Research Article

Molecular targeting of BCl-2 in oral cancer cells by nordentatin: An in silico docking and molecular dynamics study

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Abstract: Background: Oral cancer is a major problem in dentistry and remains a major global health issue. In oral cancer, BCL-2, which plays a role in regulating cell death, is overexpressed, thus leading to its progression and resistance to chemotherapy. Clausena excavata contains a coumarin called nordentatin, which has been shown to have anticancer properties. Particularly with regard to its function as a BCL-2 inhibitor, the underlying molecular mechanism is yet unknown. Objectives: The aim of this study was to determine the potential of nordentatin as a BCL-2 protein inhibitor through molecular docking and then validate it with molecular dynamics. Materials and Methods: Nordentatin's ADMET and bioactivity were estimated in silico. AutoDock Vina was used for molecular docking against BCL-2 using methotrexate as a comparison ligand and venetoclax as a reference ligand. PyMOL and Biovia Discovery Studio were then used for interaction analysis. Complex stability was assessed using a YASARA molecular dynamics simulation. Results: Nordentatin exhibits potential as an anti-inflammatory, anti-angiogenic, antioxidant, and anticancer drug based on bioactivity predictions. ADMET analysis showed that this compound is readily absorbed in the intestine, has moderate clearance, limited penetration into the central nervous system, and low toxicity, thus indicating a favorable pharmacokinetic and safety profile. Molecular docking results show that nordentatin has a fairly strong binding affinity for BCL-2 with a value of −7.4 kcal/mol, which is mainly influenced by hydrophobic and electrostatic interactions. Molecular dynamics simulations indicate that the BCL-2–nordentatin complex remains stable, with an RMSD value below ±2 Å, a compact Rg profile, and favorable RMSF fluctuations. Conclusion: The results indicate that nordentatin binds strongly and stably to BCL-2, thus offering potential for development as a BCL-2 inhibitor for oral cancer therapy.

Keywords: Nordentatin, BCL-2 inhibitor, oral cancer, molecular docking, molecular dynamics.

Introduction

Oral cancer continues to be a significant global health concern. Current treatments for oral cancer, such as surgery, radiation, chemotherapy, immunotherapy, and hormonal therapy, have improved clinical outcomes; however, they are very toxic to healthy tissues, often cause severe side effects and lower the patient quality of life. As a result, there is an urgent need for new therapeutic medications that specifically target important biochemical pathways involved in the development of oral cancer. Active phytochemicals are one of the most promising supplementary therapies since they have shown promise in treating and managing several types of human cancer [1-3].

Bcl-2 is an important regulator of cell death. The mechanism by which BCL-2 modulates apoptosis is not known, although the protein is thought to generate homodimers and heterodimers that are important for

its biological activities through multiple protein-protein interactions. The cell sensitivity to apoptosis is regulated by the homodimer and heterodimer forms of the BCL-2 family genes. The BCL-2 family can be separated into two opposing groups: proapoptotic (BAD, BAX, BID, BCL-Xs, BAK, BIM, PUMA, and NOXA) and antiapoptotic (MCL-1, BCL-XL, and BCL-2). In contrast, antiapoptotic proteins, located in the outer mitochondrial membrane, endoplasmic reticulum, and nuclear membrane, facilitate pore formation and regulate ion transport. Increased production of cytochrome c and apoptosis-inducing factor (AIF) leads to apoptosis. In contrast, antiapoptotic proteins (localizing to outer mitochondrial membrane, endoplasmic reticulum and nuclear membrane) enhance pore formation and ion transport. Specifically, they control the Ca2+ concentration within the cell. These proteins inhibit apoptosis by blocking the release of AIF and cytochrome c, which are precursors of caspases [4, 5]. Therefore, it can be concluded that cell fate depends on the ratio and interaction between proapoptotic and antiapoptotic proteins. Therefore, overexpression of the antiapoptotic BCL-2 protein promotes cancer survival and progression [4]. This reduces the effectiveness of apoptosis-based treatments such as chemotherapy and radiotherapy due to increased apoptosis resistance caused by BCL-2 deregulation. Therefore, drug therapy targeting the BCL-2 family for cancer treatment is a promising approach. Therefore, extensive knowledge of the function and interactions of these molecules is needed [6, 7].

