Indonesian Journal of Cancer Chemoprevention, February 2023
ISSN: 2088–0197
e-ISSN: 2355-8989

# Bioinformatic and Molecular Docking Study of Zerumbone and Its Derivates against Colorectal Cancer

**Riri Fauziyya[1*], Winni Nur Auli[1], Nisa Yulianti Suprahman[1], Sarmoko[1], Arif Ashari[2], Kalista Alsadila[3], Lanita Agustin[3], Safia Fazila[3], Miralda Zahra[3] Esteria Christina Pane[3], Sukrasno[4]**

[1]Department of Pharmacy, Faculty of Science, Sumatera Institute of Technology, South Lampung 35365, Indonesia
[2]Department of Chemistry, Faculty of Science, Sumatera Institute of Technology, South Lampung 35365, Indonesia
[3]Undergraduate Program in Department of Pharmacy, Faculty of Science, Sumatera Institute of Technology, South Lampung 35365, Indonesia
[4]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia

## Abstract

The prevalence of colorectal cancer (CRC) is ranked third among all cancer types in both men and women, highlighting the urgency for drug exploration. Zerumbone and its derivatives have gained attention for their ability to inhibit angiogenesis, invasion, and metastasis and have been tested for their efficacy against various cancer cells. This study aimed to investigate the potential targets and mechanism of action of zerumbone derivatives in colon cancer invasion and migration. Bioinformatic analysis was conducted using STITCH and STRING to identify potential target genes, and molecular docking was used to search for anticancer candidates from 20 zerumbone derivatives. The results revealed that six proteins were targeted by zerumbone derivatives, including XIAPBIR3 (1TFT), AKT1 (3O96), JAK2 (6VGL), HASP90AA (2XJX), MDM2 (4MDN), and XIAPBIR2 (4KJU). Compound 4 was found to have a lower binding energy than zerumbone as well as AZD5363 (pan-Akt inhibitor) when interacting with the protein target AKT1. This makes it the most promising candidate among the zerumbone derivatives for treating colorectal cancer. Further development, such as the addition of an amine functional group, is expected to improve the potency of this molecule through the formation of hydrogen bonds and other interactions with lower bond energy.

**Keywords:** *bioinformatic, molecular docking, zerumbone derivatives, colorectal cancer.*

## INTRODUCTION

Cancer is a leading cause of death worldwide, with colorectal cancer (CRC) being the third most prevalent among both men and women (Siegel, *et al*., 2022). The Global Burden of Disease Study in 2019 placed the mortality and disease burden of CRC in second rank. In Indonesia, CRC ranks sixth, with 17,368 reported cases (The Global Cancer Observatory, 2020).

Submitted: February 12, 2023
Revised: March 10, 2023
Accepted: March 14, 2023
Published online: April 10, 2023

*Corresponding author: rr.fauziyya@gmail.com

One of the biggest challenges in cancer therapy is resistance to chemotherapeutic agents, leading to the need for drugs that can protein target involved in carcinogenesis (Housman, *et al.*, 2014; Antolin, *et al*., 2016).

Phytochemical compounds have shown promise in treating various human diseases, including lempuyang wangi (*Zingiber zerumbet*), a traditional ingredient with anti-cancer properties. Zerumbone, an active compound in lempuyang wangi, has been shown to suppress the proliferation and survival of cancer cells by altering molecular pathways such as NF-κB, Akt, and IL-6/JAK2/STAT3 and their downstream targets (Girisa, *et al*., 2019). Zerumbone has been derivatized and has been tested for its activity against several types of cancer cells (Chinh, 2019; Takashi, *et al*., 2013 and Songsiang, *et al*., 2010). However, identification of action potential targets of zerumbone derivatives against colorectal cancer cells and their activity has never been tested.

Bioinformatics, incorporating disciplines such as mathematics, statistics, biochemistry, computational genetics, and molecular biology, can predict target genes and their mechanisms of action (Edi, 2017). Optimization of identification and prediction of binding mode and affinity can be done *in silico* with docking (El-Hachem, *et al*., 2017). This study aims to investigate the potential targets of zerumbone derivatives for inhibiting colon cancer cells and understand the mechanism of action.

