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Original Research Article

Cancer Stem Cells as Potential Targets of Phytotoxic Alkaloids from the Toxic Plants-Phytotoxins Database

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ABSTRACT

Targeting cancer stem cells (CSCs) in anticancer discovery is very difficult due to the resistance of CSCs to conventional drugs. Different CSC targets, such as the ABC cassette, surface markers, signal cascade, and tumour microenvironment, are involved in the interruption of cell signalling pathways that are critical for the survival and functioning of the CSC population. The study aimed to identify potential drug-like phytotoxic alkaloids with anticancer activity from the toxic plants-phytotoxins (TPPTs) database. A total of 1586 phytotoxins were filtered to obtain 653 alkaloids. Lipinski's properties and the TPSA were predicted for drug likeness and toxicity based on various organ endpoints. Compounds that obeyed Lipinski's rule of five, with moderate or no toxicity were selected. The 11 drug-like phytotoxic alkaloids obtained from the filtering were docked on an isomerase-perdeuterated E65Q-TIM protein (ID: 7AZA; resolution = 1.10 Å) co-crystallized with phosphoglycolohydroxamate. The best binding poses were ranked using their binding energies (E) and inhibition constants (Ki). An evaluation of the protein—ligand's best conformational poses allowed us to identify three indole alkaloids (catharanthine, (-)-eburnamonine and apovincamine) with significant protein—ligand binding interactions for (-)-eburnamonine (E = −8.03 kcal/mol; Ki = 1.30 µM), catharanthine (E = −8.229 kcal/mol; Ki = 0.910 µM) and apovincamine (E = −8.40 kcal/mol; Ki = 0.701 µM). All the docked ligands could bind more efficiently to the target than phosphoglycolohydroxamate. The efficient inhibition of perdeuterated E65Q-TIM in CSCs using phytotoxic alkaloids provided more insights into understanding the mechanisms of the anticancer activity of phytotoxic alkaloids.

Keywords: Cancer Stem Cells, Phytotoxic Alkaloids, Molecular Docking, Drug-Likeness

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Introduction

Alkaloids are diverse class of secondary metabolites and represent the toxic components of many of the well-known poisonous plants.[1] They represent a highly diverse group of compounds and about 3000 distinct of them have been characterized from fungus, plants and animals together.[2] They are low molecular weight organic nitrogenous chemical entity, often chemically classified into pyrrolidines, pyridines, tropanes, pyrrolizidines, isoquinolines, indoles, quinolines, terpenoids and steroids.[3] It has been reported that alkaloids impart a restraining effect on the topoisomerase enzyme, thus stalling DNA replication and consequently cell death.[4] Therefore, alkaloids have been a base for drug development for various ailments such as anti-inflammatory, antibacterial, and antitumor.[5] Some of these activities have been harnessed for the design and development of important chemotherapeutic agents. The plant-based alkaloids have proven efficacy in oncogenesis suppression.[5] The toxic plants-phytotoxins (TPPTs) database comprises phytochemicals such as allergens, allelochemicals, fatal toxins, biopesticides and hallucinogens, that constitute a category of natural compounds with various toxic effects and diverse molecular structures.

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Cancer is a complex and diverse group of disease and researchers have identified various drug targets for different types of cancer.[6] Available data showed that millions of cancer cases exist worldwide despite available management options such as surgery, radiation, immunotherapy and chemotherapy. Challenges such as poor and late detection, resistance to medication and toxicities have limited the eradication of cancer.[7] Since several alkaloids and nitrogen-containing molecules such as vincristine, vinblastine, thiotepa, nitrosoureas, folate antagonists, purine and pyrimidine antagonists are known cytotoxic agent, there is high potential of identifying more potent, broad spectrum cytotoxic compounds from natural alkaloids.[5,8]

The cancer management strategies and the targeted-design of new chemical entities have been challenged by lack of selectivity and specificity. Several targets such as the kinases, cancer stem cells (CSCs), tubulin/microtubules, tumour vasculature and monoclonal antibodies have been explored for the design of new anticancer therapies.[7] Drug targets are specific molecules or pathways that play a crucial role in the growth and spread of cancer cells and targeting these molecules or pathways can lead to the development of anti-cancer drugs.[9] The CSCs have been implicated in the myelogenous leukaemia, brain, prostate, melanoma, colon, lung and ovarian cancers.[10]

