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In silico Assessment of Galanthamine Alkaloids as Cytotoxic Agents and Brd4 Inhibitors

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Cancer is associated with high mortality. The potential of Galanthamine alkaloids as cytotoxic agents and Brd4 inhibitors was investigated. Selected alkaloids were screened for cytotoxic properties using the Cell Line Cytotoxicity Predictor (CLCPred). The drug-likeness, physicochemical and pharmacokinetic properties of the compounds were determined using SwissADME. The interactions of the ligands with the Brd4 protein were investigated using SwissDock. Lastly, the toxicity of the compounds was investigated using SwissADME. The compounds showed cytotoxic potential against bone marrow neuroblastoma at Pa>0.5. All the compounds satisfied Lipinski's, Verber's, and Muegge's conditions for drug-likeness. The binding energy of the alkaloids with Brd4 ranged between - 7.22 and - 7.82 kcal mol^{-1}. Lycoramine with a binding energy of -7.82 kcal mol^{-1} had comparable binding energy to those of the standard drug, doxorubicin (-7.91 kcal mol^{-1}), and Brd4 inhibitors: Pelabresib (-7.96 kcal mol^{-1}) and Birabresib (-8.43 kcal mol^{-1}). The compounds were non-AMES toxic, non-carcinogens, and weak inhibitors of the human ether-a-go-go related gene (hERG). Galanthamine alkaloids showed potential for treating human bone marrow neuroblastoma. The results of this study have laid a foundation for subsequent in vitro and in vivo studies to establish the predicted activity.

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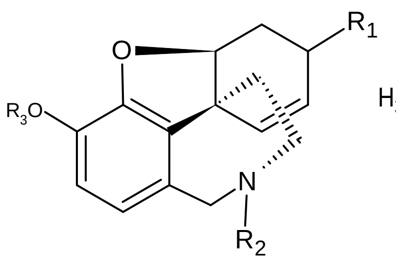
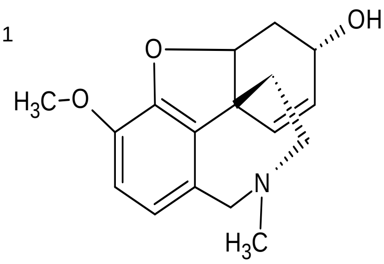
1. INTRODUCTION

Cancer is a leading cause of death worldwide. Data from the World Cancer Research Fund International indicates that there are 18.1 million cancer cases worldwide in the year 2020 [1]; about 10 million persons died from the disease the same year [2]. The global burden has been projected to rise to 27.5 million new cancer cases and 16.3 million cancer deaths by 2040 [3]. The rapidly increasing incidence of cancer is due to various factors, including infections, genetic mutations, unhealthy diet, smoking, physical inactivity, and environmental pollutants [4]. The treatment options currently available for cancer include surgery, chemotherapy, radiation therapy, gene therapy, and hormonal therapy [5]. Despite these therapeutic options, cancer mortality remains high. Furthermore, most of the chemotherapeutic agents currently in use have serious side effects (including hair loss, cardiac toxicity, gastrointestinal lesions, and neurologic dysfunction, among others) since they tend to attack normal cells alongside the cancerous cells [6]. These factors have necessitated the continuous search for new, cheaper, and less toxic anticancer agents. Herbs play a key role in treating diseases in traditional medicine. Phytochemicals are responsible for the biological activities displayed by plants [7] and are reported to be less toxic than their synthetic counterparts [8]. Some phytochemicals of pharmacological importance used in cancer treatment include taxol, vincristine, vinblastine, topotecan, irinotecan, and etoposide [9]. Up to 60% of drugs currently used in cancer treatment are natural products or their derivatives [10].

