.

Table 4 Lipinski's rule of five violation

Molecular weight	≤500 g/mol
Lipophilicity (MLOGP)	≤4.15
Hydrogen donors	≤5
Hydrogen acceptors	≤10

MLOGP, moriguchi octanol-water partition coefficient and molecular weight.

the three anthocyanins were in the acceptable range (–7.0<XLOGP3<5.0).

Water solubility is another physicochemical property of compounds to estimate oral or parenteral administration [28]. Water solubility (log S) of anthocyanins is estimated based on three models that are ESOL Model, Ali Model, and SILICOS-IT in the SwissADME tool. Log S values of C3A, P3G, and P3A were found to be –1.36, –1.52, and –2.05, respectively, by the SILICOS-IT model. As a result, anthocyanins were suitable for oral administration as they are water-soluble.

As the number of rotatable bonds increases, the flexibility of the molecules increases and, thus, the binding efficiency of the candidate molecules to the active sites of the biomolecules increases [29]. In our results, the number of rotatable bonds of P3A and P3G is 4. The number of rotatable bonds of known inhibitors is 3. Herewith, anthocyanins are more flexible than known inhibitors and show better binding properties in docking analyses.

Three anthocyanins are not substrates of P-gp. However, known inhibitors of ALK5 receptor are substrates of P-gp. Therefore, this feature increases the possibility of anthocyanins being drug candidates. The binding of molecules to P-gp may decrease the accumulation of molecules in the cell and cause drug resistance to transported the out of the cells [24]. Except for the P-gp substrate, anthocyanins do not show inhibitory properties for CYP enzymes. Consequently, the biotransformation of drugs metabolized by CYP enzymes will not be inhibited by anthocyanins. Thus, increased drug accumulation will be prevented, and the side effects of drugs will be reduced [24].

WLOP and TPSA values allow us to learn about HIA and BBB [30]. Anthocyanins have low HIA and do not pass through the BBB. Nanoparticle carrier systems should be used to increase the absorption of anthocyanins from the gastrointestinal tract.

In the literature, NPs are commonly used compounds for the treatment of different diseases such as cardiovascular diseases, multiple sclerosis, and infectious [31]. Furthermore, in-silico tools are of great importance for the investigating of novel compounds with anticancer properties. Alam and Khan [32] carried out the 3D-QSAR, ADME/Tox, and docking studies of flavone analogs that have anticancer activity. They have tested in-silico Tankyrase inhibition activity of these flavone analogs. Their results reveal that the eight flavone analogs (F2, F3, F8, F11, F13, F20, F21, and F25) are fit for Tankyrase inhibition activity with IC50 values as 1.59, 1, 0.62, 0.79, 3.98, 0.79, 0.63, and 0.64, respectively [32]. Rai *et al.* [33] carried out TGFβ-receptor related in-silico study (comparative molecular docking and ADME studies) on FDA-approved drugs. They have found that the compounds found in *Curcumin* have a potential against TGFβ-receptors [33]. Furthermore, Kandagalla *et al.* [29] studied the molecular docking analysis of 142 curcumin analogs against ALK5 receptor. Their results reveal that six curcumin analogues show ALK5-inhibiting activity with significant binding energy (between –10.0 and –10.2 kcal mol^{-1}). These six analogs are defined as promising compounds against ALK5 receptor [29]. In our study,

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we have carried out with different ligands (C3A, P3G, and P3A) on the same receptor (ALK5). Anthocyanins are polyphenol-based secondary metabolites. The most common of anthocyanidins (aglycone part of anthocyanins) are cyanidin, pelargonidin, peonidin, malvidin, and delphinidin [34]. When sugars (such as glucose, rhamnose, xylose, and arabinose) bond to anthocyanidins, the physicochemical and biological properties of anthocyanins change. In addition, sugars bond to anthocyanidins as monosaccharide, disaccharide, or trisaccharide form. They attach to the 3- or 5,7-position on the A- or C-ring, respectively [34]. These chemical properties make C3A, P3G, and P3A higher binding energies to ALK5 receptor than the known ALK5 inhibitors. In terms of study limitations, the number of compounds (anthocyanins) tested by using ADME properties is 21. The results of these three of 24 data were evaluated as effective against ALK5; on the other hand, the number of compounds could be increased to obtain more promising results against the same receptor (ALK5).

Conclusion

Computational methods provide early stages of the drug design procedure and understanding the chemical properties of compounds in a virtual manner. Therefore, these methods can make this process simple and economical for drug discovery. Future studies on the novel computational methods and in-vitro and in-vivo experiments will need to further researches to clarify the effect of C3A, P3G, and P3A on TGF-β studies.

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Conflicts of interest

There are no conflicts of interest.

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