Molecular and Cellular Biomedical Sciences, Vol.7 No.2, July 2023, p.58-108
Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i2.330

RESEARCH ARTICLE

MCBS
Mol Cell Biomed Sci. 2023; 7(2): 81-9
DOI: 10.21705/mcbs.v7i2.330

Bioactive Compounds from *Penicillium* sp. Inhibit Antiapoptotic Bcl-2, Bcl-X_L and Mcl-1: An *in silico* Study

Adhie Massardi, Sandy Samsul Bahry, Nur Anindya Rahmawati, Carissa Azmi Shabirah, Artini Pangastuti

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia

Background: Antiapoptotic Bcl-2 proteins are overexpressed in cancer cells, leading to inhibition of apoptosis and the development of therapeutic resistance. Targeting only one type of antiapoptotic protein may have limited efficacy in cancer therapy. Anticancer drugs capable of inhibiting Bcl-2, Bcl-X_L and Mcl-1 simultaneously are necessary to be explored. *Penicillium* sp. produces various bioactive compounds with anticancer, antibacterial, and antiviral activities. The aim of this research was to determine the best bioactive compound candidates for inhibiting Bcl-2, Bcl-X_L, and Mcl-1 proteins.
Materials and methods: Molecular docking analysis was conducted to estimate the binding affinity of *Penicillium* sp. bioactive compounds with Bcl-2, Bcl-X_L, and Mcl-1 proteins. Compounds with the lowest binding energies were visualized using PyMol and Ligplot+ and further subjected to drug-likeness testing based on Lipinski's rule of five.
Results: Bioactive compounds with the highest binding affinities were verruculogen and wortmannin. Wortmannin complied with Lipinski's rule of five. Meanwhile, verruculogen violated one out of the five rules by having a molecular weight >500 Da. Both compounds could be used as oral drugs.
Conclusion: Verruculogen and wortmannin from *Penicillium* sp. show significant potential as oral anticancer drug candidates.

Keywords: *Bcl-2, Bcl-X_L, Mcl-1, Penicillium sp., in silico*

Introduction

The antiapoptotic B-cell lymphoma protein 2 (Bcl-2) family members play an important role in the regulation of apoptosis in various types of cancer cells, including lung cancer, breast cancer, and acute lymphocytic leukemia.[1] In cancer cells, Bcl-2 proteins are overexpressed, leading to inhibition of apoptosis and the development of therapeutic resistance.[2] The potential of a protein to facilitate interactions with antiapoptotic proteins and promote programmed cell death is correlated with the Bcl-2 homology (BH)3 domain.[3] Mutations in one or more of the key residues in the BH3 domain can abolish binding with Bcl-2 family proteins and impede the proapoptotic function of a protein.[4]

Apoptosis failure often involves multiple antiapoptotic proteins, as evidenced by the identification of Bcl-2, Bcl-X_L, and Mcl-1 proteins in some myeloma cells. Previous

Submission: March 4, 2023
Last Revision: May 11, 2023
Accepted for Publication: May 17, 2023

Corresponding Author:
Adhie Massardi
Department of Biology
Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret
Jl. Ir. Sutami 36 A, Surakarta 57126, Indonesia
e-mail: adhiemassardi004@gmail.com

Cell and Biopharmaceutical Institute

studies have shown that inhibiting either Mcl-1 or Bcl-X_L alone is not sufficient to induce apoptosis in cancer cells. In fact, inhibition of Bcl-2 and Bcl-X_L can even increase Mcl-1 expression, resulting in prolonged survival of cancer cells. Notably, cancer cells often express three antiapoptotic proteins, namely Bcl-2, Bcl-X_L, and Mcl-1, but their expressions vary greatly depending on the type of cancer. Therefore, targeting only one type of antiapoptotic protein may have limited efficacy in cancer therapy.[5] It is evident that cancer cells exhibit drug resistance even with single inhibition of antiapoptotic proteins, indicating their dependence on multiple antiapoptotic proteins for survival.[6]

Numerous anticancer drugs have been developed to target the Bcl-2 protein family. Navitoclax is an oral drug that binds to Bcl-2 and Bcl-X_L but does not bind to Mcl-1.[7] However, strong inhibition of Bcl-X_L by navitoclax can result in thrombocytopenia due to decreased platelet count. As a result, venetoclax was developed as a specific Bcl-2 inhibitor to reduce its binding to Bcl-X_L, and thereby maintaining platelet count.[8] Nevertheless, both navitoclax and venetoclax have been shown to increase the expression of Mcl-1, leading to the cancer cell resistance to these drugs.[9] Therefore, exploration of drugs capable of inhibiting Bcl-2, Bcl-X_L and Mcl-1 simultaneously is necessary to overcome resistance and improve therapeutic outcomes in cancer treatment.

Penicillium sp. is a fungus known for its role as a decomposer and has been isolated from various environments, including terrestrial environments, food products, and soil.[10] Previous studies reported that *Penicillium* sp. produced a wide range of bioactive compounds with potential anticancer, antibacterial, and antiviral activities. For instance, the culture filtrate of *Penicillium citrinum* has been shown to exhibit cytotoxic effects in A549 and MCF-7 cell lines.[11] Additionally, bioactive fraction isolated from *Penicillium rubens* has been found to induce apoptosis through upregulation of proapoptotic Bax and downregulation of antiapoptotic Bcl-2 expression.[12] Other compounds derived from *Penicillium* sp., such as L-asparaginase and polyketide derivatives have demonstrated anticancer activity as well.[13] Penicitrine A produced by *P. citrinum* from marine sediments has been shown to induce apoptosis in A-375 cells by downregulating Bcl-2 and upregulating Bax.[14] Moreover, approximately 48 bioactive compounds from *Penicillium* sp. have been identified as anticancer agents with diverse mechanisms of action.[15] Therefore, the aim of this research was to determine the best bioactive compound candidates for inhibiting Bcl-2, Bcl-X_L and Mcl-1 proteins.

