Potential Deoxycytidine Kinase Inhibitory Activity of Amaryllidaceae Alkaloids: An In Silico Approach

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Background: Plants of the Amaryllidaceae family have been under intense scrutiny for the presence of a couple of alkaloidal secondary metabolites with enduring cytotoxic activity, such as pancratistatin (1), 7-deoxypancratistatin (2), narciclasine (3), 7-deoxynarciclasine (4), trans-dihydonarciclasine (5), and 7-deoxy-trans-dihydonarciclasine (6). Nevertheless, preclinical evaluation of these alkaloids has been put on hold because of the limited quantity of materials available from isolation. Aim: To explore the underlying cytotoxic molecular mechanisms of the Amaryllidaceae alkaloids (1–6) and to assess their absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles using chemoinformatic tools. Materials And Methods: AutoDock 4.0 software along with different in silico chemoinformatic tools, namely PharmMapper, Molinspiration, MetaPrint2D, and admetSAR servers, were used to assess the druggability of the Amaryllidaceae alkaloids (1–6). Results: Deoxycytidine kinase (dCK) (PDB: 1P60) was predicted as a potential target with fitting score of 5.574. In silico molecular docking of (1–6) into dCK revealed good interactions, where interesting hydrogen bonds were observed with the amino acid residues—Gly-28 and Ser-35—located in the highly conserved P-loop motif. This motif plays a special role in dCK function. Contrary to (1), in silico pharmacokinetic results have shown good absorption and permeation and thus good oral bioavailability for (2–6). Conclusion: The in silico docking data have proposed that the reported cytotoxic activity of the Amaryllidaceae alkaloids (1–6) could be mediated through dCK inhibition. In addition, the ADMET profile of these alkaloids is promising and thus (1–6) could be candidates for future drug development.

Keywords: Amaryllidaceae alkaloids, cytotoxicity, deoxycytidine kinase, in silico

INTRODUCTION

Over the past several decades, the search for natural products in marine and terrestrial environments has led to the discovery of a number of biologically active alkaloids. Of those are the Amaryllidaceae alkaloids, which are structurally related compounds primarily isolated from the plants of the family Amaryllidaceae.[1] Among these, pancratistatin (1), 7-deoxypancratistatin (2), narciclasine (3), 7-deoxynarciclasine (4), trans-dihydonarciclasine (5), and 7-deoxy-trans-dihydonarciclasine (6) constitute an emblematic group. They are characterized by their highly oxygenated phenanthonidine core with contiguous chiral centers on one ring [Figure 1]. Amaryllidaceae alkaloids are known to have antitumor and antiviral activities along with other interesting biological activities.[2] Pancratistatin, which was isolated in 1984 by Pettit et al.[3] from the roots of the Hawaiian Pancratium littorale, has shown promising in vitro antineoplastic activity.[4] Furthermore, pancratistatin and other Amaryllidaceae alkaloids...
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also proved to induce apoptosis against a large panel of cancer cell lines.  

Interestingly, pancratistatin has an insignificant cytotoxic effect on noncancerous cell lines.  

Nevertheless, the biochemical mechanism by which pancratistatin induces apoptosis in cancer cells is still unknown.  

This can be attributed to the limited quantity of material available from either isolation or total syntheses.  

Fascinatingly, the in silico prediction of leads’ molecular mechanisms is now widely accepted, where different computational tools are used to mimic the involved biological systems.  

It was, therefore, hoped that the molecular mechanism of pancratistatin could be assessed without having to undergo the costly and tedious wet conventional experiments.  

Our preliminary in silico investigations revealed that deoxycytidine kinase (dCK) is a potential target for pancratistatin.  

This target is a crucial enzyme in deoxyribonucleoside salvage pathway and is involved in apoptosis inhibition.  

dCK is activated on deoxyribonucleic acid (DNA) damage, and the degradation products are recycled to aid in DNA repair and apoptosis inhibition.  

On the other hand, inactivation of dCK causes DNA replication stress and subsequent cell cycle arrest, resulting in apoptosis induction.  

Thus, dCK inhibition could be envisioned as a potential strategy for cancer therapy.  

We herein report the unprecedented use of an in silico approach to uncover the molecular mechanism of the anticancer properties of pancratistatin and its derivatives as a dCK inhibitor and, thus, as apoptosis inducers.

Materials and Methods

Preparation of ligand and protein structures

ChemDraw Ultra 12 software (CambridgeSoft) was used for ligand preparation and optimization.  

The crystal structure of the predicted target protein was retrieved from the Protein Data Bank and optimized using Swiss-PdbViewer 4.1.0 software (Swiss Institute of Bioinformatics).

Biological activity

The potential protein targets for the tested compounds were predicted using PharmMapper server.  

On the other hand, for each compound, Molinspiration server was used to predict drug-likeness properties such as G-protein–coupled receptor (GPCR) ligands, ion channel modulators (ICM), kinase inhibitors (KI), nuclear receptor ligands (NRL), protease inhibitors (PI), and enzyme inhibitors (EI).

