In Silico Evaluation of the Potential Anti-Apoptotic Effects of Diarylheptanoids from *Curcuma comosa* Roxb. Rhizomes by Targeting the Caspase-3 Network in Neurodegenerative Disorders

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Abstract

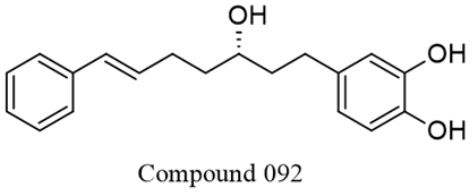
Apoptosis is a vital biological process known as programmed cell death. It is characterized by a series of well-orchestrated events that lead to the elimination of cells in a controlled manner. Compound-092, (3S)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6E)-6-hepten-3-ol, was examined for its cytoprotective effects on C6 astroglial cells in the context of oxidative stress induced by hydrogen peroxide (H_2O_2) exposure. The mechanism by which compound-092 protects against H_2O_2-induced apoptotic signaling involves the prevention of increases in phosphorylated p53 levels, the Bax/Bcl-2 ratio, and cleaved caspase-3 expression. In this study, we focus on the caspase-3 network, which was obtained from the STRING database. The proteins in the network, caspase-3, PARP1, XIAP, and BIRC2, were selected for docking with compound-092 using the PyRx 0.8 program. The docking results showed that compound-092 can bind with the four proteins with binding energies of -7.10, -6.17, -7.30, and -6.11 kcal/mol, respectively. The overall activity of compound-092 would then depend on the balance between its inhibitory effects on caspase-3 and any potential interactions with other apoptotic regulators like XIAP and PARP1. This balance will dictate whether the compound ultimately promotes cell survival or inadvertently allows for unchecked cellular proliferation in pathological conditions. Compound-092 exhibited a significant cytoprotective effect on C6 astroglial cells by simultaneously promoting the anti-apoptotic activity of all four proteins. Additionally, compound-092 contributed to the inhibition of elevated phosphorylated p53 levels and the Bax/Bcl-2 ratio.

Keywords: *Curcuma comosa, Compound-092, Diaryheptanoids, Anti-Apoptosis, Caspase-3 network, Molecular docking,*

1. Introduction

The slow, cumulative loss of neurons in the central nervous system (CNS) is a common feature of a group of disorders known as neurodegenerative diseases (NDs). There is presently no cure for these NDs, which include amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), and Alzheimer's disease (AD) (Singh et al., 2021). Programmed cell death, also known as apoptosis (Goyal et al., 2024), is a process of cell death that is frequently seen in a variety of biological processes, such as immune responses, synaptogenesis, and embryogenesis. Numerous morphological changes are associated with apoptosis, including chromatin condensation, nuclear material compartmentalization into vesicular apoptotic bodies, oligonucleosomal DNA fragmentation, and nuclear and cytoplasmic compartment shrinkage (Porter, & Jänicke, 1999). The recruitment of cell-surface death receptors and apoptotic stimuli, or direct disruption of the mitochondria and subsequent activation of the proteolytic cascade, including executioner caspases, can both cause target cell apoptosis within a tissue through cell signaling activation. Apoptosis is essential for the elimination of redundant or damaged cells, thereby contributing to the maintenance of homeostasis. Excessive apoptosis can be detrimental, as evidenced by neuronal cell death in neurodegenerative diseases. Apoptosis has been observed in both acute and chronic neurological disorders (Gorman, 2008). Numerous drug groups, including cholinesterase inhibitors (ChEIs) like donepezil, tacrine, and rivastigmine, demonstrate neuroprotective activity in neuronal cultures and animal models, especially in the context of Alzheimer's disease (Moreira et al., 2022; Arias et al., 2005).