Medicinal plants are believed to be a source of unique bioactive substances which may be helpful in the treatment of cancer. Wild shrub Clausena excavata (Burm. f.) is a member of the Rutaceae family. It is most frequent in Southeast Asia, China, India, Malaysia, Thailand and Indonesia [8]. The plant has shown several biological activities such as antibacterial, antifungal, analgesic, anti-inflammatory, anti-viral, antioxidant, immune-modulator and anticancer activity [9]. Phytochemical investigations revealed that the major bioactive chemicals of the plant responsible for its medicinal benefits are alkaloids and coumarins. Nordentatin is a coumarin with potent anti-inflammatory and anti-cancer activity [9].

Even though it has been shown to fight cancer, the way nordentatin might work as a BCL-2 inhibitor is still not well known. To fill this gap, molecular docking and molecular dynamics simulations were used to look at how it interacts with BCL-2. Venetoclax, a clinically licensed BCL-2 inhibitor [4, 10], was used as the positive control. Methotrexate was used as a comparison ligand in this study, because it has been proven to be able to change transcription in cancer cells by binding to the BCL-2 promoter region. Methotrexate also exhibits various anticancer activities in several cancer types [11]. The aim of this study was to determine the interaction of nordentatin with BCL-2 and the stability of the complex through molecular docking and dynamics, thus determining its potential as an oral anticancer candidate.

Materials and Methods

Bioactivity Prediction

The biological activity of nordentatin in relation to cancer was predicted by PASS Online server (https://www.way2drug.com/passonline/) [12]. Then, its activities were investigated to clarify its role in oral cancer pathways, particularly those linked to oxidative stress, apoptosis, and cell proliferation. Then, some essential activities linked to the expectations of nordentatin were studied, such as antineoplastic effects, impacts on HIF1A and NOS2 expression, free radical scavenging, and effects on BCL-2-associated apoptosis regulation. These actions were highlighted because they have a significant role in modulating the tumor microenvironment, inhibiting angiogenesis, inducing apoptosis and delaying the growth of tumor in oral squamous cell carcinoma.

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Prediction

The absorption, distribution, metabolism, excretion and toxicity (ADMET) features of Nordentatin were evaluated in silico using the pkCSM pharmacokinetics server, which predicts pharmacokinetic and toxicity parameters using graph-based signatures. Predicted pharmacokinetic properties provided a complete ADMET profile for nordentatin, including aqueous solubility, Caco-2 cell permeability, human intestinal absorption, skin permeability, P-glycoprotein substrate/inhibitor status, volume of distribution, plasma protein binding, blood–brain barrier (BBB) penetration, central nervous system (CNS) permeability, cytochrome P450 enzyme interactions, hepatotoxicity, and acute and chronic rat toxicity [13].

Ligand Preparation

The three-dimensional (3D) chemical structures of nordentatin and methotrexate were retrieved from the PubChem database in SDF format, venetoclax was native ligan from BCL-2 (PDB ID: 6O0K). All ligands were converted and saved in PDB format using BIOVIA Discovery Studio 2021. In AutoDock Tools version 1.5.6, ligand preparation was carried out by defining rotatable bonds and assigned atom types. The prepared ligands were saved in PDBQT format for subsequent molecular docking analysis [14-16].

Protein Preparation and

The crystal structure of human B-cell lymphoma 2 (BCL-2) protein was downloaded from RCSB Protein Data Bank (PDB ID: 6O0K). The native ligands were extracted from the protein structures using BIOVIA Discovery Studio 2021. Proteins were prepared using AutoDock Tools 1.5.6 by eliminating water molecules, adding polar hydrogen atoms, and assigning Kollman charges. The generated protein was stored in the PDBQT format [14-16].

Molecular Docking

Molecular docking with AutoDock Vina was used to estimate the binding mechanism and affinity of nordentatin and methotrexate with BCL-2. The binding site was identified by re-docking the native ligand venetoclax into the BCL-2 protein structure (PDB ID: 6O0K). The top ranking docking poses were selected according to binding energy (kcal/mol) and good interactions with critical residues. The docking results were displayed and analyzed with PyMOL 2.5 and BIOVIA Discovery Studio 2021.

Molecular Dynamics (MD) Simulation

Molecular dynamics (MD) simulations were performed with YASARA (version 25.1.13) for 50.00 nanoseconds with 501 snapshots with the AMBER 14 force field. We put each compound in a cubic simulation cell with periodic boundary conditions and injected TIP3P water molecules to break it down. We also added Na^+ and Cl^- ions to make the system neutral. The steepest descent method was utilized to lower energy, and subsequently the system was brought to equilibrium at 310 K under NVT conditions. We did production MD simulations for 50 ns with a time step of 2.0 fs. Long-range electrostatics were calculated with particle mesh Ewald (PME) technique. We used a threshold of 8 Å for van der Waals interactions. The trajectory was analysed with YASARA's built-in analysis capabilities. The following methods were used: root mean square deviation (RMSD), root mean square fluctuation (RMSF) and monitoring of hydrogen bonds.