Bromodomain-containing protein 4 (Brd4) is a key mediator of gene expression in cancers. For example, Brd4 expression is significantly up-regulated in NSCLC tissues and NSCLC cell lines with high invasion and metastasis potentials [11]; Brd4 was highly over-expressed in primary and metastatic melanoma tissues and essential for melanoma tumor growth [12]; Brd4 maintains c-Myc expression, thereby promoting aberrant self-renewal of AML cells [13]; Brd2 and Brd4 were significantly elevated in glioblastoma [14]; and Brd4 regulated breast cancer metastasis by modulating extracellular matrix gene expression [15]. Results from several studies indicate that when the expression of Brd4 is inactivated or deregulated, cancer development is inhibited. For example, Brd4 inhibitors suppressed tamoxifen-resistant breast cancer cells growth [16]; impaired cell invasion, accelerated cell apoptosis, and inhibited cell proliferation in NSCLC cell lines [11]; demonstrated anti-leukemic effect in several human AML cell lines [17]; and strongly attenuated melanoma cell proliferation in vitro and *In vivo* [12]. Owing to the significant relationship between Brd4 expression and cancers, Brd4 is a promising therapeutic target in many malignancies [18]. A significant amount of effort has been put into developing pharmacological inhibitors of Brd4 (some of these inhibitors have progressed to clinical and preclinical phases) [19,20].





Lycoramine

Compound	R_1	R_2	R_3
Galanthamine	βOH	Me	Me
Epinorgalanthamine	αOH	H	Me
Narwedine	O	Me	Me
Norgalanthamine	βOH	H	Me
Sanguinine	βOH	Me	H

Fig. 1. Structures of Galanthamine Alkaloids under investigation

Galanthamine, an isoquinoline alkaloid, was first isolated from *Galanthus nivalis* and *Galanthus woronowii*, members of the Amaryllidaceae family [21]. Galanthamine and its derivatives have since been isolated from several *Crinum* species [22]. Galanthamine was approved by the Food and Drug Administration (FDA) for treating mild to moderate stages of Alzheimer's Disease [23]. This investigation is an attempt at re-purposing this drug and its derivatives for possible use as anticancer agents. In this study, the potentials of Galanthamine-type alkaloids as cytotoxic agents and inhibitors of Brd4 were evaluated. The structures of the compounds investigated are presented in Fig. 1.

2. METHODOLOGY

2.1 Prediction of Cytotoxicity of Compounds

The cytotoxic properties of the compounds were predicted using the online web service tool, Cell Line Cytotoxicity Predictor (http://www.way2drug.com/cell-line/). This tool uses the structural formula of compounds to predict their cytotoxic properties in normal and cancer cell lines. The cytotoxicity of the alkaloids (expressed as Pa and Pi values) was predicted by pasting their SMILE formats in the appropriate portal on the webtool server. Compounds with Pa > 0.5 are likely to possess good activity against a particular cell line; Pi expresses the probability that a compound would be inactive [24].

2.2 Prediction of Physicochemical and Pharmacokinetic Properties of Compounds

The Physicochemical and Pharmacokinetic properties of the compounds were predicted by pasting their SMILE formats in the SwissADME online Web server (https://www.swissadme.ch). The drug-likeness of the compounds was assessed based on the cutoff points set by Lipinski, Veber and Muegge [25,26,27].

2.3 Molecular Docking

2.3.1 Ligand preparation

The alkaloids selected for investigation in the current study are Galanthamine (1), Epinorgalanthamine (2), Lycoramine (3), Narwedine (4), Norgalanthamine (5), and Sanguinine (6); while Birabresib (7), and Pelabresib (8), and Doxorubicin (9) were selected as standards against which the cytotoxicity and Brd4 inhibitory properties of the compounds were measured. Doxorubicin is a standard drug used in cancer treatment, while Birabresib (MK-8628/OTX015) and Pelabresib (CPI-0610) are selective inhibitors of Bromodomain and Extra-Terminal (BET) proteins (BRD2, BRD3, and BRD4) [28,29]. The structures of the alkaloids and the standards were downloaded from the PubChem database in the SDF format. The structures were converted into the mol2 format before docking [30].