It has been reported that diarylheptanoids extracted from the rhizome of *Curcuma comosa* Roxb. have many biological activities (Boonmee et al., 2011; Winuthayanon et al., 2009; Pabuprapap et al., 2022). Especially biological effects on the growth of P388 leukemic cells in mice. Compound.092, (3S)-1-(3,4-dihydroxyphenyl)-7-phenyl-(6E)-6-hepten-3-ol, which has a catechol group, was the most effective diarylheptanoid at stopping the growth of P388 leukemic cells, with an IC_{50} of 4 μM through mechanisms involving DNA breakage and apoptosis induction. Apoptotic cell death is characterized by chromatin condensation, apoptotic body formation, DNA fragmentation, and the externalization of plasma membrane phosphatidylserine. This compound elevated caspase-3 activity approximately fivefold compared to the untreated control, reduced intracellular levels of glutathione, and disrupted mitochondrial transmembrane potential (Viriyaadhammaa et al., 2024). In contrast, Vattanarongkup and colleagues reported that compound-092, derived from the same herb, was examined for its cytoprotective effects on C6 astroglial cells in the context of oxidative stress induced by hydrogen peroxide (H_2O_2) exposure. The mechanism by which compound-092 protects against H_2O_2-induced apoptotic signaling involves the prevention of increases in phosphorylated p53 levels, the Bax/Bcl-2 ratio, and cleaved caspase-3 (Vattanarongkup et al., 2016). Khin Aung and colleagues also discovered that compound-092 demonstrated a neuroprotective effect by suppressing apoptotic cell death through a reduction in phospho-p53 and cleaved caspase-3 expression. The findings indicate that the diarylheptanoid compound-092 derived from *C. comosa* exhibits anti-apoptotic properties and may be further explored as a potential therapeutic agent for oxidative stress-related neuronal disorders (Aung et al., 2022).



Compound 092

Figure 1 Structures of diarylheptanoid from *Curcuma comosa* Roxb. (compound-092)

The apoptosis cell death process involves the caspase-3 protein (Dou et al., 2023), as indicated by the information provided above. Given our focus on networks that control apoptosis, we searched the STRING database (Szklarczyk et al., 2023) using the keywords “caspase-3” and “Homo sapiens” to retrieve a relevant network. Subsequently, we clustered the network using k-means clustering, resulting in a small network of caspase-3 (Figure 2).

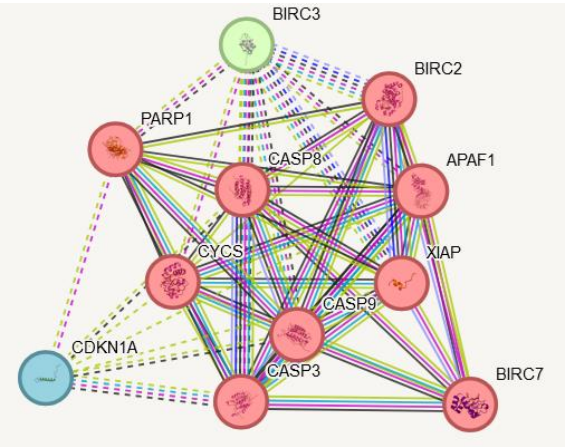


Figure 2 Caspase-3 network in red color connection

By applying k-means clustering, this complexity can be simplified into smaller, more manageable sub-networks. This allows for focused analysis of specific interactions or functional modules within the larger network. K-means clustering helps identify groups of proteins that may function together or share similar biological roles. Smaller networks derived from k-means clustering are easier to interpret than a large, dense network. Caspases are a family of cysteinyl aspartate-specific proteases that found in all multicellular organisms. They play a key role in controlling apoptosis (Chowdhury et al., 2008). Caspase-3 is the inactive precursor form of the enzyme, also known as procaspase-3. It exists as a zymogen, meaning it requires modification to become active. In this state, it is a roughly 32 kDa protein. It is present in the cytoplasm of cells, ready to be activated when an apoptotic signal is received. Cleaved caspase-3 is the active form of the enzyme. When a cell receives signals to undergo apoptosis, caspase-3 is cleaved (cut) by other caspases (such as caspase-8 or caspase-9, depending on the apoptotic pathway). This cleavage occurs at specific aspartate residues. The cleavage removes the pro-domain and results in two subunits, a large subunit (approximately 17-20 kDa) and a small subunit (approximately 10-12 kDa), which then associate to form the active enzyme. Active, cleaved caspase-3 is the executioner (Liu et al., 2017).

