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Investigation of the Bcl-2 Inhibition Potential of Drugs Withdrawn from the Clinic Using in silico Methods

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ABSTRACT

Apoptosis is essential for tissue homeostasis, and its dysregulation is a hallmark of cancer. The B-cell lymphoma-2 (Bcl-2) protein family, particularly known for its anti-apoptotic role, contributes to tumor survival and chemoresistance. Venetoclax, a selective Bcl-2 inhibitor, has shown notable clinical success; however, resistance and toxicity highlight the need for alternative strategies. This study aimed to explore the inhibitory potential of withdrawn drugs on Bcl-2 using *in silico* methods to identify candidates for repurposing in anticancer therapy. Withdrawn drugs were retrieved from the DrugBank database and screened via molecular docking using MzDOCK software, with venetoclax as the reference inhibitor. Compounds displaying higher or comparable affinities were analyzed for key interactions, including hydrogen bonds, hydrophobic contacts, and salt bridges within the Bcl-2 binding pocket. Plicamycin showed the strongest binding affinity (-11.5 kJ/mol), surpassing venetoclax (-10.3 kJ/mol). It not only shared common binding residues with the reference molecule but also formed additional stabilizing interactions. Other drugs, such as depreotide, piperacetazine, and phenolphthalein, also demonstrated favorable binding profiles, supporting the feasibility of repurposing withdrawn compounds. Several withdrawn drugs retain valuable scaffolds for targeting Bcl-2. Plicamycin, despite historical toxicity concerns, emerges as a strong candidate for further preclinical evaluation. Modern delivery systems, such as nanoparticle formulations, may improve efficacy while reducing adverse effects. These findings underscore the promise of computational drug repurposing for anticancer discovery and encourage re-examination of withdrawn drugs within contemporary therapeutic contexts.

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Keywords: *Bcl-2, Drug repurposing, In silico, Molecular docking, Plicamycin*

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1. Introduction

Apoptosis is a tightly regulated form of programmed cell death essential for maintaining tissue homeostasis and preventing malignant transformation [1]. This process is orchestrated through two main pathways: the extrinsic pathway, initiated by death receptor signaling, and the intrinsic pathway, which is regulated by mitochondrial outer membrane permeabilization (MOMP) [2,3]. The B-cell lymphoma-2 (Bcl-2) family of proteins is a central regulator of the intrinsic pathway, where the balance between pro-apoptotic (Bax, Bak, Bid) and anti-apoptotic members (Figure 1.)

(Bcl-2, Bcl-xL, Mcl-1) determines cell fate [4]. Aberrant overexpression of Bcl-2 not only prevents apoptosis but also contributes to chemoresistance, tumor progression, and poor patient outcomes in hematological malignancies and solid tumors [5,6]. While alternative mechanisms such as p53 dysregulation, caspase inhibition, and alterations in autophagy pathways also contribute to apoptosis resistance, Bcl-2-mediated inhibition of MOMP represents one of the most prominent survival strategies in cancer cells [5,7]. Therefore, pharmacological inhibition of Bcl-2 has been considered a promising therapeutic approach.

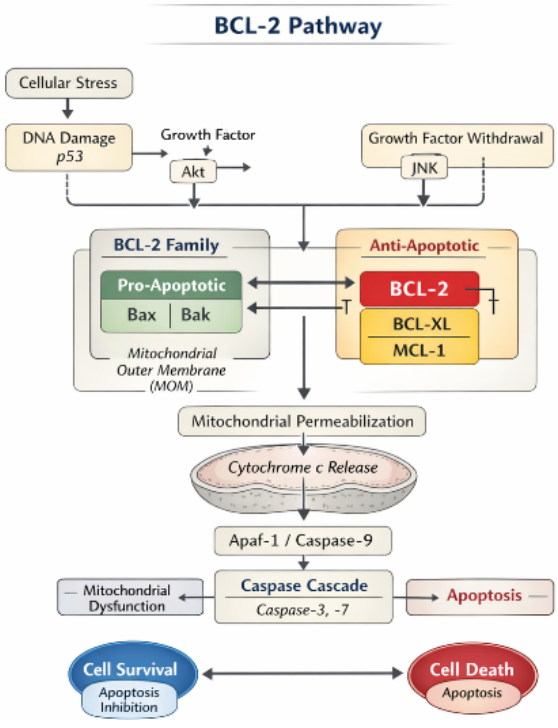


Figure 1. Apoptotic map including Bcl-2.

Several small-molecule inhibitors targeting Bcl-2 have entered clinical evaluation. Navitoclax (ABT-263), a dual Bcl-2/Bcl-xL inhibitor, demonstrated antitumor activity but was limited by dose-dependent thrombocytopenia due to Bcl-xL inhibition [8]. Venetoclax (ABT-199), designed as a selective Bcl-2 inhibitor, overcame this limitation and became the first Bcl-2 inhibitor approved for chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML), showing remarkable clinical efficacy [7-10]. Beyond these, ongoing clinical studies are exploring the potential of combining Bcl-2 inhibitors with other targeted therapies, immune checkpoint inhibitors, and conventional chemotherapy to overcome resistance and broaden therapeutic applicability [11].