2.3.2 Protein preparation

The Brd4 protein (PDB ID: 4JBX) was obtained from the protein databank. A Ramachandran plot of the protein was obtained using the PROCHECK server. The Ramachandran plot affirms the accuracy and reliability of the target protein, showing percentages of the protein backbone falling within the allowed and disallowed regions.

2.3.3 Molecular docking

The interaction of the ligands with the target protein was investigated using the SwissDock Server (http://www.swissdock.ch/) [31], which sends a link containing the docking results for each compound to the user's email. The prediction (output file) gives information on the estimated binding energy, the full fitness score, and cluster rank, among others for each of the possible binding mode of the ligand. The lowest binding mode was selected as the one where the most favourable interaction exists between the protein and the ligand. After docking, Chimera was used to visualize and analyze the interactions between the protein and the ligands [32].

2.3.4 Prediction of toxicity of alkaloids

The toxicity of the compounds were assessed using ADMESAR (http://lmmd.ecust.edu.cn/admetsar1/predict/) by pasting their SMILE formats in the specified portal on the webpage [33].

3. RESULTS

The potential cytotoxic properties of the compounds reported as Pa and Pi values are in Table 1. Pa indicates the probability that a compound would be active against a particular cell line while Pi estimates the likelihood that the compound would be inactive. Table 1 shows that at Pa > 0.5, all the galanthamine alkaloids (except lycoramine (3)) demonstrated potential for cytotoxicity against the bone marrow neuroblastoma cell line (SH-SY5Y), with Pa values ranging between 0.588 and 0.657. The standard drug, Doxorubicin showed no likelihood for cytotoxicity at Pa>0.5. The results also indicated that none of the alkaloids showed potential for activity against the normal cancer cell lines.

Table 2 shows that all the compounds satisfied Lipinski's, Verber's, and Muegge's rules for drug-likeness. The compounds had MlogP values ranging between 1.50 and 1.83.

Table 1. Prediction of cytotoxicity of compounds on cancer cell lines (at Pa > 0.5)

Ligands	Cancer Cell Line (Pa>0.5)				Normal Cell Line (P>0.5)
	Pa	Pi	Cell line	Cancer Cell Full name	
Galanthamine	0.623	0.003	SH-SY5Y	Bone marrow neuroblastoma	No activity
Epinorgalanthamine	0.657	0.003	SH-SY5Y	Bone marrow neuroblastoma	No activity
Lycoramine	No activity	No activity	No activity	-	No activity
Narwedine	0.588	0.003	SH-SY5Y	Bone marrow neuroblastoma	No activity
Norgalanthamine	0.657	0.003	SH-SY5Y	Bone marrow neuroblastoma	No activity
Sanguinine	0.624	0.003	SH-SY5Y	Bone marrow neuroblastoma	No activity
Doxorubicin	No activity	No activity	No activity	-	No activity

Table 2. Physiochemical properties and drug likeness of the five inhibitors

Alkaloids	Physicochemical Properties							Drug likeness		
	MW	HBA	HBD	MR	TPSA (Aº)	NRB	MlogP	Lipinski	Veber	Muegge
1	287.35	4	1	84.05	41.93	1	1.74	Yes	Yes	Yes
2	273.33	4	2	79.15	50.72	1	1.50	Yes	Yes	Yes
3	289.37	4	1	84.52	41.93	1	1.83	Yes	Yes	Yes
4	285.34	4	1	83.09	38.77	1	1.66	Yes	Yes	Yes
5	273.33	4	1	79.15	50.72	1	1.50	Yes	Yes	Yes
6	273.33	4	2	79.58	52.93	0	1.50	Yes	Yes	Yes

MW- Molecular weight; HBA- Hydrogen Bond Acceptor; MR- Molar refractivity ; TPSA- Total Polar Surface Area; NRB – No of Rotatable Bonds