2. Objective

The objective of this study was to evaluate the binding affinity of compound-092 from *C. comosa* against four proteins (Caspase-3, PARP1, XIAP, and BIRC2) in the caspase-3 network to gain deeper insight into its anti-apoptotic properties.

3. Materials and Methods

3.1 Target proteins for docking

We obtained Caspase-3 (PDB ID: 1NMS), PARP1 (7KK3), XIAP (5M6E), and BIRC2 (7TRM) from the Protein Data Bank (www.rcsb.org). We screened the protein using PROCHECK (https://www.ebi.ac.uk/ thornton-srv/software/PROCHECK/) to assess the stereochemical quality of the protein structure. Protein preparation involved the addition of polar hydrogen atoms or missing residues, removal of water molecules, and generation of states at pH 7.0 ± 0.5 for the het atom. Finally, we redocked the x-ray ligand into its respective protein and determined the RMSD between the docking position and the x-ray ligand. The binding site position for docking to 1NMS is X = -9.919, Y = -4.666, Z = 22.012, 7KK3 is X = -38.825, Y = 7.235, Z = -6.811, 5M6E is X = -14.023, Y = -18.450, Z = -6.117, and for 7TRM it is X = -2.071, Y = -46.254, Z = -9.529. The box dimension is 25 × 25 × 25 Å.

3.2 Compounds for docking study and docking process

We obtained the compound-092 structure from PubChem by first drawing a molecular structure and then conducting a database search. After identifying the correct structure, we found and downloaded the 3D structure. Next, the structure was subjected to energy minimization using the MMFF94 method in the Avogadro program. Molecular docking studies were performed using AutoDock Vina implemented in PyRx 0.8 to evaluate and validate the binding interactions between ligands and the target protein. In PyRx, the ligands and protein structures were initially converted to PDBQT format, with all water molecules removed and polar hydrogens added. The grid box was centered on the active site residues with dimensions of X × Y × Z Å, encompassing the entire binding pocket of each protein mentioned in Section 3.1. AutoDock Vina parameters were set to their default values, with exhaustiveness of 8, and the top 9 poses were generated for each ligand.

4. Results and Discussion

4.1 Redock accuracy evaluation

The x-ray ligand of each protein was redocked into its parent protein with the setup conditions, and the results are shown in Table 1.

From the redocking results in Table 1, the RMSD of four proteins are below 2 Å, ranging from 0.098 to 0.356 Å. Redocking refers to the process of re-evaluating a ligand's binding pose to a target protein using molecular docking software, typically after the initial docking has been performed. The accuracy of redocking is crucial for validating docking predictions and improving drug discovery processes (Agarwal, & Smith, 2023). An RMSD value below 2 Å signifies a good docking procedure.

