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Computational Investigation of Compounds of *Allium cepa* as Potential Inhibitors of Transforming Growth Factor-beta Signaling in Cancer

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Abstract

Transforming growth factor-beta (TGF-β) plays a crucial role in cancer during development and metastasis. The TGF-β signaling pathway begins with the binding of active TGF-β to TGF-β receptor type II, which ultimately leads to the expression of target genes in the nucleus. In this study, 56 compounds from *Allium cepa* were docked against transforming growth factor-beta receptor I and II (TGFBR I and II) to identify small molecular weight compounds capable of binding firmly to the kinase domain of the target proteins and inhibiting them in the process. For each protein target, five compounds with the highest binding affinities were identified and reported. From the results, three compounds; petunidin 3-glucoside-5-(6″-acetylglucoside) (-12.106 kcal/mol and -11.899 kcal/mol), myricetin (-11.66 kcal/mol and -13.924 kcal/mol), and fisetin (-10.61 kcal/mol and -12.76 kcal/mol) showed robust binding affinities to both protein targets (TGFBR I and TGFBR II, respectively). The ADMET profiling carried out on the identified compounds indicated promising ADMET properties. These compounds could be exploited as antiviral agents that disrupt the TGF-β signaling. However, further investigations using *in vitro* and *in vivo* techniques must be carried out to validate these findings.

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Introduction

The transforming growth factor-beta (TGF- β) is a family of structurally similar proteins that control several cellular processes such as cell division, differentiation, epithelial-mesenchymal transition (EMT), and movement [1]. The crucial role of the TGF-β signaling pathway has been identified in different forms of cancer during development and metastasis [2]. The TGF-β signaling pathway begins with the binding of active TGF-β to TGF-β receptor type II. The receptor is modified in the process and this conformational change causes the type II receptor to become active and subsequently phosphorylate type I TGF-β receptor [3]. As a result, downstream transcription factors are phosphorylated and activated in a series of reactions leading to the expression of target genes in the nucleus [4, 5].

TGF-β is considered a druggable target because an increase in the cellular concentration and/or activity of TGF-β in tumor cells and their environment facilitates tumorigenesis by initiating EMT and increasing tumor cell proliferation, thus, blockade of the TGF-β signaling pathway may provide therapeutic interventions to inhibit tumor progression and metastasis [3]. There is a constantly increasing profile of drugs, including large and small molecular weight compounds that target and inhibit the TGF-β pathway and may be useful for therapeutic intervention in cancer [6]. A series of strategies, including the use of ligand traps, small molecule inhibitors, antisense oligonucleotides, and small molecule receptor kinase inhibitors are engaged to interrupt the progression of the TGF-β signaling pathway [7, 8].

Considering this, different low molecular weight drugs that target the kinase domain of the type of receptor have been recently developed [9-13]. Computer-aided protocols such as structural screening of different small molecular weight compounds and evaluation of the binding pose of ligands to protein targets have been integrated into the modern-day drug discovery [14]. Molecular docking is a computer-aided procedure that is targeted at predicting the binding pose and affinity of a ligand to a target protein [15]. Molecular docking, as with many other computer-aided tools, has become essential and has relatively easier application in the field of drug discovery [16]. Herein, a concise molecular binding study was carried out on compounds of *Allium cepa* to investigate the inhibitory potentials of these compounds against TFGBR I and II. The most promising compounds in terms of binding affinity are reported together with the corresponding ADMET profile.

Materials and Methods

Ligands and protein targets

The 2D structures of the secondary metabolites of *A. cepa* were mined from an online repository in sdf format, incorporated into Maestro (Schrodinger suite), and prepared using the functional ligprep tool (Fig. S1). Subsequently, the protein targets (Transforming growth factor-beta receptor I and II) were retrieved in 3D format and prepared using the protein preparation wizard. Grid boxes were generated for both target proteins in preparation for molecular docking.

Molecular docking

The molecular docking procedure was carried out with the protein targets treated as rigid bodies and the ligands rotatable. The procedure was automated using a glide script on Schrodinger suite. A total of 56 compounds from *A. cepa* were examined for the degree of firmness exhibited when bound to the kinase domain of the target protein. The procedure was automated with two different glide scripts in maestro. Initially, standard precision was employed to screen the compounds and score them based on their characteristic mode of binding. Subsequently, the top-scoring compounds were subjected to more rigorous extra precision to correctly investigate the binding affinities of the compounds to the protein targets.