Drug repositioning is an effective strategy that involves evaluating existing or previously approved drugs for new therapeutic indications, and it particularly accelerates the development process of anti-cancer drugs. This approach relies on examining drug-target interactions using publicly available databases, clinical data, bioinformatics methods, and AI-based analyses. By leveraging existing pharmacokinetic and safety information, development time and costs are reduced, and new therapeutic options can be identified more quickly [9,10]. Since 1950, numerous drugs have been withdrawn from the market due to safety, efficacy, quality, and commercial reasons. The most significant reason for drug withdrawals is safety concerns, with drug-related toxicity being particularly prominent. The most frequently reported reasons for drug withdrawals are liver toxicity (24%) and cardiovascular toxicity (19%), while other safety concerns such as allergic reactions, teratogenicity, psychiatric and hemorrhagic effects, drug abuse, and overdose also play a significant role[11]. A striking example is thalidomide (Figure 2), which was withdrawn from the market in the 1960s due to severe teratogenic effects, yet later repurposed successfully as an immunomodulatory and anti-angiogenic agent for the treatment of erythema nodosum leprosum and multiple myeloma [12,13]. The thalidomide case demonstrates that even drugs with a history of serious adverse events may harbor therapeutic potential when reassessed systematically. Plicamycin was withdrawn from the market in 2000 due to its serious and potentially fatal adverse effects, including life-threatening bleeding and organ damage. Furthermore, the development of safer and more effective treatment alternatives also contributed to the discontinuation of the drug [14]. Depreotide was withdrawn from the market not due to safety or efficacy issues, but for commercial reasons, due to decreased use because of more advanced imaging techniques becoming available in clinical practice [15]. Piperacetazine was withdrawn from the market due to the risk of serious neurological and hepatic adverse effects, and its declining commercial viability as safer and more effective alternatives became more widely available. Thus, exploring withdrawn drugs against critical molecular targets such as Bcl-2 can open novel avenues in anticancer therapy development [16].

In this study, we focused on exploring the potential of withdrawn drugs as inhibitors of Bcl-2 using computational approaches. By evaluating their binding affinities and dynamic stability within the Bcl-2 binding pocket, this research aims to identify promising scaffolds that could be further optimized for anticancer therapy, thereby contributing to the development of novel therapeutic strategies targeting apoptosis resistance.

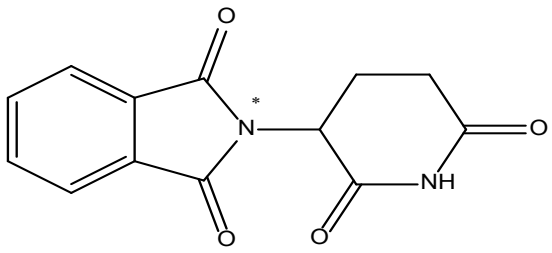


Figure 2. Thalidomide Chemical Structure.

2. Materials and Methods

2.1. Ligand Library Preparation

The ligand library used in this study was created using the DrugBank [14] database, a comprehensive online resource containing detailed information on approved, investigational, and withdrawn drugs. Data coded 5.1.13 consists of a 45.6 kB sdf file with a last update date of 03/01/2025. To investigate the potential for repurposing previously withdrawn drugs, compounds categorised as withdrawn in DrugBank were specifically selected. Database entries were downloaded in Structure Data File (.sdf) format to ensure compatibility with the MzDOCK V2.3 molecular modelling software.

2.2. Protein Determination and Preparation

To investigate the Bcl-2 inhibitory properties using HTS or docking studies, the clinically available molecule Venetoclax was used as a reference. PDB ID: 6O0K (https://www.rcsb.org/structure/6O0K) (Figure 3.), from which Bcl-2 and the ligand were co-crystallized, was selected. Using MzDOCK [18] software, all atoms were subjected to MMF94 forces, and all water molecules were deleted.

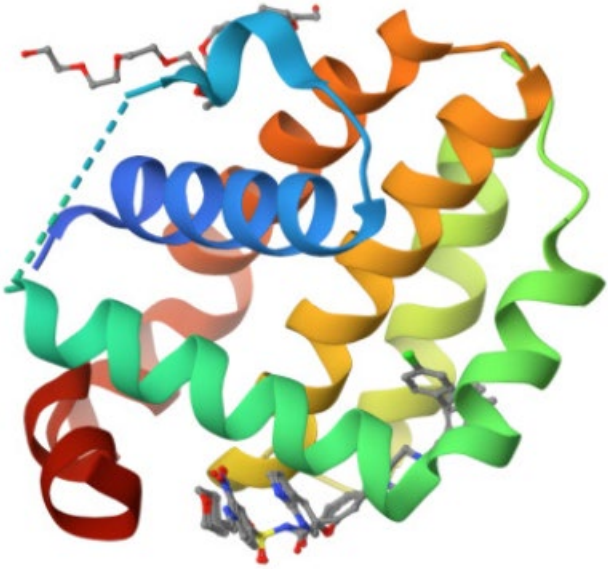


Figure 3. 3D structure of PDB ID:6O0K.