Table 3. Pharmacokinetics properties of Investigated Compounds

Ligands	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor
1	High	Yes	Yes	No	No	No	No
2	High	Yes	Yes	No	No	No	No
3	High	Yes	Yes	No	No	No	No
4	High	Yes	Yes	No	No	No	Yes
5	High	Yes	Yes	No	No	No	No
6	High	Yes	Yes	No	No	No	No

The pharmacokinetic properties of the compounds are in Table 3. The compounds possess high gastrointestinal absorption, can penetrate the blood-brain barrier, and are substrates for Permeability glycoprotein (Pg-p).

The Ramachandran plot (Fig. 2) shows that 94.5% of the target protein's residue (4JBX) falls within the preferred area (a protein model of excellent quality has 90% of its residue in the preferred areas.

Table 4 shows the results obtained on docking the ligands with 4JBX. The binding energy of the alkaloids ranged between -7.22 and - 7.82 kcal mol^{-1}. Lycoramine with a binding energy of -7.82 kcal mol^{-1} had comparable binding energy to those of the standards, Pelabresib (-7.96 kcal mol^{-1}), Birabresib (-8.43 kcal mol^{-1}) and doxorubicin (-7.91 kcal mol^{-1}). Information on the hydrogen bond interactions between the ligands and the protein residues is provided in Table 1, while Fig. 3 shows the orientation of the compounds in the protein binding pockets.

The results of the *in silico* toxicity screening of the compounds are in Table 5. The compounds are non-carcinogens, non-AMES toxic, and weak Inhibitors of Human Ether-a-go-related genes. In addition, they have high fish toxicity and are not readily biodegradable.

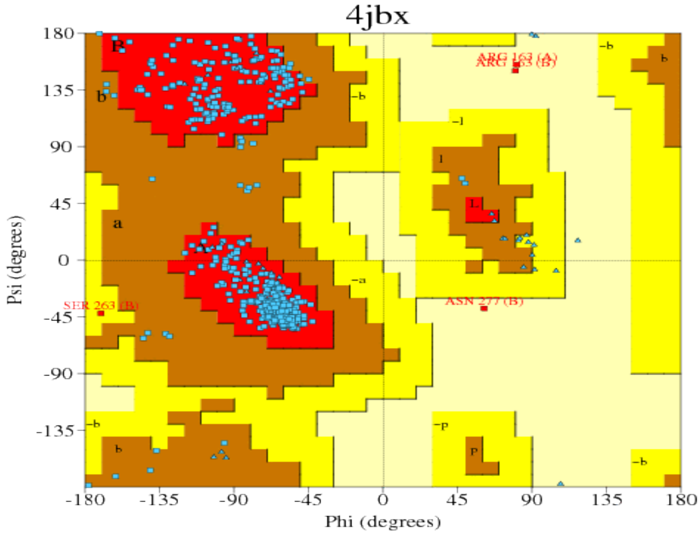


Fig. 2. The Ramachandran plot

Table 4. Docking results of BET (4BJX) with selected Phytoconstituents

Compound	Binding Affinity (kcal/mol)	Fullfitness	H-bonds	Length (A°)
Galanthamine	-7.57	-608.3592	N…O ASN135	7.597
			N…O CYS136	6.926
			N…O MET132	8.646
Epinorgalanthamine	-7.31	-611.628	H12…O ASP88	5.865
Lycoramine	-7.82	-605.99664	N…O ASP88	7.009
Narwedine	-7.42	-600.20276	N…O CYS136	7.040
			N…O ASN135	7.760
			O1…HN GLN84	7.023
			O1…HN GLN85	7.393
Norgalanthamine	-7.22	-609.8246	H12…O ASP88	5.959
			O2…HN GLN84	8.223
Sanguinine	-7.40	-618.19995	N…O PHE79	8.345
Birabresib	-8.43	-310.7484	H21…O MET132	6.681
			H21…O MET105	7.674
			N3…O LYS91	6.987
			N2…O ASP88	7.235
			N2…O PRO82	7.596

