RESEARCH ARTICLE

Inositol Hexakisphosphate ($InsP_6$) Induces Apoptosis via Caspase-Dependent Pathways: Molecular Docking Insights

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Abstract

BACKGROUND: Inositol hexakisphosphate ($InsP_6$) exhibits anticancer activity, especially by inducing intrinsic and extrinsic apoptotic pathways. However, there is still no molecular docking evidence that directly examines $InsP_6$ interactions with either upstream or downstream apoptotic regulators. Therefore, the current study was conducted to investigate the molecular docking of $InsP_6$ to caspases as upstream/downstream apoptotic regulators.

METHODS: Ligands including $InsP_6$, $InsP_5$, $InsP_4$, histone deacetylase inhibitor, and caspase inhibitors were retrieved from PubChem, while target proteins (histone, caspase-8, caspase-2, and caspase-3) were obtained from the Protein Data Bank. Ligand toxicity was predicted using ProTox-3.0, and physicochemical properties were analyzed with SwissADME. Ligand structures were energy-minimized using PyRx with the Universal Force Field, while proteins were prepared by removing water molecules and non-essential heteroatoms in BIOVIA Discovery Studio. Molecular docking was conducted using CB-Dock 2.0, with binding poses selected based on the lowest Vina score, and ligand–protein interactions were visualized in Discovery Studio.

RESULTS: Molecular docking results showed that $InsP_6$ bound strongly to histone, caspase-8, caspase-2, and caspase-3 with affinities comparable to reference inhibitors, forming multiple hydrogen bonds with key active-site residues. $InsP_6$, $InsP_5$, and $InsP_4$ exhibited several similar binding sites to caspase-3, with only minor differences in binding affinity.

CONCLUSION: $InsP_6$ shows strong binding to histone, caspase-8, caspase-2, and caspase-3 based on *in silico* results, supporting its role in inducing both extrinsic and intrinsic apoptotic pathways. Taken together, $InsP_6$ could be a potential inducer of apoptosis in cancer cells.

KEYWORDS: cancer, apoptosis, $InsP_6$, $InsP_5$, $InsP_4$, caspase, *in silico*, molecular docking

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Introduction

Inositol hexakisphosphate ($InsP_6$) has been identified as a potent anticancer compound by targeting crucial biological pathways.(1,2) Recent studies have highlighted anticancer potential of $InsP_6$, which includes inhibition of cell proliferation, blockade of the cell cycle, and induction of apoptosis.(3) These effects are linked to its ability to control key signaling pathways and to activate apoptosis-related proteins. Therefore, $InsP_6$ is considered a promising natural agent with potential anticancer activity, particularly through its role in inducing apoptosis in cancer cells.(4) The potential of $InsP_6$ has been validated across multiple cancer cell types. In HeLa cells, $InsP_6$ inhibits cell growth and induces apoptosis through caspase activation and suppression of the Akt-nuclear factor-kappa B (NF-κB) survival pathway.(5) In HT-29 colorectal cancer cells, anticancer activity occurs through the suppression of phosphoinositide 3-kinases (PI3K) and Akt, key regulators of cell survival and proliferation.(6) Meanwhile, in leukemia cell lines A230, $InsP_6$ causes G2/M phase cell cycle arrest.(7) Complementing these findings, studies in prostate cancer mouse models demonstrated that $InsP_6$ reduces tumor growth, progression, and aggressiveness through several pro-apoptotic mechanisms.(8)

Beyond $InsP_6$ alone, synergistic and derivative effects have also been observed. Combination of histone with $InsP_6$ enhances pro-apoptotic activity in oral and nasopharyngeal carcinoma HONE-1 cells.(9) Combining $InsP_6$ with histone reduced the concentration required to induce apoptosis in HeLa cells by almost 10-fold compared to $InsP_6$ alone.(5) Moreover, structurally related compounds such as $InsP_5$ and $InsP_4$ have shown anticancer activity. $InsP_5$ suppresses angiogenesis and tumor progression by inhibiting hypoxia-inducible factor (HIF)-1α and vascular endothelial growth factor (VEGF).(10) In addition, $InsP_4$ exerts anticancer potential by interfering with molecular mechanisms that promote metastasis and tumor cell proliferation.(11)