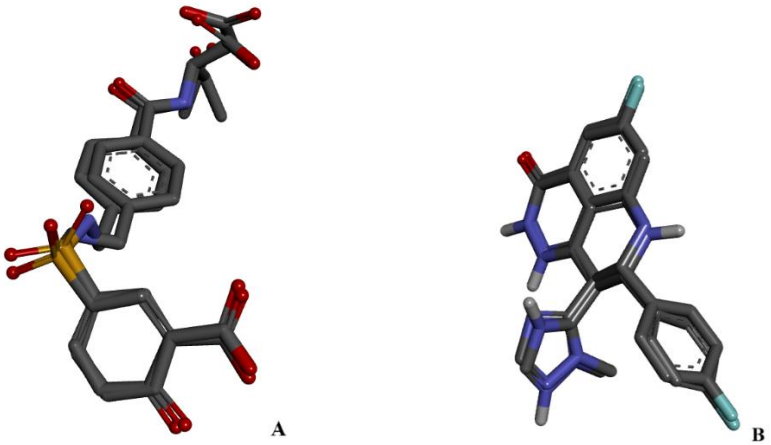
Table 1 The RMSD of redock results

X-ray ligand of proteins	RMSD (Å)
1NMS	0.356
7KK3	0.179
5M6E	0.238
7TRM	0.098

4.2 Docking with Caspase-3 (1NMS), PARP1 (7KK3), XIAP (5M6E), and BIRC2 (7TRM)

4.2.1 Docking with Caspase-3 (1NMS)

Caspase-3 is a critical enzyme in the family of cysteine-aspartic acid proteases (caspases), playing a pivotal role in the process of apoptosis, or programmed cell death. It is encoded by the *CASP3* gene and is primarily known for its function as an executioner caspase, making it one of the final steps in the apoptotic cascade that leads to cell death (Porter, & Jänicke, 1999). Compound-092 exhibited a binding energy of -7.1 kcal/mol with 1NMS, compared to the inhibitor ligand (5-[4-(1-Carboxymethyl-2-oxo-propylcarbamoyl)-benzylsulfamoyl]-2-hydroxy-benzoic acid), which was -7.55 kcal/mol (Figure 4). Several amino acids interact with both compound-092 and the x-ray inhibitor in a similar manner. These are CYS163, ARG207, TYR204, TRP214, TRP206, and PHE250. This suggests that compound-092 could serve as a potential caspase-3 inhibitor.



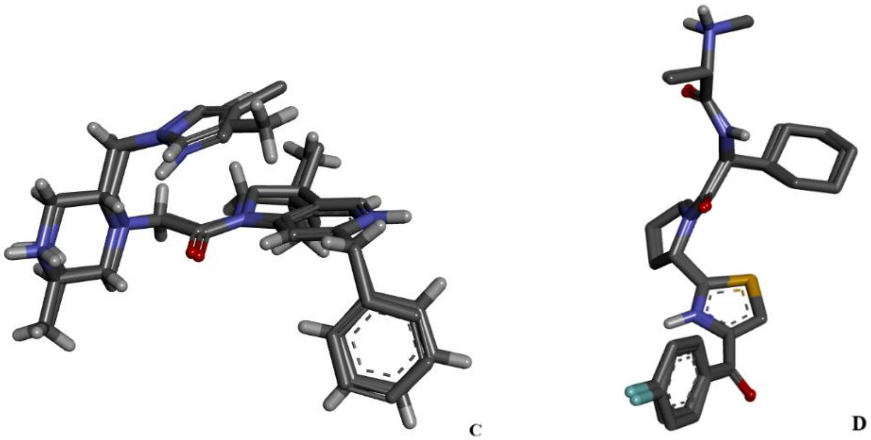


Figure 3 Redock X-ray ligand structure of (**A**) Caspase-3 (1NMS), (**B**) PARP1 (7KK3), (**C**) XIAP (5M6E) and (**D**) BRIC2 (7TRM)