## METHODS

### Bioinformatic Study

The identification of the potential targets of zerumbone was conducted through bioinformatics analysis. The NCBI database (https://www.ncbi.nlm.nih.gov/gene) was utilized to identify target genes, while direct target proteins of zerumbone were identified using the STITCH database (http://stitch.embl.de) and indirect target proteins were identified by STRING-DB v11.0 (https://string-db.org). A Venn diagram was created to combine direct and indirect target proteins of zerumbone using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/fat/). The intersecting gene in the Venn diagram represented the molecular target of zerumbone that regulates colorectal cancer cells. Protein-protein interactions were visualized using STRING-DB v11.0 (https://string-db.org) and were inputted into Cytoscape software. The top 20 hub genes were identified by utilizing the Cytohubba plugin in Cytoscape software, with degree score as the parameter (Hermawan, *et al*., 2021).

### Molecular Docking

Thirty-three derivatives of Zerumbone were selected from previous studies (Chinh, 2019; Takashi, *et al*., 2013; and Songsiang, *et al*., 2010). Our analysis found that 21 of these derivatives were compliant with Lipinski's Rule of Five (RO5) (Auli, *et al.*, in press), which suggests their potential as drugs. The selected derivatives include 1A (or B), 1B (C), 2A (D), 2B (E), 3 (F), 4 (G), 5 (H), 6A (I), 6B (J), 6C (K), 6D (L), 7A (M), 7B (N), 8 (O), 9A (P), 9B (Q), 9C (R), 10 (S), 17 (T), 19 (U), and 20 (V) (Figure 1).

The crystal structure of the target bound to each native ligand was obtained from the Protein Data Bank (PDB) database (https://rcsb.org). Crystal structure was validated (re-docked) using native ligand to get the position of binding site residues seen from grid box parameter. The 3-dimensional structure of zerumbone and its derivatives was generated using Avogadro software and further refined using Gaussian 16, Revision D.01. The optimized zerumbone derivatives were then used as inputs for molecular docking using Autodock 1.5.6, which utilized the Lamarckian genetic algorithm. The docking was performed in a rigid and flexible protein environment, with 100 independent runs conducted per ligand. The Gibbs energy of the substrate-enzyme complex was calculated and compared to that of the native