ADMET and drug-likeness

The absorption, distribution, metabolism, excretion, and toxicity profiles, which include the water-solubility, drug-likeness, pharmacokinetics, and toxicity profile of the lead compounds were determined using online *in silico* predictive tool; pkCMS and Swiss ADME server. The predicted pharmacokinetic characteristics are human intestinal absorption, blood-brain barrier (BBB) permeability, skin permeability, hERG I and II inhibition, water solubility, and potential inhibition of selected cytochrome P450 (CYP) isoforms. The toxicity profile was also evaluated, and the properties evaluated were the LD50 and hepatotoxicity.

Results and Discussion

To identify small molecular weight antiviral agents, several naturally occurring compounds in *A. cepa* were docked against the kinase domain of transforming growth beta receptors I and II to investigate the molecular binding interactions of these compounds to the target receptors. The top-scoring compounds against TGFBR I and II are presented in Tables 1 and 2, respectively. These compounds exhibit good binding affinities to the protein targets analyzed with docking scores in the range of -9.425 kcal/mol to -12.106 kcal/mol against TGFBR I and -11.899 kcal/mol to -13.924 kcal/mol against TGFBR II (Table S1; S2). In both cases, the reported compounds exhibited lower docking scores (higher binding affinity) to both protein targets than the standard co-crystallized ligands. Petunidin 3-glucoside-5-(6″-acetylglucoside) had the most promising binding affinity to transforming growth factor-beta receptor type I with a docking score of -12.106 kcal/mol and reacted with 6 amino acids (ASP351, LYS337, ASN338, LYS335, ASP290, and HIS283) in the substrate-binding domain of the protein. Myricetin, on the other hand, had the highest binding affinity to TGFBR II. In the same vein, the compound had a docking score of -13.924 kcal/mol and formed hydrogen bond interactions with HIS 328, GLU290, and ASP397. Hydrogen bonding is the major form of interaction observed in the ligand-receptor complexes analyzed. Above others, three compounds; Petunidin 3-glucoside-5-(6″-acetylglucoside) (-12.106 kcal/mol and -11.899 kcal/mol), Myricetin (-11.66 kcal/mol and -13.924 kcal/mol), and Fisetin (-10.61 kcal/mol and -12.76 kcal/mol) showed robust binding affinities to both protein targets (TGFBR I and TGFBR II, respectively) and they could be explored as antivirals with inhibitory potentials against both type and type receptors in the TGF-β signaling pathway.

Table 1 Top-scoring compounds against receptor type I.

Sr. No.	Ligands	TGFBR I
1	Petunidin 3-glucoside-5-(6″-acetylglucoside)	-12.106
2	Myricetin	-11.66
3	Malvidin-3-glucoside	-11.064
4	Isorhamnetin	-10.664
5	Fisetin	-10.61
6	Standard ligand	-9.425

Table 2 Top-scoring compounds against receptor type II.

Sr. No.	Ligands	TGFBR II
1	Myricetin	-13.924
2	Quercetin	-13.35
3	Delphinidin 3-glucoside	-13.197
4	Fisetin	-12.76
5	Petunidin 3-glucoside-5-(6″-acetylglucoside)	-11.899
6	Standard co-ligand	-10.831

ADMET profile

The predicted water solubility (in Log mol/L) of the test compounds ranged between -3.181 and -2.835 (Table 3). All values fall within the soluble class (-4 < water solubility < -2) and are hence soluble. The caco-2 permeability prediction for the compounds ranged from moderate to low. None of the test compounds had a high Caco-2 permeation. Permeation of human epithelial colorectal adenocarcinoma cell (caco-2) is a widely used model to predict the absorption of oral drug candidates. All the test compounds have logkp value less than -2.5 and have moderate skin permeation rates. Petunidin 3-glucoside-5-(6″-acetylglucoside) had poor human intestinal absorption (12.12%). Contrarily, Fisetin had the highest human intestinal absorption rate (83.75%). Quercetin (77.207%), isorhamnetin (76.014%), and myricetin (65.93%) had acceptable absorption rates. An orally administered drug must possess the structural orientation that enables it to cross the lumen of the intestine.

The predicted distribution rate of the test compounds showed that the log-BB of all compounds ranged from -1.039 to -2.504 (Table 3). None of the compounds would permeate the blood-brain barrier (BBB) given that a log-BB value of 0.3 or higher is required for easy blood-brain barrier permeation and that a log-BB of less than -0.1 would not permeate the BBB. Blood-brain barrier permeation is useful when designing psychoactive drugs. In designing drugs that are not meant to target the brain, it is safe that these drugs do not cross the BBB to avoid adverse drug reactions. The predicted pharmacokinetic profiles of the lead compounds showed that three of the compounds; petunidin 3-glucoside-5-(6″-acetylglucoside), malvidin 3-glucoside and delphinidin 3-glucoside, have no inhibitory effect on the selected cytochrome P450 isoforms. Myricetin, fisetin, quercetin, and isorhamnetin showed inhibitory activity against CYP1A2, while fisetin alone showed an inhibitory potential against

Table 3 Pharmacokinetic profiles of the lead compounds.