The anticancer effects of $InsP_6$ are primarily mediated through the regulation of apoptotic pathway. Regulation of extrinsic and intrinsic apoptotic pathways has been reported as a key mechanism through which $InsP_6$ contributes to its anticancer activity.(12) The extrinsic pathway is initiated by extracellular signals that bind to death receptors, leading to activation of caspase-8 through death-inducing signaling. In contrast, the intrinsic pathway is triggered by intracellular stress such as DNA damage or oxidative imbalance.(13,14) Caspase-2 contributes to apoptosis by responding to DNA damage, functioning as a tumor suppressor, and maintaining genomic stability as well as cell cycle regulation.(15,16) Both pathways lead to the activation of executioner caspases, particularly caspase-3, which facilitates mitochondrial cytochrome c release and drives the execution phase of apoptosis.(17)

Although the anticancer activity of $InsP_6$ has been reported through caspase activation, there is still no molecular docking evidence that directly examines $InsP_6$ interactions with upstream apoptotic regulators such as caspase-8 in the extrinsic pathway, caspase-2 as a sensor of DNA damage in the intrinsic pathway, and caspase-3 in the execution phase. Therefore, this study aimed to perform molecular docking analysis of $InsP_6$ with histones and key caspase proteins to clarify its potential binding mechanisms. This approach is expected to enhance the understanding of $InsP_6$ effectiveness and provide new insights into its pro-apoptotic and anticancer properties.

Methods

Ligand and Protein Data Mining

All ligand data including Canonical Simplified Molecular Input Line Entry System (SMILES) of Inositol Hexakisphosphate ($InsP_6$) (Pubchem CID: 890), CID: Inositol-(1,3,4,5,6)-Pentakisphosphate ($InsP_5$) (Pubchem CID: 17754035), Inositol-(1,4,5,6)-Tetrakisphosphate ($InsP_4$) (Pubchem CID: 443266), histone deacetylase inhibitor (Pubchem CID: 5353484), Z-IETD-FMK caspase-8 inhibitor (Pubchem CID: 25108681), Z-VDVAD-FMK caspase-2 inhibitor (Pubchem CID: 25108684), and Ac-DEVD CMK caspase-3 inhibitor (Pubchem CID: 9959259) were obtained and downloaded in sdf format. Ligands were selected based on structural relevance to the target compounds for subsequent analysis.

Target protein data of histone (PDB ID: 8JCC), caspase-8 (PDB ID: 5L08), caspase-2 (PDB ID: 1PYO), caspase-3 (PDB ID: 3KJF) were retrieved and downloaded in PDB format. Proteins were selected based on good resolution (2.0–2.5 Å), absence of mutations, 90% residues in favored regions, and 0% in disallowed regions.

$InsP_6$ Toxicity Prediction and Physicochemical Analysis

$InsP_6$ toxicity prediction was conducted using ProTox-3.0 (Environmental Protection Agency, Washington DC, USA) at (https://tox.charite.de/protox3/). Canonical SMILES was input to detect toxicity class, hepatotoxicity, immunotoxicity, mutagenicity, and cytotoxicity.

$InsP_6$ analysis was conducted using SwissADME (Molecular Modeling Group, Lausanne, Switzerland) at (http://www.swissadme.ch). Canonical SMILES was input to analyze molecular weight, molar refractivity, lipophilicity, water solubility, number of heavy atoms, rotatable bonds, hydrogen bond acceptors, and hydrogen bond donors.

Ligand and Protein Preparation

Each ligand was prepared by performing energy minimization with PyRx-Virtual Screening Tool (FastSpring, Amsterdam, Netherlands). Briefly, each ligand structure was imported into the workspace and subjected to energy minimization using the Universal Force Field (UFF). Ligand optimization was performed using the conjugate gradient method with a maximum of 200 steps. Convergence was considered achieved when the energy gradient was below 0.01 kcal/mol·Å, ensuring that the ligands adopted the most stable 3D conformation. Minimized ligand structures were then exported and saved in PDB format for further analysis.

Meanwhile, each protein was prepared by removing water molecules and bound ligands using Biovia Discovery Studio 2016 (Dassault Systèmes, Vélizy-Villacoublay, France). Briefly, crystal structures of the proteins were obtained from PDB loaded into the software. Water molecules, co-crystallized ligands, and other non-essential heteroatoms were removed to prevent interference during the docking process. The cleaned and optimized protein structures was then saved and further converted into PDB format.