Materials and methods

Protein and Ligand Preparation

The protein structures of Bcl-2 (ID: 6qgh), Bcl-X_L (ID: 4qnq), and Mcl-1 (ID: 6ne5) were obtained from the Protein Data Bank (PDB) (www.rcsb.org) and prepared using PyMol 2.5 (Schrödinger, New York, NY, USA). Any existing ligands and water molecules were removed, and the proteins were converted into a dockable PDB format. Forty-eight bioactive compounds from *Penicillium* sp. with known anticancer properties[15] were retrieved from the PubChem database (pubchem.ncbi.nlm.nih.gov) in SDF format and converted to pdbqt format for docking. All compounds were energetically minimized to increase the accuracy of the docking process.

Molecular Docking

Molecular docking analysis was conducted using PyRx-0.9.2. (SourceForge, San Diego, CA, USA) to estimate the binding affinities of bioactive compounds from *Penicillium* sp. with Bcl-2, Bcl-X_L, and Mcl-1. The docking coordinates for the proteins were set as follows: Bcl-2 – center x=-2.15, y=2.83, z=8.74; Bcl-X_L – center x=52.31, y=9.52, z=-13.96; Mcl-1 – center x=2.38, y=-39.17, z=63.71. The docking method was validated by redocking the bound compound from PDB to their respective proteins, and the root mean square deviation (RMSD) of the crystal and docked conformation was calculated.[16] Compounds with the lowest binding energies were visualized using PyMol and Ligplot$^+$ V.2.2. (EMBL-EBI, Cambridgeshire, UK) and further subjected to drug-likeness testing.

Drug-likeness Test

Compounds with the lowest binding energies were selected and their simplified molecular input line entry system (SMILES) notations were obtained. These SMILES notations were then entered into Admetsar 2.0 (http://lmmd.ecust.edu.cn/admetsar2) for prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties. The results were subsequently analyzed for compliance with Lipinski's rule of five, which assesses the drug-likeness of compounds based on their physicochemical properties.

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i2.330

Results

Molecular Docking Results

In this study, a total of 49 ligands were examined, comprising 48 bioactive compounds from *Penicillium* sp. and one native ligand. Navitoclax was used as a native ligand of Bcl-2 and Bcl-X_L, while $C_{39}H_{39}Cl_2N_5O_4$ was used as a native ligand of Mcl-1. Among the ligands tested, wortmannin and verruculogen showed the highest binding affinities for Bcl-2, Bcl-X_L, and Mcl-1, respectively. However, wortmannin and verruculogen displayed lower binding affinities compared with the native ligand of Bcl-2 and Mcl-1. Wortmannin had a lower binding affinity, while verruculogen had a higher binding affinity compared with the native ligand of Bcl-X_L (Table 1). The RMSD values for the native ligands of Bcl-2, Bcl-X_L, and Mcl-1 were 0.315, 0.206, and 0.000 Å, respectively (Figure 1).

Based on docking visualization data, navitoclax was found to stretch along the P2-P4 pockets in the BH3-binding groove of Bcl-2 and Bcl-X_L. Verruculogen and wortmannin were bound to the P2 and P4 pockets of Bcl-2, respectively (Figure 2A). Both navitoclax and verruculogen interacted with Glu152, Phe153, Met115, Leu137, Val133, Glu136, Ala149, and Phe112 residues in Bcl-2 through hydrophobic bonds. Navitoclax and verruculogen also interacted with Asp111 through hydrophobic bond and hydrophilic interaction, respectively (Figure 2B, Figure 2C). Both navitoclax and wortmannin interacted with Trp144,

Table 1. Binding affinity of bioactive compounds from *Penicillium* sp. with Bcl-2, Bcl-X_L, and Mcl-1 proteins.

Compound	Binding Affinity (kcal/mol)		
	Bcl-2	Bcl-X_L	Mcl-1
Cetrimonium	-5.3	-5.1	-6.5
Emodin	-7.2	-7.1	-7.0
Gliotoxin	-7.2	-6.7	-5.4
Hadacidin	-3.9	-3.9	-3.7
Secalonic acid D	-8.0	-7.1	-4.9
Maculosin	-7.3	-7.3	-8.1
Penicillide	-7.4	-7.9	-7.5
Mycotoxin 81	-8.7	-8.6	-10.3
Obtusin	-6.7	-6.1	-6.2
Chembl305250	-7.8	-7.6	-9.7
Methylenolactocin	-6.0	-5.8	-6.1
Griseofulvin	-7.4	-7.2	-7.0

Table 1. Binding affinity of bioactive compounds from *Penicillium* sp. with Bcl-2, Bcl-X_L, and Mcl-1 proteins (cont.).