Compounds	A	B	C	D	E	F	G
Water solubility (log mol/L)	-2.89	-2.915	-2.835	-3.181	-2.925	-2.874	-3
$CaCO_2$ permeability (log Papp in 10^{-6} cm/s)	-1.443	0.095	0.116	0.058	-0.229	-1.121	-0.003
Human intestinal absorption (%)	12.12	65.93	46.812	83.752	77.207	32.504	76.014
Skin permeability (log kp)	-2.735	-2.735	-2.735	-2.735	-2.735	-2.735	-2.735
BBB permeability (log BB)	-2.504	-1.493	-1.887	-1.039	-1.098	-2.156	-1.135
CYP2D6 substrate	No	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	No
CYP1A2 Substrate	No	Yes	No	Yes	Yes	No	Yes
CYP2C19 substrate	No	No	No	No	No	No	No
CYP2C9 substrate	No	No	No	Yes	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	No
Total clearance (log ml/min/kg)	0.233	0.422	0.676	0.421	0.407	0.571	0.508
hERG I inhibitor	No	No	No	No	No	No	No
hERG II inhibitor	Yes	No	Yes	No	No	Yes	No
Hepatotoxicity	No	No	No	No	No	No	No
LD50 (mol/kg)	2.491	2.497	2.569	2.465	2.471	2.586	2.407

A = Petunidin 3-glucoside-5-(6''-acetylglucoside), **B** = Myricetin, **C** = Malvidin 3-glucoside, **D** = Fisetin, **E** = Quercetin, **F** = Delphinidin 3-glucoside, **G** = Isorhamnetin.

CYP2C9. Be that as it may, none of the compounds is an inhibitor of CYP2D6, CYP3A4, and CYP2C19. Interactions with any of the CYP isoforms and potential subsequent inhibition would result in a drug-drug reaction.

The predicted toxicity profile of the lead compounds showed that none of the compounds is an inhibitor of the potassium channels encoded by hERG I (human ether-a-go-go gene 1); however, petunidin 3-glucoside-5-(6″-acetylglucoside), malvidin 3-glucoside and delphinidin 3-glucoside are inhibitors of hERG II channels. Consequently, none of the drugs showed predicted toxic activity on the liver. The anthocyanins (petunidin 3-glucoside-5-(6″-acetylglucoside), malvidin 3-glucoside and delphinidin 3-glucoside) exhibited potential inhibition of hERG II channels whose inhibition could lead to a potential ventricular arrhythmia. The predicted clearance rate (hepatic plus renal in log(ml/min/kg)) showed that malvidin 3-glucoside acetate has the highest clearance rate (0.676) per minute of all compounds analyzed. Contrarily, petunidin 3-glucoside-5-(6″-acetylglucoside) had the lowest clearance rate (0.233) per minute. The total clearance is a combination of hepatic clearance and renal clearance and is important for estimating the dosage of drugs in order to have a stable therapeutic concentration [17].

The high binding affinities exhibited by the compounds for the TGF-β signaling pathway target proteins in this study is an indication of the inhibitory potential of these compounds against these essential macromolecules and their possible roles as therapeutic agents against cancer. It was observed in this study that all the reported compounds had significantly higher binding affinity for the target proteins than the standard co-crystallized ligands. This could be linked to the aromatic rings contained in the structure of these compounds. Among the amino acid residues in the active site of TGFBR I, specific characteristic binding and interaction with HIS283 is consistent with a previous study [18]. In addition to the robust inhibitory potential exhibited by the compounds, a significant number of them possessed promising ADMET properties. Howbeit, lead optimization might be required to improve the pharmacokinetic profiles of some of the compounds and still maintain the binding integrity of the compounds to the target proteins. Precisely, ADMET profiling is an analytic procedure used for determining whether a compound can be easily absorbed, transported to their target site of action via systemic circulation, metabolized and easily removed from the body via excretory organs.

Conclusions

Fifty-six compounds from *A. cepa* were engaged in this study to examine the binding energy exhibited by the compounds. The compounds reported in this study showed promising molecular interactions with the target receptors as well as moderate ADMET properties. Above others, Petunidin 3-glucoside-5-(6″-acetylglucoside), Myricetin, and Fisetin showed impressive binding to both TGFBR I and TGFBR II and could be considered as antiviral agents. However, investigative *in vitro*

and *in vivo* analyses are essential to validate these findings.

Conflict of Interest

The authors declare that they have no conflict of interest

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