Molecular Docking Analysis

Molecular docking analysis was performed using CB-Dock 2.0 (https://cadd.labshare.cn/cb-dock2/php/index.php). Prepared ligand and protein were input to detect ligand-protein interactions and possible bindings. Molecular docking was performed using CB-Dock2.0, which automatically detects up to five potential binding cavities and applies AutoDock Vina as the docking engine. For each cavity, multiple binding poses were generated, and the best pose was selected based on the lowest Vina score binding affinity (kcal/mol) and cavity suitability. Visualization of three-dimensional (3D) and two-dimensional (2D) interactions were carried out using Biovia Discovery Studio 2016.

Results

Toxicological Profiles and Physiochemical Properties of InsP$_6$

Toxicity prediction using the ProTox-III platform classified InsP$_6$ into toxicity Class IV, suggesting relatively low acute toxicity. Furthermore, InsP$_6$ was predicted to be inactive across all evaluated toxicity endpoints (Table 1). Based on its physicochemical properties, InsP$_6$ was identified as a highly polar and water-soluble compound. The compound exhibited a high number of hydrogen bond donors and acceptors, which contributed to its strong hydrophilicity. This was further supported by its negative log P value, indicating low lipophilicity (Table 2). Following toxicity prediction, docking analysis was conducted to examine protein–ligand interactions.

Table 1. Toxicity prediction profiles of InsP$_6$ using Pro-Tox III.

Toxicity Test	Ligand
	InsP$_6$
Toxicity Class (LD$_{50}$)	Class 4 (1500 mg/kg)
Hepatotoxicity (probability)	Inactive (0.91)
Immunotoxicity (probability)	Inactive (0.99)
Mutagenicity (probability)	Inactive (0.71)
Cytotoxicity (probability)	Inactive (0.78)

LD$_{50}$: lethal dose for 50% of the test population. Substances in Class I (LD$_{50}$ ≤ 5 mg/kg) and Class II (5 mg/kg < LD$_{50}$ ≤ 50 mg/kg) are considered fatal if ingested. Class III compounds (50 mg/kg < LD$_{50}$ ≤ 300 mg/kg) are categorized as toxic, whereas Class IV (300 mg/kg < LD$_{50}$ ≤ 2000 mg/kg) is regarded as harmful. Class V substances (2000 mg/kg < LD$_{50}$ ≤ 5000 mg/kg) may still pose harmful effects, while Class VI (LD$_{50}$ > 5000 mg/kg) is classified as non-toxic.

Molecular Docking Interaction of InsP$_6$ and Histone

Molecular docking results showed that InsP$_6$ had a stronger binding Vina score with the histone compared to histone protein-histone deacetylase inhibitor (Table 3). InsP$_6$

Table 2. Physiochemical properties of InsP$_6$ using SwissADME web-based platform.

Physiochemical	Ligand
	InsP$_6$
Molecular Weight (g/mol)	660.04
Number of Heavy Atoms	36
Number of Rotatable Bonds	12
Number of HBA	24
Number of HBD	12
Molar Refractivity	101.27
Lipophilicity (Log P)	-4.78
Water Solubility (Log S / ESOL)	3.34

HBA: Hydrogen Bond Acceptors; HBD: Hydrogen Bond Donors; Log P: logarithm of the partition coefficient; Log S: Logarithm of the aqueous solubility; ESOL: Estimated Solubility.

Table 3. Molecular docking interactions of histone protein complex with InsP$_6$ and histone deacetylases inhibitor.

Ligand	Vina Score (kcal/mol)	Hydrogen Bond Details	Van Der Waals Bond	Other Bonds
InsP$_6$	-5.7	ARG17, GLY11, GLY9, GLY2, LYS5,SER1	ARG3, GLY4, LEU10, LYS16,	N/A
Histone deacetylase inhibitor	-5.5	ARG45, GLY41	ILE46, ILE50, ILE34, SER47, THR54, VAL43,	Pi-Sigma: ALA38 Amide-Pi Stacked: LEU37

formed multiple hydrogen bonds with key histone residues, including ARG17, GLY2, GLY11, GLY9, LYS5, and SER1. In contrast, histone protein and histone deacetylase inhibitor exhibited fewer hydrogen bonds, involving only ARG45 and GLY41 (Figure 1).

Molecular Docking Interaction of InsP$_6$ and Caspase-8

InsP$_6$ demonstrated a strong binding affinity with caspase-8. The affinity was comparable to the Z-IETD–caspase-8, the reference inhibitor (Table 4). Molecular docking analysis showed that InsP$_6$ interacted with caspase-8 through hydrogen bonding with ARG47 and LYS121 (Figure 2).

