VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 1381-1395 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

In Silico Molecular Docking Analysis of Adhatodine and Caffeic Acid: Exploring Their Mode of Action on Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL Proteins

Lakshmi Praba S.[1*] and Ashok K.[2]

[1]Department of Zoology, Presidency College, Chennai 600 005, Tamil Nadu, India
[2]Department of Microbiology and Biotechnology, Bharath Institute of Higher Education and Research (BIHER), Chennai 600 073, Tamil Nadu, India

**Corresponding Author*

Received: 2nd July, 2023; **Accepted:** 22nd November, 2023; **Published online:** 30th December, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.154

Abstract: This study investigated the potential interactions between certain proteins (Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl-XL) and two ligands (Adhatodine and Caffeic acid). Protein structures were obtained from the Protein Data Bank, while ligand structures were created using ACD ChemSketch software. Docking, a computational technique, was employed to predict binding interactions between proteins and ligands. The results were analyzed using PyMOL, and the presence of binding sites and hydrogen bonds was determined. Lipinski rule analysis and *in silico* toxicity analysis were also performed. The findings suggest potential therapeutic applications for Adhatodine and Caffeic acid as natural therapeutic agents, particularly for Caspase 3, Bax, and Bcl-2 interactions. Experimental validation is needed to confirm the predicted binding affinities and interactions.

Keywords: Protein-ligand interactions, Docking, Binding affinity, Molecular docking, Binding site, Hydrogen bonds, Lipinski rule, *In silico* toxicity analysis, Therapeutic agents, Caspase-3, Caspase-9, Bax, Bcl-2, Bcl-XL, Adhatodine, Caffeic acid

Citation: Lakshmi Praba S. and Ashok K.: *In silico* molecular docking analysis of adhatodine and caffeic acid: exploring their mode of action on Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL proteins. Intern. J. Zool. Invest. 9(2): 1381-1395, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.154

This is an Open Access Article licensed under a Creative Commons License: Attribution 4.0 International (CC-BY). It allows unrestricted use of articles in any medium, reproduction and distribution by providing adequate credit to the author (s) and the source of publication.

Introduction

In the field of drug discovery and development, understanding the interactions between small molecules (ligands) and target proteins is crucial for designing effective therapeutic interventions (Shi *et al.*, 2023). Molecular docking is a computational technique widely used to predict and study these interactions *in silico*, providing valuable insights into the mode of action of ligands (Yeşilkaynak *et al.*, 2023). This study focuses on two specific ligands, Adhatodine and Caffeic acid,

and their docking against several key proteins involved in apoptotic pathways, namely Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL. By analyzing the docking results, we aim to elucidate the binding mechanisms and predict the mode of action of these ligands, potentially uncovering their therapeutic potential in targeting apoptotic proteins.

The primary objective of this study was to investigate the mode of action of Adhatodine and Caffeic acid by performing *in silico* molecular docking against Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL proteins. The specific objectives are as follows:

(i) *Perform molecular docking simulations*: Utilize molecular docking software to simulate the binding of Adhatodine and Caffeic acid with the target proteins, considering their three-dimensional structures.

(ii) *Analyze docking results*: Evaluate the docking results to identify potential binding sites, binding affinities, and specific protein-ligand interactions. This analysis will provide insights into the ligand-protein binding mechanisms.

(iii) *Predict mode of action*: Based on the docking results and subsequent analysis, predict the mode of action of Adhatodine and Caffeic acid in relation to Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL proteins. This prediction will shed light on how these ligands interact with the apoptotic proteins and potentially modulate their function.

(iv) *Assess therapeutic potential*: Discuss the implications of the predicted mode of action in terms of therapeutic interventions. Assess the potential of Adhatodine and Caffeic acid as lead compounds for developing novel drugs targeting apoptotic pathways.

By accomplishing these objectives, we aim to enhance our understanding of the molecular interactions between Adhatodine, Caffeic acid, and the apoptotic proteins, thereby contributing to the development of more effective therapeutic strategies for various diseases associated with apoptosis dysregulation.

Materials and Methods

To predict the mode of action of the ligands, Adhatodine and Caffeic acid docked against Caspase-3, Caspase-9, Bax, Bcl-2 and Bcl XL proteins *in silico* molecular docking studies were carried out. The following database and tools were used.

Databases: Protein Data Bank

Tools: RasMol, Chem Sketch, ArgusLab and PyMol Viewer.

Results and Discussion

For molecular docking studies of proteins *viz.,* Caspase-3, Caspase-9,Bax, Bcl-2 and Bcl XLagainst the ligands, Adhatodine and Caffeic acid,the details are given below:

Retrival of Protein Structure from PDB Database:

Caspase-3 : PDB ID-1CP3

Caspase-9 : PDB ID-1JXQ

Bax : PDB ID-4BDU

Bcl-2 : PDB ID-1G5M

Bcl XL : PDB ID-7XGF

The three-dimensional structures of Caspase-3 (1CP3), Caspase-9 (1JXQ), Bax (4BDU), Bcl-2 (1G5M), and Bcl XL (7XGF) were downloaded from the PDB database. The compound structures of Adhatodine and Caffeic acid (ligands) were drawn using ACD ChemSketch. The Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL 3-D structures were docked with the inhibitors using the ArgusLab tool. The docking results were analyzed using the PyMol visualization tool. In silico docking studies revealed the interactions between the two ligands and the five proteins using the *in silico* molecular docking method to calculate the Docking Score between them. These results indicate the presence of a binding site between the proteins Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL, and the ligands Adhatodine and Caffeic acid (Table 1; Figs. 1 to 24). The docking is further supported by the formation of hydrogen bonds between them. The results of the Lipinski rule suggest that the