Since CSCs are vital for metastasis and tumour progression due to prolonged proliferation potential, permanent elimination of CSCs is vital in checking cancer progression.[11] However, this has remained a challenge due to the resistance of CSCs to conventional drugs, their low proliferation rate, improved DNA damage repair, overexpression of antiapoptotic proteins and multidrug resistance transporters.[12] The protection offered by the CSCs microenvironment can also be targeted to interrupt cell signalling pathways that sustains the CSCs.[13] Several

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promising chemical entities have failed to actualize this at advanced stages of clinical trials.[14] Therefore, this study explored this target to identify drug-like phytotoxic alkaloids that could target CSCs and achieve significant improvement in the chemotherapy of human cancers.

Materials and Methods

Chemical dataset
A library of phytotoxins were obtained from a newly developed, freely available (https://www.agroscope.admin.ch/agroscope/en/home/publications/apps/tppt.html) database of Toxic Plants–PhytoToxins (TPPT) which comprises phytochemicals such as allergens, allelochemicals, fatal toxins, biopesticides and hallucinogens, that constitute a category of natural compounds with various toxic effects and diverse molecular structures.[15] The TPPTs database contains 1586 SMILES of phytotoxins linked to 844 plant species from where the Phyto alkaloids were filtered.[15]

Preparation of chemical dataset
The SMILES of the TPPT were converted to MOL file format using ACD/ChemSketch v.12.0 and clustered based on the phytochemical class to obtain a cluster of 653 phytotoxic alkaloids.

Prediction of Lipinski's parameters
The Lipinski's parameters such as the number of hydrogen bond acceptor (HBA), number of hydrogen bond donor (HBD), octanol-water partition coefficient (log P(o/w) and the molecular weight and other parameter such as topological polar surface area (TPSA) were also computed by Swiss ADME web server.[16]

Prediction of toxicity endpoints
The toxicity parameters such as hepatotoxicity, carcinogenicity, immunotoxicity, mutgenicity, cytotoxicity was predicted for the compounds that passed the Lipinski's Ro5 using ProTox II (ProTox II v. 3.0).[17] Compounds that have no toxicity against the target organs and with high LD_{50} (group 5 or 6 toxicity profile) were selected for further studies. The toxicity cut-off was placed at oral LD > 2000 mg/kg and toxicity classes of 5 and 6.

Molecular docking studies
Ligands
The ligands selected for molecular docking included the drug-like phytotoxic alkaloids that passed all the pre-screening prediction such as the ADME and toxicities as shown in Table 1.

Drug targets
The 3D co-crystallized protein (resolutions < 2.0 Å) complexed with a ligand representing important pathways in cancer cell in human beings was obtained from the protein data bank (www.rcsb.org).[18] The targets were selected based on the stem cell as potential target for cancer management.[11-14] The target is an isomerase perdeuterated E65Q-TIM protein (ID: 7AZA) complexed with phosphoglycolohydroxamate ligand, expressed in *E. coli* and resolution of 1.10 Å.[18]

Preparation of protein target
The protein structure was prepared using the Autodock Tools 1.5.6, a part of MGL Tools molecular visualization interface. The protein was pre-treated by removing the water molecules and other non-essential components. The missing atoms were checked and repaired. A sufficient number of polar hydrogen atoms and Kollman charges were added.[19] The prepared protein molecule was saved for molecular docking study.