2.3. High Throughput Virtual Screening Studies

The ligand library was prepared and submitted to the programme as .sdf. In this way, a list of multiple ligands was also provided as input to perform virtual screening analyses.

Then, the ligands were energetically minimized with the force field selected by the user. MMFF94 force fields were marked. The steepest descent algorithm is applied for energy minimization. The chance to generate ligand enantiomers for SMILES is also enabled. A batch script for each file format was used to generate a pdbqt 3d structure for docking.

The target structure is taken as input in PDB format and refined to correct the bond order, add hydrogen atoms. MzDOCK provides the user with several options, including the possibility to add or remove hydrogen, change partial charges (i.e. Kollman or Gasteiger charges), delete or keep all heteroatoms, and Gasteiger was chosen. Ions and cofactors were manually selected to be retained in the final target structure, and venetoclax was selected. It is possible to select structural or functional crystallographic waters to be protected, but no selection was made because no structure was found to be protected.

2.4. Molecular Docking Studies

The 15 compounds with the best docking results in the HTS study were further docking. During this docking, the compounds were saved in .sdf format. They were then subjected to MMF94 for ligand preparation. Redocking was performed using the venotoclax residue field in PDB ID: 600K, and images were then captured in PyMOL software.

2.5. *In silico* Toxicity Prediction

In silico toxicity profiling of the studied compound was performed using the Deep-PK platform (https://biosig.lab.uq.edu.au/deeppk), a deep learning-based pharmacokinetic and toxicity prediction system. The molecular structure of the compound was uploaded in digital format and processed through the toxicity prediction module, which integrates multiple pretrained neural network models trained on curated experimental datasets covering mutagenicity, carcinogenicity, ecotoxicity, nuclear receptor signalling, stress response pathways, and organ-specific toxicities.

The platform generated predictions for a comprehensive set of toxicity endpoints, including AMES mutagenesis, carcinogenicity, drug-induced liver injury (DILI), hERG channel blockade, nuclear receptor (NR) signalling pathways, stress response (SR) pathways, acute and chronic rodent toxicity, aquatic toxicity models (Daphnia magna, fathead minnow, Tetrahymena pyriformis), biodegradation, bioconcentration factor, and respiratory and skin sensitization endpoints.

Predicted values were reported either as probabilistic scores (for classification-based models) or as continuous numerical outputs (for regression-based models), depending on the endpoint architecture. For each prediction, model applicability was evaluated based on whether the compound fell within the model training domain and molecular weight (MW) training range, as provided by the Deep-PK platform. Endpoints outside the training domain were interpreted with caution due to potential extrapolation effects. The resulting toxicity outputs were compiled and formatted in accordance with Elsevier journal table standards for scientific reporting.

3. Results and Discussion

The high cost, lengthy duration, and low success rates of traditional drug development processes have increased the need for alternative strategies. In this context, drug repurposing stands out as an effective approach based on identifying new therapeutic uses for existing drugs. This method reduces the cost and time burden of the drug discovery process by enabling the evaluation of approved, developing, or discontinued drugs in new indications. Furthermore, *in silico* methods such as machine learning, data mining, and computational modelling play a significant role in identifying potential candidates. Drug repurposing also includes the re-evaluation of drugs that have failed in clinical trials due to insufficient efficacy for different diseases. In this study, virtual screening was used to investigate how the drug interacts with a novel target protein for a Bcl-2 inhibitor. Our study revealed that the reasons for the withdrawal of the examined drugs from the market differed and stemmed from various toxicity profiles and safety issues. In this context, targeted drug delivery systems offer promising approaches to overcoming these limitations. Direct delivery of active ingredients to the target region can significantly reduce unwanted side effects by increasing therapeutic efficacy and reducing the exposure of healthy tissues to the drug. In recent years, nanostructures and nanoparticles have attracted increasing attention in drug development studies due to their advantages such as increased bioavailability, development of targeted therapy strategies, controlled drug release, and improved therapeutic outcomes [19].

Table 1 shows the docking results of the closest 15 commercially withdrawn drugs according to the docking score of venotoclax reference drug. It is understood that the molecule with a better docking score than the venetoclax reference belongs to the plicamycin molecule with a value of -11.5 kJ/mol. Depreotide has the same docking score as venotoclax, with a value of -10.3 kJ/mol. Other compounds with calculated docking scores have docking scores like the reference compound.