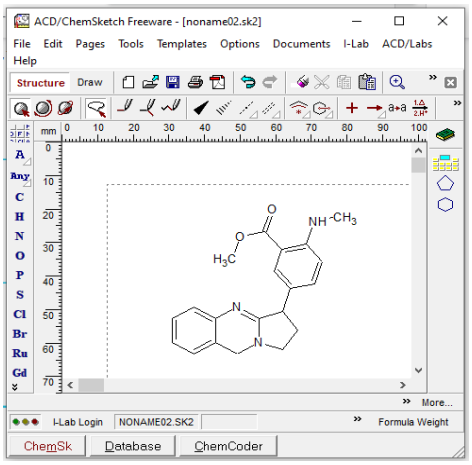

Fig. 1: 2D structure of Adhatodine.

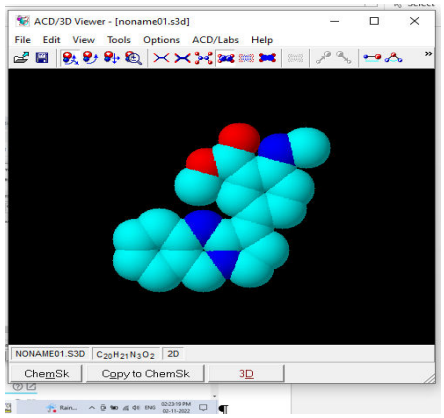

Fig. 2: 3D structure of Adhatodine.

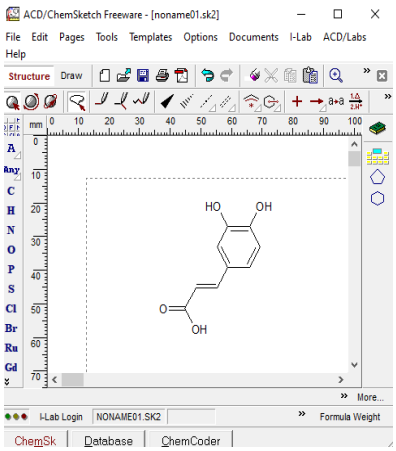

Fig. 3: 2D structure of Caffeic acid.

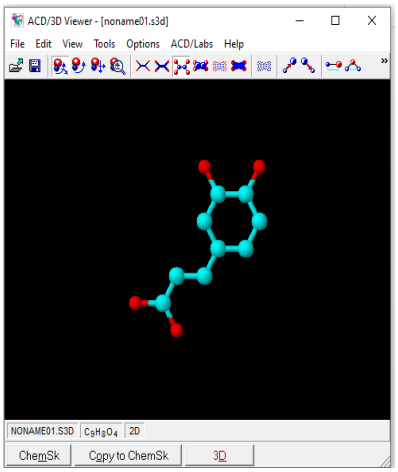

Fig. 4: 3D structure of Caffeic acid.

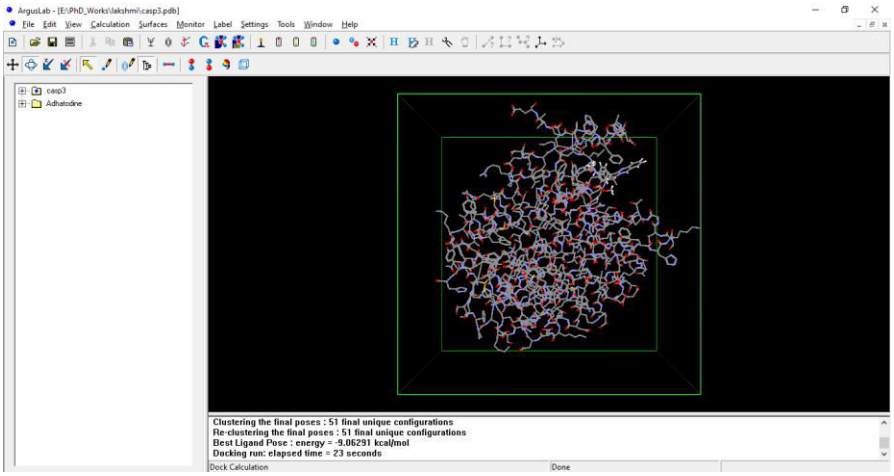

Fig. 5: Docking pose of Caspase-3 docked with Adhatodine using ArgusLab.