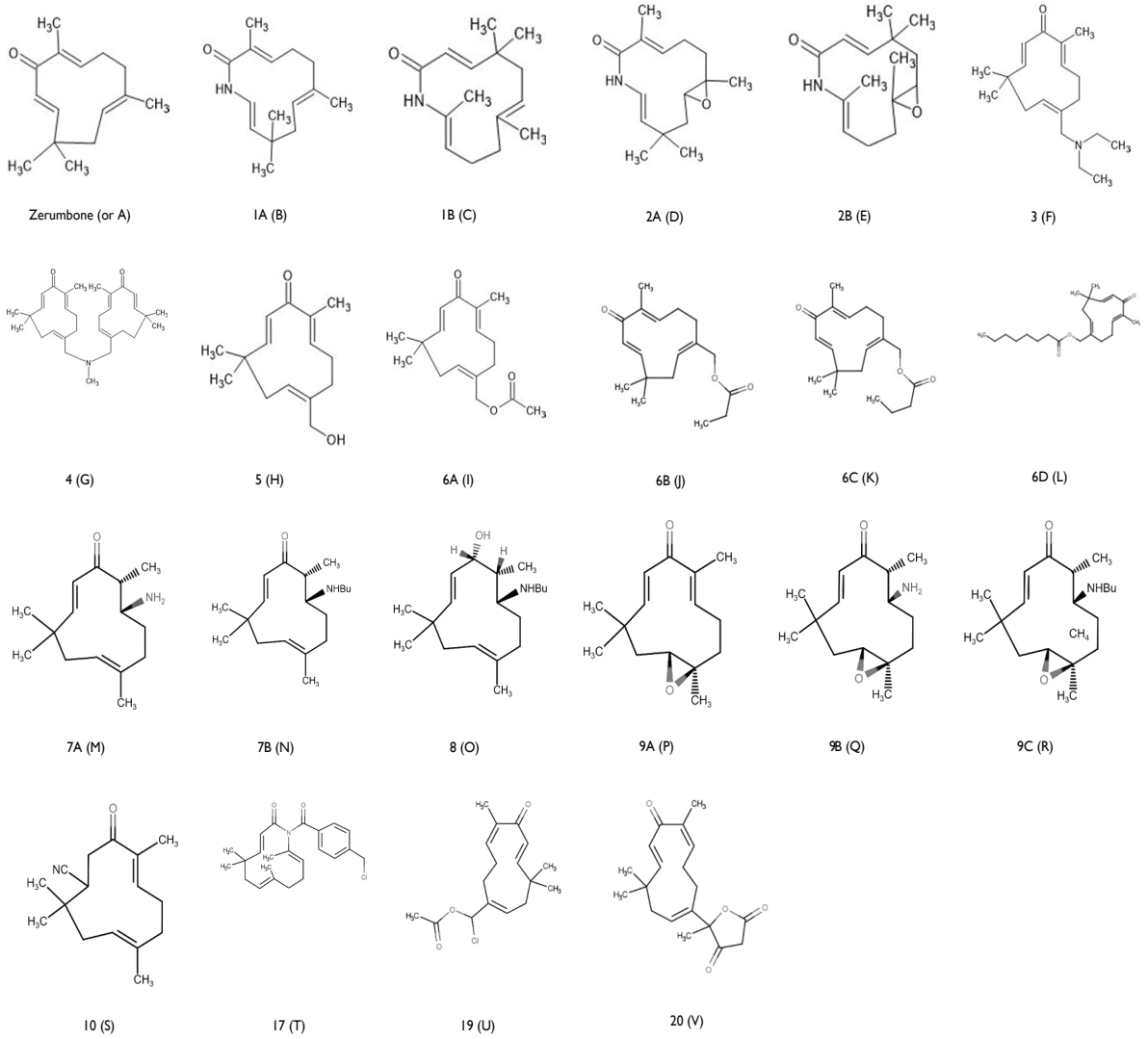


**Figure 1. Structure of zerumbone and derivates.**

ligand. The binding modes of the compounds and target proteins were analyzed using the Discovery Studio Visualizer software.

## RESULTS

### Bioinformatic Study

A bioinformatics study identified twenty potential targets of zerumbone against colorectal cancer regulatory genes (Figure 2). However, crystal structures of each target bound to a native ligand or a small molecule inhibitor were only available for six proteins: XIAPBIR2 (PDB ID 4KJU), XIAPBIR3 (PDB ID 1TFT), AKT1 (PDB ID 3O96), JAK2 (PDB ID 6VGL), HASP90AA (2XJX), and MDM (4MDN). As a result, these six proteins were selected to continue with the molecular docking process.

### Molecular Docking

A molecular docking study was conducted to evaluate the binding affinity of zerumbone and its derivatives to six potential targets in colorectal cancer. The results, including the binding energy of

the native ligand, zerumbone, and its derivatives, can be found in Figure 3.

The molecular docking results showed that some of the zerumbone derivatives had a lower binding energy than zerumbone for all of the target proteins. Out of the target proteins tested, AKT1 was found to be the most druggable target, as some of the agents (such as capivarsetib) targeting it have undergone clinical trials. In this study, therefore, we focused on the potential of zerumbone derivatives to target AKT1.