Results

Bioactivity and ADMET Prediction

The results show a probability of activity (Pa) >0.7, indicating a stronger confidence that nordentatin exhibits the predicted activities for several biological activities related to anticancer mechanisms. These activities include antineoplastic activity, HIF-1α inhibition, NOS2 inhibition, free radical scavenger, hepatoprotectant and chemopreventive. The absorption profile showed high gastrointestinal absorption (94.5%). Distribution parameters show poor BB penetration and poor CNS penetration. For the metabolism parameters, nordentatin is predicted not to be a substrate of CYP2D6 and CYP3A4. It is anticipated that the chemical may inhibit CYP1A2, CYP2C19, and CYP2C9, but not CYP2D6 or CYP3A4. Moderate clearance is shown in excretion. Toxicity prediction includes AMES toxicity and hepatotoxicity parameters. The predicted biological activities from the Online PASS analysis and detailed ADMET prediction results of nordentatin are summarized in Table 1.

Tabel 1: Predicted Biological Activities and ADMET Properties of Nordentatin.

Category	Probable activity	Pa	Pi	Prediction
Biological activity	Hepatic disorders treatment	0,.863	0.003	Predicted
	HIF1A expression inhibitor	0.857	0.008	Predicted
	CYP2C12 substrate	0.851	0.024	Predicted
	Antineoplastic	0.823	0.009	Predicted
	Free radical scavenger	0.798	0.003	Predicted
	CYP2A11 substrate	0.798	0.004	Predicted
	Spasmolytic, urinary	0.785	0.005	Predicted
	Nitric oxide antagonist	0.765	0.003	Predicted
	CDP-glycerol Glycerophosphotransferase inhibitor	0.766	0.038	Predicted
	Spasmolytic	0,725	0,006	Predicted
	Aspulvinone dimethylallyltransferase inhibitor	0.759	0.045	Predicted
	CYP2C9 substrate	0.719	0.010	Predicted
	CYP2C substrate	0.715	0.015	Predicted
	NOS2 expression inhibitor	0.701	0.003	Predicted
	Hepatoprotectant	0.703	0.007	Predicted
	Chemopreventive	0.701	0.007	Predicted
ADMET	**Property**	**Predicted value**		**Interpretation**
Absorption	Water solubility (log mol/L)	-4.424		Low solubility
	Caco-2 permeability (log Papp)	1.269		Moderate permeability
	Human intestinal absorption (%)	94.5		High absorption
	Skin permeability (log Kp)	3.227		Low skin permeability
	P-gp substrate	Yes		Likely efflux by P-gp
	P-gp I/II inhibitor	No		Not inhibitor
Distribution	VDss (human) (log L/kg)	0.651		Moderate distribution
	Fraction unbound (Fu)	0.193		Moderate plasma protein binding
	BBB permeability (log BB)	0.016		Poor BB penetration
	CNS permeability (log PS)	-1.822		Poor CNS penetration

Metabolism	CYP2D6 substrate	No	Not a substrate
	CYP3A4 substrate	No	Not a substrate
	CYP1A2/CYP2C19/CYP2C9 inhibitor	Yes	Inhibitory effect predicted
	CYP2D6/CYP3A4 inhibitor	No	No inhibition
Excretion	Total clearance (log ml/min/kg)	0.868	Moderate clearance
	Renal OCT2 substrate	No	Not a substrate
Toxicity	AMES toxicity	No	Non-mutagenic
	Max.tolerated dose (log mg/kg/day)	-0.077	Within safe limit
	hERG I/II inhibition	No	Low cardiotoxicity risk
	Oral rat acute toxicity (LD50, mol/kg)	2.423	Moderate toxicity
	Oral rat chronic toxicity (LOAEL)	1.399	Moderate
	Hepatotoxicity	No	Non-hepatotoxic
	Skin sensitization	No	Non-sensitizer

Note: Pa (Probability "to be Active") = the probability that the compound is active in a particular biological activity; Pi (Probability "to be Inactive") = the probability that the compound is inactive in that biological activity. The higher the value of Pa (often >0.7), the more confident the prediction is that the compound will have the activity. The value of Pi provides an estimate of inactivity complementary to that of Pa.