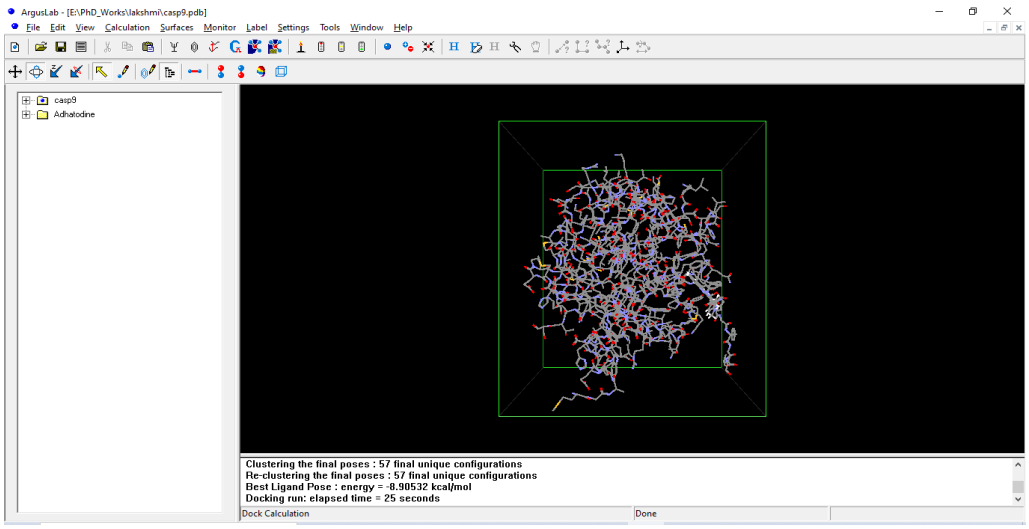

Fig. 6: Docking pose of Caspase-9 docked with Adhatodine using ArgusLab.

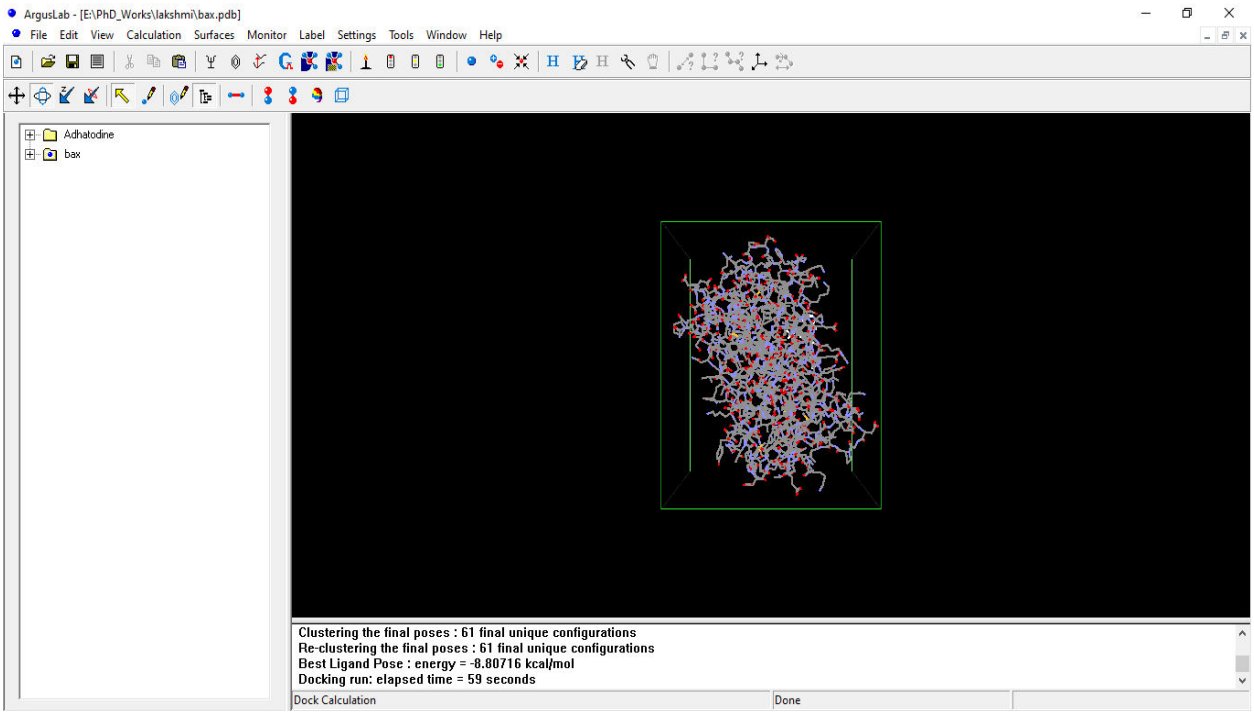

Fig. 7: Docking pose of Bax docked with Adhatodine using ArgusLab.