Molecular Docking Interaction of InsP$_6$ and Caspase-2

Molecular docking analysis showed that InsP$_6$ bound to the active site of caspase-2 with a high binding affinity, forming hydrogen bonds with ARG156, GLU287, GLN129, LYS225, and TYR221. This binding interaction was comparable to Z-VDVAD, which formed hydrogen bonds with ASN232, ARG231, THR233, and TYR273 (Figure 3, Table 5).

Molecular Docking Interaction of InsP$_6$, InsP$_4$, InsP$_5$, and Caspase-3

InsP$_6$ also exhibited strong binding affinity to caspase-3. Similarly, the small difference in Vina scores between the InsP$_6$–caspase-3 and protein–inhibitor binding supported this finding, suggesting that InsP$_6$ bound to caspase-3 with an affinity comparable to that of the reference inhibitor (Table 6). In addition, InsP$_6$ formed hydrogen bonds with several residues of caspase-3, including ARG207, ARG64, CYS163, HIS121, and SER120 (Figure 4).

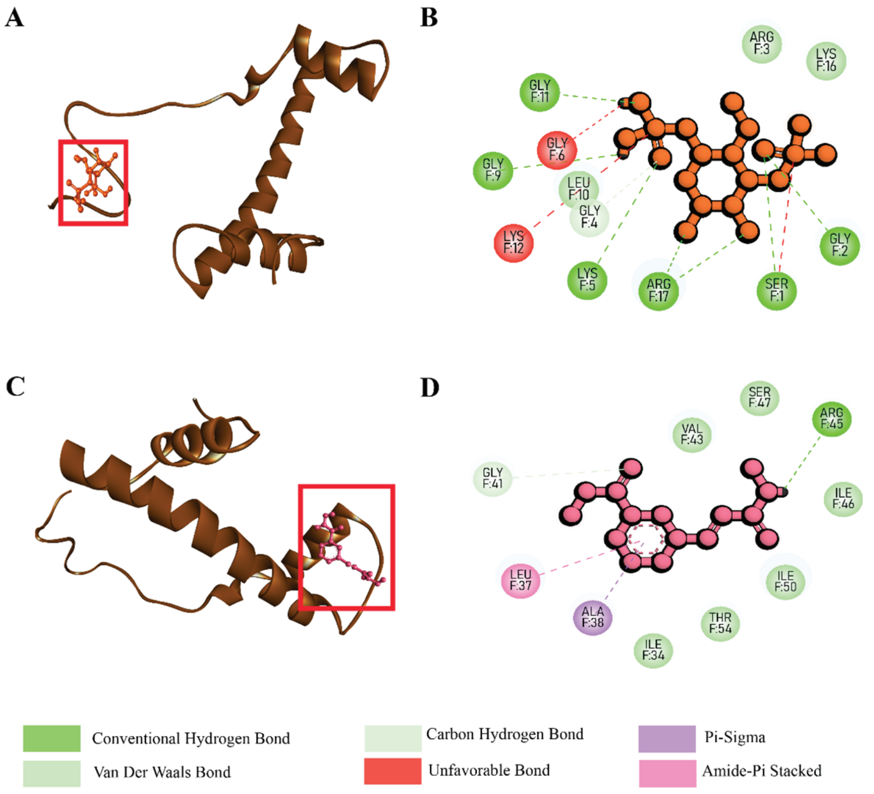

Figure 1. 3D and 2D interaction between histone protein complex and two ligands: InsP$_6$ and histone deactylase inhibitor. The red rectangle highlights the binding sites of both ligands within the active site of the histone protein complex (brown colour with ribbon pattern). A: 3D interaction of InsP$_6$ with the histone protein complex. B: 2D interaction of InsP$_6$ with the histone protein complex. C: 3D interaction of the histone deacetylase inhibitor with the histone protein complex. D: 2D interaction of the histone deacetylase inhibitor with the histone protein complex.

Table 4. Molecular docking interactions of caspase-8 with ligand target InsP6 and Z-IETD-FMK.

Ligand	Vina Score (kcal/mol)	Hydrogen Bond Details	Van Der Waals Bond	Other Bonds
$InsP_6$	-7.3	ARG47, LYS121	CYS131, GLN125, GLU110, GLN46, LEU124, LEU133, MET43, PHE122, SER113	Attractive Charge: ASP135, GLU50, GLU111, GLU116
Z-IETD-FMK	-8.6	ASN168, GLU111, GLN125, GLN49, SER170,	ARG47, ASP2, GLU50, GLU55, GLU56, GLU126, GLN46, GLN107, GLU116, LEU54, LEU42, PHE45, SER129, SER4, SER113	Alkyl/Pi-Alkyl: ILE174, LEU7 Halogen (Fluorine): CYS131, GLU110

All binding of $InsP_6$, $InsP_5$, and $InsP_4$ with caspase-3 showed a similar binding ability. Vina scores showed only slight differences among the three ligands (Table 6). $InsP_6$, $InsP_5$, and $InsP_4$ had some similar hydrogen and Van Der Waals bond residues (ARG207, ALA162, GLY122, CYS163, HIS121, and TRP206) (Figure 4).