Molecular docking

Molecular docking was performed using AutoDock 4.0 software (Molecular Graphics Laboratory) based on Lamarckian Genetic Algorithm.  

Polar hydrogen atoms were added to the protein target and Kollman united atomic charges were computed.  

All hydrogen atoms were added to the ligands before the Gasteiger partial charges were assigned.  

The cocrystal ligand was removed and the bond orders were checked.  

The target’s grid map was calculated and set to 60 × 60 × 60 points with grid spacing of 0.375 Å.  

The grid box was then allocated properly in the target to include the active residue in the center.  

The default docking algorithms were set in accordance with the standard docking protocol.  

Docking results having less than 1.0 Å in positional root-mean-square deviation were clustered together, and the results were retrieved as binding energies.  

Poses that showed the lowest binding energies were visualized using molecular operating environment and University of California San Francisco chimera.

Physicochemical properties

LogP, topological polar surface area (TPSA), and the number of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs) for the tested leads were estimated using Molinspiration server.

Pharmacokinetics and toxicity

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) were estimated using admetSAR and MetaPrint2D online servers.

Results

Biological activity and molecular docking

PharmMapper server predicted that dCK (PDB: 1P60) is the best target in terms of fit score (5.574) for pancratistatin (1).  

Having nearly similar chemical scaffolds, we propose dCK as a potential target of the other Amaryllidaceae alkaloids (2–6).  

Docking results revealed that alkaloids (1–6) docked nicely into the active pocket and showed good biochemical interactions with essential amino acid residues [Figure 2].  

Herein, (1) ($E = -7.00$ kcal/mol)
docked into the highly conserved P-loop motif located at the amino acid residues—Gly-28 and Ser-35—in which this motif plays an essential role in dCK function. As for (2), a derivative of (1), a better binding affinity ($E = -7.20 \text{ kcal/mol}$) and a superior interaction were observed. With a similar binding affinity to (1), (2) has showed an additional hydrogen bond with Arg-128. Interestingly, Arg-128 is noted to have a chief anchor function, as it interacts via its NH$_2$ group with the 5’-hydroxyl group of deoxycytidine and with Glu-35, which is strictly conserved within the dCK enzyme.$^{[28]}$ On the other hand, (3) and its derivatives (4, 5, and 6) have shown favorable interactions over the aforementioned compounds. The binding energies were $-7.81$, $-7.79$, $-7.35$, and $-7.21$ for (3), (4), (5), and (6), respectively. In addition, (3) and its derivatives (4, 5, and 6) have shown a hydrogen bonding with Arg-128 along with biochemical interactions with the residues of the conserved P-loop.

Molinspiration server was used to assess the physicochemical properties based on Lipinski’s rule of five.$^{[30]}$ With the exception of (1) (TPSA = 148 and HBD = 6), all the other compounds have shown good absorption and permeation and thus good oral bioavailability, as its predicted physicochemical properties were in agreement with Lipinski’s rule of five [Table 1]. The compounds have also been predicted to be GPCR ligands, ICM, KI, NRL, PI, and EI, which were retrieved as bioactivity scores [Table 2]. Herein, scores more than 0.00 indicate high activity, between 0.00 and $-0.5$ indicate moderate activity, and less than $-0.5$ indicate inactivity.$^{[31]}$

For ADMET profile prediction, we used Metaprint2D and admetSAR web-based servers. Having a glance at the Metaprint2D results [Figure 3], we could see that the tested compounds show a diverse pattern of metabolic transformations based on the normalized occurrence ratio (NOR). With the exception of (3) and (4), the metabolic transformation of the 1,3-dioxolane moieties in the four compounds (1, 2, 5, and 6) represented good sites for metabolism. Herein, the methylene group was predicted to undergo dealkylation and hydroxylation, whereas both oxygen atoms were predicted to undergo only dealkylation. As for (3) and (4), the –NH and –OH groups were predicted to undergo oxidative–deamination and sulfation, respectively. The compounds (1), (3), and (5) that bare phenol moieties were estimated to undergo a number of metabolic transformations. Both (1) and (5), which contain the highly metabolized –OH group, were predicted to undergo five transformations, namely glucuronidation, methylation, sulfation, glucosidation, and hydroxylation. Results of admetSAR revealed that all six compounds do not cross the blood–brain barrier (BBB). Further, these compounds were predicted not to penetrate through Caco-2 cell line. Obviously, all compounds did not show any acute toxicity and mutagenic effect with respect to the ADMET test data [Table 3].