Compound 4 (G) showed the lowest binding energy of -10.27 kcal/mol to AKT1 compared to zerumbone and all derivatives making it the most potent derivative. Figure 4 shows the interactions between AKT1 and the native ligand IQO. The interaction created two conventional hydrogen bonds and one H-bond with TYR A:326 and THR A:211. Additionally, there were interactions such as C-H bond, Pi-cation, Pi-anion, Pi-sigma, Pi-Pi stacked, alkyl, and Pi-Alkyl. However, the HBD of the inhibitor had an unfavorable bond with

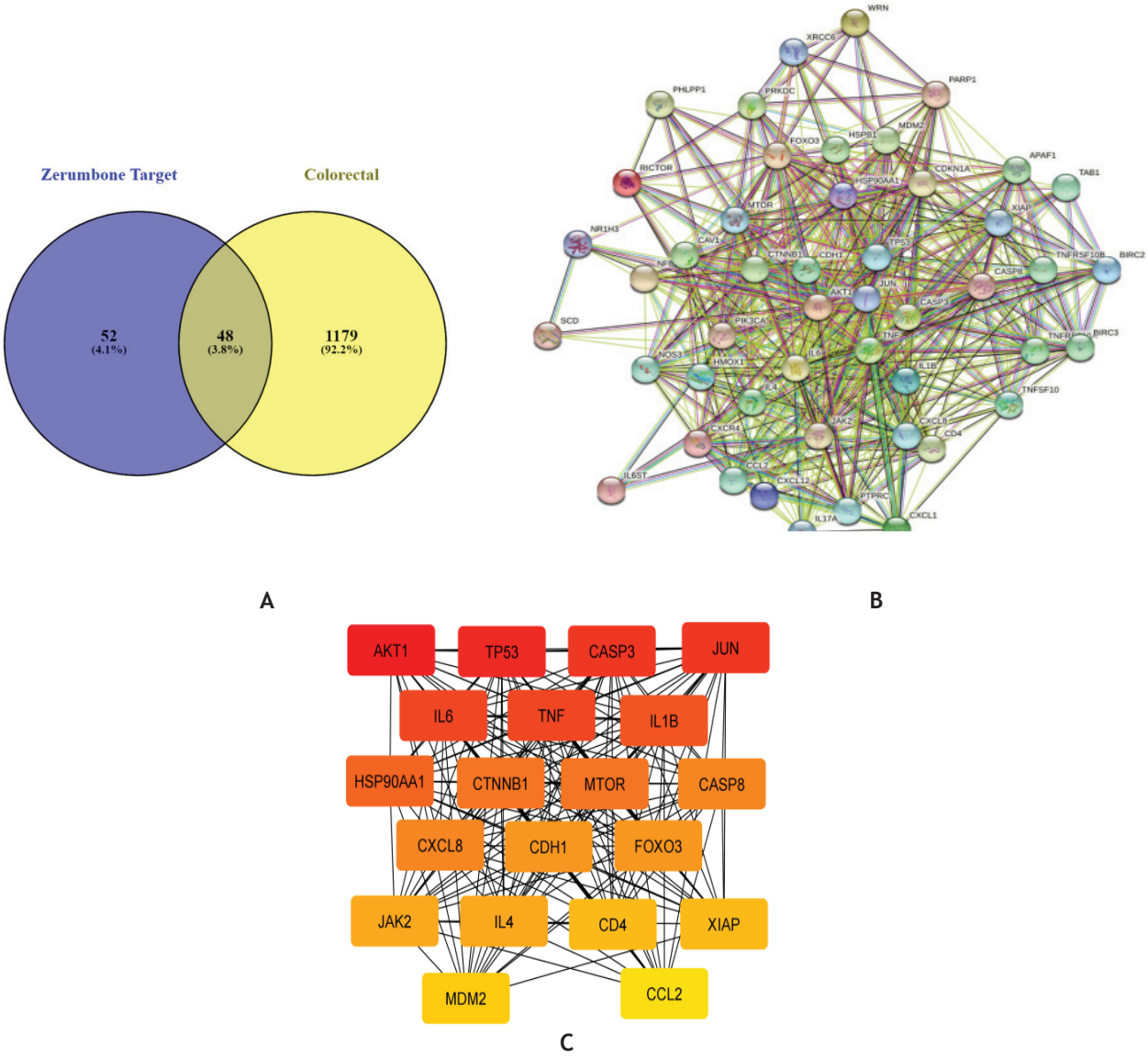


**Figure 2. (A) Venn diagram of Zerumbone potential targets and colorectal cancer regulatory genes; (B) A network of protein-protein interaction between Zerumbone targets; (C) Top 20 genes based on biological interaction degree score, analyzed by Cytohubba.**