Molecular Docking

Redocking of venetoclax as a native ligand was carried out at the binding site of the BCL-2 protein (PDB ID: 6O0K) for docking validation. A gridbox was obtained with center size x = –12.55, y = 1.304, and z = –10.744 and dimensions x = 18, y = 20, and z = 20. The ligand redocking process successfully positioned the ligand in the optimal position, which confirmed that the docking parameters were in the correct binding site (Figure 1A). The obtained gridbox was used as a guide for docking nordentatin and methotrexate compounds. Molecular docking was performed using the defined grid box parameters for redocking of native ligands and receptors, with the optimal binding poses of all ligands shown in Figure 1B.

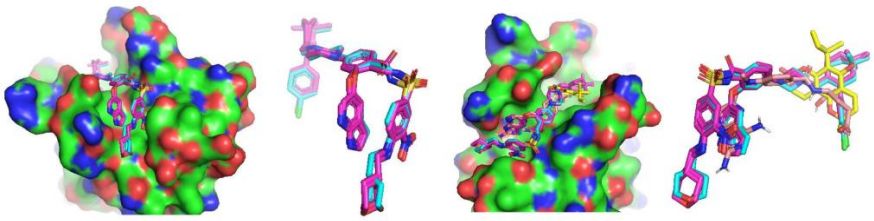

Figure 1: A. Redocking of the native ligand venetoclax into BCL-2. The best re-docked pose is shown in blue, while the crystal ligand conformation is represented in pink. B. Superimposition of all ligands at the best binding site position. The native ligand is shown in magenta, the redocked ligand in blue, nordentatin in yellow, and methotrexate in peach.

The binding affinity of nordentatin in the present study was -7.4 kcal/mol. The control ligand methotrexate showed a high binding affinity of -8.0 kcal/mol. Nordentatin binding is stabilized via hydrophobic interactions with Tyr108 (5.56 Å) and Leu137 (5.09–5.06 Å) and electrostatic interactions with Asp111 (4.05 Å). On the other hand, methotrexate established five hydrogen bonds (2.25–3.59 Å), which helped a lot to its stability, and also five more hydrophobic interactions (4.64–5.33 Å). The reference BCL-2 inhibitor Venetoclax exhibited the highest binding affinity (−12.0 kcal/mol) and formed four hydrogen bonds with Gly145 (3.03 Å), Ala100 (3.05 Å), Arg107 (3.54 Å), and Tyr202 (4.15 Å) and 15

hydrophobic interactions with residues including Tyr202, Phe104, Val156, Ala100, Ala149, Met115, Arg146, Val148, and Phe112, while no electrostatic interactions were observed (Figure 2, Table 2).

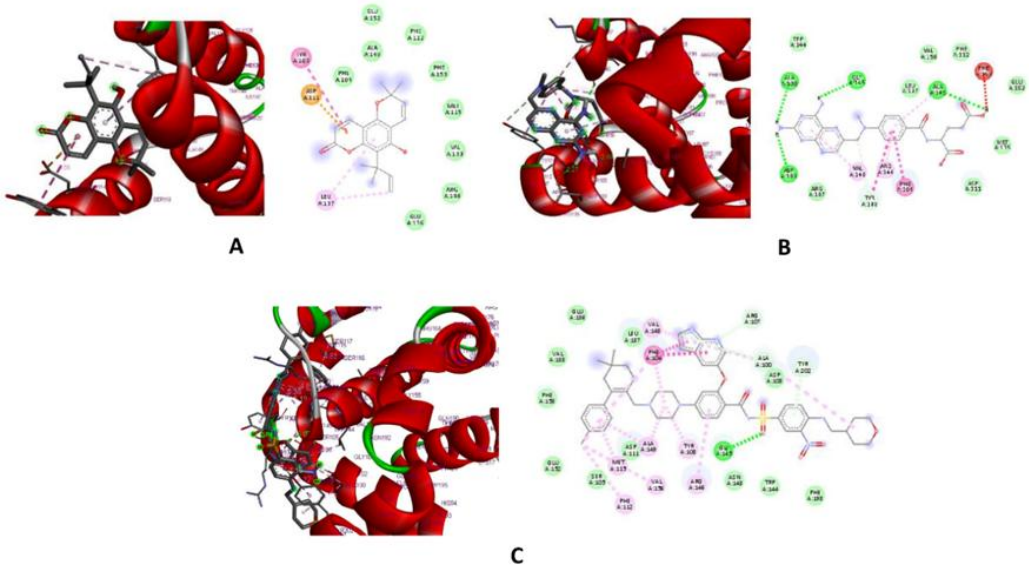

Figure 2: A. Interaction between nordentatin ligand and BCL-2 receptor, B. Interaction between methotrexate and the BCL-2 receptor, C. Interaction between venetoclax and the BCL-2 receptor.