Compound	Binding Affinity (kcal/mol)		
	Bcl-2	Bcl-X_L	Mcl-1
Mycophenolic acid	-7.2	-6.8	-7.9
Sch 642305	-7.5	-7.3	-7.0
Wortmannin	-11.1	-10.3	-11.4
Citromycin	-7.1	-6.5	-6.8
5,8-tetradecadienal	-5.7	-5.2	-6.6
Duclauxin	-8.4	-8.1	-8.3
Vermistatin	-6.8	-7.1	-7.8
Pyrenocine A	-5.7	-5.6	-6.2
Chaetoglobosin A	-9.7	-10.0	-8.6
Neoxaline	-8.4	-7.2	-6.2
Epolactaene	-7.2	-7.5	-7.5
Isochromophilone IV	-7.5	-7.6	-9.0
Leteusin A	-7.6	-7.2	-8.5
Fumagillin	-7.6	-7.2	-7.8
Trichodimerol	-7.9	-7.1	-7.0
Topopyrone C	-7.9	-7.6	-7.6
Trachspic acid	-7.0	-6.3	-6.8
Sequoiatone A	-7.1	-6.3	-7.4
Quinolactacin A	-7.6	-7.0	-7.3
Penostatin A	-7.6	-6.7	-8.5
Fellutanine B	-8.8	-8.1	-9.5
Psychrophilin D	-9.2	-8.7	-8.4
Rubratoxin B	-8.9	-7.8	-7.3
Oxazine	-4.2	-3.7	-3.8
Verruculogen	-11.9	-10.5	-11.1
Dehydoaltenusin	-7.5	-6.9	-7.0
Penicillone	-5.8	-5.5	-6.4
Penicillenone	-7.0	-6.6	-7.7
Quinolinone A	-7.0	-6.5	-8.0
Penochalasin A	-9.6	-9.5	-10.1
5S-HETE di-endoperoxide	-6.7	-6.1	-7.7
Phalluside-1	-6.9	-7.0	-6.2
Kurilensoside F	-7.7	-7.5	-7.2
Leptosphaerone	-6.7	-6.7	-6.7
Cyclopiazonic acid imine	-8.2	-7.8	-7.7
Eupenifeldin	-8.3	-8.1	-7.5
Native ligand	-12.3	-10.4	-13.2

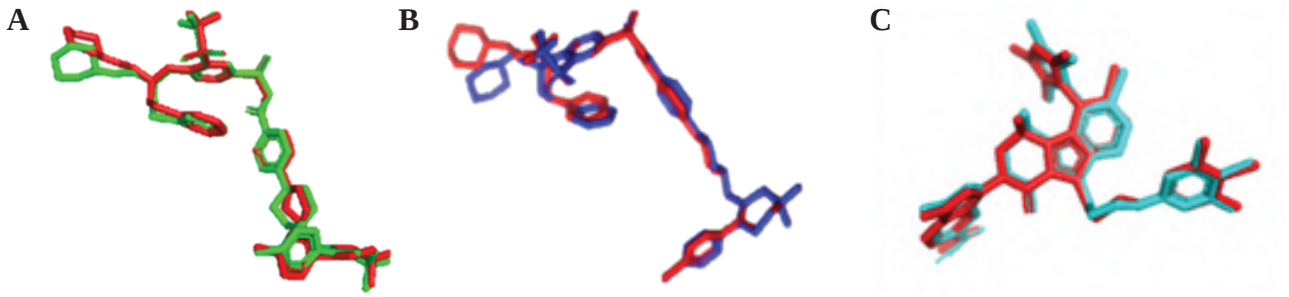

Figure 1. Molecular docking validation of native ligands of antiapoptotic proteins. A: Bcl-2; B: Bcl-X_L; C: Mcl-1.

Glu209, Gly145, Val148, Ala100, Phe198, Tyr202 and Leu201 residues in Bcl-2 through hydrophobic bonds (Figure 2B, Figure 2D).

Verruculogen bound to the P4 pocket of Bcl-X_L (Figure 3A). Both navitoclax and verruculogen interacted with Glu129, Val126, Leu130, Phe146, Phe105, and Leu108 through hydrophobic bonds (Figure 3B, Figure 3C). Verruculogen also interacted with Ala104 through hydrophilic interaction (Figure 3C). Wortmannin, on the other hand, bound to the Bcl-X_L amino acid residues in the P2 pocket (Figure 3A) and interacted with the same amino acid residues as navitoclax, namely Tyr101, Phe97, Ala93, Val141, Tyr195, Phe191, and Trp137 through hydrophobic bonds. Navitoclax and wortmannin also interacted with Gly138 through hydrophobic bond and hydrophilic interaction, respectively (Figure 3B, Figure 3D).

Verruculogen and wortmannin were found to bind to the P2 pocket of Mcl-1 (Figure 4A). Both $C_{39}H_{39}Cl_2N_5O_4$ and verruculogen interacted with His224, Thr266, Phe228, Met250, Leu267, Phe254, Val253, and Phe270 through hydrophobic bonds (Figure 4B, Figure 4C). On the other hand, $C_{39}H_{39}Cl_2N_5O_4$ and wortmannin was bound to Phe254, Val253, Ala227, Phe228, Phe270, and Leu267 through hydrophobic bonds (Figure 4B, Figure 4D). Verruculogen interacted with Arg263 through hydrophobic bond, while $C_{39}H_{39}Cl_2N_5O_4$ and wortmannin interacted with Arg263 through hydrophilic interaction (Figure 4B-D).

Analysis of Drug-likeness Properties

Wortmannin complied with Lipinski's rule of five. Meanwhile, verruculogen violated one out of the five rules, since its molecular weight (MW) >500 Da (Table 2). The Lipinski's rule of five parameters were as follows: MW≤500 Da, lipophilicity ≤5, the hydrogen bond donor ≤5, the hydrogen bond acceptor ≤10, partition coefficient (logP) ≤5, and the rotational bond count ≤10.

Discussion

There are approximately 48 bioactive compounds from *Penicillium* sp. that have been reported to exhibit anticancer effects.[15] The validity of the molecular docking procedure was assessed using the RMSD value. The docking pose is generally considered to be near native if the RMSD from the original pose is ≤2.0 Å[17], while RMSD values ≥10 Å can introduce noise in the scoring model[18] due to the ligand being far from the binding site. Further examination of the docking approach is warranted based on this validation.