Table 1. Drug-likeness properties of selected phytotoxic alkaloids

ID	Chemical class	HBA	HBD	Log P(o/w)	TPSA	Weight
1	Indole	2.00	0.00	4.153	34.47	336.435
2	Piperidine	2.00	2.00	1.376	32.26	143.230
3	Piperidine	2.00	1.00	1.644	23.47	157.257
4	Pyridine	0.00	0.00	1.3560	44.01	137.138
5	Indole	2.00	0.00	3.411	25.24	294.398
6	Quinolizidine	3.00	1.00	1.5310	26.71	250.386
7	Piperidine	3.00	3.00	1.795	53.16	293.455
8	Isoquinoline	4.00	4.00	2.738	72.72	271.316
9	Terpenoids	3.00	2.00	2.807	43.70	345.527
10	Indole	2.00	1.00	3.3630	45.33	336.435
11	Piperidine	2.00	2.00	1.376	32.26	143.23

Apovincamine (**1**), conhydrine (**2**), *N*-methylpseudoconhydrine (**3**), trigonelline (**4**), (-)-eburnamonine (**5**), retamine (**6**), palustridiene (**7**), norcoclaurine (**8**), dihydroatisine (**9**), catharanthine (**10**), pseudoconhydrine (**11**)

Molecular docking
The molecular docking adopted blind docking model using the AutoDockTools-1.5.6. The ligands were assigned torsions using the default settings.[20] The potential grid maps were executed using AutoGrid module with 50 hybrid GA-LS runs and population size of 300, 2.5 million energy evaluations and 27000 generations. A root means square deviation of 2.0 Å was set to group the clusters while other parameters were at default. The docking protocol was validated by re-docking the native ligands into the proteins using the Lamarckian Genetic Search algorithms. The binding poses visualization was performed using Discovery Studio Visualizer v17.2.0.16349 and protein-ligand interaction profiler webserver.

Results and Discussion

Distribution of alkaloids database
The distribution of alkaloids (Figure 1) used in the study showed that 17.3% of the alkaloids were of the isoquinoline group comprising 79 isoquinoline, 24 benzylisoquinoline/opium and 10 erythrinan isoquinoline. The alkaloids in the miscellaneous class (9.18%) comprises 5 acridines/acridones and quinazolines each, 1 indolizidine and purine each, 6 purine/methylxanthines and pyrrolidines each, 16 pyridines and quinoline each and 4 pyrimidines. The indole alkaloids (11.18%) comprises 4 indoles, 5 β-carbolines and ergolines each, 56 monoterpenes, 2 pyrolo-indole and 1 ergot alkaloids. Other classes included; 24 amarylidaceae (3.67%), 38 piperidines (5.82%), 66 pyrrolizidines (10.11%), 52 quinolizidines (7.96%), 83 steroids (12.71%), 78 terpenoids (11.94%), 36 tropanes (5.51%) and 30 amines (4.59%).

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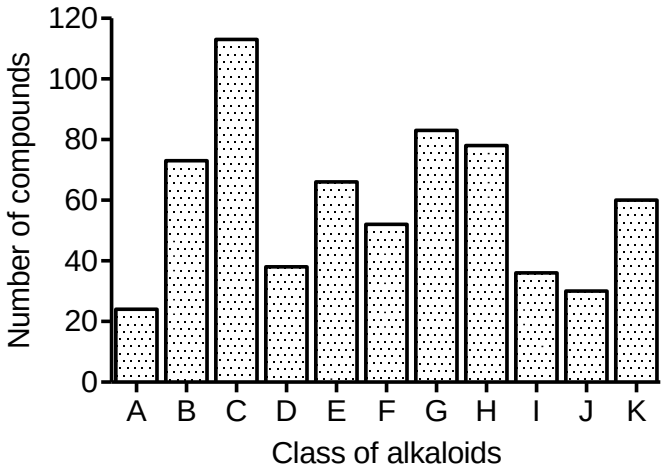

Figure 1: Distribution of alkaloids in different chemical classes; Amarylidaceae (A), indole (B), isoquinoline (C), piperidine (D), pyrrolizidine (E), quinolizidine (F), steroids (G), terpenoids (H), tropane (I), amines (J) and miscellaneous (K)

Chemical data set
The chemical data set of 1586 phytotoxic compounds were filtered for alkaloids and alkaloid-like compounds by manually separating them from the entire database. A total of 653 alkaloids were obtained and used to form a new data set. The compounds were energy minimized and subjected to a conformational search using the appropriate force field. The best conformers (dE = 0) of each compound were converted to the SMILES notations then used for all the *in-silico* studies following the systematic scheme (Figure 2).