Table 2: Ligand binding affinities at the optimal binding site locations for the BCL-2 receptor, along with specific interactions with surrounding amino acid residues.

Ligands	Binding Affinity (kcal/mol)	Distances (Å) and interacting amino acid residues		
		hydrogen bond	hydrophobic interaction	electrostatic interaction
Nordentatin	-7.4	-	Tyr108 (5,56) Leu137 (5,09) Leu137 (5,06)	Asp111 (4,05)
Methotrexate	-8.0	Asp103 (2,27) Ala100 (2,89) Gly145(2,31) Ala149 (2,25) Tyr108 (3,59)	Phe104 (5,06) Tyr108 (5,22) Arg146 (5,34) Ala149 (4,64) Val148 (5,24)	-
Venetoclax	-12.0	Gly145 (3,03), Ala100 (3,05), Arg107 (3,54), Tyr202 (4,15)	Tyr202 (4,32), Phe104 (5,58), Phe104 (5,40), Val156 (4,58), Ala100 (5,08), Ala149 (4,89), Met115 (4,83), Ala149 (5,23), Arg146 (5,38), Ala100 (5,15), Val148 (4,65), Phe104 (5,48), Phe104 (5,19), Tyr108 (5,12), Phe112 (4,56)	-

Molecular dynamics simulation

Molecular dynamics shows the RMSD Cα value of the apo BCL-2 protein with a mean value of 1.37 Å (range 0.36–2.12 Å); the BCL-2–nordentatin complex displayed a mean RMSD of 1.67 Å (range 0.32–2.32 Å); the BCL-2–methotrexate complex displayed a mean RMSD of 1.74 Å (range 0.38–2.70 Å); and the BCL-2–venetoclax complex, a known inhibitor, yielded a mean RMSD of 1.49 Å (range 0.38–2.02 Å). The RMSD Cα value can be seen in figure 3 and table 3.

The apo protein exhibited a mean Rg of 14.69 Å (range 14.52–14.89 Å); the BCL-2–nordentatin complex showed a mean Rg of 14.67 Å (range 14.49–14.90 Å); the methotrexate complex displayed a slightly higher mean Rg (14.74 Å; range 14.54–14.97 Å); and the venetoclax complex (14.69 Å; range 14.55–14.88 Å).

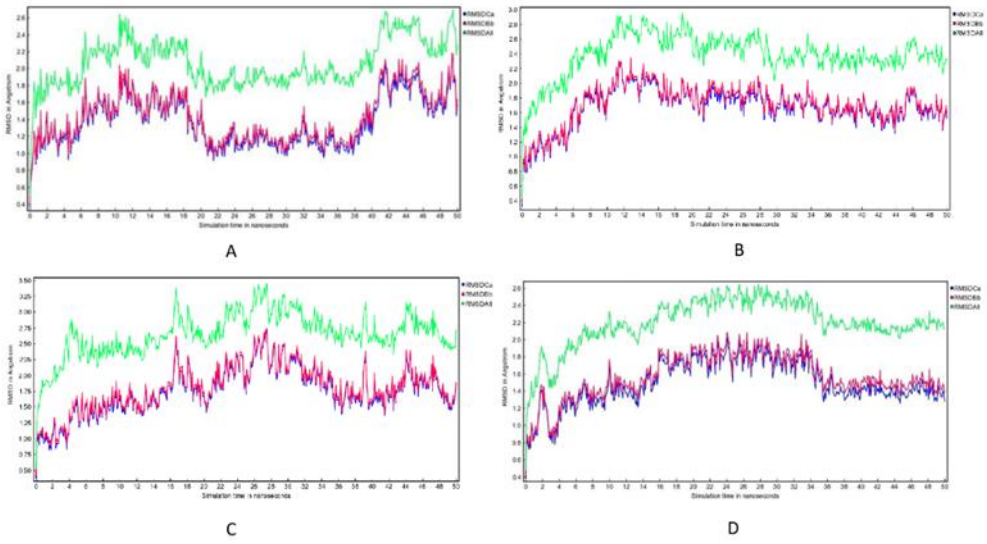

Figure 3. Root mean square deviation (RMSD) profiles of BCL-2 (A), the BCL-2–nordentatin complex (B), the BCL-2–methotrexate complex (C), and the BCL-2-venetoclax complex (D)

Table 3. RMSD Cα and Radius of Gyration (Rg)