Verruculogen and wortmannin exhibited the lowest binding energies compared with other compounds isolated from *Penicillium* sp. Verruculogen showed a lower binding energy than wortmannin when forming bonds with Bcl-2. Interestingly, both verruculogen and wortmannin demonstrated lower binding affinities than navitoclax when interacting with Bcl-2, despite having relatively lower binding energies. It is worth noting that substances with higher binding affinities have greater protein inhibitor potential.[19] The current findings also showed higher binding affinity of verruculogen with Bcl-X_L when compared with the native ligand of Bcl-X_L, suggesting its potential as a potent inhibitor for this target.

Navitoclax was found to extend along the P2-P4 pockets in the BH3-binding groove of both Bcl-2 and Bcl-X_L. P2 and P4 pockets of Bcl-2 proteins are commonly targeted for therapeutic interventions.[20] Interestingly, verruculogen and wortmannin were found to bind to the P2 and P4 pockets of Bcl-2, respectively. Furthermore, verruculogen was bound to the P4 pocket, while wortmannin was bound to the P2 pocket of Bcl-X_L. The difference in binding may be attributed to a mutation in Bcl-2 family proteins that alters the properties of their active sites. In Mcl-1, the binding of BH3 to the P4 pocket is less significant compared with Bcl-2 and Bcl-X_L due to

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i2.330

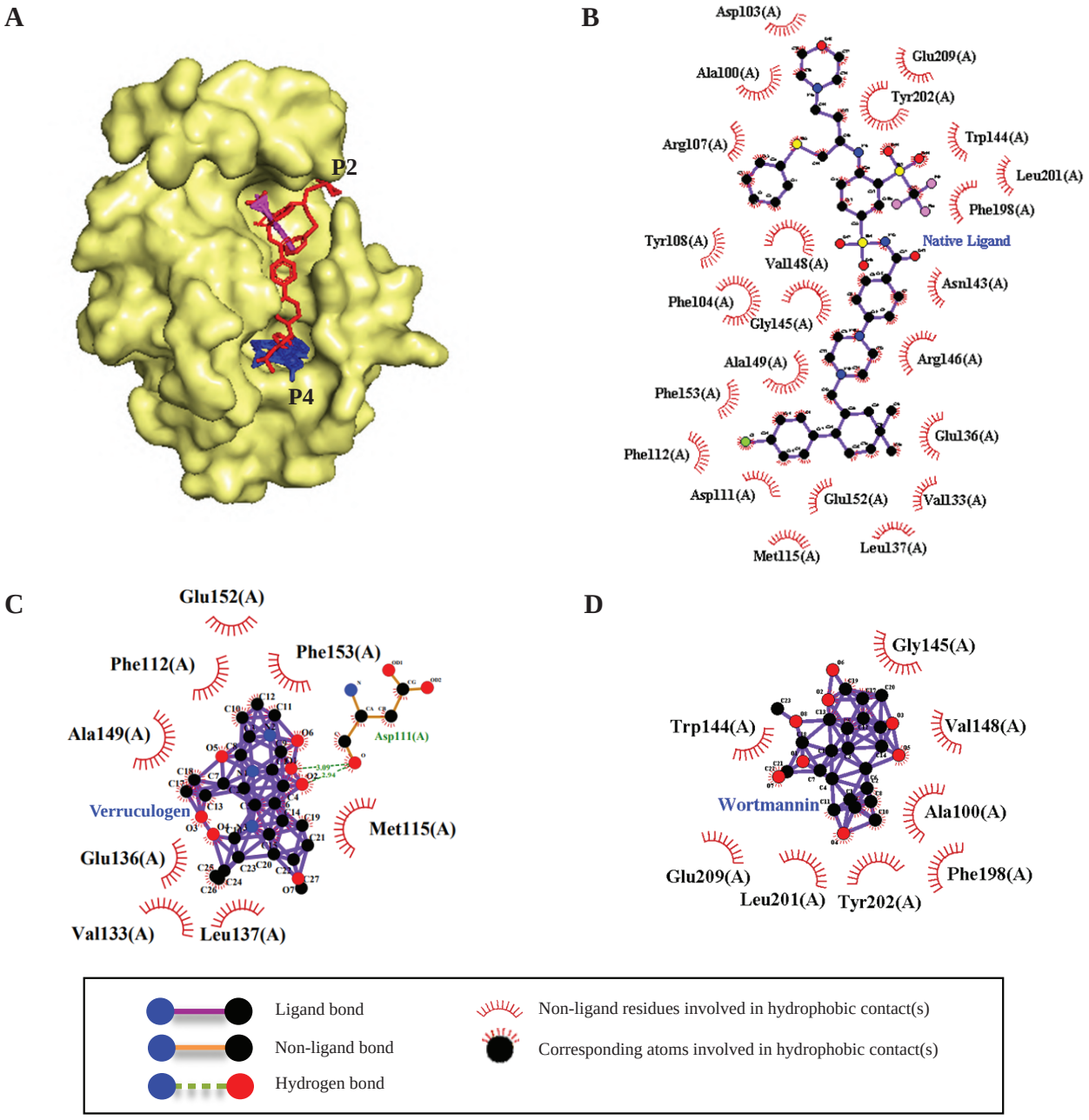

Figure 2. Interaction of bioactive compounds from *Pencillium* sp. with Bcl-2. A: The binding positions of navitoclax (red), verruculogen (purple), and wortmannin (blue) in the Bcl-2 pocket; B: Amino acid residues that interacted with navitoclax; C: Amino acid residues that interacted with verruculogen; D: Amino acid residues that interacted with wortmannin.

