Pharmacoinformatic Analysis of the Phytocompound Epigallocatechin Gallate for Potential Bcl-2 Inhibitor

Balasundari A.*

Abstract

Objectives: The B-cell lymphoma-2 (Bcl-2) is a protein that is particularly though to be involved in preventing apoptosis. Bcl-2 family members also essential for cancer survival, and its over expression has been linked to tumor genesis, development and resistance to anticancer treatments. The Bcl-2 family of members that inhibit apoptosis is a promising target for the creation of anticancer drugs. Methods: The study was based on computational approach using different phytocompounds for evaluating their potential against Bcl-2 protein. The compound namely epigallocatachin gallate and their similar compounds derived from Pubchem and three known inhibitor of Bcl-2 was selected. Molecular docking was conducted systematically using Pyrex and Biovia to determine the binding affinities between bioactive compounds. The pharmacological characteristics and toxicity of the compounds, analyzed by using Swiss ADME and ADMETlab 2.0. Results: The docking results revealed that some phytocompounds were the best inhibitor of Bcl2 protein, especially (2R,3R)-2-(3,4,5-trihydroxyphenyl) chroman-3-yl 3,4,5-trihydroxybenzoate. Perfectly binds inside the pocket of target protein like standard drug. The pharmacological studies also revealed that non toxicity and carcinogenicity. Conclusion: This computational study finding might help develop potential drug to combat standard drugs. The findings could aid in the creation of affordable, all-natural cancer treatments.

Keywords: Bcell lymphoma 2, Epigallocatechin gallate, Venetoclax, Phytocompounds.

INTRODUCTION

One of the characteristics of cancer is the capacity of cancer cells to evade an apoptosis or programmed cell death and continues to grow, which is a significant focus of the development of cancer therapies. The bcl-2 family of proteins controls apoptosis. The Bcl-2 gene is located on chromosome 18, and much B-cell leukemia and lymphomas have Bcl-2 gene translocation to other chromosomes. Increased production of the Bcl-2 protein as a result may prevent cancer cells from dying. Also known as lymphoma 2 proteins or B-cell leukemia. Thus, approaches designed to interfere with Bcl-2 protein activity have been carefully researched in order to create new cancer treatments.

*Author for Correspondence
Balasundari A.*
E-mail: sundarimb@gmail.com

Student, Department of bioinformatics, Bionome, Bangluru, India

Apoptosis is controlled by members of the bcl-2 family, although these proteins also serve other purpose; they interact with both cell death inducers and inhibitors. Collectively, they control and orchestrate the intrinsic apoptosis pathway, which is the mechanism by which mitochondria cause cell death [1]. The intrinsic process is regulated by keeping a delic balance between two sets proteins in the mitochondrial membrane, anti-apoptotic proteins like Bcl-2 and Bcl-X and pro-apoptotic proteins like Bax and Bak. Pro-apoptotic proteins

are prevented from acting in healthy cells by the anti-apoptotic proteins, which bind to them. Bcl-2 and Bcl-X, however, are each stopped in turn if a cell is injured or if it quits receiving cues for survival. After that, Bax and Bak are free to punch a number of mitochondrial channels, enabling molecules from the mitochondria, such cytochrome C, to seep into the cytoplasm. Cytochrome C that has spilled binds to Apf-1 proteins to form a substance that triggers the caspase cascade. Cancer cells are able to prevent apoptosis, which allows them to live and grow. Designing targeted therapeutics based on better apoptosis control requires an understanding of how pathways and the caspase cascade operate [2].

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The primary bioactive component of green tea polyphenols is catechins (flavan-3-oils), which is regarded as an affordable, easily used, and safe phytochemical. The four main green tea are (-)-epicatechin (EC), (-)-epigallocatechin (ECG), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG). EGCG has drawn the most attention for its inhibitory effects on cancer development at all stages, including inhibition, promotion and progression [3]. Through activating caspase-dependent internal (mitochondrial) and extrinsic (death receptor) mechanisms, EGCG causes B lymphoma cell apoptosis [4]. These results imply that EGCG may have therapeutic potential for B lymphoma.

BH3-mimetic drugs are a novel family of anticancer drugs that bind to pro-survival proteins like Bcl-2 in the same way as BH3-only proteins and limit Bcl-2's capacity to bind BAX or BAK. Since Bcl-2 exists coupled to natural BH3-only proteins, BH3-mimetics can also displace these endogenous apoptosis activators [5, 6].