Prediction of drug-likeness properties of alkaloids
The drug-likeness properties of the phytotoxic alkaloids were predicted using SwissADME. The selection was based on the Lipinski rule of five in addition to the TPSA of the compounds and compounds with not more than one violation of the rule were selected for further studies. It was observed that 157 phytotoxic alkaloids violated all the Lipinski's Ro5 and were designated as not drug-like for oral bioavailability.[16,21] The drug-likeness prediction left behind 496 alkaloids for further optimization.

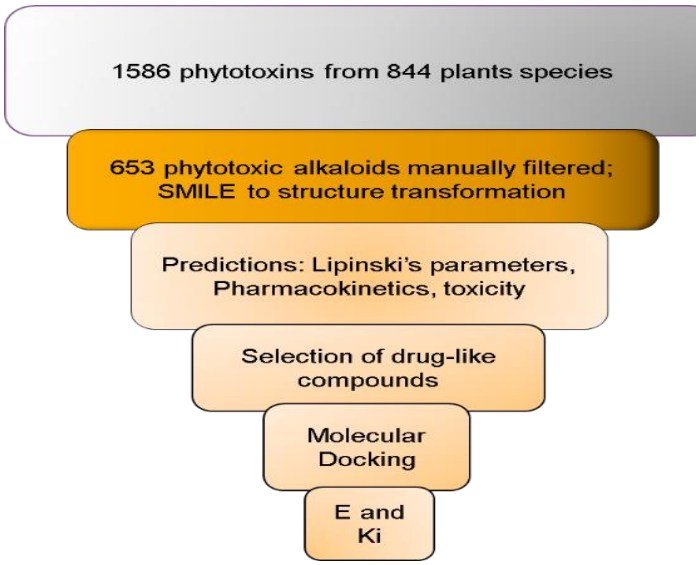

Table 2: Predicted toxicity of selected phytotoxic alkaloids

Figure 2: Schematic representation of the *in-silico* studies

Prediction of Toxicity of compounds
The toxicity of the compounds that passed the Lipinski's Ro5 were predicted. The prediction was targeted at the hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity and the estimation of the LD_{50}. [17,22] Based on the toxicity profiles, eleven compounds with no predicted potential toxicity on the target organs were selected for further studies as shown in Table 2.

Molecular docking studies
The identified alkaloids (Figure 3) were docked to a stem cell-based protein, 7AZA using blind docking and the binding energies, inhibition constants and the amino acids of the protein responsible for interaction were identified for the best poses. The binding parameters of the ligand compared with the native ligand are shown in Table 3.

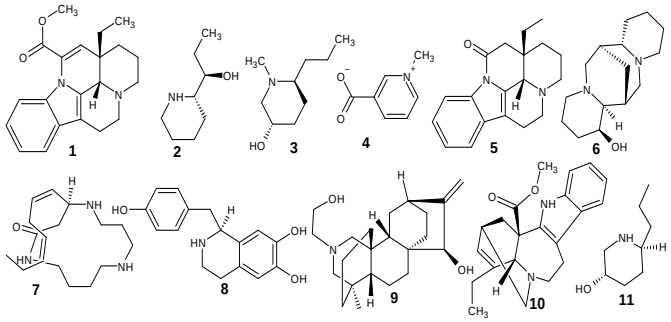

Figure 3: Chemical structures of selected drug-like alkaloids

Binding interactions of phytotoxic alkaloids with protein 7AZA
The amino acids involved in the binding of the apovincamine, an indole alkaloid with the protein target are shown in Figure 4 for the best posed interactions. Classical interactions such as the hydrogen bond, van der Waals, alky and pi-alkyl were formed with ala236, val233, ile172, glu97, lys13 and gly235 of the target protein (Figure 4B). In drug discovery, the use of large and chemically diverse compound libraries for computational and biological screening is one of the most widespread strategies. [23] Molecular docking is the application of various in silico methods for selecting promising compounds from chemical databases. [15] It can be regarded as the computational counterpart of experimental biological evaluation methods, such as high-throughput screening. [21] This has stimulated the use of virtual screening as a fast and cost-effective method for the evaluation of a variety of compound collections, usually ligand- or structure-based approach.