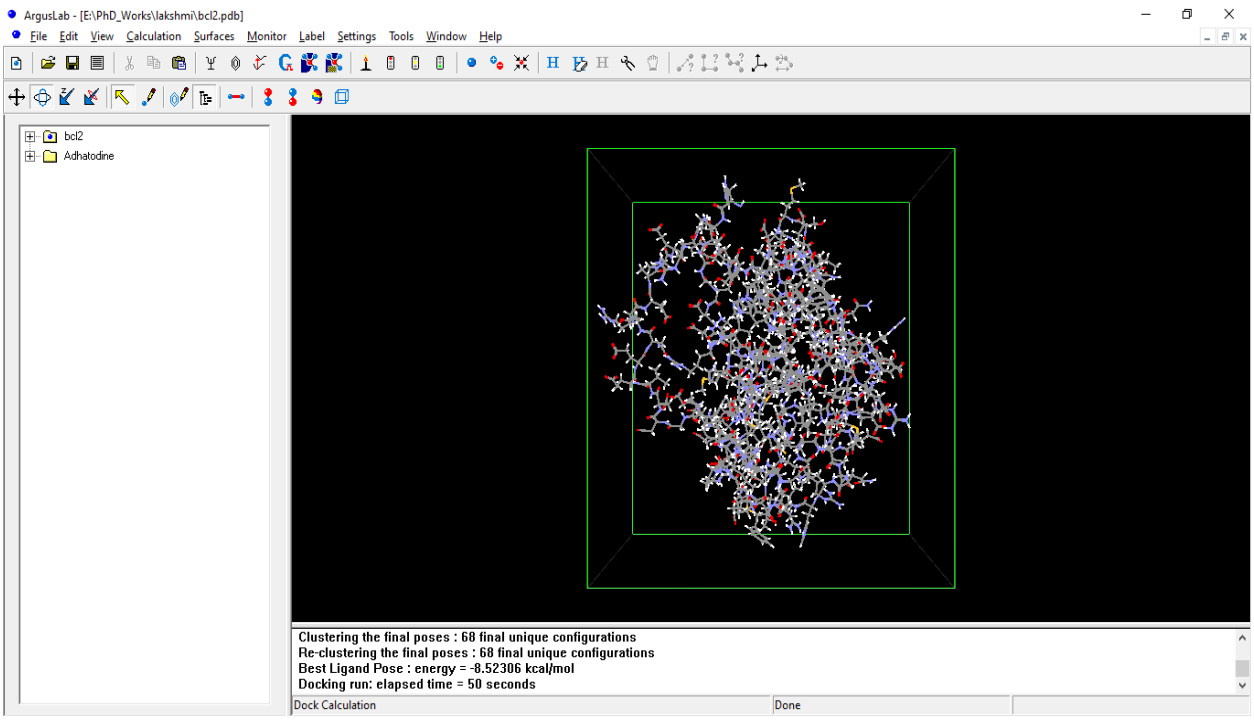

Fig. 8: Docking pose of Bcl-2 docked with Adhatodine using ArgusLab.

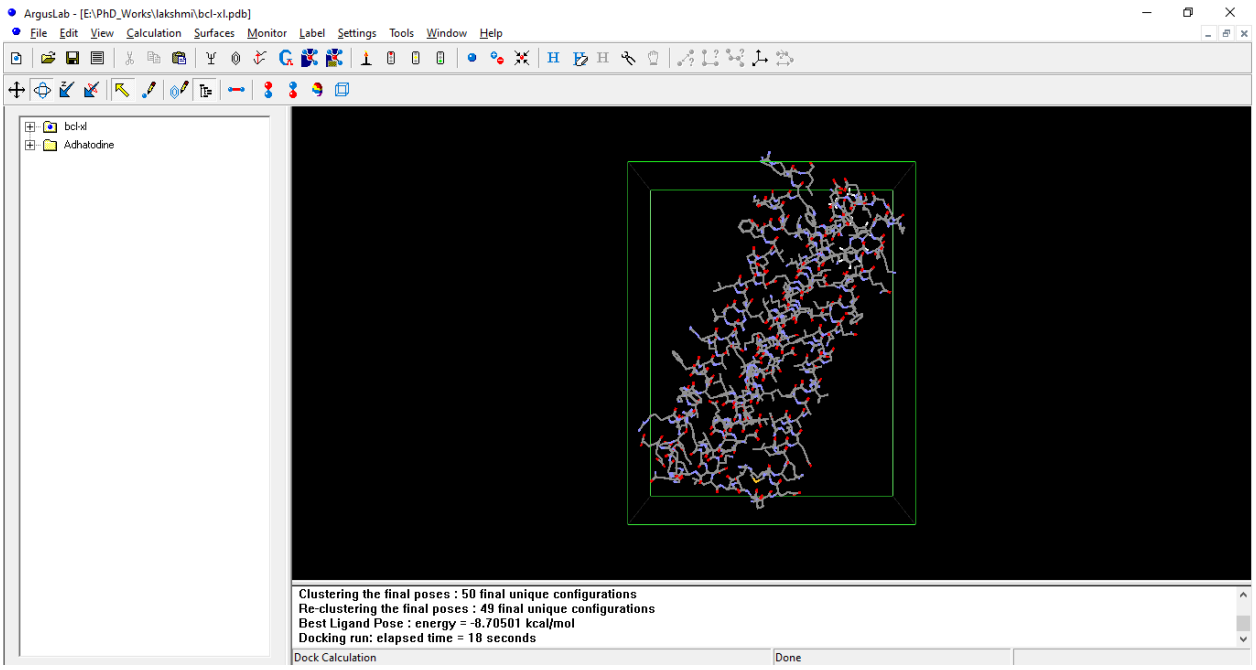

Fig. 9: Docking pose of Bcl-XL docked with Adhatodine using ArgusLab.