Parameter	Complex	Mean	Min	Max	Range
RMSD Cα	BCL-2 (Apo)	1.37	0.36	2.12	1.76
	BCL-nordentatin	1.68	0.32	2.32	2.00
	BCL-methotrexate	1.75	0.38	2.70	2.33
	BCL-2 - venetoclax	1.49	0.38	2.02	1.64
Radius of Gyration (Rg)	BCL-2 (Apo)	14.69	14.52	14.89	0.38
	BCL-nordentatin	14.67	14.49	14.91	0.41
	BCL-methotrexate	14.74	14.54	14.97	0.43
	BCL-2 – venetoclax	14.69	14.55	14.88	0.33

The RMSF analysis showed that BCL-2 has some flexible areas, especially between residues 30–40 and 100–120, which are loop regions The apoprotein showed the highest fluctuations in these loops (Figure 4).

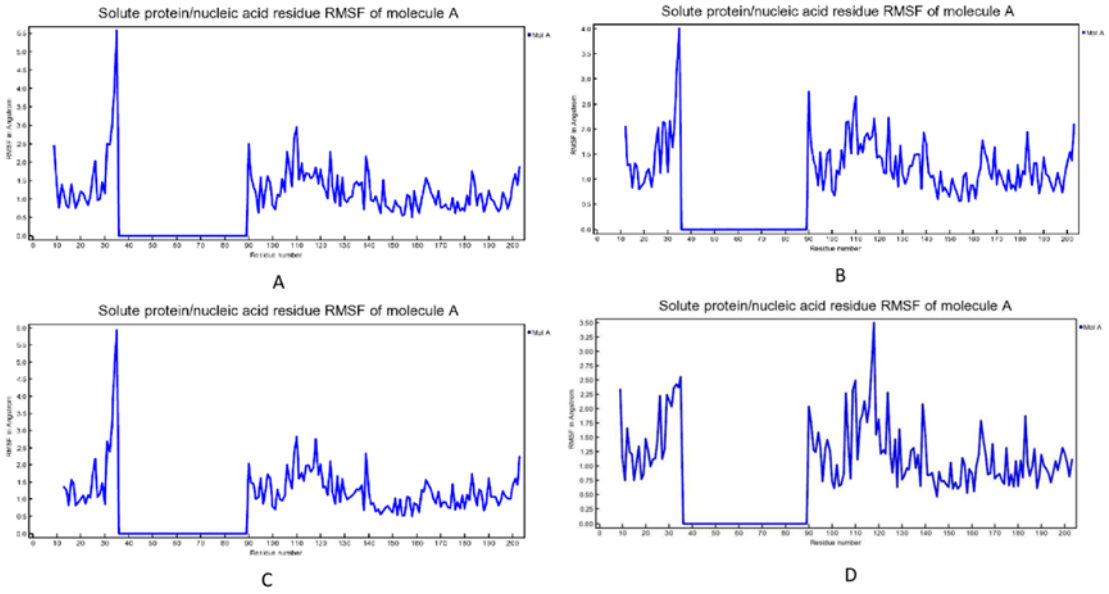

Figure 4. Root mean square fluctuation (RMSF) profiles of BCL-2 (A), the BCL-2–nordentatin complex (B), the Bcl-2–methotrexate complex (C), and the BCL-2–venetoclax complex, demonstrating the flexibility of the protein and its complexes at the residue level.

Discussion

Online PASS prediction suggested that nordentatin has a good potential to be an anticancer drug, with anticipated antineoplastic action consistent with inhibition of BCL-2, a recognized therapeutic target in solid and hematological malignancies [17]. Inhibition of HIF-1α predicted to inhibit angiogenesis and tumor adaptation to hypoxia which are critical in OSCC progression [18] and also inhibition of NOS2 (iNOS) predicted to inhibit inflammation and oxidative stress that drive OSCC proliferation and metastasis and can also inhibit angiogenesis [19]. The free radical scavenger activity of Nordentatin proved its capacity to quench or scavenge free radicals, therefore inhibiting oxidative damage to biological systems. This pathway is one of the antioxidant actions identified [20].