mutagenesis in this protein.[21] Moreover, wortmannin had a lower binding energy compared with verruculogen when interacting with Mcl-1. Additionally, the type of interaction between the ligands and Arg263[22], an amino acid residue crucial for BH3 binding to Mcl-1, was found to be different in verruculogen and wortmannin, as revealed by the current finding. The intermolecular interactions between the ligands and the proteins were stabilized by hydrogen bonding[23], which is known to improve the binding affinity of ligands to proteins.[24] Verruculogen and wortmannin interacted with amino acid residues of Bcl-2, Bcl-X_L, and Mcl-1 in a similar manner with those of the native ligands. These findings suggest that both substances have the potential to inhibit Bcl-2, Bcl-X_L, and Mcl-1 from blocking cell death

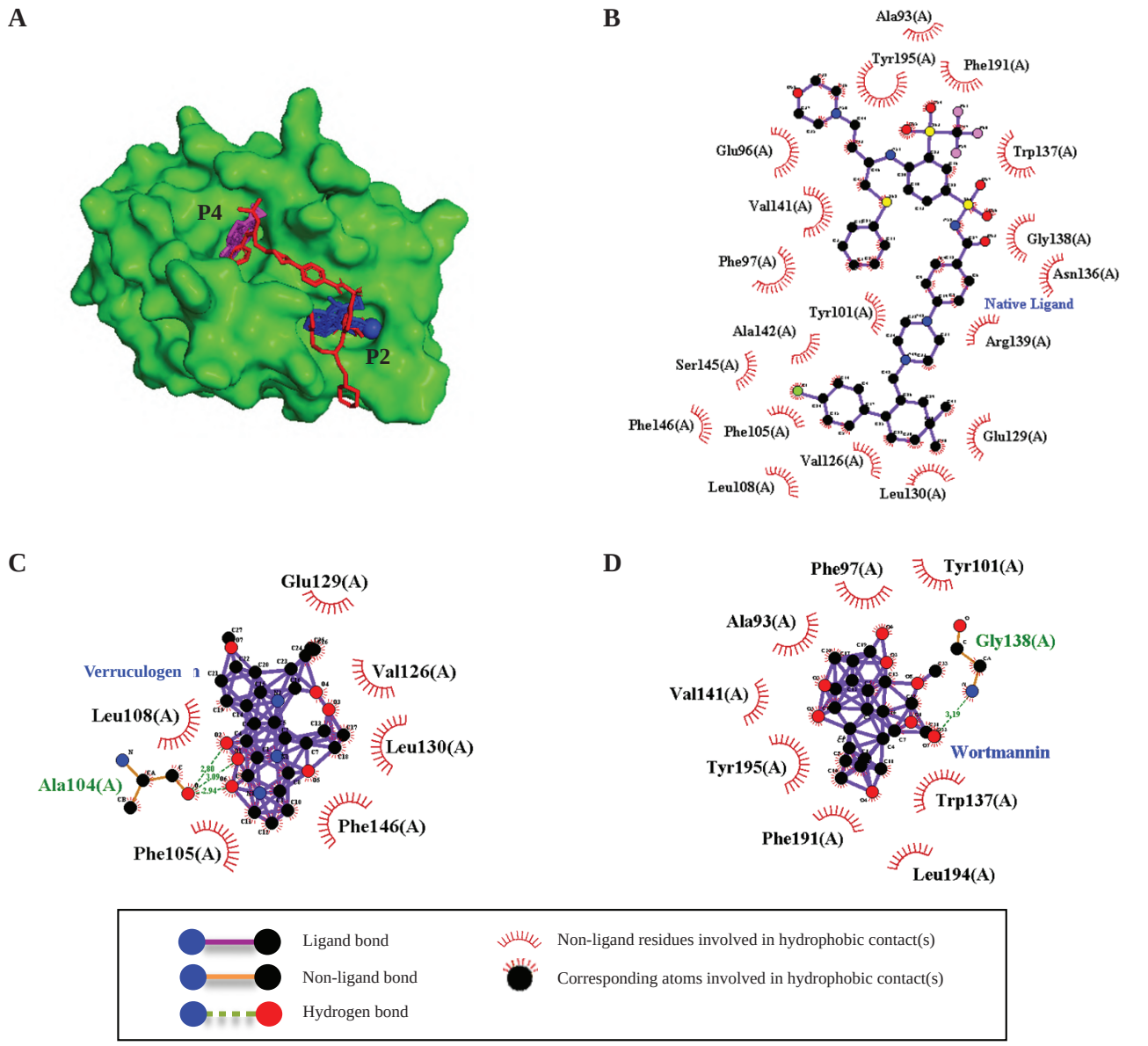

Figure 3. Interaction of bioactive compounds from *Pencillium* sp. with Bcl-X_L. A: The binding positions of navitoclax (red), verruculogen (purple), and wortmannin (blue) in the Bcl-X_L pocket; B: Amino acid residues that interacted with navitoclax; C: Amino acid residues that interacted with verruculogen; D: Amino acid residues that interacted with wortmannin.

by occupying the BH3-binding groove of the antiapoptotic proteins, thereby rendering the proteins inactive, ultimately allowing cells to undergo apoptosis.