**Discussion**

dCK is an interesting target to be considered for therapeutic targeting in cancer, being involved in

| Compounds | MlogP | TPSA | natoms | MW | HBA | HBD | nrotb |
|-----------|-------|------|--------|----|-----|-----|-------|
| 1         | −1.81 | 148.71 | 23 | 325 | 9 | 6 | 0 |
| 2         | −1.51 | 128.48 | 22 | 309 | 8 | 5 | 0 |
| 3         | −1.12 | 128.48 | 22 | 307 | 8 | 5 | 0 |
| 4         | −0.83 | 108.25 | 21 | 291 | 7 | 4 | 0 |
| 5         | −1.11 | 128.48 | 22 | 309 | 8 | 5 | 0 |
| 6         | −1.11 | 128.48 | 22 | 309 | 8 | 5 | 0 |

TPSA = topological polar surface area, natoms = number of atoms, MW = molecular weight, HBA = hydrogen bond acceptor, HBD = hydrogen bond donor, nrotb = number of rotatable bonds

| Compounds | GPCR | ICM | KI | NRL | PI | EI |
|-----------|------|-----|----|-----|----|----|
| 1         | 0.10 | 0.08 | −0.18 | −0.10 | 0.18 | 0.27 |
| 2         | 0.07 | 0.02 | −0.12 | −0.12 | 0.23 | 0.29 |
| 3         | 0.30 | 0.15 | −0.10 | 0.07 | 0.17 | 0.31 |
| 4         | 0.26 | 0.09 | −0.04 | 0.04 | 0.21 | 0.33 |
| 5         | 0.24 | 0.17 | −0.07 | −0.11 | 0.46 | 0.27 |
| 6         | 0.24 | 0.17 | −0.07 | −0.11 | 0.46 | 0.27 |

GPCR = G-protein–coupled receptor, ICM = ion channel modulators, KI = kinase inhibitors, NRL = nuclear receptor ligands, PI = protease inhibitors, EI = enzyme inhibitors
DNA repair and apoptosis.\cite{17,18} On the basis of the in silico pharmacophore mapping, we propose dCK as a possible target of pancratistatin. It is worth noting that dCK is highly expressed in thymus and bone marrow indicating its great role in hematopoiesis.\cite{14} Furthermore, it has been reported that dCK has a crucial role in both DNA replication and repair process pertained with its constitutive expression throughout the cell cycle.\cite{32}

Our in silico molecular docking results revealed that alkaloids (1–6) are possible dCK inhibitors. On the basis of the superlative knowledge of being cytotoxic to a panel of cancerous cell lines,\cite{6} we attempted to explore their anticancer mechanisms at the molecular level. It is noteworthy that DNA damage induces rapid hyperphosphorylation and activation of p53.\cite{33} This promotes DNA repair through dCK activation, leading to increase in the salvage pathway of deoxynucleoside triphosphate (dNTP) production.\cite{34} Furthermore, dCK inactivates cyclin-dependent kinase-1 (Cdk1),\cite{16} which induces G2/M transition and mitosis.\cite{35} Therefore, inhibition of dCK by these alkaloids would hopefully lead to induction of apoptosis in cancer cells.

As most lead compounds fail to meet the pharmacokinetic criteria of a drug at the final stages of the drug discovery pipeline, it is advantageous to assess the physicochemical drug-likeness properties and ADMET profiles using advanced in silico tools. Molinspiration server was used to assess the physiochemical properties based on Lipinski's rule of five.\cite{30} Poor absorption or permeation is more likely when there are more than 5 HBDs, 10 HBAs, the molecular weight is greater than 500 Da, and the calculated LogP (CLogP) is greater than 5 (or MlogP > 4.15). Moreover, good bioavailability is more likely for compounds with ≤10 rotatable bonds (nrotb) and TPSA of ≤140 Å.\cite{36} Interestingly, all six compounds were predicted to be EI (score > 0.00) [Table 2]. In addition, (4), (5), and (6) have shown good activities such as GPCR and PI (score >0.00). From these results, we can confidently explain the predicted drugability of the tested compounds as dCK inhibitors. Nevertheless, other potential activities could be hopefully medicated via other pathways as
PI or GPCR. Other ADMET properties such as BBB penetration, human intestinal absorption, human colon carcinoma cell (Caco-2) permeability, and Ames test were estimated via admetSAR. Having nearly similar chemical scaffolds, we expect that most of their ADMET patterns are quite similar. All six compounds are not expected to cross the BBB, as more polar compounds have lower lipid solubility, limiting their
BBB penetration. Furthermore, these compounds were predicted not to penetrate through Caco-2 cell line, which has also been proposed for the prediction of the BBB permeability of drugs. Herein, our tested compounds could be of low oral bioavailability and need further structural optimization. On the other hand, the metabolic performance of the compounds on cytochrome P450 (CYP) enzyme had been predicted on to 2C9, 2D6, 3A4, and 1A2 isoenzymes. It is worth noting that the drug that undergoes CYP-mediated oxidative biotransformation is responsible for the large number of clinically significant drug interactions during multiple drug therapy.

**CONCLUSION**

To conclude, the cytotoxic mechanism of the studied Amaryllidaceae alkaloids (1–6) as dCK inhibitors and their ADMET profiles have been addressed using in silico approach. Wet experiments are needed to confirm these findings.

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**Conflicts of interest**

There are no conflicts of interest.

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