Discussion

$InsP_6$ has been reported to require relatively high concentration for inducing apoptosis in cancer cells.(5,9) In addition, in the present study for toxicity prediction, $InsP_6$ was classified to have a low acute toxicity at specific dosage levels (Table 1), suggesting a relatively safe toxicity profile for $InsP_6$. From the physicochemical profile, $InsP_6$ has been shown to have high polarity and hydrogen bonding capacity (Table 2), which might cause limited membrane permeability, leading to reduction of absorption and transport across biological membranes.(19-21) Therefore, formulation strategies had been pursued to improve permeability of $InsP_6$. One reported approach involved binding $InsP_6$ to histones.(5,9) In the present study, molecular docking analysis indicates that $InsP_6$ exhibited stable binding to the histone, comparable to the binding observed with the control (Table 3). Previous studies indicate that histone binding can facilitate the cellular uptake of $InsP_6$. Moreover, the binding of histone was shown to increase potential of $InsP_6$ in inducing apoptosis, so that a high concentration of $InsP_6$ was no longer required.(5,9) Collectively, these findings

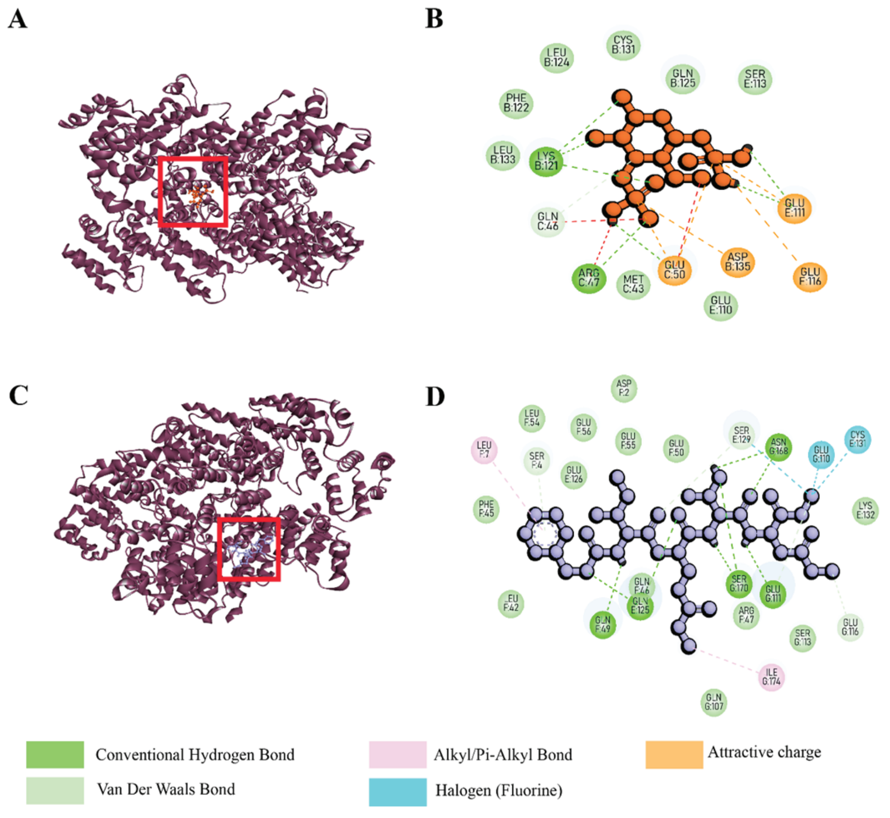

Figure 2. 3D and 2D interaction between caspase-8 protein complex and two ligands: $InsP_6$ and Z-IETD-FMK. The red rectangle highlights the binding sites of both ligands within the active site of the caspase-8 protein complex (magenta colour with ribbon pattern). A: 3D interaction of $InsP_6$ with the caspase-8 protein complex. B: 2D interaction of $InsP_6$ with the caspase-8 protein complex. C: 3D interaction of the Z-IETD-FMK with the caspase-8 protein complex. D: 2D interaction of the Z-IETD-FMK with the caspase-8 protein complex.

suggest that histone play an important role in enhancing pro-apoptotic efficacy of InsP_6.