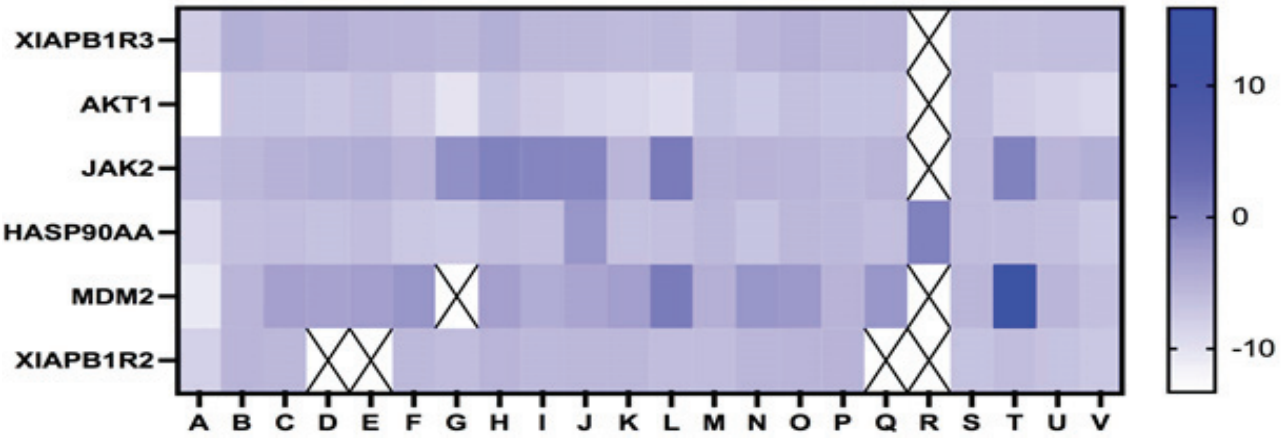


**Figure 3. Heatmap representation of binding energy interaction of compounds to protein target.** (A) native ligand; (B) zerumbone; (C-V) all zerumbone derivatives (compound 1A to 20).

ARG A:273, GLN A:79, and ASN A:54, resulting in a higher calculated bond value. In contrast, the interaction between AKT1 and zerumbone was facilitated only through alkyl and pi-alkyl interactions and no hydrogen bonds were formed. Compound 4 showed lower binding energy, which was facilitated by C-H bond, Pi-sigma, alkyl, and pi-alkyl interactions.

## DISCUSSION

Zerumbone has the potential to inhibit proteins involved in the progression of colon cancer. In our study, we conducted a bioinformatics analysis to examine zerumbone and its derivatives and discovered potential targets such as XIAPBIR3, AKT1, JAK2, HSP90AA, MDM2, and XIAPBIR2. The X-chromosome-linked inhibitor of apoptosis protein (XIAP) blocks the activity of pro-apoptotic proteins caspase 3, 7, and 9, thus promoting cancer cell survival. The inhibitor of apoptosis protein contains three BIR domains (BIR1, BIR2, and BIR3), with BIR2 binding to caspases 3 and 7 and BIR3 binding to caspase 9 (Mudde, Booth and Marsh, 2021).

Janus Kinase (JAK) is a non-receptor tyrosine kinase activated by growth factor or cytokine binding to its receptor. Upon activation, JAK phosphorylates and activates cytokine receptors, further triggering signal transduction pathways. *In vivo* experiments have demonstrated that inhibiting JAK can slow the progression of solid tumors (Qureshy, *et al.*, 2020), including colorectal cancer cells (Xiong, *et al*., 2008).