Penicillium brasilianum and *Penicillium verruculosum* are known to produce verruculogen, a compound with potential anti-tumor activity. *In vitro* studies have also demonstrated that verruculogen reduces the proliferation of Sf-9 cells with minimal effects on mammalian cells[25] as evidenced by its IC_{50} value of 9.8 M.[26] The effect of verruculogen in cancer cells is limited. On the other hand, *Penicillium funiculosum* is known to produce wortmannin. Treatment with wortmannin at a dose of 50 nM has been shown to inhibit the growth of MCF-7 cells and trigger apoptosis after 24 hours of exposure.[24] A study has also revealed that wortmannin can block the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway, thereby preventing the expression of Bcl-2 and Bcl-X_L in primary acute myeloid leukemia (AML) cells.[25] The PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway plays a crucial role in cell survival, growth,

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i2.330

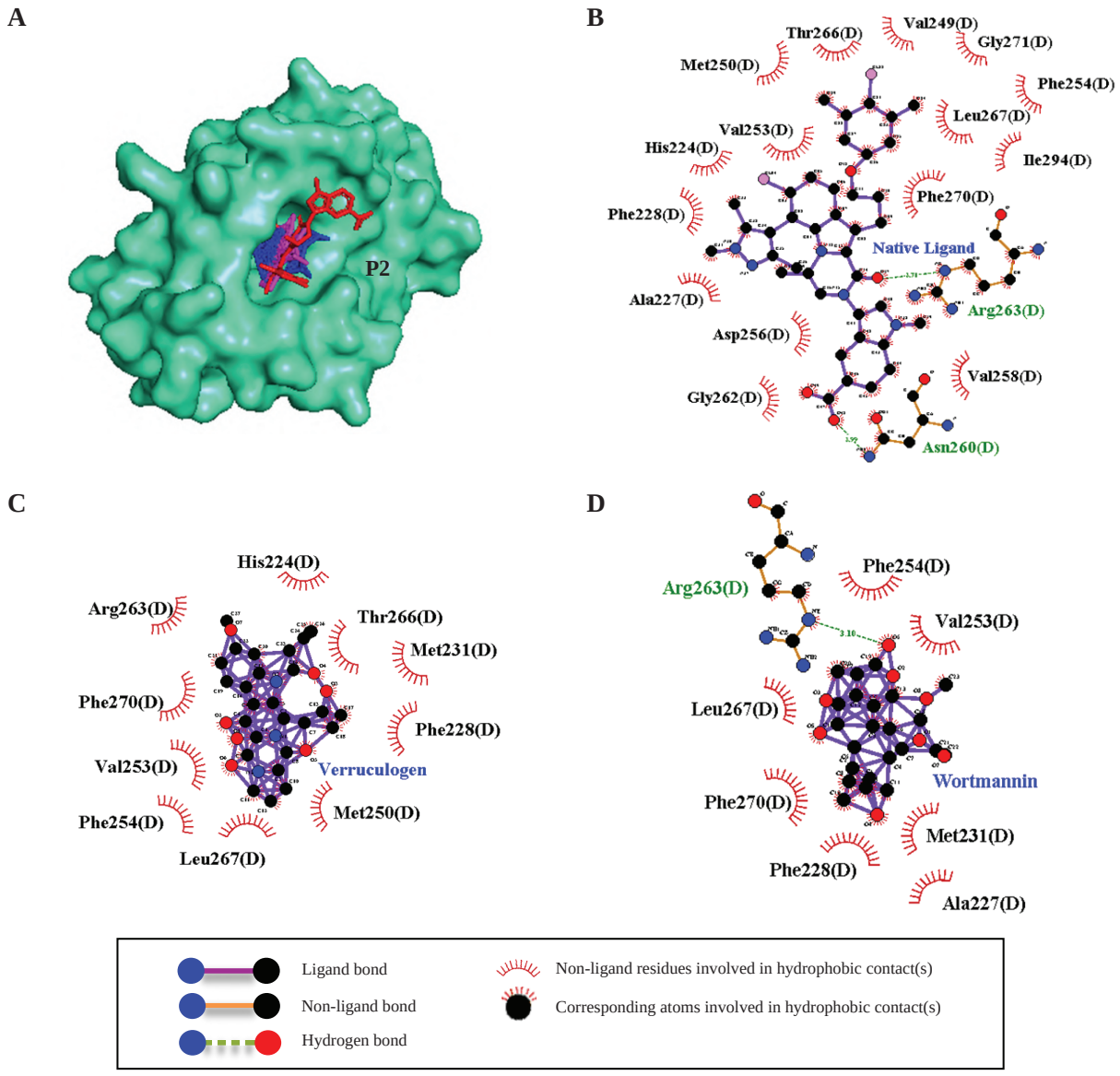

Figure 4. Interaction of bioactive compounds from *Pencillium* sp. with Mcl-1. A: The binding positions of $C_{39}H_{39}Cl_2N_5O_4$ (red), verruculogen (purple), and wortmannin (blue) in the Mcl-1 pocket; B: Amino acid residues that interacted with $C_{39}H_{39}Cl_2N_5O_4$; C: Amino acid residues that interacted with verruculogen; D: Amino acid residues that interacted with wortmannin.

and proliferation in various tumor cells, as well as in physiological settings.[27] *In vivo* studies have shown that wortmannin treatment increases apoptotic cells and inhibits autophagy through the PI3K pathway.[28]

A crucial step in developing a compound into a potential drug candidate is drug-likeness assessment.[29] This assessment is based on parameters derived from the structure and physicochemistry of the drug candidates themselves.[30] Lipinski's rule of five is widely used to differentiate between drug-like compounds and non-drug molecules.[31] Lipinski's rule of five criteria includes MW≤500 Da, lipophilicity ≤5, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, and logP≤5. A compound should adhere to three or more rules, so it can be considered as a drug candidate.[32,33] Lipinski's rule of five is well-known for predicting the performance of absorption, distribution, metabolism, and excretion (ADME) properties of drug candidates.[34]

The concentration of a molecule and its intestinal absorption are inversely correlated with its MW, which is indicative of its size.[31] High molecular size may hinder

Table 2. Drug-likeness test results of verruculogen and wortmannin against the Lipinski's rule of five.

