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Identification of therapeutic phytochemicals targeting B-cell lymphoma 2 (BCL2) as anti-acute myeloid leukemia agents: An in-silico approach

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Abstract

Background: Acute myeloid leukemia (AML) is a deadly cancer. B cell lymphoma 2 (BCL2) is frequently upregulated in AML and plays a vital role in the viability of both AML and AML stem cells. This study aimed to identify novel phytochemicals against BCL2 and evaluate their pharmacokinetics and toxicity prediction using in-silico tools.

Methods: In-silico screening of phytochemicals against BCL2 active site using the PyRx0.8 AutoDock tool, followed by in silico pharmacokinetic and toxicity predictions was performed. Protein-protein interaction analysis was performed using the STRING database for assessing the interactions between BCL2 and neighboring interacting proteins.

Results: In total, 1106 terpenoid compounds were screened to evaluate their binding affinity toward BCL2. Five natural compounds demonstrated strong binding to the BCL2 protein after extensive screening, detailed interaction analysis, and visual inspections. Notably, these compounds had higher binding energies than the positive control (venetoclax). In addition, these compounds were found to bind to key BCL2 residues and possess good drug-like properties.

Conclusions: The identified phytochemicals represent an important initial step in drug discovery for AML management. Experimental validation is required to optimize the identified phytochemicals as potential BCL2 inhibitors.

Introduction

Leukemia is a type of cancer that originates from hematopoietic stem cells or progenitor cells. It is a heterogeneous group of diseases that affects the normal development and function of blood cells. Acute myeloid leukemia (AML) is a particularly aggressive form of leukemia characterized by the abnormal growth and proliferation of myeloid progenitor cells. AML is a significant health problem worldwide, with a reported global incidence of over one million cases per year and resulting mortality rate of more than 100,000 [1,2]. Chemotherapy is still the most commonly used primary treatment option for AML [3,4]. Despite this treatment option, patients with AML have a five-year survival rate of only approximately 28%, indicating poor outcomes. Globally, the overall survival rate for individuals with AML remains poor [5].

The B cell lymphoma 2 (BCL2) protein family, which is involved in the regulation of apoptotic cell death, has been linked to the pathogenesis and progression of various cancers [6,7]. In AML, BCL2 is commonly upregulated and plays a crucial role in promoting the survival of both AML and AML stem cells [8,9]. As BCL2 is a central regulator of the intrinsic apoptotic pathway involved in the development and progression of several malignancies, inhibition of this protein represents a promising therapeutic strategy for developing novel anticancer agents [10]. Venetoclax, also known as ABT-199, is a newly discovered small-molecule inhibitor that can be administered orally and has been designed specifically to target BCL2 [11]. While the majority of patients respond to venetoclax treatment, the extent and duration of this response remain suboptimal [12].

Terpenes, a class of organic compounds derived primarily from plants and trees, exhibit notable biological properties such as analgesic and anticonvulsant properties. Numerous studies have shown that certain terpenes can reduce inflammation symptoms by inhibiting pro-inflammatory cytokine release [13]. Terpenes also have antimicrobial, antiviral, antioxidant, and analgesic properties, aid in digestion, and have a variety of other biological activities. Their application ranges from influencing food health factors to acting as components in anti-cancer therapeutic modalities [14].

Computer-aided drug design (CADD) has been widely adopted by biologists and chemists as an essential component of a comprehensive drug discovery approach [15]. CADD is extensively utilized in the pharmaceutical industry to reduce costs and expedite the early-stage development of biologically novel and active compounds. Moreover, CADD is integral to drug discovery, design, and analysis processes [16]. Phytochemicals are plant-derived compounds that are often more chemically diverse and safer than commercially available synthetic medications. Additionally, they often possess a variety of pharmacological properties, including antibacterial, anticancer, antioxidative, and anti-inflammatory activities [17,18]. This study aimed to identify novel hits from phytochemicals, specifically terpenoid compounds, that can potentially be used as BCL2 inhibitors to combat AML.

Methods

Target protein retrieval and preparation

The crystal structure of BCL2 protein in complex with venetoclax was retrieved from the PDB database (PDB ID: 6O0K) [19]. The first step in preparing the protein was to eliminate both water and co-crystallized ligand, which in this case was venetoclax. The structure was then saved in the .pdb format.

Protein-protein interaction (PPI) analysis

PPI analysis was performed using the STRING database for BCL2 and neighboring interacting proteins to gain a better understanding of the protein-interacting partners [20].