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MATERIALS AND METHODS
Protein Structure Retrieval
The 3D structure of Bcl-2 protein was retrieved from protein data bank ((http://www.rcsb.org)using PDB ID: 1G5M and used as target protein receptor. Figure 1 shown molecular structure of Bcl-2 isoform 1. NMR spectroscopy has been used to identify the structure of two Bcl-2 isoforms that vary from one another by two amino acids. Bcl-2 or Bcl-X chimaeras, where a shorter loop from Bcl-X was substituted for a piece of the putative unstructured loop of Bcl-2, were used to identify the structures since wild-type Bcl-2 exhibited poor behavior in solution. Two Bcl-2 isoforms share a six-alpha helical shape with

the homologous protein Bcl-X, which also has a hydrophobic groove on its surface. The binding grooves electrostatic potential and structural topology change between the Bcl-2 and Bcl-X structures, despite the fact that their overall folds are the same [11]. Bcl-2 isoform 1 was employed in the study for more research.
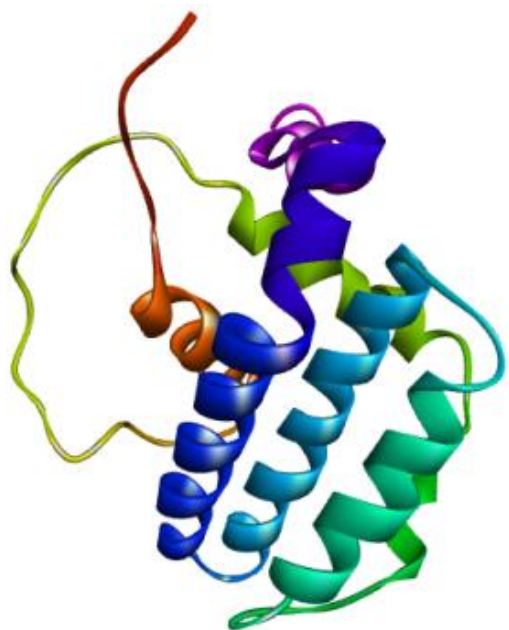

Figure 1. Molecular structure of Bcl-2 Isoform 1.

Ligand Library Preparation

The structure of epigallocatechin gallate-related compounds were retrieved in structure data format (SDF) from the Pubchem database (http://pubchem.ncbi.nlm.nih.gov/). For the goal of molecular docking, fourteen chemicals were chosen and employed as ligands. Table 1 displays the phytocompounds together with their Pubchem ID. Using Autodock tools, the ligand and protein molecules were then transformed to dockable pdbqt format.

Selection of Standard Drugs

With the brand name VENCLAXTA, the first United States of Food and Drug selectively oral Bcl-2 inhibitor for the treatment of CLL is Venetoclax (ABT-199). Pro-apoptotic BAX and BAK proteins can be activated to cause apoptosis because it displaces pro-apoptotic BH3-only proteins from Bcl-2. Similar to ABT-737, Bcl-2 and Bcl-X are inhibited by this drug. Although it is orally bioavailable, navitoclax suppresses Bcl-2 and Bcl-X [12]. Because the Bcl-2 protein is the sole one we are targeting in this study, Venetoclax, ABT-737 and Navitoclax which are typical pharmacological compounds, were tried.

Molecular Docking

To locate lead compounds with required biological activity, small-molecule libraries are docked to macromolecules via virtual molecular screening. The use of this *in silico* approach in computer aided drug creation is widely recognized [13]. Autodock and Autodock Vina were among the ligands that were virtually screened using PyRex virtual screening software with the Lamarckian genetic algorithm (LGA) serving as the score function [14]. Docking was done on the Bcl-2 protein (PDB ID: 1G5M) for 14 phytocompounds and 3 standard drugs to determine the best binding energy value. In order to dock the ligands, the active site dimensions were established as grid size of centre X=52.5217, centre Y=58.3366 and centre Z=45.6200 (XYZ axis). The maximum exhaustiveness of 10 was computed for each ligand. Charges were assigned to the protein and ligand structures by Autodock Vina before to the docking procedure. The produced ligands offer a great deal of potential as therapeutic candidates.

Drug Likeness Evaluation
Swiss ADME

The top phytocompounds with the highest docking scores were then further chosen using Lipinski's rule of five [15]. With the use of Swiss ADME, the compounds drug similarity was assessed. This rule includes information on the use of ligands as drugs and covers molecular characteristics crucial to a drugs pharmacokinetics in the human body. A potential drug candidate should be "drug-like", which refers to having properties that are comparable to those of well-established medications. Using the Swiss ADME programmed, bioavailability radar was created after six physiochemical parameters, including solubility, molecular size, polarity, lipophilicity, saturation and flexibility, were taken into consideration while evaluating drug-likelihood for potential candidates (http://www.swissadme.ch/).