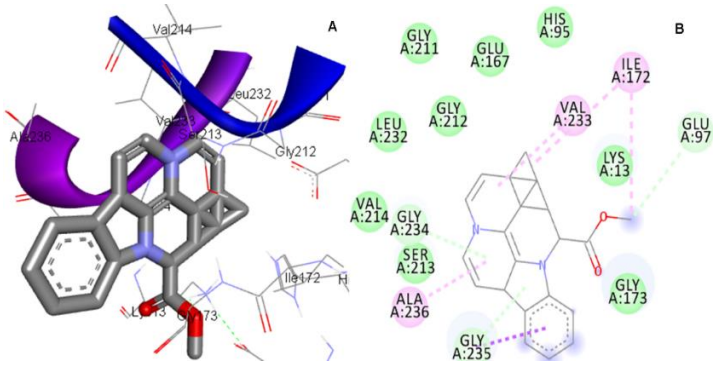

Figure 4: 3D (A) and 2D (B) representation of the theoretical binding pose with isomerase perdeuterated E65Q-TIM protein

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ID	Hepatoxic	Carcinogen	Immunotox	Mutagen	Cytotoxic	LD_{50} (mg/kg)	Class
1	No	No	No	No	No	2200	5
2	No	No	No	No	No	3500	5
3	No	No	No	No	No	3500	5
4	No	No	No	No	No	3720	5
5	No	No	No	No	No	3000	5
6	No	No	No	No	No	3000	5
7	No	No	No	No	No	2303	5
8	No	No	No	No	No	3350	5
9	No	No	No	No	No	3500	5
10	No	No	No	No	No	2100	5
11	No	No	No	No	No	3500	5

Apovincamine (**1**), conhydrine (**2**), *N*-methylpseudoconhydrine (**3**), trigonelline (**4**), (-)-eburnamonine (**5**), retamine (**6**), palustridiene (**7**), norcoclaurine (**8**), dihydroatisine (**9**), catharanthine (**10**), pseudoconhydrine (**11**)

Table 3: Binding parameters of phytotoxic alkaloids on 7AZA target

Ligands	E (kcal/mol)	Ki (µM)
Native	-5.470	98.060
Apovincamine (**1**)	-8.400	0.701
Conhydrine (**2**)	-6.850	9.590
N-methylpseudoconhydrine (**3**)	-7.380	3.860
Trigonelline (**4**)	-5.820	54.410
(-)-Eburnamonine (**5**)	-8.030	1.300
Retamine (**6**)	-7.810	1.890
Palustidiene (**7**)	-6.004	45.390
Dihydroatisine (**9**)	-7.234	3.908
Catharanthine (**10**)	-8.229	0.910
Pseudoconhydrine (**11**)	-7.660	2.094

E = Binding energy; Ki = Inhibition constant

In this study, the discovery of potent anticancer agents was initiated on phytochemically diverse phytotoxins obtained from freely available database Toxic plants-phytotoxins (TPPT) containing 1586 phytotoxins linked to 844 plant species using different filtration strategies and in silico molecular docking. The phytotoxins were initially clustered into different phytochemical groups to obtain 653 alkaloids containing diverse chemical classes. It is imperative to mention that alkaloids have been found to possess anticancer activities. [4,5] For instance, several anticancer compounds such as colchicine, vincristine and vinblastine are alkaloids and have been modified to different functionalities. [5]