Table 2 and Table 3 show the 3D docking visualization and the distance values of the bonds captured with amino acids. According to these results, it was observed that the plicamycin molecule captured common amino acids with the amino acids captured by venetoclax. Venotoclax molecule, which is a Bcl-2 inhibitor used in the clinic, is calculated to be able to establish various bonds with Phe104, Asp111, Met115, Leu137, Asn143, Arg146,

Val148, Phe153, Tyr202, Tyr202, Asp103, Tyr108, Phe104 amino acids in the activity area and shown in Table 2.

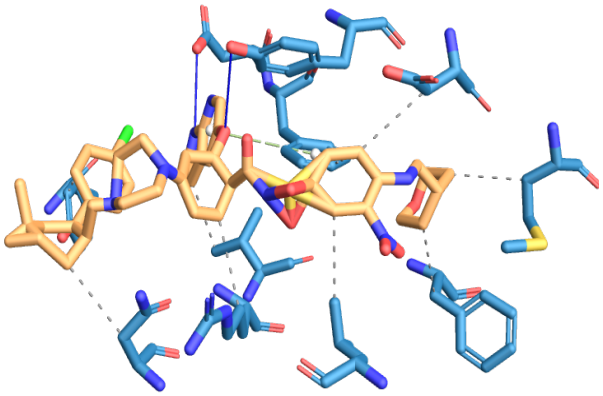
Table 1. Molecular Docking Results

Drug Name	Docking Score (kJ/mol)
Venotoclax *	-10.3*
Plicamycin	-11.5
Depreotide	-10.3
Piperacetazine	-9.8
Phenolphthalein	-9.6
Tetracycline	-9.5
Novobiocin	-9.5
Oxyphenisatin	-9.3
Terfenadine	-9.2
Troglitazone	-9.2
Medrogestone	-9.2
Nefazodone	-9.0
Glibornuride	-9.0
Indoramin	-9.0
Mibefradil	-8.9
Fluclorolone acetonide	-8.9
RMSD 2.3 Å	

*Clinically used Bcl-2 inhibitor

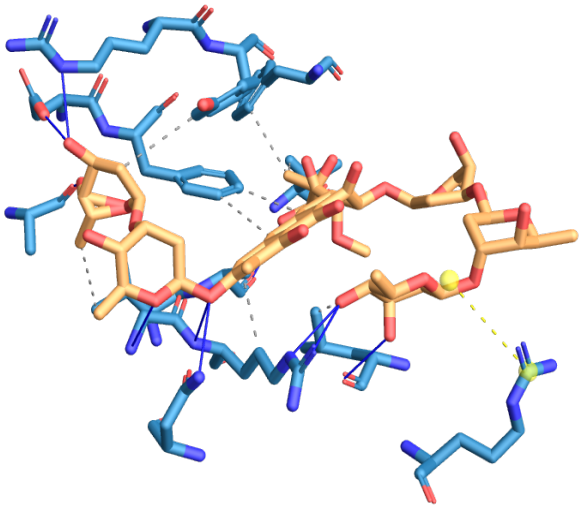
The plicamycin molecule, which we think may have Bcl-2 inhibition potential from the data in the docking results in our study, is one of the amino acids in the target area of the reference molecule venotoclax, Phe104, The results in Table 3 show that Tyr108, Phe112, Leu137, Arg146, Val148, Val156, Ala100, Asp103, Arg107, Leu137, Asn143, Gly145, Arg146, Ala149, Arg139 make one or more bond types with amino acids and shown in Table 3.

Table 2. Venotoclax Database



Interaction	Residue	Distance(Å)
hydrophobic	Phe104	3.49
hydrophobic	Asp111	3.88
hydrophobic	Met115	3.65
hydrophobic	Leu137	3.79
hydrophobic	Asn143	3.9
hydrophobic	Arg146	3.92
hydrophobic	Val148	3.65
hydrophobic	Phe153	3.92
hydrophobic	Tyr202	3.69
hbond	Asp103	2.81
hbond	Tyr108	3.58
pi-stacking	Phe104	5.04

Table 3. Plicamycin Database



Interaction	Residue	Distance(Å)
hydrophobic	Phe104	3.68
hydrophobic	Phe104	3.31
hydrophobic	Tyr108	3.94
hydrophobic	Phe112	3.72
hydrophobic	Leu137	3.74
hydrophobic	Arg146	3.58
hydrophobic	Val148	3.83
hydrophobic	Val156	3.6
hbond	Ala100	2.76
hbond	Asp103	1.95
hbond	Arg107	2.64
hbond	Leu137	2.55
hbond	Asn143	2.47
hbond	Gly145	3.2
hbond	Arg146	2.27
hbond	Arg146	3.57
hbond	Ala149	2.33
salt-bridge	Arg139	4.78

This study reveals the potential for re-evaluation of drugs withdrawn from clinical use as Bcl-2 inhibitors. According to our findings, plicamycin showed higher binding energy compared to the reference venetoclax. This result suggests that plicamycin may be effective in targeting apoptotic pathways. Recent clinical and translational studies emphasize that although venetoclax shows high clinical efficacy in AML and CLL, resistance mechanisms are a serious obstacle [20,21]. This resistance is caused by factors other than Bcl-2 mutations, such as intracellular pathway remodelling, mitochondrial adaptation and tumor microenvironment. Therefore, the development of alternative or combined Bcl-2 inhibitors is of clinical importance.