ADMET (absorption, distribution, metabolism, excretion and toxicity) analysis supported the therapeutic potential by predicting high intestine absorption and moderate Caco-2 permeability which are extensively used indications of oral medication absorption. In addition, the chemical was expected to be a substrate but not an inhibitor of P-glycoprotein, suggesting the potential for transporter-mediated efflux without transporter inhibition. Efflux transporters, such as P-glycoprotein, are important regulators of the intestinal absorption and permeability of drugs [21]. Distribution assays demonstrated low BBB and CNS penetration and moderate plasma protein binding (Fu=0.193) consistent with medicines with logBB < 0.3. Nordentatin is not predicted to be a substrate of CYP2D6 or CYP3A4 but may inhibit CYP1A2, CYP2C19 and CYP2C9 indicating a risk for potential medication-drug interactions since these CYPs are important enzymes for drug metabolism. Moderate clearance was predicted and no renal OCT2 substrate activity was seen. Toxicity profiling revealed good safety, including negative AMES mutagenicity, no hepatotoxicity and low risk of cardiotoxicity due to the absence of hERG inhibition [22]. The predicted acute oral toxicity (LD_{50} = 2.423 mol/kg) and chronic toxicity (LOAEL = 1.399 log mg/kg bw/day) of the compound calculated by the pkCSM pharmacokinetics server are in low-to-moderate toxicity ranges based on OECD and EPA thresholds indicating acceptable safety margins for the future development.

Overall, these results imply that nordentatin has a favorable ADMET profile and may be a suitable option for a safe and bioavailable anticancer agent. Table 2 lists the expected ADMET properties of nordentatin.

The free binding energies (ΔG) are consistent with the AutoDock Vina binding affinity scores, where greater negative values represent stronger interaction between ligand and target protein [23]. Molecular docking between BCL-2 and nordentatin proteins yielded binding affinities of -7.4 kcal/mol and -8.0 kcal/mol for BCL-2 and methotrexate and -12.0 kcal/mol for BCL-2 and venetoclax, suggesting a stronger association for the reference ligand (venetoclax). Interaction profiling shows that nordentatin involves a limited number of contacts (hydrophobic and electrostatic), with the amino acid residues dominated by hydrophobic interactions involving residues Tyr108 and Leu137 and one electrostatic interaction with Asp111. Methotrexate creates more hydrogen and hydrophobic interactions, but venetoclax creates the largest network of hydrogen bonds and hydrophobic contacts. The predominance of nonpolar residues in the BCl-2-nordentatin interaction suggests that the binding pocket is hydrophobic and that the ligand-binding mechanism is probably hydrophobic-driven. Previous studies have reported that hydrophobic interactions are one of the main factors controlling the stability of biomolecular complexes through their contribution to Gibbs free energy (ΔG). Although most studies have been conducted on protein-protein systems, this mechanism remains relevant in protein-ligand interactions due to the similarity of the underlying noncovalent interaction principles [24]. In addition, the binding affinity value of -7.4 kcal/mol suggests strong stability and important role of noncovalent interactions such as hydrophobic and van der Waals forces to Gibbs free energy (ΔG) [25]. Electrostatic interactions with Asp111 also have a role in orienting the ligand in the binding site although to a lesser extent than hydrophobic interactions [26]. The present analysis shows the absence of hydrogen bonding and the small contribution of polar contacts. This means that the thermodynamically favorable binding stability is mainly owing to the hydrophobic interactions that occur.

This interaction pattern is related to the relative binding affinity, and the molecular dynamics (MD) simulations still show a stable binding mode of nordentatin with fewer interactions, which supports its potential for further optimization. The average RMSD value of the BCL-2–nordentatin complex was 1.675, which is lower than the BCL-2–methotrexate complex with an average of 1.749. The best RMSD value is achieved for the BCL-2 – venetoclax complex with an average of 1.490. The structural stability of the protein and its complexes was confirmed by RMSD analysis over the simulation. Although transient fluctuations were observed, all complexes maintained conformational consistency, with RMSD values generally below ~2 Å, indicating that ligand binding did not cause significant structural perturbation throughout the trajectory, consistent with previous MD studies where sustained low RMSD reflects stable protein-ligand interactions [27].

All the complexes were in their stable state as shown by the radius of gyration (Rg). Nordentatin (mean Rg 14.67 Å) showed a compactness profile similar to venetoclax (14.69 Å), with somewhat better compactness than methotrexate (14.74 Å) (Table 4). We performed Rg assays to examine the stability and compactness of BCL-2 and its ligand complexes in a thorough way. Larger changes in Rg in molecular dynamics simulations indicate that the protein is becoming more flexible or begins to unravel. Small Rg fluctuations over long periods often imply that the overall protein structure and compactness are still maintained. A lower value of Rg shows a more compact and stable complex structure. This suggests a less flexible structure of the simulation [28].