The 653 phytotoxic alkaloids were screened for drug-likeness properties using the descriptor-based model. In this approach, relevant drug-like features such as the log P, molecular weight, number of hydrogen bond acceptor atoms and number of hydrogen bond donor atoms were calculated. On application of the Lipinski's rule of five (Ro5), compounds that failed to obey the Lipinski's rule were dropped due to lack of potential for oral bioavailability. Lipinski's Ro5 is an important parameter used in drug discovery to forestall discovery failures during the later stages of trials. Lipinski's rule of five, also known as Pfizer's Ro5 or simply the rule of five (Ro5), is a rule of thumb to evaluate drug-likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would likely make it an orally active drug in humans.[16] Lipinski's rule states that, in general, an orally active drug has no more than one violation certain criteria.[21] The criteria included; no more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen, and oxygen–hydrogen bonds), no more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms), a molecular mass of less than 500 Daltons, and a calculated octanol-water partition coefficient (log P) that does not exceed 5.[16,21] In this study, stricter criteria of compliance to all the rules was set to ensure that compounds that passed at this stage would not have chances of failure at the later stage of development.[22]

Apart from pharmacokinetic properties of molecules, toxicity is another factor that may lead to failure of molecules along the drug discovery route. The study further predicted the toxicity profiles of the compounds that passed the Lipinski's Ro5. The toxicity indices such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity as well as the LD_{50} were predicted. With an LD_{50} > 2000 mg/kg and no toxicity recorded, eleven compounds were selected with potential to be developed as chemotherapeutic agents.[16,17,21]

To get insights into the potential of anticancer activity of the eleven selected phyto-toxic alkaloids, which included apovincamine (**1**), conhydrine (**2**), *N*-methylpseudoconhydrine (**3**), trigonelline (**4**), (-)-eburnamonine (**5**), retamine (**6**), palustridiene (**7**), norcoclaurine (**8**), dihydroatisine (**9**), catharanthine (**10**), pseudoconhydrine (**11**) and belonging to indole, isoquinoline, piperidine, pyridine, quinolizidine and terpenoid pharmacophores were docked to a target protein. The protein is an isomerase perdeuterated E65Q-TIM protein (ID: 7AZA) complexed with phosphoglycolohydroxamate ligand, expressed in *E. coli* and resolution of 1.10 Å. All the alkaloids docked showed negative binding energies higher than the native ligand. Specifically, apovincamine and catharanthine, both indole alkaloids and retamine, a quinolizidine alkaloid possess highest negative binding energy compared with the native and other ligands with corresponding Ki of 0.701, 0.910 and 1.89 µM respectively.

The eleven phytotoxic alkaloids belonging to the indole, isoquinoline, piperidine, pyridine, quinolizidine and terpenoid chemical class passed the drug-likeness prediction with LD_{50} > 2000 mg/kg. Molecular docking study showed that phytotoxic alkaloids, like colchicine,

vincristine and vinblastine, binds effectively to an isomerase perdeuterated E65Q-TIM protein (ID: 7AZA) complexed with phosphoglycolohydroxamate. There are strong hydrogen and hydrophobic interaction between the ligands and the target protein. Of the eleven alkaloids docked to the protein, apovincamine, catharanthine and retamine showed more efficient binding than other ligands. Several amino acid residues interacted efficiently with the functional groups of the ligands.[23,24] The study has demonstrated the *in-silico* potential of phytotoxic alkaloids to be further developed as inhibitors of cancer stem cells.[25]

Conclusion

The current findings have successfully provided important insights into some sterically favourable interactions of the docked drug-like phytotoxic alkaloids with an isomerase perdeuterated E65Q-TIM protein implicated in cancer disease. The Glu167A/234A/235A, gly212A, ser213A, val214A, leu232A, Ile172A, and val233A of perdeuterated E65Q-TIM protein were found to favourably interact with the phytotoxic ligands. Three compounds, apovincamine, catharanthine and retamine were found to bind more effectively and efficiently and could be further optimized for development.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original. Any liability for claims relating to this article will be borne by us.

Author Contributions

Conceptualization, C.O.N. and O.K.D.; methodology, C.O.N.; software, S.H.B. and M.U.U.; validation, O.K.D.; formal analysis, O.K.D.; investigation, S.H.B. and M.U.U.; writing—original draft preparation, O.K.D.; writing—review and editing, C.O.N.; visualization, S.H.B and M.U.U.; supervision, C.O.N.; All authors have read and agreed to the published version of the manuscript

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