The anticancer properties of plicamycin were previously reported, but its use in the clinic was limited due to its high toxicity profile [21]. Today, drug carrier nanotechnologies have brought the therapeutic potential of such molecules back to the agenda. Nanoparticle-based systems (liposomes, polymeric nanoparticles, targeted carriers) have been shown to increase tumor-specific distribution by reducing the toxicity of classical chemotherapeutics [22,23]. However, the potential toxicity risks of polymeric nanocarriers themselves should

also be considered [24]. Therefore, the re-evaluation of plicamycin should be considered in conjunction with controlled release and targeted drug delivery strategies.

Table 4. Deep-PK (biosig.lab.uq.edu.au). Values represent predicted probabilities or continuous outputs depending on the endpoint.

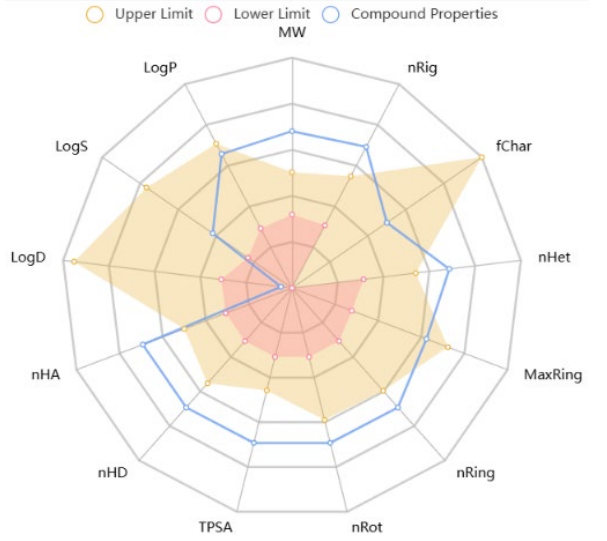
Endpoint	Predicted value	In training domain	MW in training domain
AMES Mutagenesis	0.065	Yes	–
Avian Toxic	1.0	No	–
Bee Toxic	0.0	No	–
Bioconcentration Factor	-961037.33	No	No
Biodegradation	0.01	No	–
Carcinogenesis	0.004	No	–
Crustacean Toxic	1.0	No	–
Drug-Induced Liver Injury (DILI I)	0.206	No	–
Eye Corrosion	0.0	No	–
Eye Irritation	0.001	No	–
Maximum Tolerated Dose	-1.47	Yes	No
Drug-Induced Liver Injury II	0.674	No	–
hERG Blocker	1.0	Yes	–
Daphnia magna	4.05	Yes	No
Micronucleus Toxicity	1.0	No	–
NR-AhR	0.0	No	–
NR-AR	0.134	Yes	–
NR-AR-LBD	0.721	No	–
NR-Aromatase	1.0	No	–
NR-ER	0.11	No	–
NR-ER-LBD	0.0	Yes	–
NR-GR	0.868	No	–
NR-PPARγ	0.0	No	–
NR-TR	0.999	No	–
Tetrahymena pyriformis	-7.5E10	No	No
Rat acute toxicity	3.78	Yes	Yes
Rat chronic oral toxicity	161.75	No	No
Fathead minnow	9.4E7	No	No
Respiratory toxicity	1.0	No	–
Skin sensitisation	0.0	No	–
SR-ARE	0.007	No	–
SR-ATAD5	0.0	Yes	–
SR-HSE	0.0	No	–
SR-MMP	0.904	No	–
SR-p53	1.0	No	–

Other molecules (depreotide, piperacetazine, phenolphthalein) also showed remarkable binding scores [25].

In particular, the fact that depreotide is a somatostatin analogue suggests that it may contribute through additional mechanisms that suppress tumor growth. This supports the exploratory potential of drug repositioning.

In conclusion, our findings are consistent with the central role of the Bcl-2 family in cancer cell survival and suggest that it is rational to re-evaluate molecules with strong binding profiles such as plicamycin. In future studies, *in vitro* and *in vivo* validation tests of these molecules, followed by integration with nanoparticle-based drug delivery systems, may lead to redefinition of their safety and efficacy. Thus, both existing treatment options can be expanded, and drug development costs can be reduced [26-29].

Table 5. Data related to the ADMELab3.0 application.