InsP_6 has been demonstrated to induce anticancer effects through the extrinsic apoptotic pathway, with caspase-8 playing a central role.(4,6,28) Molecular docking results indicated that InsP_6 bound strongly to the active site of the caspase-8 protein (Table 4). These results suggest that InsP_6 may support the potential of InsP_6 as an anticancer

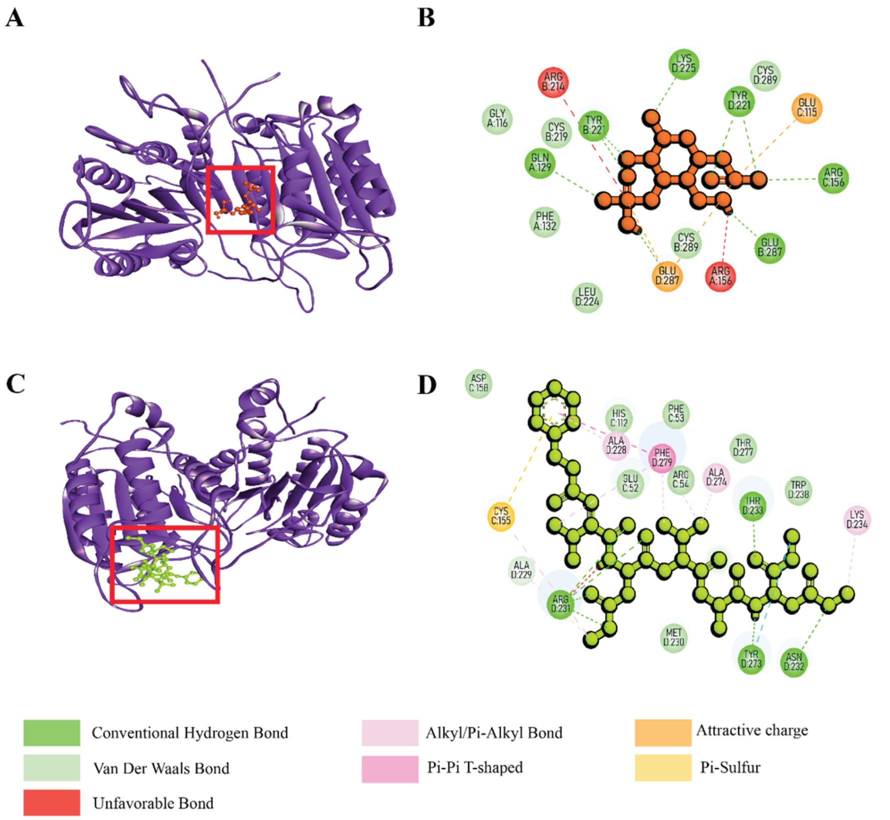

Figure 3. 3D and 2D interaction between caspase-2 protein complex and two ligands: InsP_6 and Z-VDVAD. The red rectangle highlights the binding sites of both ligands within the active site of the caspase-2 complex (purple colour with ribbon pattern). A: 3D interaction of InsP_6 with the caspase-2 protein complex. B: 2D interaction of InsP_6 with the caspase-2 protein complex. C: 3D interaction of the Z-VDVAD with the caspase-2 protein complex. D: 2D interaction of the Z-VDVAD with the caspase-2 protein complex.

Table 5. Molecular docking interactions of caspase-2 with InsP_6, and Z-VDVAD.

Ligand	Vina Score (kcal/mol)	Hydrogen Bond Details	Van Der Waals Bond	Other Bonds
InsP_6	-6.8	ARG156, GLN129, GLU287, LYS225, TYR221	CYS289, CYS219, GLY116, LEU224, PHE132	Attractive Charge: GLU287, GLU115
Z-VDVAD	-7.3	ASN232, ARG231,THR233, TYR273	ALA229, ARG54, ASP158, GLU52, HIS112, MET230, PHE53, TRP238, THR277,	Alkyl/Pi-Alkyl: ALA274, ALA228, LYS234 Pi-Pi T-shaped: PHE279 Pi-Sulfur: CYS155

Table 6. Molecular docking interactions of caspase-3 with InsP_6, InsP_5, InsP_4, and Ac-DEVD-CMK.