Compound	MW (Da)	logP	H-Bond Acceptor	H-Bond Donor	Rotatable Bonds
Verruculogen	511.58	2.86	8	2	2
Wortmannin	428.44	2.54	8	0	3

the absorption of a molecule in the intestine. Verruculogen (MW>500 Da) might have a lower intestinal absorption compared with wortmannin (MW<500 Da). To enhance solubility in water and optimize absorption, the logP value, which determines the solubility factor of a compound, can be increased.[31] Verruculogen and wortmannin both exhibited lipophilic characteristics, as per the test result. A logP value in the range of 0 to 3 (0<logP<3) corresponds to the optimal oral bioavailability for both compounds.[35] However, excessively high logP values result in poor solubility in water, while very low logP values hinder the penetration of the compound through the lipid bilayers of the cell membranes.[34]

Our findings revealed that both verruculogen and wortmannin exhibited a low number of hydrogen bond acceptor (<10) and hydrogen bond donor (<5). In a lipophilic environment, an excess of hydrogen bond acceptor and hydrogen bond donor can decrease permeability.[36] The number of bond rotations in a molecule can serve as an indicator of its flexibility and oral absorption potential. A reduced number of bond rotation signifies a modest conformational change in the protein upon ligand binding.[31] Notably, both wortmannin and verruculogen exhibited a favorable number of rotatable bonds (<10).

Wortmannin complied with Lipinski's rule of five, as determined by drug-likeness evaluations. However, verruculogen violated one out of the five rules by having a MW>500 Da. Nevertheless, compounds that violate fewer than two of Lipinski's rule of five criteria can still be considered as potential oral drugs.[32] Therefore, these compounds may have potential applications as oral anticancer drugs.

Conclusion

Verruculogen and wortmannin, bioactive compounds derived from *Penicillium* sp., show significant potential as anticancer drug candidates. These compounds could inhibit antiapoptotic Bcl-2, Bcl-X_L, and Mcl-1 by binding to the BH3-binding groove, thereby inducing apoptosis. Furthermore, both verruculogen and wortmannin exhibit potential as oral drugs, making them attractive candidates for further investigation in anticancer drug development.

Authors Contribution

AM and AP were involved in concepting and planning the research. AM and CAS performed the data acquisition. SSB and NAR calculated the experimental data and performed the analysis. SSB and CAS drafted the manuscript. SSB designed the figures and tables. AM and AP aided in interpreting the results. All authors took parts in giving critical revision of the manuscript and have read and approved the final manuscript.

References

1. Campbell KJ, Tait SWG. Targeting BCL-2 regulated apoptosis in cancer. Open Biol. 2018; 8(5): 180002. doi: 10.1098/rsob.180002.
2. Kang MH, Reynolds CP. Bcl-2 inhibitors: Targeting mitochondrial apoptotic pathways in cancer therapy. Clin Cancer Res. 2009; 15(4): 1126-32.
3. Shamas-Din A, Brahmbhatt H, Leber B, Andrews DW. BH3-only proteins: Orchestrators of apoptosis. Biochim Biophys Acta. 2011; 1813(4): 508-20.
4. Shamas-Din A, Kale J, Leber B, Andrews DW. Mechanisms of action of Bcl-2 family proteins. Cold Spring Harb Perspect Biol. 2013; 5(4): a008714. doi: 10.1101/cshperspect.a008714.
5. Kim S, Park HS, Oh BH. Computational design of an apoptogenic protein that binds BCL-xL and MCL-1 simultaneously and potently. Comput Struct Biotechnol J. 2022; 20: 3019-29.
6. Abdul Rahman SF, Azlan A, Lo KW, Azzam G, Mohana-Kumaran N. Dual inhibition of anti-apoptotic proteins BCL-XL and MCL-1 enhances cytotoxicity of nasopharyngeal carcinoma cells. Discov Oncol. 2022; 13(1): 9. doi: 10.1007/s12672-022-00470-9.
7. de Vos S, Leonard JP, Friedberg JW, Zain J, Dunleavy K, Humerickhouse R, *et al*. Safety and efficacy of navitoclax, a BCL-2 and BCL-XL inhibitor, in patients with relapsed or refractory lymphoid malignancies: Results from a phase 2a study. Leuk Lymphoma. 2021; 62(4): 810-8.
8. Han Z, Liang J, Li Y, He J. Drugs and clinical approaches targeting the antiapoptotic protein: A review. Biomed Res Int. 2019; 2019: 1212369. doi: 10.1155/2019/1212369.
9. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, *et al*.