Physicochemical Properties	
Parameters	**Value**
Molecular Weight (MW)	1084.47
Volume	1032.006
Density	1.051
HydrogenBond Acceptor (HBA)	24
HydrogenBond Donor (HBD)	11
Number of Rotatable Bonds	15
Number of Rings	8
TPSA (Å^2)	358.2
logS	-2.688
logP	1.31
logD (pH 7.4)	1.705
Acidic pKa	4.352
Basic pKa	5.860
Melting Point (°C)	176.19
Boiling Point (°C)	302.03
Drug Similarity and Medicinal Chemistry Parameters	
Parameters	**Result**
QED	0.11
SA Score	Hard
GASA	Hard
Fsp3	0.769
MCE-18	220.13
NP Score	1.395
Lipinski	Rejected
Pfizer	Accepted
GSK	Rejected
Golden Triangle	Rejected
PAINS	0
Alarm NMR	2
BMS Rule	1
Chelating Rule	0

Absorption Properties	
Parameters	**Result**
Caco-2 Permeability	-6.196
MDCK Permeability	-5.145
PAMPA	+++
P-gp Inhibitor	---
P-gp Substrate	+++
Human Intestinal Absorption	---
Oral Bioavailability (20%)	---
Oral Bioavailability (30%)	---
Oral Bioavailability (50%)	+++
Distribution Characteristics	
Parameters	**Result**
PPB (%)	84.2
VDss	0.074
Free Fraction (Fu)	19.0%
BBB Permeability	---
OATP1B1 Inhibitor	+++
OATP1B3 Inhibitor	+++
BCRP Inhibitor	---
MRP1 Inhibitor	--
BSEP Inhibitor	++
Metabolic Characteristics	
Parameters	**Result**
CYP1A2 inhibitor	---
CYP1A2 substrate	---
CYP2C19 inhibitor	---
CYP2C19 substrate	---
CYP2C9 inhibitor	---
CYP2C9 substrate	---
CYP2D6 inhibitor	---
CYP2D6 substrate	---
CYP3A4 inhibitor	---
CYP3A4 substrate	+
CYP2B6 inhibitor	+++
CYP2B6 substrate	---
CYP2C8 inhibitor	+++
Human Liver Microsomal Stability (HLM)	+++
Features of Elimination	
Parameters	**Value**
Plasma Clearance (CLplasma)	-0.517
Half-life ($T_1/2$, hours)	9.082
Toxicity Predictions	
Parameters	**Probability**
hERG Blockade	0.026
hERG Blockade (10 μM)	0.288
DILI	0.961
AMES Mutagenicity	0.984
Rat Acute Oral Toxicity	0.176
FDAMDD	0.327
Skin Sensitisation	0.996
Carcinogenicity	0.001
Human Hepatotoxicity	0.613
Drug-Induced Nephrotoxicity	0.994
Drug-Induced Neurotoxicity	0.000
Ototoxicity	1.000
Hematotoxicity	0.087
Genotoxicity	0.978
Immunotoxicity (RPMI-8226)	0.938
Cytotoxicity (A549)	0.903
Cytotoxicity (HEK293)	0.593

In silico toxicity analyses conducted using the Deep-PK platform indicate that the plicamycin molecule possesses a safety profile characterized by generally low risk in terms of genotoxicity and carcinogenicity, but containing potential toxicity signals directed towards specific organ-specific and molecular targets (Table 4). In particular, the low predicted values obtained for AMES mutagenicity (0.065) and carcinogenicity (0.004) suggest that plicamycin has a low potential to cause DNA damage and that the risk of direct toxic effects on genetic material may be limited. These findings suggest that the molecule may exhibit a relatively safe profile inter oncogenic risk for long-term use scenarios.

In contrast, a positive prediction (1.0) for hERG channel blockade suggests that plicamycin may have the potential to interact with cardiac ion channels. As hERG inhibition is pharmacologically associated with QT interval prolongation, arrhythmia, and potential sudden cardiac events, this result should be considered a critical cardiotoxicity risk in plicamycin's clinical development process. In this context, validation using patch-clamp electrophysiology studies and cardiomyocyte-based *in vitro* models appears mandatory.

Signals observed at the extremes of drug-induced liver injury (DILI I and DILI II) suggest that plicamycin may have the potential to form toxic metabolites during hepatic metabolic processes. This situation indicates that reactive intermediate products, which may be formed via cytochrome P450 enzymes, could trigger mechanisms of oxidative stress, mitochondrial dysfunction, and cellular damage at the hepatocyte level. Therefore, further validation studies involving hepatocyte cultures, microsomal stability tests, and mitochondrial toxicity analyses are required for plicamycin.

Results obtained in nuclear receptor (NR) and stress response (SR) pathways indicate that plicamycin may interact with regulatory pathways such as NR-TR (thyroid receptor), NR-GR (glucocorticoid receptor), and SR-p53. These interactions suggest that the molecule may have not only cytotoxic effects but also indirect effects on endocrine system regulation, cell cycle control, and apoptotic signaling mechanisms. This situation indicates that plicamycin may carry risks such as chronic systemic toxicity and disruption of homeostatic balance in long-term exposure scenarios.