Ligand	Vina Score (kcal/mol)	Hydrogen Bond Details	Van Der Waals Bond
InsP_6	-6.8	ARG207, ARG64, CYS163, HIS121, SER120	ALA162, GLY122, SER63, SER205, THR62, TRP206, TYR204
InsP_5	-6.4	ARG207, CYS163, HIS121, SER205, TYR204,	ALA162, GLN161, GLY122, MET61, PHE256, SER120, SER63, TRP206, THR166, THR62,
InsP_4	-6.0	ARG207, ARG64, CYS163, HIS121, SER205, SER120, THR62	ALA162, GLY122, GLN161, MET61, SER209, TRP206, TYR204
Ac-DEVD-CMK	-6.9	ARG207, ARG64, ASN208, THR62, TRP214	CYS163, HIS121, HIS257, LYS210, PHE250, PHE256, SER205, SER249, SER251, TRP206, TYR204

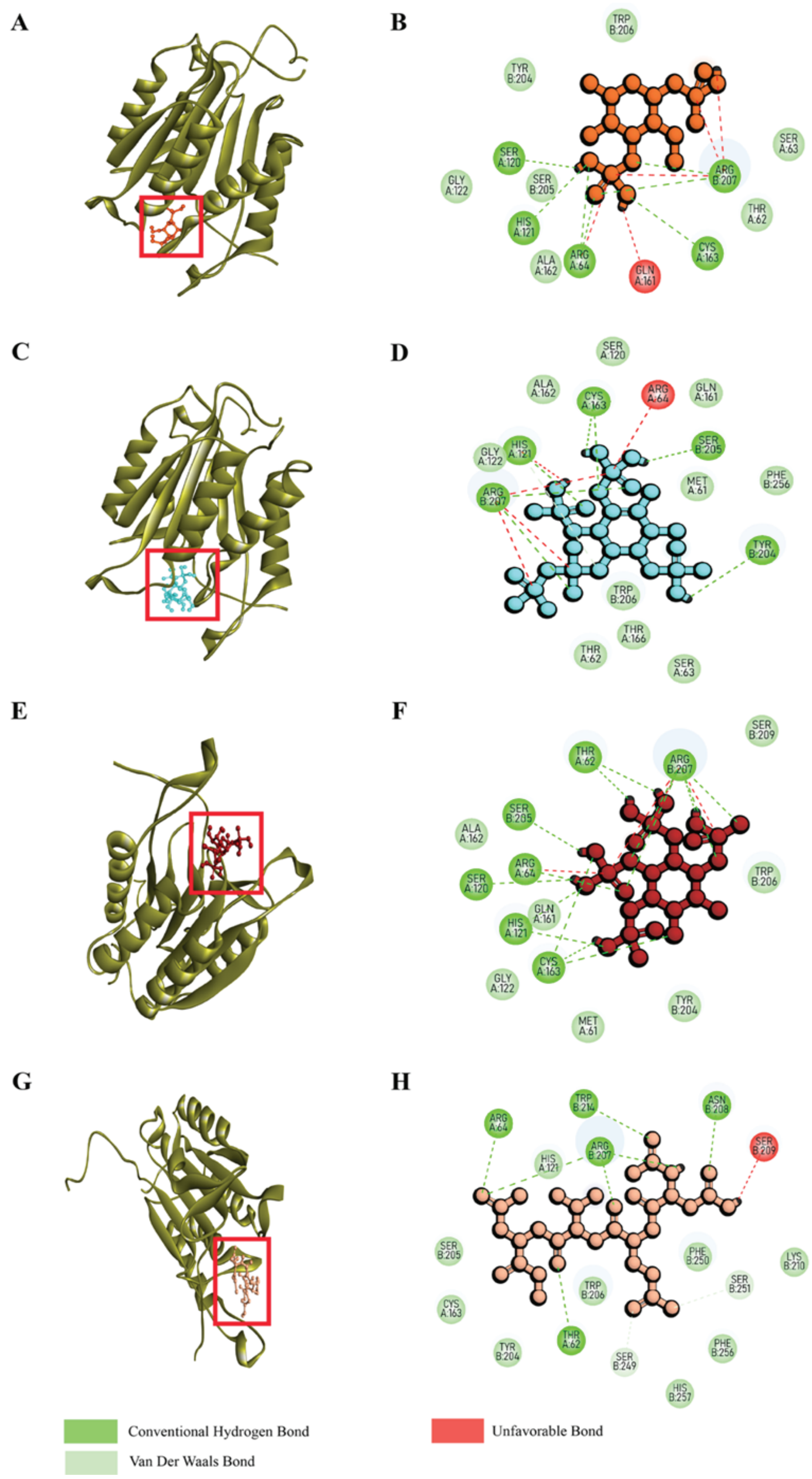

Figure 4. 3D and 2D interaction between caspase-3 protein complex and three ligands: InsP$_6$, InsP$_5$, InsP$_4$, and Ac-DEVD-CMK. The red rectangle highlights the binding sites of ligands within the active site of the caspase-3 protein complex (yellow colour with ribbon pattern). A: 3D interaction of InsP$_6$ with the caspase-3 protein complex. B: 2D interaction of InsP$_6$ with the caspase-3 protein complex. C: 3D interaction of InsP$_5$ with the caspase-3 protein complex. D: 2D interaction of InsP$_5$ with the caspase-3 protein complex. E: 3D interaction of InsP$_4$ with the caspase-3 protein complex. F: 2D interaction of InsP$_4$ with the caspase-3 protein complex. G: 3D interaction of Ac-DEVD-CMK with the caspase-3 protein complex. H: 2D interaction of Ac-DEVD-CMK with the caspase-3 protein complex.

agent by targeting key component of the extrinsic apoptosis pathway. This outcome reflects previous findings that confirm InsP$_6$ can induce apoptotic cell death in HT-29 colorectal cancer cells by increasing the expression and activity of caspase-8.(22)

Several apoptotic processes are influenced by alterations in mitochondrial membrane potential mediated by caspase-2, which has been reported to be activated by its specific trigger.(29) In this study, InsP$_6$ exhibits strong binding to active site of caspase-2 through hydrogen, Van der

Waals, and attractive charge interactions (Figure 3, Table 5). These results suggest that $InsP_6$ has the potential to modulate caspase-2 activity within the intrinsic apoptotic pathway.(23) In this pathway, caspase-2 could initiate apoptosis by cleaving BH3 Interacting Domain (BID) into truncated BID (tBID), which in turn promotes mitochondrial outer membrane permeabilization (MOMP). This event triggers the release of cytochrome c, leading to the formation of the apoptosome and subsequent activation of downstream effector caspases, including caspase-3.(24,25)