4.2.2 Docking with PARP1 (7KK3)

Poly (ADP-ribose) polymerase 1 (PARP1), also known as NAD^+ ADP-ribosyltransferase 1, is a critical enzyme encoded by the *PARP1* gene in humans. It is the most abundant member of the PARP family, responsible for approximately 90% of the NAD^+ consumed by this enzyme family. PARP1 is predominantly located in the cell nucleus, although it can also be found in the cytosol (Ko, & Ren, 2012). It is primarily involved in detecting and repairing DNA damage, particularly single-strand breaks (SSBs). Upon sensing DNA damage, PARP1 binds to the site of injury and synthesizes poly(ADP-ribose) (PAR), which serves as a signal to recruit other DNA repair proteins (Bastos et al., 2024; El Hassab et al., 2025). An important step in apoptosis is the interaction between caspase-3 and PARP1. During apoptosis, activated caspase-3 cleaves PARP1; PARP1 is one of the most well-known substrates of caspase-3. This cleavage prevents PARP1 from carrying out its DNA repair functions (Li et al., 2022). For PARP1 inhibitors, there are 3 different major types, and their impact on the caspase-3/PARP1 interaction can vary. First, most PARP1 inhibitors are catalytic inhibitors. These molecules attach to the PARP1 catalytic domain preventing it from making PAR chains. These inhibitors do not directly prevent the physical interaction (binding) between caspase-3 and PARP1. Caspase-3 can still bind to and cleave PARP1 even if PARP1's catalytic activity is inhibited. The cleavage would still occur. Additionally, if an inhibitor prevents PARP1 from attaching to DNA, which is where it normally lives and works, it might have a smaller effect on the interaction with caspase-3. However, this is less direct than a caspase-3 inhibitor, and cleavage could still occur if the two proteins encounter each other. The third type is PARP inhibitors, also called "PARP trappers" (e.g., olaparib, talazoparib). These drugs stop PARP1 from catalyzing reactions and also trap PARP1 on DNA. This trapping can actually increase DNA damage (Zhou et al., 2020). The docking result showed that compound-092 was able to bind to PARP1(7KK3) with a binding energy of -6.17 kcal/mol, which was close to the inhibitor, talazoparib (-6.99 kcal/mol). Figure 5 depicts the shape of compound-092. The amino acids TYR889, TYR907, GLY863, TYR896, and HIS862 that interact with 7KK3 are similar to the x-ray inhibitor. The likelihood of compound-092 inhibiting PARP1 function is high. This aligns with the findings of Viriyaadhammaa and co-workers, who reported that compound-092 increases apoptosis. This could be due to the compound having a reactive group capable of binding to various protein active sites. In contrast, compound-092 could affect the anti-apoptotic side by partially blocking caspase-3, preventing it from cleaving PARP1 and thus increasing cell survival, or it may involve other pathways, such as p53 and Bax/Bcl2.

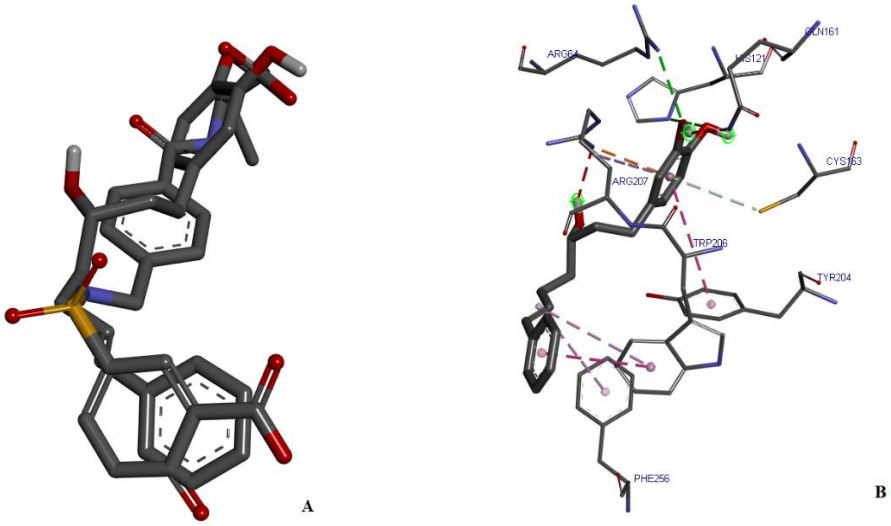


Figure 4 (**A**) Compound-092 superimposed with 1NMS x-ray ligand, (**B**) Compound-092 interacted with amino acids in the binding size of 1NMS