The high toxicity values observed in ecotoxicity models (Daphnia magna, fathead minnow and Tetrahymena pyriformis) indicate that plicamycin may have significant toxic effects on biological systems from an environmental perspective. These results support the notion that antibiotic-derived molecules pose a potential risk in environmental settings, particularly in terms of disrupting microbial balance, biological accumulation in aquatic ecosystems, and trophic chain effects. In this context, plicamycin should be evaluated not only from a clinical toxicity perspective but also from the perspectives of environmental pharmacology and ecotoxicology.

The ADMELab3.0 application was used to examine properties such as physicochemical properties and toxicity

predictions in more detail *in silico*. The results are given in Table 5. ADMET analyses revealed that plicaminin did not exhibit classic oral drug characteristics due to its high molecular weight and non-Lipinski-like structure. However, its high PAMPA permeability and approximately 9-hour half-life suggest that its systemic efficacy may be maintained. Toxicity estimates indicated high mutagenicity, genotoxicity, hepatotoxicity, and nephrotoxicity potential. Conversely, the low risk of hERG blockade can be considered a positive feature in terms of cardiac safety. Furthermore, the predicted high cytotoxicity in the A549 cell line suggests that plicaminin may exert a strong antiproliferative effect on tumor cells.

In general, the *in silico* data obtained indicate that plicamycin is safe in terms of genetic toxicity but exhibits a profile that requires careful monitoring in terms of cardiotoxicity (hERG), hepatotoxicity (DILI) and toxicity mechanisms related to endocrine/stress response. These results are important for early risk stratification in the preclinical development process of pilicamycin, clearly defining critical safety areas that need to be supported by targeted *in vitro* validation tests and selective *in vivo* toxicity models. In this context, Deep-PK outputs can be considered a strategic decision support tool for guiding rational experimental design in the development process of plicamycin.

4. Conclusion

This study shows that *in silico* re-evaluation of drugs withdrawn from the clinic may reveal promising candidates, especially for the Bcl-2 protein involved in apoptosis regulation. Plicamycin was identified as the most promising candidate due to its strong binding energy and stable interactions. The results of the study reveal that the selected molecules, especially plicamycin, should be carried to further biological studies (*in vivo, in vitro*).

Conflict of Interest

There are no conflicts of interest in this work.

Declaration of Generative AI

The authors confirm that no generative AI technologies were used in the preparation, writing, or data analysis of this manuscript.

References

[1] Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicologic Pathology, 35*(4), 495–516. https://doi.org/10.1080/01926230701320337

[2] Hanahan, D., and Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

[3] Tait, S. W. G., and Green, D. R. (2010). Mitochondria and cell death: Outer membrane permeabilization and beyond. *Nature Reviews Molecular Cell Biology, 11*(9), 621–632. https://doi.org/10.1038/nrm2952

[4] Youle, R. J., and Strasser, A. (2008). The BCL-2 protein family: Opposing activities that mediate cell death. *Nature Reviews Molecular Cell Biology, 9*(1), 47–59. https://doi.org/10.1038/nrm2308

[5] Cory, S., Huang, D. C. S., and Adams, J. M. (2003). The Bcl-2 family: Roles in cell survival and oncogenesis. *Oncogene, 22*(53), 8590–8607. https://doi.org/10.1038/sj.onc.1207102

[6] Delbridge, A. R. D., Grabow, S., Strasser, A., and Vaux, D. L. (2016). Thirty years of BCL-2: Translating cell death discoveries into novel cancer therapies. *Nature Reviews Cancer, 16*(2), 99–109. https://doi.org/10.1038/nrc.2015.17

[7] Adams, J. M., and Cory, S. (2018). The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell Death and Differentiation, 25*(1), 27–36. https://doi.org/10.1038/cdd.2017.161

[8] Wilson, W. H., O'Connor, O. A., Czuczman, M. S., LaCasce, A. S., Gerecitano, J. F., Leonard, J. P., … Sharman, J. P. (2010). Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: A phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *The Lancet Oncology, 11*(12), 1149–1159. https://doi.org/10.1016/S1470-2045(10)70261-8

[9] Souers, A. J., Leverson, J. D., Boghaert, E. R., Ackler, S. L., Catron, N. D., Chen, J., … Elmore, S. W. (2013). ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nature Medicine, 19*(2), 202–208. https://doi.org/10.1038/nm.3048

[10] Kowsalya, K., Vidya, N., Halka, J., Preetha, J. S. Y., Saradhadevi, M., Sahayarayan, J. J., ... & Arun, M. (2025). Plant glycosides and glycosidases: classification, sources, and therapeutic insights in current medicine. *Glycoconjugate Journal, 42*(2), 107–124. https://doi.org/10.1007/s10719-025-10180-3

[11] Pan, R., Hogdal, L. J., Benito, J. M., Bucci, D., Han, L., Borthakur, G., … Konopleva, M. (2014). Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. *Cancer Discovery, 4*(3), 362–375. https://doi.org/10.1158/2159-8290.CD-13-0609