Phytochemical library preparation

In this study, the Plant Secondary Compound DataBase (PSC-db) [21] was used; a total of 1106 terpenoid compounds were obtained from this database. The compounds were downloaded in the .sdf format, minimized, and prepared for further screening.

Virtual screening (VS)

In this study, the screening process involved the utilization of the PyRx0.8 AutoDock Vina program [22] to identify the binding between prepared phytochemicals (terpenoid compounds) and BCL2 protein. PyRx0.8 is an open source VS application used in CADD techniques to screen libraries of compounds against the target protein. The ligand-protein complex exhibiting the highest negative binding energy was selected for further investigation, and the binding interactions were subsequently analyzed. The grid box of the BCL2 active site was set as X= -11.003, Y= 1.56, and Z= -9.00 for the screening of the prepared compounds.

Estimation of the physicochemical and drug-likeness properties

The physicochemical and drug-likeness properties of the 10 selected compounds were estimated using the SwissADME web server [23].

Results

This study included the analyses of PPI interaction of BCL2 and molecular docking-based VS of 1106 terpenoid compounds to identify potent natural

inhibitors of BCL2. PPI analysis revealed that BECN1, BAX, TP53, BAD, BCL2L11, BIK, BBC3, BID, BCL2L1, and FKBP8 were the closest proteins that interacted with BCL2.

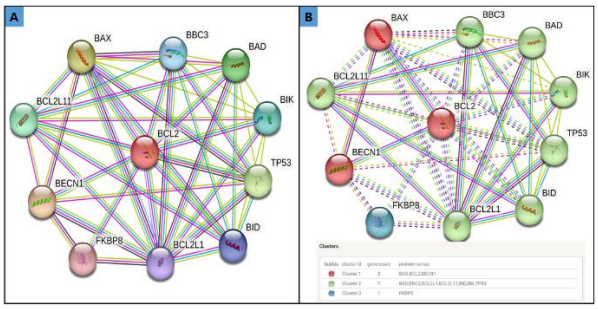

Figure 1: Protein-protein interaction analysis. 10 neighboring proteins interacting with BCL2 **(A)**, and k-means clustering of the network of interacting proteins **(B)**.

Clustering analysis showed that these interacting proteins belonged to the following three distinct groups or clusters: BAX, BCL2, and BECN1 in one cluster; BAD, BBC3, BCL2L1, BCL2L11, BID, BIK, and TP53 in the second cluster; and FKBP8 alone in the third cluster (Figure 1 and Table 1).

clustering method	cluster number	cluster color	gene count	protein name
k-means	1	Red	3	BAX
			3	BCL2
			3	BECN1
	2	Green	7	BAD
			7	BBC3
			7	BCL2L1
			7	BCL2L11
			7	BID
			7	BIK
			7	TP53
	3	Blue	1	FKBP8

Table 1: Clusters of 10 interacting proteins with BCL2.

Screening of the prepared library of 1106 terpenoid compounds, in which the compounds were mainly carotenoids and apocarotenoids, diterpenoids, hemiterpenoids, monoterpenoids, sesquiterpenoids, steroids, and triterpenoids, was performed by targeting the binding pocket of BCL2. In this study, venetoclax was used as a reference control for analyzing the binding patterns and positions of the docked compounds. Visual inspection was performed to assess the binding efficiencies of both venetoclax and the top-screened ligands. Based on the findings, the top ten terpenoid compounds were identified, as shown in Table 2 (Figure 2).

The physicochemical and drug-like properties of the selected compounds were evaluated, and nearly all compounds demonstrated drug-like characteristics, indicating their potential to be developed as drug molecules (Table 3).

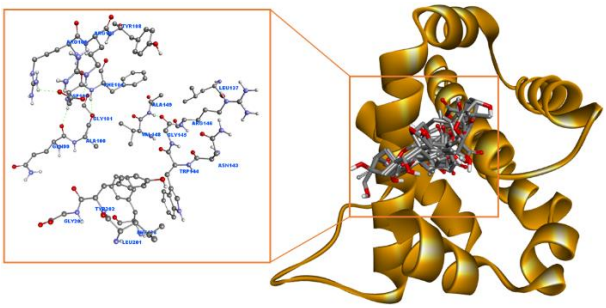

Figure 2: Binding sites residues of the BCL2 protein and docked complex of selected terpenoids in its active site.