Toxicity Prediction

Toxicity screening studies are one of the most important steps in discovering and developing of new active compound or medicines for disease treatment [16]. The ADMETlab 2.0 was used to predict the toxicity of the ligand in this study (http://admetmesh.scbdd.com/service/screenin/cal).

RESULTS
Retrieval of Ligands

The compounds having similar structures as that of Epigallocatechin gallate were selected as a candidate drug library for the present investigation. The information about the ligands included in the study is documented in Table 1.

Table 1. List of Epigallocatechin gallate similar compounds retrieved from Pubchem and standard drugs (selective Bcl-2 inhibitors).

| S.N.	Pubchem ID	Chemical Name
1	65064	(-)-Epigallocatechin gallate
2	120953261	Epigallocatechin gallate 4'-palmitate
3	162642698	(+/-)-Epigallocatechin Gallate-13C3
4	44195666	Epigallocatechin gallate 4-palmitate
5	76308050	[131I]-Epigallocatechin gallate
6	73425507	Epigallocatechin gallate 4'-stearate
7	76959811	Epigallocatechin gallate 3-stearate
8	76961109	Epigallocatechin gallate 3'-stearate
9	101127473	Epigallocatechin metabolite m6'
10	1287	5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate
11	45093080	(R)-5-(3,4-Dihydroxybenzyl)dihydrofuran-2(3H)-one
12	9873850	Inoveolegcg
13	16747763	(2R,3R)-2-(3,4, 5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate
14	92850118	Epigallocatechin metabolite M4
Standard drug		
15	49846579	Venetoclax
16	11228183	ABT-737
17	24978538	Navitoclax

Molecular Docking

Using molecular docking software, all 14 of the chosen phytocompounds were docked with the Bcl-2 protein. It was discovered that some phytocompounds had a greater affinity for the target protein. Using Biovia Discovery studio, an interaction study was carried out utilizing the maximum negative binding energy. The distances between various amino acid interactions were determined. After docking

of the selected phytocompounds with Bcl-2, it was found that of all the 14 phytocompounds, (2R,3R)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate has the highest binding energy of 8.7 Kcal/mol followed by 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate, (+/-)- epigallocatechin gallate-13C3 and (-)-epigallocatechin gallate with binding energy of 8.3, 8.1 and 8.1 respectively and also have 7,10,10 and 9 number of various interaction respectively (Table 2). The least amount of binding energy is found in epigallocatechin gallate 3'-stearate, which has 6.2 Kcl/mol. From the results of interaction between the phytocompounds and Bcl-2 protein (Figure 2), (2R,3R)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate has the maximum binding energy and a large number of interactions, making it an ideal starting point for developing a Bcl-2-targeting medicinal lead chemical. while the amino acids ARG A:6, TYR A:9, TRP A:195, ASN A:11, ASN A:182, ASP A:196, HIS A:186 as shown in Table 2 are the most important residue for potential drug targeting Bcl-2 protein. Based on their high binding affinities, seven of the top 17 compounds were chosen for further study. While the amino acids in EGCG phytocompounds are comparable to those in regular drugs like ASN, GLU and ASP they are attached to the OH group in phytocompounds rather than the H group in standard drugs. The bonding attraction between these chemicals differs significantly from one another.

Table 2. Docking results of phytochemicals and standard drugs with Bcl2 protein.

| S.N.	Chemical Name	Binding Energy	Total Number of Interaction	Hydrogen bonds
1	(-)-Epigallocatechin gallate	-8.1	9	OH- ALA H- HIS H- GLY
2	Epigallocatechin gallate 4'-palmitate	-7.4	16	OH- SER H- ASP
3	(+/-)-Epigallocatechin Gallate-13C3	-8.1	10	H- ASN H- GLU
4	Epigallocatechin gallate 4-palmitate	-7.3	15	OH- SER H- ASP
5	[131I]-Epigallocatechin gallate	-6.7	9	OH- ASP OH- GLU OH- SER
6	Epigallocatechin gallate 4'-stearate	-7.1	14	OH- GLU
7	Epigallocatechin gallate 3-stearate	-7.3	19	OH- LYS H- GLU H- GLY H - ASP
8	Epigallocatechin gallate 3'-stearate	-6.2	8	H- ARG
9	Epigallocatechin metabolite m6'	-7.1	5	H- TYR
10	5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate	8.3	10	OH- ASN H- GLY
11	(R)-5-(3,4-Dihydroxybenzyl)dihydrofuran-2(3H)-one	-7	6	OH- ASN
12	Inoveolegcg	-7.8	10	OH- ASP OH- GLU OH- SER
13	(2R,3R)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate	-8.7	7	OH- ASP OH- HIS OH- ASN H- TRP
14	Epigallocatechin metabolite M4	-7.4	6	OH- ASN
15	Venetoclax	-9.5	11	H- ASP H- GLU
16	ABT-737	8.8	10	H- ASN H- GLU
17	Navitoclax	8	15	H- GLU H- SER