Compound	Binding Affinity (kcal/mol)	Fullfitness	H-bonds	Length (A°)
			O…HN GLN85	7.274
			O…HN GLN84	6.826
			H14…O GLN85	5.640
			H14…O TRP81	5.371
			H14…O PRO86	5.560
Pelabresib	-7.96	-607.5853	H15…O GLY143	8.512
			N…HN MET149	7.843
			N…HN ALA150	8.225
Doxorubicin	-7.91	-572.25885	O10…HN LYS141	7.493
			H19…O ASN93	7.161
			H19…O LEU92	4.974
			H16…O LEU92	4.174
			H19…O LYS91	6.219
			O9…HN ASP88	5.018
			H23…O LYS91	6.931
			H21…O PRO86	6.818
			O9,,,HN GLN85	6.424
			H21…O GLN85	7.349

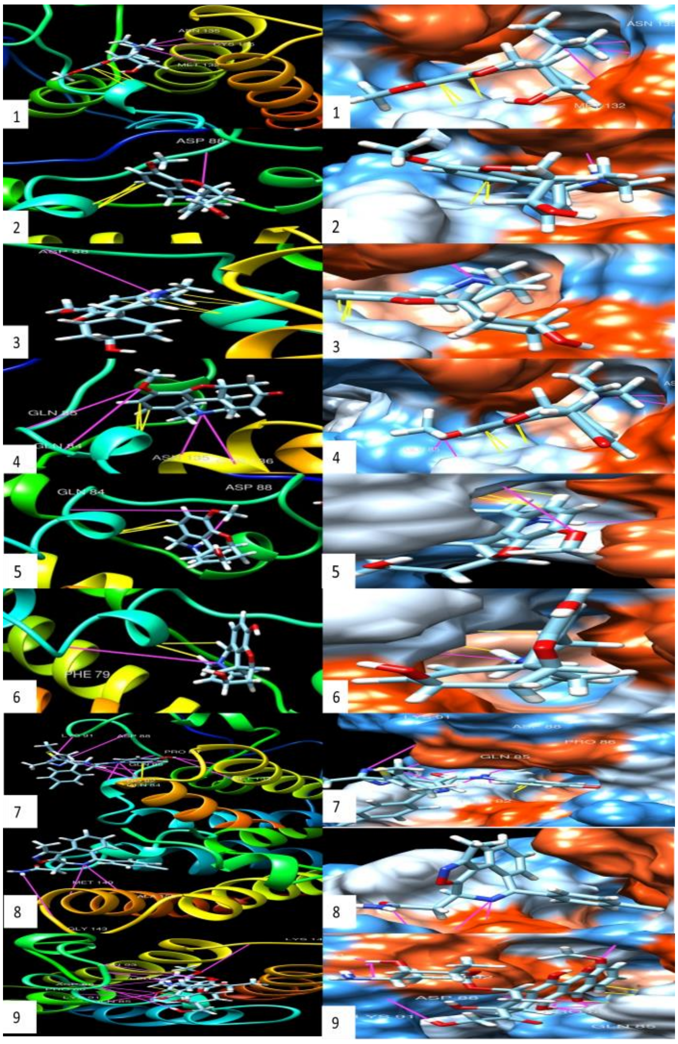


Fig. 3. Docking poses of the compounds within the protein binding pockets

Table 5. Prediction of toxicity of compounds

Compound	Human Ether-a-go-go-Related Gene	AMES Toxicity	Carcinogens	Fish Toxicity	Biodegradation
Galanthamine	Weak inhibitor	Non AMES toxic	Non-carcinogens	High FHMT	Not ready biodegradable
Epinorgalanthamine	Weak inhibitor	Non AMES toxic	Non-carcinogens	High FHMT	Not ready biodegradable
Lycoramine	Weak inhibitor	Non AMES toxic	Non-carcinogens	High FHMT	Not ready biodegradable
Narwedine	Weak inhibitor	Non AMES toxic	Non-carcinogens	High FHMT	Not ready biodegradable
Norgalanthamine	Weak inhibitor	Non AMES toxic	Non-carcinogens	High FHMT	Not ready biodegradable
Sanguinine	Weak inhibitor	Non AMES toxic	Non-carcinogens	High FHMT	Not ready biodegradable