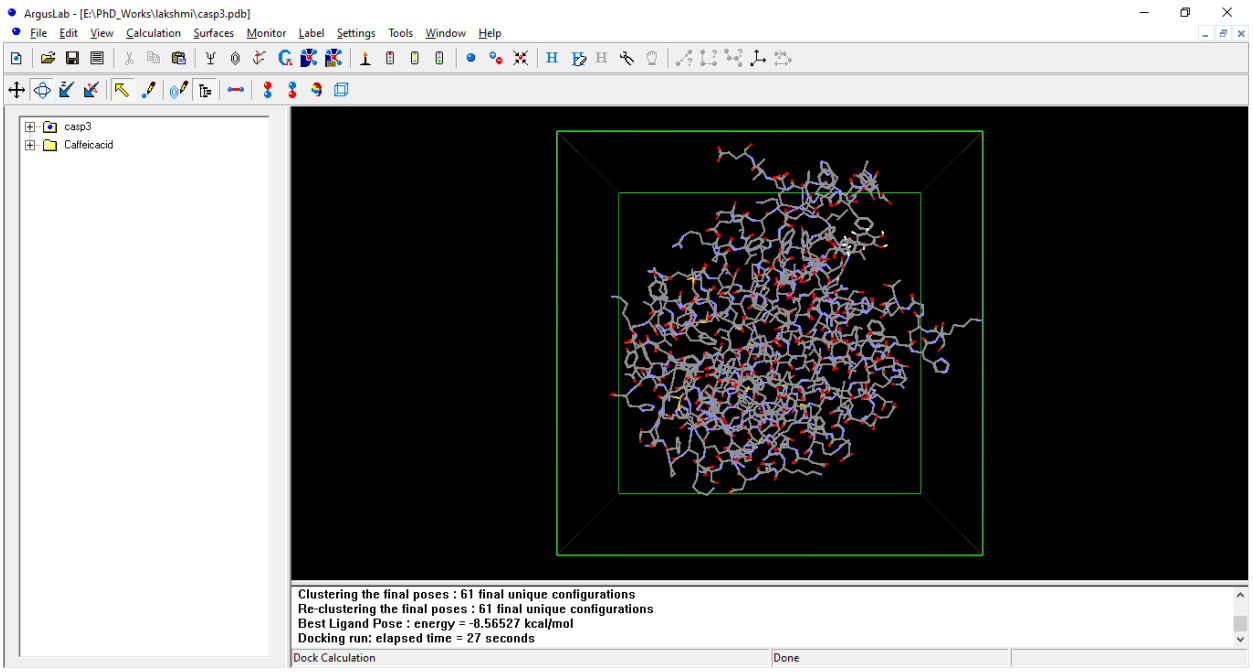

Fig. 10: Docking pose of Caspase-3 docked with Caffeic acid using ArgusLab.

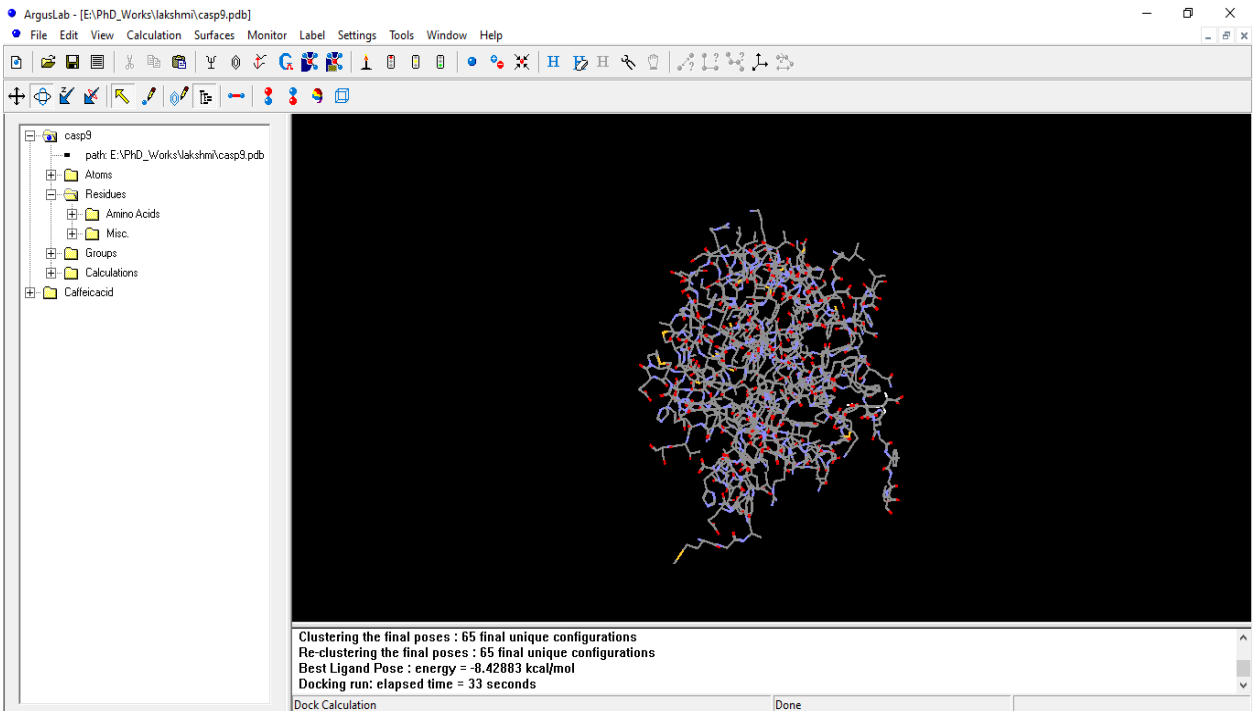

Fig. 11: Docking pose of Caspase-9 docked with Caffeic acid using ArgusLab.