RMSF analysis indicated localized flexibility in BCL-2 with peaks at residues 30–40 and 100–120 corresponding to loop regions (Figure 5). The RMSF profile evidently shows that the complex with

comparatory ligand methotrexate shows the highest fluctuations in the loop area even more than that of the apo state which indicates the increased flexibility of the residues and minimum stabilization effect. The apo protein exhibits rather substantial fluctuations indicating an innate flexibility in the absence of ligand. In contrast, the residue fluctuations are significantly reduced for the case of nordentatin binding as observed by the decrease in the value of RMSF, which shows local stabilization in the loop region. It is suggested that the stabilization is increased by hydrophobic contacts with residues Tyr108 and Leu137 and electrostatic interactions with Asp111 that would effectively limit the mobility of the residues. Meanwhile, the reference inhibitor venetoclax generally shows the least RMSF profile in terms of the ability of maintaining the protein structure. These observations are generally supported by molecular dynamics studies where RMSF profiles of apo proteins and ligand bound complexes are used as a measure of residue flexibility and interaction stability, with lower RMSF values being indicative of structural rigidification by strong protein ligand interactions [29].

Molecular docking and dynamics investigations showed stable complex formation between nordentatin and BCl-2 proteins. Docking suggested the relevance of hydrophobic interactions and electrostatic contributions. This stability was validated by MD with converging RMSD, steady Rg and low residue fluctuation (RMSF) inside the binding pocket. These studies reveal that nordentatin binds well and maintains the structural stability of the complex, indicating its potential as a possible inhibitor of BCL-2 proteins.

Limitations and future work

Taken together, this study provides novel evidence supporting nordentatin as a BCL-2 inhibitor candidate by in silico analysis. However, the limitations of the computational approach mean that the conclusions should be considered predictive rather than definitive. The short simulation time (50 ns) may not be sufficient to capture the long-term conformational dynamics and the absence of experimental validation might restrict the direct biological interpretation. In future in vitro and in vivo tests, the further pharmacological action of nordentatin, especially in the treatment of oral cancer, needs to be investigated and confirmed.

Conclusion

Docking and molecular dynamics investigations indicate that nordentatin binds specifically in the pocket of BCL-2 and the stability of the complex is mostly due to hydrophobic and electrostatic interactions. These results reveal a new interaction profile of nordentatin and suggest it as a potential new natural scaffold for the development of BCL-2 inhibitors. Nevertheless, further investigations using in vitro and in vivo assays are needed to explore and confirm the pharmacological effect of nordentatin, notably in the treatment of oral cancer.

Conflicts of interest

The authors have no conflicts of interest to declare.

Author Contributions

DA; Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, and Funding acquisition. RM; Software, Formal analysis, Data curation, Writing -

Original draft preparation, and Writing - Review & Editing. DA; Visualization, Investigation, Project administration, Writing - Review & Editing. BA; Software, Writing - Reviewing and Editing. YR; Visualization, Investigation, Writing - Reviewing and Editing. SJA; Writing - Reviewing and Editing.

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الاستهداف الجزيئي لبروتين BCL-2 في خلايا سرطان الفم بواسطة النوردنتاتين: دراسة حاسوبية للارتباط الجزيئي والديناميكيات الجزيئية

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المستخلص

يُعدّ سرطان الفم من أبرز التحديات الصحية في طب الأسنان بسبب ارتفاع معدلات الإصابة وصعوبة العلاج، ويرتبط ذلك بزيادة التعبير عن بروتين BCL-2 المسؤول عن تنظيم موت الخلايا المبرمج، مما يسهم في تطور الورم ومقاومة العلاج الكيميائي. هدفت هذه الدراسة إلى تقييم النوردنتاتين، وهو مركب كومارينيّ مستخلص من نبات *Clausena excavata*، كمثبط محتمل لبروتين BCL-2 باستخدام تقنيات المحاكاة الحاسوبية والديناميكيات الجزيئية. تم تحليل خصائص الامتصاص والتوزيع والاستقلاب والإخراج والسمية(ADMET) ، إضافةً إلى النشاط الحيوي للمركب. كما أُجري الارتباط الجزيئي باستخدام برنامج AutoDock Vina ، مع تحليل التفاعلات الجزيئية بواسطة PyMOL و BIOVIA Discovery Studio، بينما استُخدم برنامج YASARA لتقييم استقرار المعقد. أظهرت النتائج أن النوردنتاتين يمتلك نشاطًا مضادًا للسرطان والالتهابات والأكسدة، مع خصائص دوائية جيدة وسُمية منخفضة. كما أظهر ألفة ارتباط جيدة مع بروتين BCL-2 بقيمة 7.4- كيلو كالوري/مول واستقرارًا ملحوظًا خلال محاكاة الديناميكيات الجزيئية، مما يجعله مرشحًا واعدًا لعلاج سرطان الفم.