The initiator caspase-8 and caspase-2 play a pivotal role in triggering apoptosis.(30,31) However, the apoptotic cascade can be attenuated by inhibitory molecules, particularly those belonging to the inhibitor apoptosis protein (IAP).(32) In the present study, $InsP_6$ exhibited strong docking interactions not only with initiator caspases but also with the effector caspase-3 (Figure 4, Table 6). Previous reports have also highlighted anticancer potential of other inositol phosphates, such as $InsP_5$ and $InsP_4$.(10,11) Molecular docking analysis showed that $InsP_6$ binds to caspase-3 at several active sites similar to $InsP_5$ and $InsP_4$, suggesting that $InsP_6$ may contribute to apoptosis induction (Figure 4, Table 6).

$InsP_6$ has been demonstrated to induce apoptosis, supported by molecular docking interactions with apoptotic proteins. The apoptosis can be induced through diverse mechanisms including caspase-dependent/independent pathways. The present results indicate that $InsP_6$ engages caspase-mediated mechanisms, highlighting its potential role in regulating apoptosis. Nevertheless, $InsP_6$ may also act through caspase-independent pathways. Therefore, the caspase-independent pathway should be explored further to have a better understanding of $InsP_6$ anticancer role in inducing apoptosis. In addition, since this study only utilized computer-based analysis, which provided results that may be useful; however, these findings need to be further confirmed and explored through *in vitro* analysis.

Conclusion

$InsP_6$ exhibited low acute toxicity, suggesting a favorable safety profile. The strong binding between histones and $InsP_6$ as confirmed by molecular docking results, could increase the potency of $InsP_6$ in inducing apoptosis. The apoptosis induced could be triggered through both caspase-dependent extrinsic and intrinsic apoptosis pathways. Taken together, $InsP_6$ may act as a potential inducer of apoptosis in cancer cells.

Authors Contribution

FS concepting and planning the research. DR, JH, AP, and KHL contributed additional ideas. FS and VEP performed FS concepting and planning the research. DR, JH, AP, and KHL contributed additional ideas. FS and VEP performed the data acquisition/collection, performed the data analysis, drafted the manuscript, designed the figures. All authors took parts in giving critical revision of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest or competing interests related to the content of this manuscript.

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