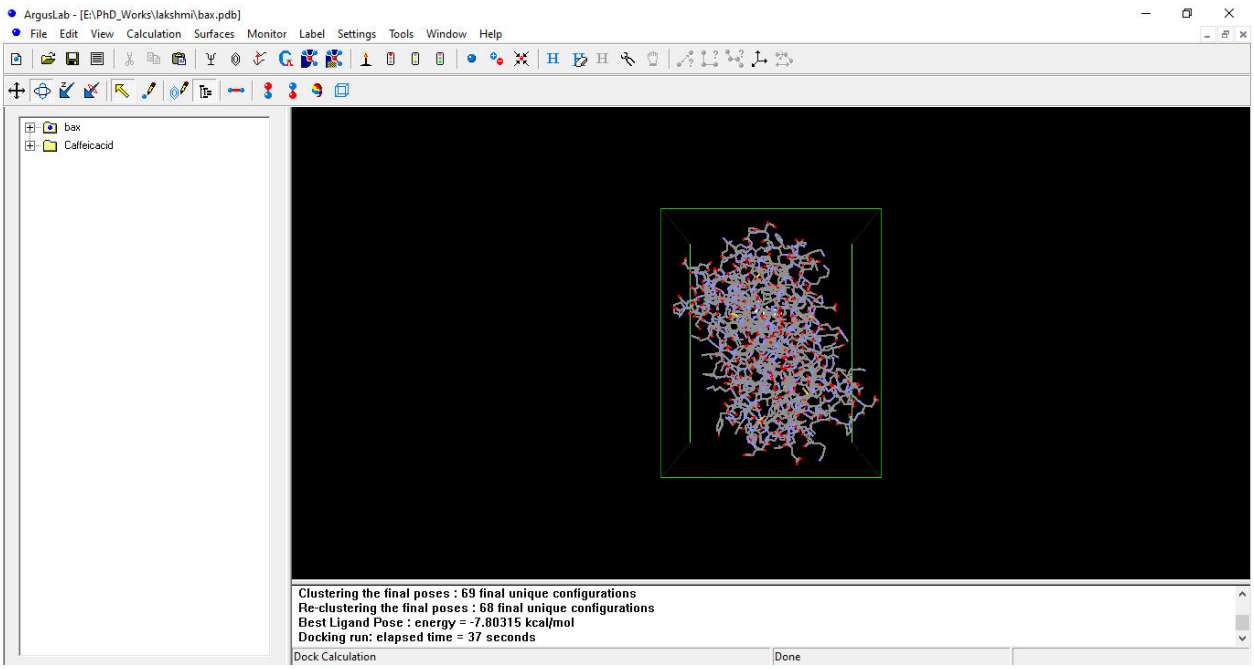

Fig. 12: Docking pose of Bax docked with Caffeic acid using ArgusLab.

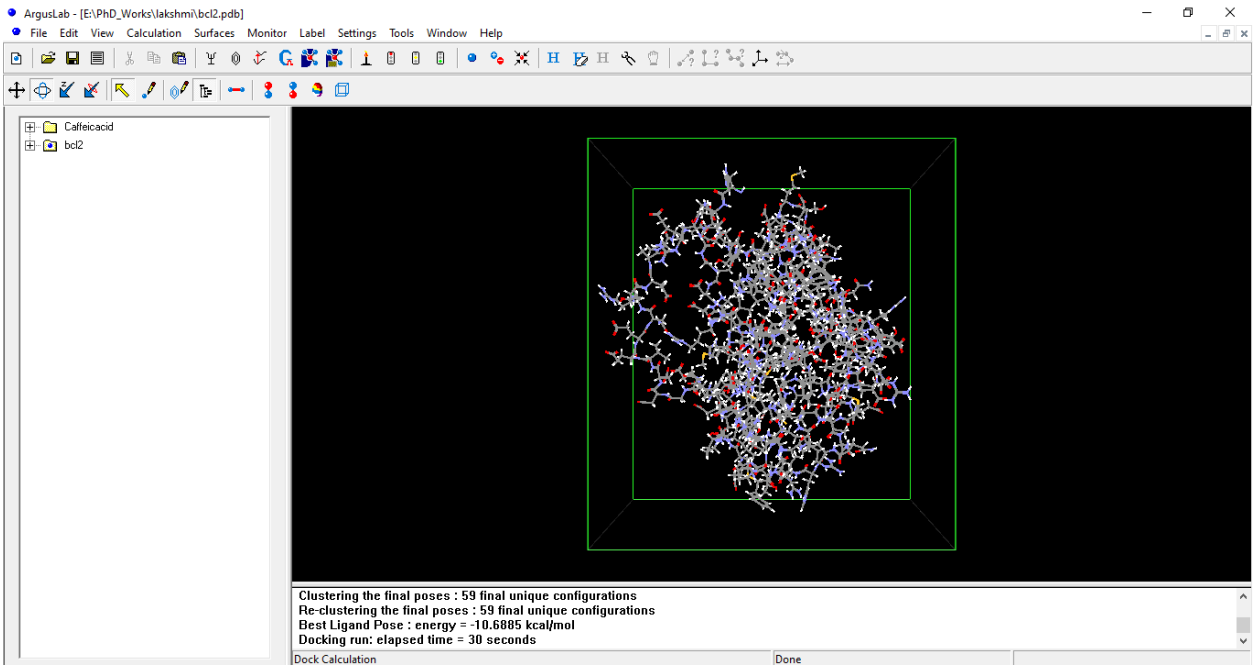

Fig. 13: Docking pose of Bcl-2 docked with Caffeic acid using ArgusLab.