4. DISCUSSION

Neuroblastoma accounts for about 8–10% of all childhood cancer cases [34]. The disease affects 11 to 13 per million children under the age of 15; with the incidence varying from 1 per million in children aged between 10-14 years and 65 per million in children less than 1 year old [35,36].

Based on various clinical parameters including age, stage, cellular differentiation/maturation, and biological markers at the time of diagnosis, children with Neuroblastoma are classified as low-risk, intermediate-risk, and high-risk (HR-NB). While the prognosis for the low-/intermediate-risk groups is favorable (with >90% overall survival), the HR-NB group has<50% five-year overall survival and poor long-term survival [37]. It is imperative, therefore to develop more effective drugs that can arrest disease progression, and ensure the long-term survival of children with HR-NB. The results of this study show that Galanthamine-type alkaloids showed good potential as cytotoxic agents against bone marrow Neuroblastoma cancer cell lines. Furthermore, the galanthamine alkaloids investigated showed high selectivity towards the cancer cell line as they did not show any potential for activity against the normal cell lines at Pa >0.5.

Lipinski's and Verber's rules measure the oral bioavailability of bioactive compounds while Muegge's rule measures the likelihood that a candidate would become a successful drug molecule from pharmacophore point calculation [38]. All the compounds studied satisfied Lipinski's, Verber's, and Muegge's conditions for drug-likeness, and are therefore potential lead agents for the treatment of Neuroblastoma. The topological polar surface area (TPSA) measures the fraction of the compound's surface area containing polar atoms. Compounds with a PSA less than 140A^2 exhibit better intestinal absorption [39], while compounds with PSA values less than 70A^2 can penetrate the blood-brain barrier [40]. All the compounds investigated had excellent intestinal absorption and can penetrate the blood-brain barrier (Table 3). However, the compounds are substrates for Permeability glycoprotein (Pg-p). Pg-p is an efflux transporter pump in the cell membrane that reduces the concentration of the drug by conveying it away from the cell membrane and cytoplasm, resulting in therapeutic failure. Therefore, for optimal performance, a drug candidate should possess excellent gastrointestinal permeability and low Pg-p liability [41]. Though lycoramine showed no activity when it was screened for cytotoxicity *In silico* (at Pa>0.5), it had the least binding energy with the protein(4JBX) among the Galanthamine-type alkaloids studied, and had comparable binding energy with the standards: Doxorubicin, Birabresib, and Pelabresib.

Cancer has often been linked to mutations. The AMES test is used to assess the mutagenic potential of chemical compounds. In the current study, all the compounds gave a negative result on the test, implying that they are not potential carcinogens. The compounds are weak inhibitors of the human ether-a-go-go-related gene (hERG). hERG can be inhibited by marketed drugs, which may lead to QT prolongation and possibly fatal cardiac arrhythmia [42].

5. CONCLUSION

The galanthamine-type alkaloids investigated demonstrated cytotoxic potential against human bone marrow neuroblastoma. All the compounds satisfied the conditions for drug-likeness. The binding energy of lycoramine with Brd4 is comparable to those of Doxorubicin, Birabresib, and Pelabresib. The compounds were non-AMES toxic, non-carcinogens, and weak inhibitors of hERG. Galanthamine alkaloids demonstrated potential for treating human bone marrow neuroblastoma. However, in vitro and in vivo studies need to be carried out to confirm the predicted activity.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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