S. No.	Compound name	Binding energy (kcal/mol)
1.	Ginkgolide A	-10.12
2.	Gibberellin A34-catabolite	-10.09
3.	Gibberellin 1	-10.05
4.	Zexbrevin B	-9.95
5.	7beta,12alpha-Dihydroxykaurenolide	-9.92
6.	Caryoptin	-9.90
7.	Echinocystic acid	-9.88
8.	Enhydrin	-9.85
9.	Paucin	-9.81
10.	Isodonal	-9.79
11.	Venetoclax (control)	-9.79

Table 2: Binding energy of selected 10 terpenoids.

Based on the visual inspection of the binding positions, five compounds (Gibberellin 1, Zexbrevin B, Ginkgolide A, Phorbol, and 7beta,12alpha-Dihydroxykaurenolide) were selected for an in-depth interaction analysis. Gibberellin 1 was found to interact with the Leu137, Ala149, Arg146, Tyr108, Trp144, Gly145, Tyr202, Arg106, Asp103, Gln99, Ala100, Val148, Phe198, and Phe104 residues of BCL2. The Arg107 and Asp103 residues were involved in H-bonding, whereas the Leu137, Arg146, Trp144, Gly145, Tyr202, Arg106, Gln99, Ala100, Val148, and Phe198 residues interacted with Gibberellin 1 via van der Waals interactions (Figure 3A). Zexbrevin B interacted to the Gly145, Trp144, Tyr202, Phe198, Val148, Arg107, Asp103, Tyr108, Ala100, and Phe104 residues of BCL2. The Trp144, Phe198, Val148, Arg107, and Tyr108 residues participated in van der Waals interactions, whereas the Gly145 and Phe104 residues H-bonded with Zexbrevin B (Figure 3B). Ginkgolide A was found to interact with the Gly145, Tyr108, Val148, Tyr202, Ala100, Phe198, Asp103, Arg107, and Phe104 residues of BCL2. The Phe198, Arg107, and Phe104 residues participated in van der Waals interactions, whereas the Gly145, Tyr108, and Asp103 residues H-bonded with Ginkgolide A (Figure 3C). Phorbol interacted with the Asp103, Val148, Ala100, Gly101, Phe104, Gly145, Asn143, Trp144, Tyr108, Arg107, Phe198, and Tyr202 residues of BCL2. The Asp103 and Ala100 residues H-bonded with Phorbol, whereas the Val148, Gly101, Phe104, Gly145, Asn143, Trp144, Tyr108, Arg107, and Phe198 residues were involved in van der Waals interactions (Figure 3D).

Compound names	MW	formula	RB	HBA	HBD	lipinski	ghose	veber	egan	muegge	bioavail
Ginkgolide A	408.4	C20H24O9	1	9	2	0	0	0	0	0	0.55
Phorbol	364.43	C20H28O6	1	6	5	0	0	0	0	0	0.55
Zexbrevin B	364.39	C19H24O7	3	7	2	0	0	0	0	0	0.55
Gibberellin 1	348.39	C19H24O6	1	6	3	0	0	0	0	0	0.56
7beta,12alpha-Dihydroxykaurenolide	332.43	C20H28O4	0	4	2	0	0	0	0	0	0.55
Caryoptin	492.56	C26H36O9	8	9	0	0	2	0	0	0	0.55
Echinocystic acid	472.7	C30H48O4	1	4	3	1	3	0	1	1	0.56
Enhydrin	464.46	C23H28O10	7	10	0	0	0	0	0	0	0.55
Paucin	468.49	C23H32O10	5	10	3	0	0	1	1	0	0.55
Isodonal	404.45	C22H28O7	4	7	1	0	0	0	0	0	0.55

Table 3: Predicted physicochemical and Drug-likeness properties of selected 10 terpenoids.

In addition, 7beta,12alpha-Dihydroxykaurenolide interacted with the Tyr202, Trp144, Gly145, Ala100, Val148, Phe198, Asp103, Arg107, Phe104, and Tyr108 residues of BCL2. The Trp144, Arg107, Phe104, and Tyr108 residues participated in van der Waals interactions, whereas the Tyr202, Gly145, and Asp103 residues H-bonded with 7beta,12alpha-Dihydroxykaurenolide (Figure 3E).

Furthermore, the control compound venetoclax interacted with the Glu152, Phe153, Val133, Glu136, Val148, Asp103, Arg107, Ala100, Asn143, Trp188, Trp144, Asn192, Leu201, Tyr202, Gly145, Arg146, Tyr108, Phe104, Ala149, Phe112, Val156, Met115, Asp111, and Leu137 residues of BCL2 (Figure 3F).