(a)

(b)
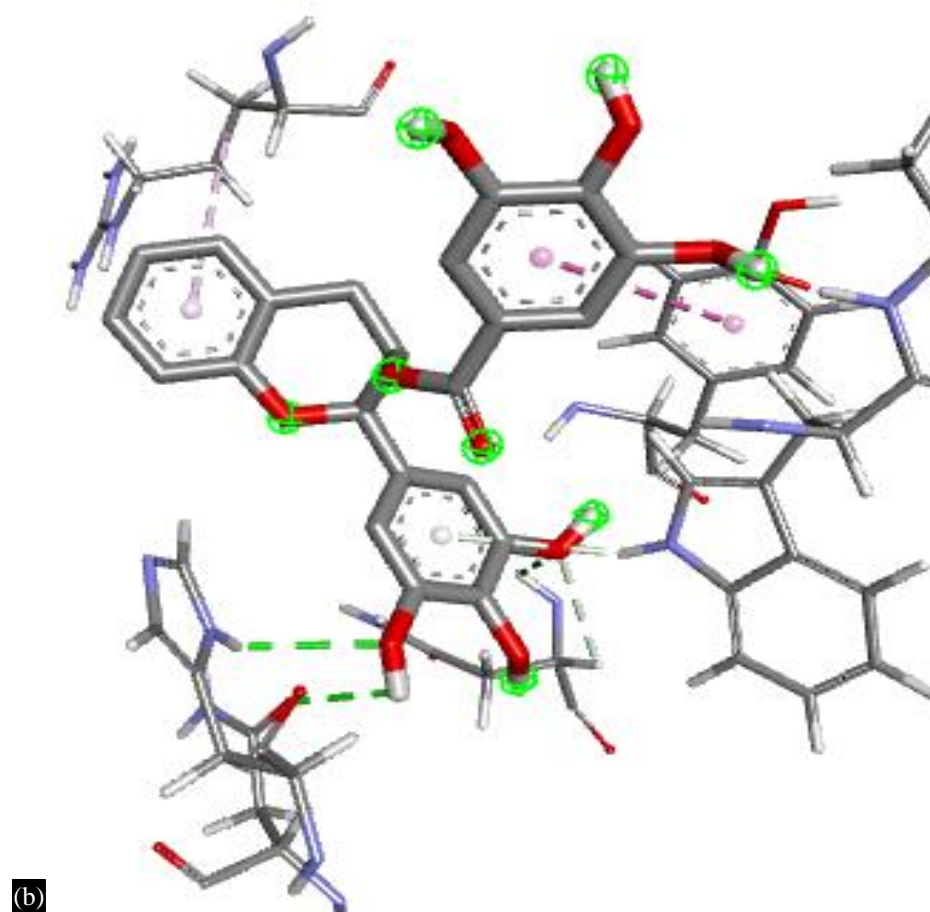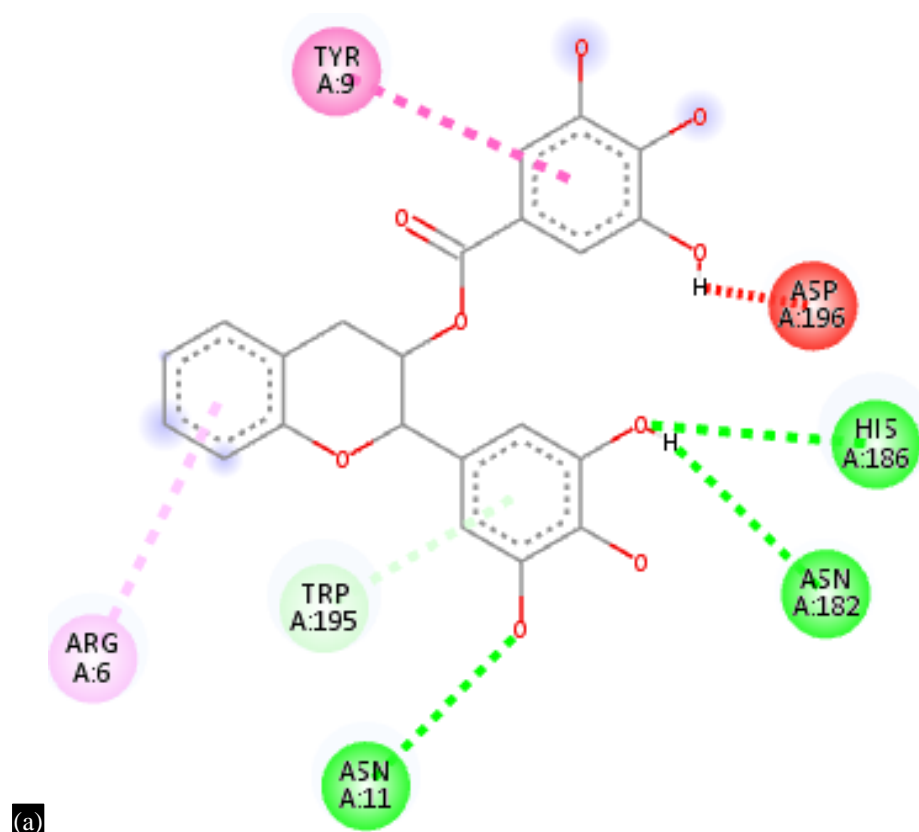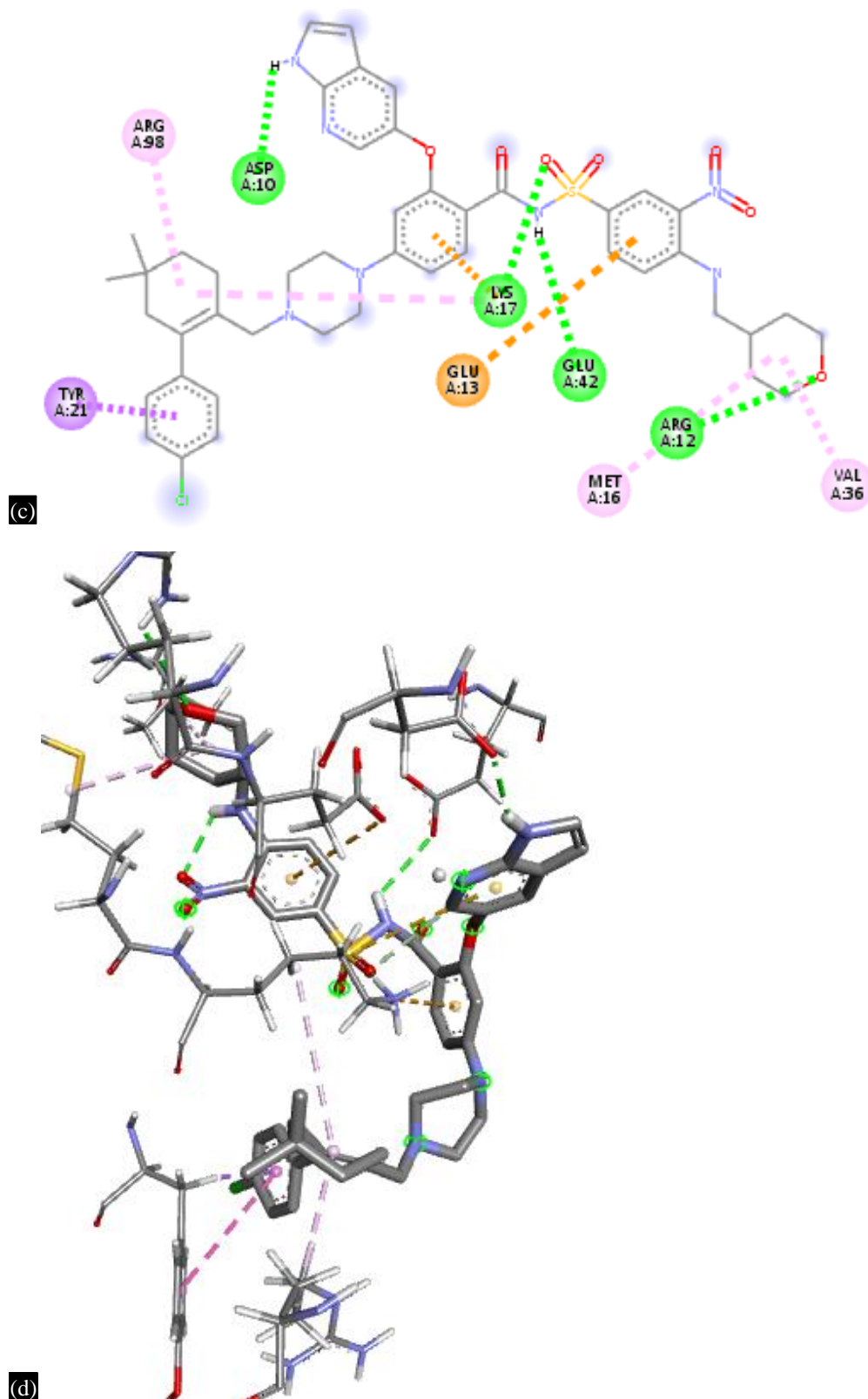
Figure 2. 2D and 3D ligand target protein interaction. (a) 2D structure showing interaction between (2R, 3R)-2-(3, 4, 5-trihydorxyphenyl) chroman-3-yl 3, 4, 5-trihydroxybenzoate and Bcl-2 protein, (b) 3D ligand–Target protein interaction between (2R, 3R)-2-(3, 4, 5-trihydroxyphenyl) chroman-3-yl 3, 4, 5-trihydroxybenzoate and Bcl-2 protein, (c) 2D structure showing interaction between Venetoclax and Bcl-2 protein, (d) 3D ligand-Target protein interaction between Venetoclax and Bcl-2 protein.