Heat shock protein 90 (HSP90) stimulates the function of proteins involved in several physiological processes, including cell cycle control and cell survival. Studies have shown that colorectal cancer patients have a statistically higher plasma level of HSP90α compared to healthy subjects and that this level is correlated with disease progression (Wei, *et al.*, 2021; Xu, *et al.*, 2014; and Drecoll, *et al.*, 2014). The ubiquitin-protein ligase MDM2 facilitates the binding of ubiquitin to proteins, initiating their degradation. Its targets include the p53 tumor suppressor protein, which is the most frequently altered protein in cancer (Kung and Weber, 2022).

One of the targeted proteins, AKT1, is a kinase activated by growth factor stimulation (Hemmings and Restuccia, 2012). Upon activation, it triggers a multi-step process, including phosphoinositide-3-kinase (PI3K), and phosphorylates downstream proteins to regulate

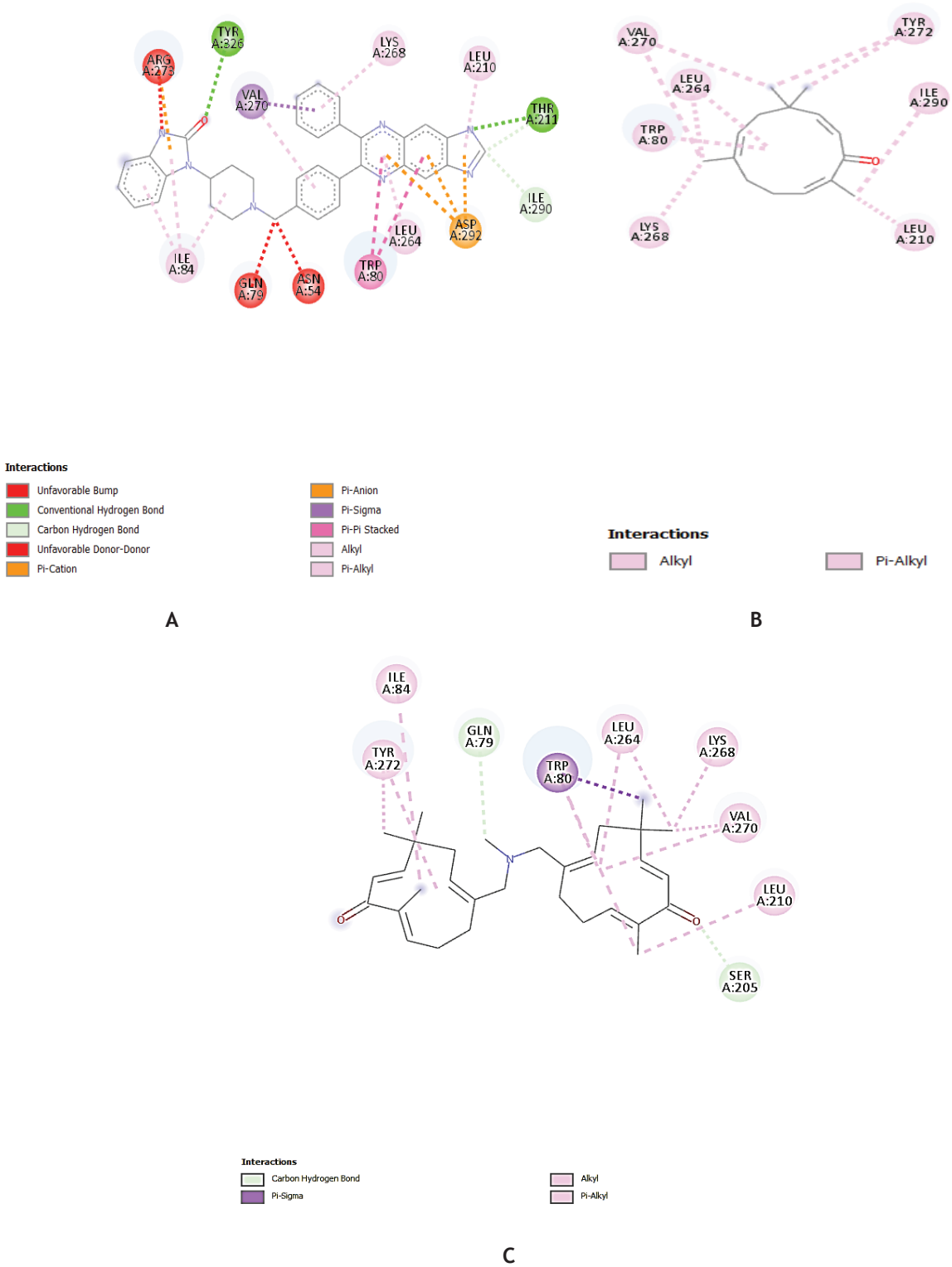


**Figure 4. Visualization of interaction compounds to AKT1.** A. native ligand IQ0; B. Zerumbone; C. Compound 4.

processes such as reduced apoptosis and increased cell proliferation. AKT1 plays a crucial role in various aspects of cancer, including tumor growth, metastasis, angiogenesis, and drug resistance (Hua, *et al.*, 2021).

The protein targets found in this study is in concordance with the published literature in which zerumbone has been shown to alter AKT and IL-6/JAK2/STAT3 signaling pathway (Girisa, *et al*., 2019). This is also supported by previous studies that shown zerumbon can inhibit proliferation and induce apoptosis in cancer cell (Rahman, *et al*., 2014; and Girisa, *et al*., 2019). The inhibition of proliferation is potentially mediated by zerumbone interaction with HSP90 and AKT1. The apoptosis signals altered by zerumbone may be caused by its affinity on XIAP-BIR2 and AKT1.

In this study, computational docking was used to evaluate the binding affinity of small molecules to proteins involved in cancer pathogenesis using Auto Dock. This process generated estimates of the binding free energy between the ligand and each target by treating the ligand and selected regions of the target as flexible conformations.