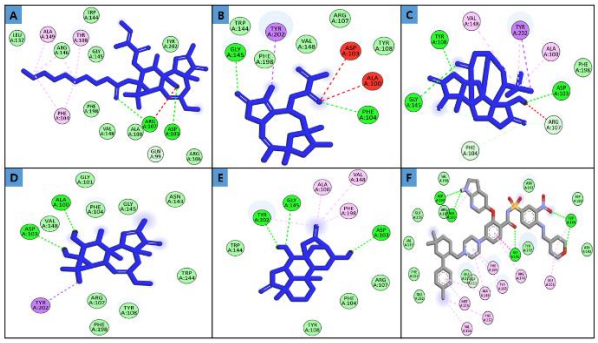

Figure 3: Interacting residues of BCL2 protein with Gibberellin 1 **(A)**, Zexbrevin B **(B)**, Ginkgolide A **(C)**, Phorbol **(D)**, 7beta,12alpha-Dihydroxykaurenolide **(E)**, and venetoclax **(F)**.

Discussion

The BCL2 family of proteins plays a crucial role in intrinsic apoptosis. In AML, excessive expression of BCL2 proteins can thwart resistance to apoptosis and chemotherapeutic agents. Therefore, investigating anti-apoptotic BCL2 inhibitors holds great promise for identifying novel pharmacological agents for cancer treatment [24]. This study screened a total of 1106 terpenoid compounds against the BCL2 protein. Among these compounds, Gibberellin 1, Zexbrevin B, Ginkgolide A, Phorbol, and 7beta,12alpha-Dihydroxykaurenolide demonstrated robust binding with BCL2. The Phe104, Met115, Leu137, Arg146, Val148, Leu137, Ala149, and Phe153 residues of BCL2 have been reported to be important in inhibitor binding [25]. Interestingly, in this study, the hit compounds (Gibberellin 1, Zexbrevin B, Ginkgolide A, Phorbol, and 7beta,12alpha-Dihydroxykaurenolide) were observed to bind to these residues of BCL2. Venetoclax binds to Glu152, Phe153, Val133, Glu136, Val148, Asp103, Arg107, Ala100, Asn143, Trp188, Trp144, Asn192, Leu201, Tyr202, Gly145, Arg146, Tyr108, Phe104, Ala149, Phe112, Val156, Met115, Asp111, and Leu137 of BCL2 Interestingly, Gly145, Tyr108, Val148, Tyr202, Ala100, Asp103, and Phe104 were observed to be the common binding residues for interactions between BCL2 and the hits (Gibberellin 1, Zexbrevin B, Ginkgolide A, Phorbol, and 7beta,12alpha-Dihydroxykaurenolide) as well as venetoclax (Figure 1A-1F).

Binding energy serves as a determinant of the degree of interaction between a protein and ligand, and a higher (negative) value of this energy signifies the successful binding of the inhibitor to its target protein [26]. Interestingly, Gibberellin 1, Zexbrevin B, Ginkgolide A, Phorbol, and 7beta,12alpha-Dihydroxykaurenolide exhibited strong binding with BCL2, with higher binding energies than that observed for venetoclax, suggesting that these hits can be used as BCL2 inhibitors to combat AML.

Phytochemicals and their derivatives found in plants have shown promise for improving cancer treatment efficacy while reducing the adverse effects. Many of these biologically active compounds occur naturally and possess significant antitumor potential [27]. Developing an effective and adverse-effect-free anticancer therapy based on phytochemicals requires initial testing of natural plant extracts for their potential anticancer biological activity. This is followed by the purification of the active phytochemicals using bioassay-guided fractionation and further evaluation of their *in vitro* and *in vivo* effects. From 1940 to 2014, approximately half of the approved anticancer drugs have originated from natural products or their derivatives [28].

Conclusion

This study screened phytochemicals, specifically terpenoid compounds against the BCL2 protein. Gibberellin 1, Phorbol, Zexbrevin B, Ginkgolide A, and 7beta, 12alpha-Dihydroxykaurenolide were observed to bind strongly with BCL2, bind to key BCL2 residues, and have good drug-like properties. These

phytochemicals have the potential to be used in the management of AML. However, further experimental validations are required to optimize their usage as BCL2 inhibitors for AML management.

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Conflicts of interest

The authors declare no conflict of interest.

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