Images were created by using Biovia Discovery Studio Visualizer and showing the interaction between the Bcl-2 protein with high affinity phytochemical namely (2R, 3R)-2-(3, 4, 5-trihydorxyphenyl) chroman-3-yl 3, 4, 5-trihydroxybenzoate (A&B) and Bcl-2 protein with Venetoclax (C&D).

Pharmacokinetic Study
ADME
In the pharmacokinetics stage of drug development, there are four steps: Absorbtion, Distribution, Metabolism and Excretion (ADME). There is sisnificant correlation between some chemical descriptors and the ADMET properties, including oral absorption, which is dependent on low molecular weight, PSA which determines fractional absorption, the penetration of the lipid membrane by passive diffusion and low molecular weight and log P are important for the body's excretion of these chemicals leftovers.

Lipinski's Rule of Five
This is the important notion in preclinical drug development in the previous decade [17]. According to this rule, if a compound breaches two or more of the following requirements, it will be poorly absorbed or impermeable: 500 molecular weight, Number hydrogen donors 5, Number of hydrogen bond acceptors 10, Log P calculated 5, Polar surface area (PSA) 140 Å.

These values were determined in this investigation using Swiss ADME for best affinity score substances. From Table 3 shown, it can be seen that (2R,3R)-2-(3,4,5trihydroxyphenyl)chroman-3-yl, 3,4,5-trihydroxybenzoate which violates one rule having PSA<140 Å2 and remaining three selected phytocompounds that violate two rules of PSA and hydrogen bond acceptor ≤ 10, because these three phytocompounds having same molecular structure and exhibit same canonical smiles. The standard drugs ABT-737 and Venetoclax are violate two rules of PSA and molecular weight <500 and Navitoclax that violate three rules of molecular weight, PSA and Log P.