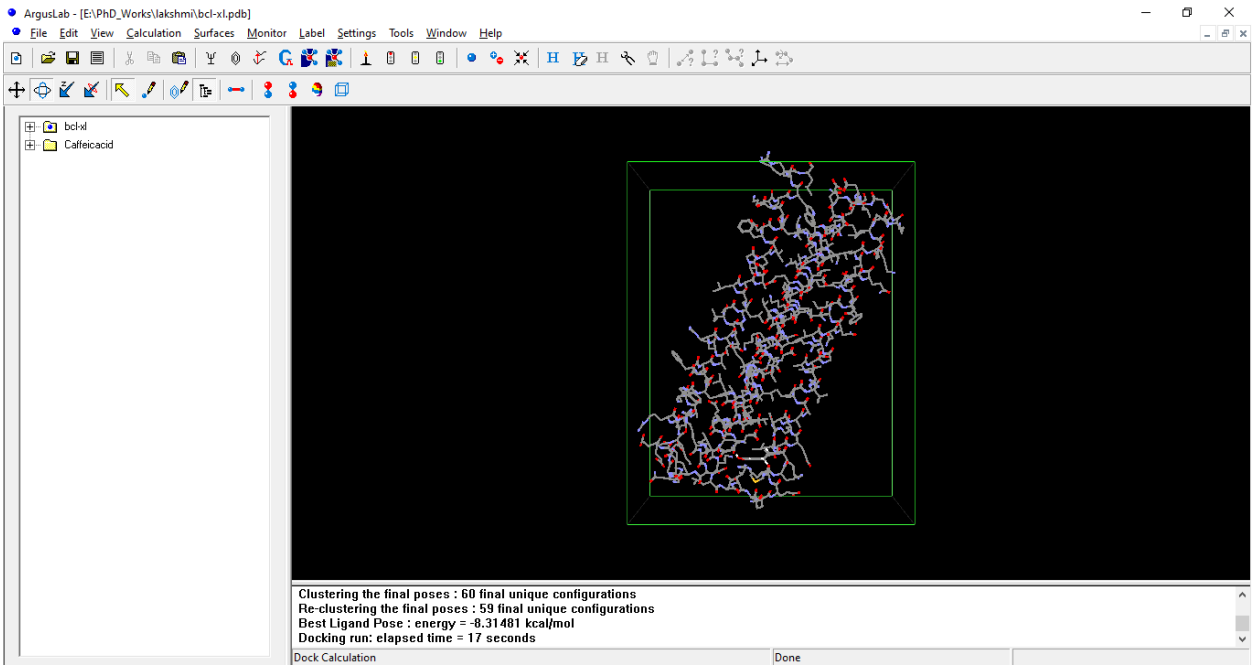

Fig. 14: Docking pose of Bcl-xl docked with Caffeic acid using ArgusLab.

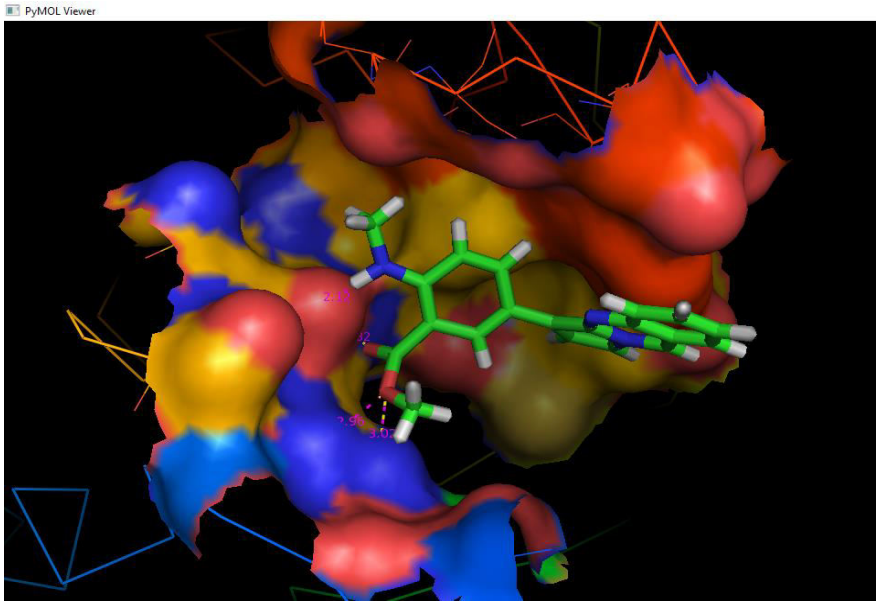

Fig. 15: Visualization of docked complex in Pymol Tool (Caspase-3 docked with Adhatodine).