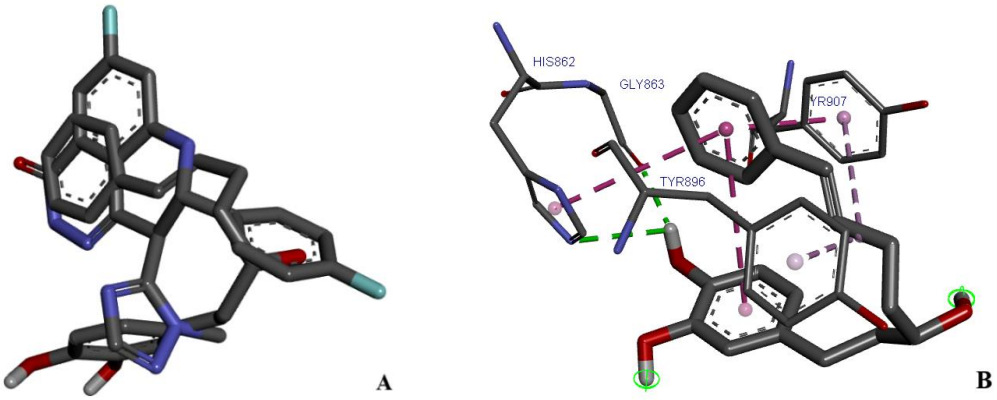


Figure 5 (**A**) Compound-092 superimposed with 7KK3 x-ray ligand, (**B**) Compound-092 interacted with amino acids in the binding site of 7KK3

4.2.3 Docking with XIAP (5M6E)

XIAP (X-linked Inhibitor of Apoptosis Protein) is a member of the inhibitor of apoptosis (IAP) family, encoded by the *XIAP* gene located on the X chromosome. It consists of three baculoviral IAP repeat (BIR) domains, a ubiquitin-associated (UBA) domain, and a RING finger domain, which contribute to its ability to inhibit caspases and regulate cellular processes (Chaudhary et al., 2016). XIAP directly binds to and inhibits caspases 3, 7, and 9, preventing their activation and subsequent induction of apoptosis. The BIR domains are critical for this inhibitory function (Shiozaki et al., 2003). The inhibitor ligand of 5M6E had a binding energy of -8.5 kcal/mol, while compound-092 had a binding energy of -7.3 kcal/mol. This suggests that the compound-092 interaction with XIAP is less stable and potentially less effective at blocking XIAP's function. However, despite its weaker binding affinity, compound-092 may still partially inhibit XIAP. Even with weaker binding, the compound might still partially inhibit XIAP. This means it reduces XIAP's activity but does not completely block it. This partial inhibition could be sufficient to have a biological effect, especially if combined with the compound's effects on caspase-3 and PARP1. It may work as a balancer for apoptosis in the caspase-3 network.

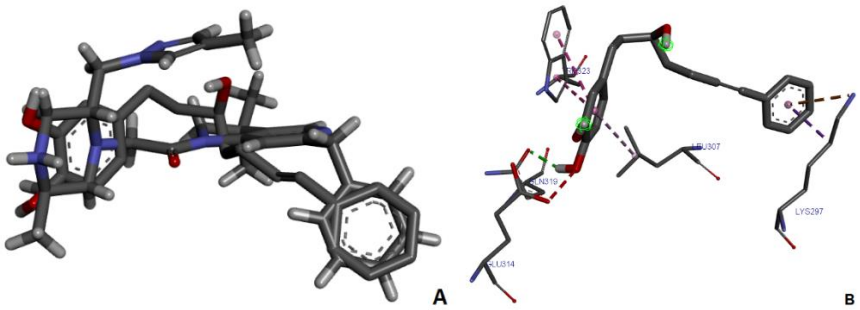


Figure 6 (**A**) Compound-092 superimposed with 5M6E x-ray ligand, (**B**) Compound-092 interacted with amino acids in the binding site of 5M6E in the 3D map

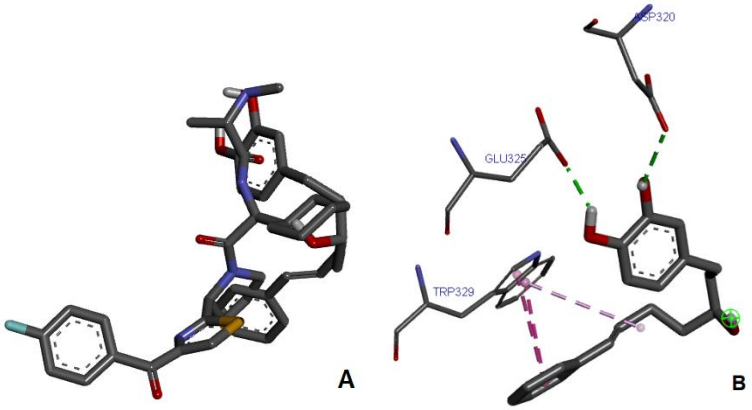


Figure 7 The picture (**A**) shows compound-092 on top of the 7TRM x-ray ligand. The picture (**B**) shows compound-092 interacting with amino acids in the binding site of 7TRM, which interacts with ASP320, GLU325, and TRP329