Table 3. Lipinski rule of five adherences of selected phytochemicals bound with Bcl-2 protein.
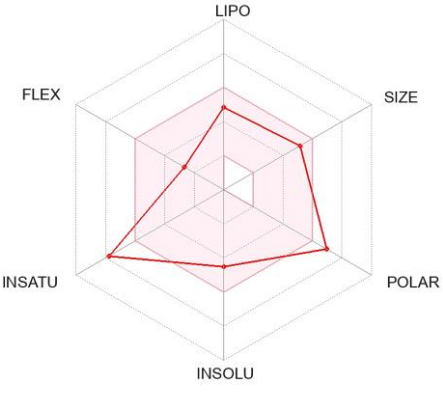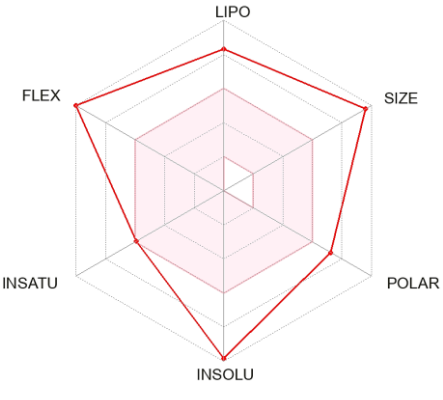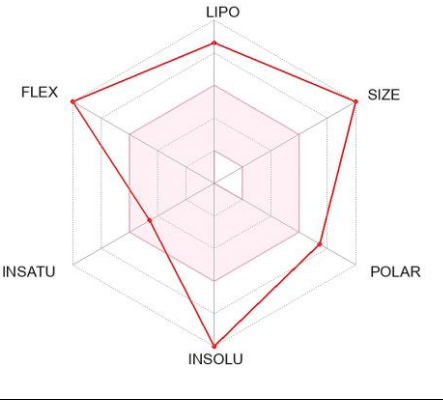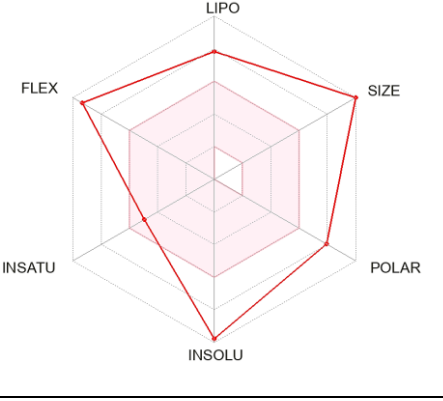
S.N.	High affinity photochemical and standard drug	Molecular weight <500	H bond donors ≤5	H bond acceptor ≤10	BBB permeant	LogP ≤5	PSA <140	No of Lipinski rule Violation
1	5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate	458.37	8	11	No	1.87	197.37	2
2	(+/-)-Epigallocatechin Gallate-13C3	458.37	8	11	No	1.87	197.37	2
3	(-)-Epigallocatechin gallate	458.37	8	11	No	1.87	197.37	2
4	(2R,3R)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate	426.37	6	9	No	1.25	156.91	1
5	ABT-737	813.43	2	7	No	5.51	164.49	2
6	Navitoclax	974.61	2	11	No	6.27	170.42	3
7	Venetoclax	868.44	3	9	No	5.57	183.09	2

Bioavailability Radar and Toxicity Prediction
Even if a medicine has a considerable impact, it may not be able to provide the required therapeutic effect within the body without careful consideration of bioavailability during the drug development process. Using the bioavailability radar map included in the Swiss ADME progarmme, one may anticipate the drug's bioavailability *in silico* [18]. As indicated in Table 4, the bioavailability and toxicity profiles of the chosen seven compounds were analyzed.

Table 4. Bioavailability Radar Plot and Toxicity Profile of the seven selected compounds. The prediction probability values are transformed into six symbols: 0.01(---), 0.1-0.3(--), 0.3-0.5(-), .0.5-0.7(+), 0.7-0.9(++) and 0.9-1.0(+++).

Name	Bioavailability radar	AMES toxicity	Oral acute toxicity	Carcinogenicity
5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate		0.153	0.01	0.01
(+/-)-Epigallocatechin Gallate-13C3		0.153	0.01	0.01
(-)-Epigallocatechin gallate		0.153	0.01	0.01

		0.341	0.121	0.01
(2R,3R)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate				
ABT-737		0.765	0.298	0.305
Navitoclax		0.01	0.464	0.349
Venetoclax		0.316	0.821	0.426

Although Venetoclax had the highest binding affinity for Bcl-2, it failed to satisfy the bioavailability requirements. The bioavailabilty radar of EGCG compounds is nearly as good as that of conventional medications. But eventhough having good affinity values, ABT-737 and Navitoclax could not meet the requirements for bioavailability and toxicity. While ABT-737 and Venetoclax were found to be positive for AMES toxicity and accurate oral toxicity, all EGCG phytocompounds showed negative values for toxicity.

Moreover, bioavailabilty and toxicity analysis of these top molecules revealed that the phytocompounds such as, (2R,3R)-2-(3,4,5-trihdoxyphenyl) chroman-3-yl 3,4,5-trihydroxybenzoate, 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate, (+/-)-epigallocatechin gallate-13C3 and (-)-epigallocatechin gallate were found to be safe and non-toxic. These finding demonstrated that EGCG phytocompounds are superior to commercially available medications and that they are also effective in inhibiting Bcl-2.