[12] Montero, J., and Letai, A. (2018). Why do BCL-2 inhibitors work and where should we use them in the clinic? *Cell Death and Differentiation, 25*(1), 56–64. https://doi.org/10.1038/cdd.2017.183

[13] Ashburn, T. T., and Thor, K. B. (2004). Drug repositioning: Identifying and developing new uses for existing drugs. *Nature Reviews Drug Discovery, 3*(8), 673–683. https://doi.org/10.1038/nrd1468

[14] DrugBank. (n.d.). *Withdrawn drug links*. https://go.drugbank.com/releases/5-1-13/downloads/withdrawn-drug-links

[15] Edo, G. I., Ali, A. B., Okolie, M. C., Orogu, J. O., Emumejaye, K., Oghroro, E. E. A., ... & Fuwape, I. A. (2026). Toxicity of nanostructures in drug delivery applications. *Pharmaceutical Science Advances*, 100117. https://doi.org/10.1016/j.pscia.2026.100117

[16] Merino, D., Gérard, A. O., Destere, A., Askenazy, F., Drici, M. D., & Thümmler, S. (2022). Antipsychotic abuse, dependence, and withdrawal in the pediatric population: A real-world disproportionality analysis. *Biomedicines, 10*(11), 2972. https://doi.org/10.3390/biomedicines10112972

[17] Bandishti, M., Pramanik, C., Roychowdury, A., Kulkarni, K. G., & Thorat, R. G. (2026). A Concise Review of Phenothiazine Based Antipsychotic Drugs and Their

Syntheses. *Journal of Heterocyclic Chemistry, 63*(1), 106–129. https://doi.org/10.1002/jhet.70115

[18] Al Khzem, A. H., & Wali, S. M. (2025). Drug Repurposing as an Effective Drug Discovery Strategy: A Critical Review. *Drug Design, Development and Therapy*, 12019–12034. https://doi.org/10.2147/DDDT.S576701

[19] Bethesda, L. (2012). Clinical and research information on drug-induced liver injury [internet]. *National Institute of Diabetes and Digestive and Kidney Diseases*.

[20] Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., … Pirmohamed, M. (2019). Drug repurposing: Progress, challenges and recommendations. *Nature Reviews Drug Discovery, 18*(1), 41–58. https://doi.org/10.1038/nrd.2018.168

[21] Singhal, S., Mehta, J., Desikan, R., Ayers, D., Roberson, P., Eddlemon, P., … Barlogie, B. (1999). Antitumor activity of thalidomide in refractory multiple myeloma. *New England Journal of Medicine, 341*(21), 1565–1571. https://doi.org/10.1056/NEJM199911183412102

[22] Vargesson, N. (2015). Thalidomide-induced teratogenesis: History and mechanisms. *Birth Defects Research Part C: Embryo Today: Reviews, 105*(2), 140–156. https://doi.org/10.1002/bdrc.21096

[23] Kabier, M., Gambacorta, N., Trisciuzzi, D., Kumar, S., Nicolotti, O., & Mathew, B. (2024). MzDOCK: A free ready-to-use GUI-based pipeline for molecular docking simulations. *Journal of Computational Chemistry, 45*(23), 1980–1986. https://doi.org/10.1002/jcc.27390

[24] Forsberg, M. (2024). SOHO State of the Art Updates and Next Questions: Mechanism of action of Venetoclax and the major mechanisms of inherent and acquired resistance to VEN. *Clinical Lymphoma, Myeloma & Leukemia, 24*(1), 1–8. https://pubmed.ncbi.nlm.nih.gov/38007372/

[25] Blombery, P., et al. (2025). Resistance to targeted therapies in chronic lymphocytic leukemia. *Leukemia, 39*(8), 2049–2060. https://doi.org/10.1038/s41375-025-02662-y

[26] Ayyad, R. R., Mansour, A. M., Nejm, A. M., Hassan, Y. A. A., & Ayyad, A. R. (2024). An overview of antibiotics used in cancer treatment and drugs that act as antimicrotubules. *Journal of Progress in Engineering and Physical Science, 3*(2), 25–32. https://doi.org/10.56397/JPEPS.2024.06.04

[27] Kawish, S. M., et al. (2024). Nanoparticle-based drug delivery platform for simultaneous administration of phytochemicals and chemotherapeutics: Emerging trends in cancer management. *Pharmaceuticals, 17*(1), e49. https://onlinelibrary.wiley.com/doi/10.1002/ppsc.202400049

[28] Hristova-Panusheva, K., et al. (2024). Nanoparticle-mediated drug delivery systems for precision cancer medicine. *Frontiers in Pharmacology, 15*, 11206252. https://pmc.ncbi.nlm.nih.gov/articles/PMC11206252/

[29] Sairam, A. B., et al. (2023). Toxicity of polymeric nanodrugs as drug carriers. *ACS Chemical Health & Safety, 30*(5), 399–408. https://doi.org/10.1021/acs.chas.3c00008