Print ISSN: 2527-4384, Online ISSN: 2527-3442
DOI: 10.21705/mcbs.v7i2.330

ABT-263: A potent and orally bioavailable Bcl-2 family inhibitor. Cancer Res. 2008; 68(9): 3421-8.
10. Park MS, Oh SY, Fong JJ, Houbraken J, Lim YW. The diversity and ecological roles of Penicillium in intertidal zones. Sci Rep. 2019; 9(1): 13540. doi: 10.1038/s41598-019-49966-5.
11. Ashoka GB, Shivanna MB. Antibacterial, antioxidant, and anticancer activities of Penicillium citrinum Thom. endophytic in Jatropha heynei. J Appl Pharm Sci. 2023; 13(3): 196–207.
12. Venkatachalam P, Nadumane VK. Modulation of Bax and Bcl-2 genes by secondary metabolites produced by Penicillium rubens JGIPR9 causes the apoptosis of cancer cell lines. Mycology. 2019; 12(2): 69-81.
13. Alhasan DAH, Al-Abedi HFH. Anticancer compounds from fungi: Review. Iraqi J Cancer Med Genet. 2021; 14(1): 25-8.
14. Fitriyanto IA, Bashari MH, Ariyanto EF. Potensi metabolit sekunder dari jamur yang berasosiasi dengan spons laut sebagai sumber senyawa antikanker. Media Kartika J Kedokt Kesehat. 2021; 4(5): 553-66.
15. Nicoletti R, Letizia Ciavatta M, Buommino E, Tufano MA. Antitumor extrolites produced by Penicillium species. Int J Biomed Pharm Sci. 2008; 2(1): 1-23.
16. Girsang E, Lister INE, Ginting CN, Khu A, Samin B, Widowati W, *et al*. Chemical constituents of snake fruit (Salacca zalacca (Gaert.) Voss) peel and in silico anti-aging analysis. Mol Cell Biomed Sci. 2019; 3(2): 122-8.
17. Bell EW, Zhang Y. DockRMSD: An open-source tool for atom mapping and RMSD calculation of symmetric molecules through graph isomorphism. J Cheminform. 2019; 11(1): 40. doi: 10.1186/s13321-019-0362-7.
18. Zheng L, Meng J, Jiang K, Lan H, Wang Z, Lin M, *et al*. Improving protein-ligand docking and screening accuracies by incorporating a scoring function correction term. Brief Bioinform. 2022; 23(3): bbac051. doi: 10.1093/bib/bbac051.
19. Anggini PW, Amanina SA, Asyura SR, Dion R. In silico study of essential oil of Bambusa vulgaris leaves as an anti beta-lactamase compound. Mol Cell Biomed Sci. 2022; 6(3): 147-54.
20. Lessene G, Czabotar PE, Sleebs BE, Zobel K, Lowes KN, Adams JM, *et al*. Structure-guided design of a selective BCL-X(L) inhibitor. Nat Chem Biol. 2013; 9(6): 390-7.
21. Garner TP, Lopez A, Reyna DE, Spitz AZ, Gavathiotis E. Progress in targeting the BCL-2 family of proteins. Curr Opin Chem Biol. 2017; 39: 133-42.
22. Denis C, Sopková-de Oliveira Santos J, Bureau R, Voisin-Chiret AS. Hot-spots of Mcl-1 protein. J Med Chem. 2020; 63(3): 928-43.
23. Widyananda MH, Pratama SK, Samoedra RS, Sari FN, Kharisma VD, Ansori ANM, *et al*. Molecular docking study of sea urchin (Arbacia lixula) peptides as multi-target inhibitor for non-small cell lung cancer (NSCLC) associated proteins. J Pharm Pharmacogn Res. 2021; 9(4): 484–96.
24. Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing. Sci Adv. 2016; 2(3): e1501240. doi: 10.1126/sciadv.1501240.
25. Boguś MI, Wrońska AK, Kaczmarek A, Boguś-Sobocińska M. In vitro screening of 65 mycotoxins for insecticidal potential. PLoS One. 2021; 16(3): e0248772. doi: 10.1371/journal.pone.0248772.
26. El-Hawary SS, Moawad AS, Bahr HS, Abdelmohsen UR, Mohammed R. Natural product diversity from the endophytic fungi of the genus Aspergillus. RSC Adv. 2020; 10(37): 22058-79.
27. Badr G, Zahran AM, Omar HM, Barsoum MA, Mahmoud MH. Camel whey protein disrupts the cross-talk between PI3K and Bcl-2 signals and mediates apoptosis in primary acute myeloid leukemia cells. Nutr Cancer. 2019; 71(6): 1040-54.
28. Turkoz Uluer E, Kilicaslan Sonmez P, Akogullari D, Onal M, Tanriover G, Inan S. Do Wortmannin and Thalidomide induce apoptosis by autophagy inhibition in 4T1 breast cancer cells in vitro and in vivo? Am J Transl Res. 2021; 13(6): 6236-47.
29. Wei W, Cherukupalli S, Jing L, Liu X, Zhan P. Fsp3: A new parameter for drug-likeness. Drug Discov Today. 2020; 25(10): 1839-45.
30. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; 7: 42717. doi: 10.1038/srep42717.
31. Fadel FZ, Tchouar N, Belaidi S, Soualmia F, Oukil O, Ouadah K. Computational screening and QSAR study on a series theophylline derivatives as aldh1a1 inhibitors. J Fundam Appl Sci. 2021; 13(2): 942–64.
32. Maurya VK, Kumar S, Prasad AK, Bhatt MLB, Saxena SK. Structure-based drug designing for potential antiviral activity of selected natural products from Ayurveda against SARS-CoV-2 spike glycoprotein and its cellular receptor. Virusdisease. 2020; 31(2): 179-93.
33. Makiyah SNN, Kita M, Setyawati I, Tasminatun S. Dioscorea alata L. tubers improve diabetes through anti-hyperglycemia, anti-inflammation, ameliorate insulin resistance and mitochondrial dysfunction. Indones Biomed J. 2022; 14(4): 365-75.
34. Zerroug A, Belaidi S, BenBrahim I, Sinha L, Chtita S. Virtual screening in drug-likeness and structure/activity relationship of pyridazine derivatives as anti-alzheimer drugs. J King Saud Univ Sci. 2019; 31(4): 595–601.
35. Domínguez-Villa FX, Durán-Iturbide NA, Ávila-Zárraga JG. Synthesis, molecular docking, and in silico ADME/Tox profiling studies of new 1-aryl-5-(3-azidopropyl)indol-4-ones: Potential inhibitors of SARS CoV-2 main protease. Bioorg Chem. 2021; 106: 104497. doi: 10.1016/j.bioorg.2020.104497.
36. Pollastri MP. Overview on the rule of five. Curr Protoc Pharmacol. 2010; Chapter 9: Unit 9.12. doi: 10.1002/0471141755.ph0912s49.