DISCUSSION

Drug design has undergone a revolution thanks to *in silico* analysis, which effectively reduces the hassle and overall cost required by the traditional drug design process. It has been successful in reducing the resources spent on drug discovery, which is Level 1 of drug designing. New prospective medications and their targets are being found and published in large quantities as a result of the development of effective bioinformatics databases, tools and software [19]. Similar to how Senbagarani Renganathan and his colleagues demonstrated the effectiveness of phytochemicals and molecular docking study of phytochemicals derived from *Prosopis juliflora* against the anti-apoptotic protein Bcl-2 [20]. In this work, innovative single-target drug of plant origin with the requisite ADME properties were tested.

The use of natural products for chemoprophylaxis and the treatment of different illnesses are rising globally. Because of its considerable inhibitory impact on cancer cells and minimal toxicity in normal cells. EGCG is one example that has garnered increasing interest. Due to the gallate group in epigallocatechin gallate (EGCG), it has been extensively documented that EGCG specifically inhibits Bcl-2 and Bcl-X when compared to other green tea phenols [21]. In this present study 14, EGCG and their similar compounds were tested, and out of these compounds (2R,3R)-2-(3,4,5-trihydroxyphenyl) chroman-3-yl 3,4,5-trihydroxybenzoate and 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate were exhibit more potent to inhibit Bcl-2 like standard drugs. Several studies have been focused only on EGCG, while this study proves EGCG similar compounds also have same capacity to act against Bcl-2.

Based on docking energy and favourable interactions with the active site residues, the ligand molecules that were already docked were chosen. The binding energy of EGCG phytocompound (2R,3R)-2-(3,4,5-trihydroxyphenyl) chroman-3-yl 3,4,5-trihydroxybenzoate was closely related to standard drug Venetoclax. Similarly5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate were seen to have matching score to standard drugs. Similarly, SiddavaramNagini et al. [22] also proposed Nimbolide as potent inhibitor of Bcl-2. Similarity, the present findings also clearly demonstrate the therapeutic importanceof EGCG derived bioactive compounds based on the molecular docking analysis. However, further in vitro and in vivo tests are required to show how well these phytocompounds suppress Bcl-2. Despite these phytocompounds identical chemical structures, a little difference allows for the creation of a powerful medication against Bcl-2. Moreover, EGCG has ben structurally changed, accorind to a research by Guang-Jian Du et al., to enhance its impact on cancer cells [18].

These phytocompounds are satisfying Lipinski's criterion and have been demonstrated to be less harmful than normal medications, despite the fact that they function well and could not fulfill pharmacokinetic regulations. This shows that these substances may undoubtedly compete with traditional medications. Venetoclax, ABT-737 and Navitoclax are far more expensive than the natural

substance EGCG on the market. It has greater side effects than other anti-cancer medications, thus phytocompounds were researched to reduce this impact and make it more affordable.

The study's major findings show that certain phytocompounds have the ability to suppress the Bcl-2 protein. The molecules such as (2R,3R)-2-(3,4,5-trihydroxyphenyl) chroman-3-yl 3,4,5-trihydroxybenzoate, 5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5-trihydroxybenzoate, (+/-)-epigallocatechin gallate-13C3, (-)- epigallocatechin gallate can be considered as the better lead molecules. Further analogues of these prospective compounds can be created using drug designing and pharmacophore modeling techniques. The *in silico* study can be validated by in vitro and in vivo testing, and if the outcomes support these findings, phytocompounds can then be chemically produced. Research, including a realism evaluation, should be done to expand upon and further test the findings of this study.

CONCLUSION

Focusing on the goal of finding an effective therapy for B lymphoma, 14 EGCG phytocompounds and three conventional medicines were evaluated using molecular docking, yielding four lead EGCG molecules. These four compounds were then subjected to pharmacokinetic analysis. This study indicated that (2R,3R)-2-(3,4,5-trihydroxyphenyl) chroman-3-yl 3,4,5-trihydroxybenzoate is the most effective chemical for inhibiting Bcl-2 and meets all drug-like properties. The development of conventional medicine-based therapy approaches will benefit from the discovery of these molecules effectiveness against the target proteins, as well as the identification of promising hits for potential lead optimization in the development of B lymphoma drugs, the researchers believe. By using molecular dynamic simulation of the protein model and experimental research on animals, it may be possible to further demonstrate the validity of these results, opening the way for the creation of effective targeted therapies for the treatment of lymphoma.

Abbreviations

Bcl-2	B Cell Lymphoma 2
EGCG	Epigallocatechin gallate
CLL	Chronic Lymphatic Leukemia
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
PSA	Polar Surface Area
PDB	Protein Data Bank

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