4.2.4 Docking with BIRC-2 (7TRM)

BIRC2 refers to Baculoviral IAP Repeat-Containing Protein 2, which is also known as cIAP1 (cellular Inhibitor of Apoptosis Protein 1). It is a member of the IAP (Inhibitor of Apoptosis Protein) family, which plays a critical role in regulating apoptosis (programmed cell death) and other cellular processes. The cIAP1 inhibits apoptosis by binding to and suppressing the activity of caspases, which are the key executioner proteins in the apoptotic pathway (Dubrez-Daloz et al., 2008). The binding energy of compound-092 to BIRC2 (7TRM) was -6.11 kcal/mol, while the binding energy of ICL-161 as the x-ray inhibitor was -8.6 kcal/mol. The docking result shows that some parts of compound-092 overlap with the ICL-161 inhibitor. Similar to XIAP, the compound might only partially inhibit BIRC2 due to its weaker binding. This means it reduces BIRC2's activity but does not completely block it. This partial inhibition could still have a biological effect, especially when combined with the compound-092's other activities. This implies that compound-092 may have some inhibition of cell apoptosis.

Table 2. Binding energy of compound-092 with 1NMS, 7KK3, 5M6E, and 7TRM proteins

Protein	Binding energy (kcal/mol) Of Compound-092	Binding energy (kcal/mol) Of X-ray structure
1NMS	-7.10	-7.55
7KK3	-6.17	-6.99
5M6E	-7.30	-8.50
7TRM	-6.11	-8.60

In summary, the docking results show that compound-092 acts as a caspase-3 inhibitor, which may prevent the activation of the key executioner caspase required for apoptotic. As a result, cells may be less likely to undergo apoptosis in response to various stimuli, potentially enhancing cell survival. Next, PARP1 is cleaved by active caspase-3 during apoptosis. Inhibiting caspase-3 would prevent PARP1 cleavage, allowing it to remain intact, which might support cell survival and repair mechanisms but could also interfere with the cellular response to DNA damage. For XIAP and BIRC2, compound-092 is partially bound with these two proteins. Since XIAP is a key inhibitor of caspases, including caspase-3, the inhibition of caspase-3 by compound-092 may alter the dynamics of XIAP's function. However, Tamanini et al. (2017) reported that XIAP and cIAP1 are constituents of the inhibitor of apoptosis protein (IAP) family and serve as crucial regulators of anti-apoptotic and pro-survival signaling pathways. Overexpression of IAPs is linked to tumor progression and treatment resistance in various malignancies. For BIRC2, it may influence either apoptosis or cell survival pathways based on its partial interaction with compound-092. According to the research of Viriyaadhammaa et al. (2024) and Vattanarongkup et al. (2016), the experiment uses different cells, and the environment in the lab is also different from the complex environment in the living organism. *In vitro*, cells are often grown in a simplified medium, lacking the complex interactions and signaling pathways present in a living organism. The overall activity of compound-092 would then depend on the balance between its inhibitory effects on caspase-3 and any potential interactions with other apoptotic regulators like XIAP and PARP1. This balance will dictate whether the compound ultimately promotes cell survival or inadvertently allows for unchecked cellular proliferation in pathological conditions. Ultimately, it is important to note that Vattanarongkup and his team have shown that compound-092 reduces the phosphorylation of p53 and the Bax/Bcl-2 ratio, both of which are implicated in cellular apoptosis. These proteins require additional exploration.

5. Conclusion

In conclusion, if compound-092 acts as a caspase-3 inhibitor, it fundamentally shifts the apoptotic landscape by promoting cell survival through the inhibition of a critical apoptotic pathway while also influencing interactions with other regulatory proteins involved in apoptosis. Further studies on compound-092 from *Curcuma comosa* would be necessary to elucidate these complex interactions and their implications for therapeutic strategies.

6. Acknowledgements

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