Among all the tested proteins, Zerumbone and almost all its derivatives showed the lowest binding energy towards AKT1. Aside from that, compared to other targets, AKT1 is considered one of the most intensively investigated target in cancer research, with several anticancer candidates targeting AKT1 currently undergoing clinical trials, including capivasertib (AZD5363), ipatasertib (RG7440), and MK-2206 (Martorana, *et al.*, 2021). Among these candidates, MK-2206 has shown promising results in treating colorectal cancer patients (NCI, 2019).

Compound 4 showed the highest potential to inhibit AKT1, but further structural modifications are necessary to develop a more potent AKT1 inhibitor. For example, incorporating an amine functional group at cyclic structure of compound 4 could potentially improve binding affinity by forming hydrogen bonds and other interactions with AKT1. However, experimental analysis such as *in vitro* analysis to targeting cells will help to decide the drug modification strategy better.

## CONCLUSION

A bioinformatic study of zerumbone's potential mechanism in suppressing colorectal cancer development identified six target proteins, including XIAPBIR3, AKT1, JAK2, HASP90AA, MDM2, and XIAPBIR2. Modifying zerumbone's structure improved its interaction and reduced binding energy with all target proteins. Among the candidates, compound 4 showed the strongest

Table 2. Interaction type between native ligand, zerumbone, and compound 4 to AKT1.

| Target (PDB ID) | Binding Characteristic | | | |
|---|---|---|---|---|
| | Native ligand | | Zerumbon | Compound 4 |
| **AKT1 (3O96)** | IQO | 1 H-bond (HBA*) with TYR A:326, 1 H-bond (HBD*) with THR A:211<br>C-H bond<br>Pi-cation<br>Pi-anion<br>Pi sigma<br>Pi-Pi stacked<br>Alkyl<br>Pi-Alkyl<br>Unfavorable donor-donor | alkyl<br>pi-alkyl | C-H bond<br>pi-sigma<br>alkyl<br>pi-alkyl |

interaction with AKT1, which is considered the most potential target protein. To further enhance the potency of the molecule, the addition of an amine functional group is expected to form hydrogen bonds and other interactions, resulting in a considerably lower bond energy and a more promising candidate.

## ACKNOWLEDGMENT

This study was supported by Institut Teknologi Sumatera Research Grant 2022 No. B/763af/IT9.C1/PT.01.03/2022.

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