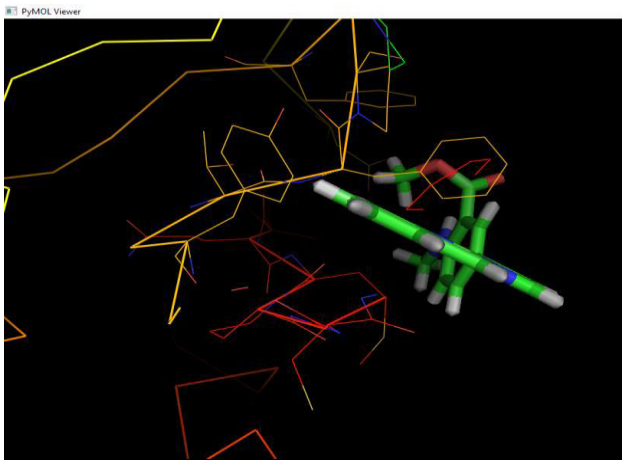

Fig. 16: Visualization of docked complex in Pymol Tool (Caspase-9 docked with Adhatodine showed no iteraction).

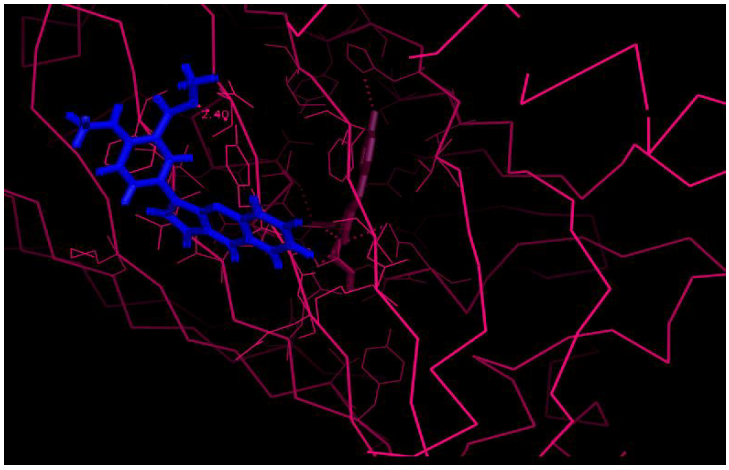

Fig. 17: Visualization of docked complex in Pymol Tool (Bax docked with Adhatodine).

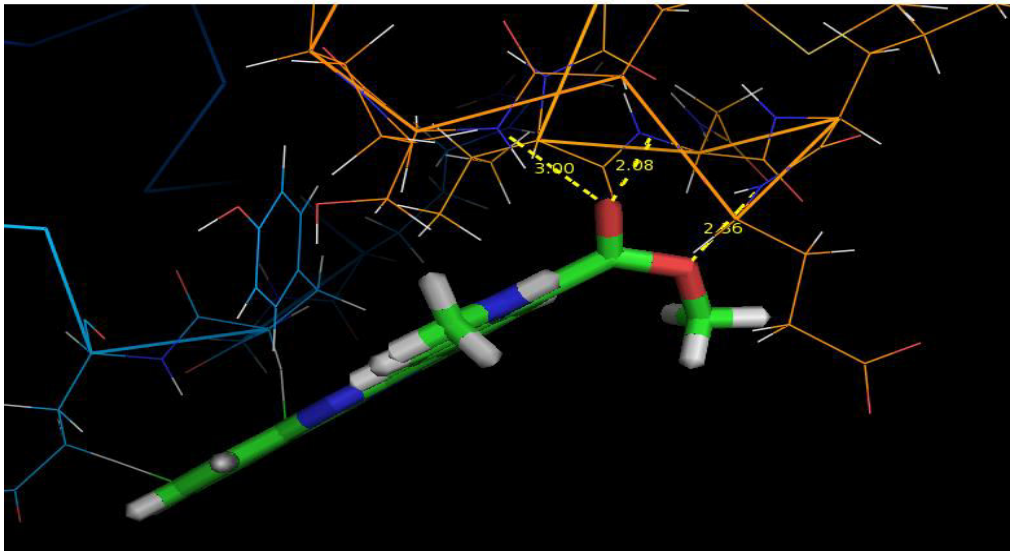

Fig. 18: Visualization of docked complex in Pymol Tool (Bcl-2 docked with Adhatodine).

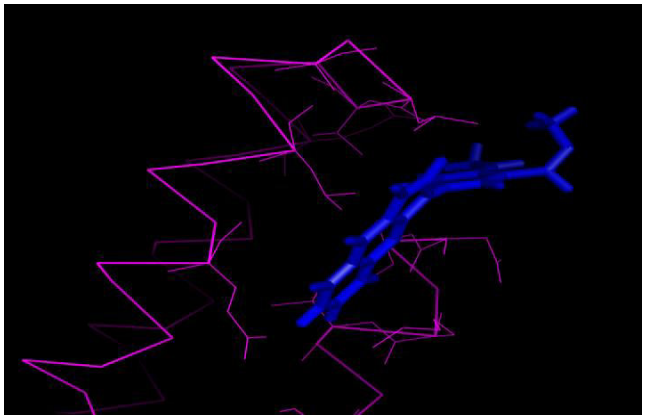

Fig. 19: Visualization of docked complex in Pymol Tool (Bcl-XL docked with Adhatodine showed no iteraction).

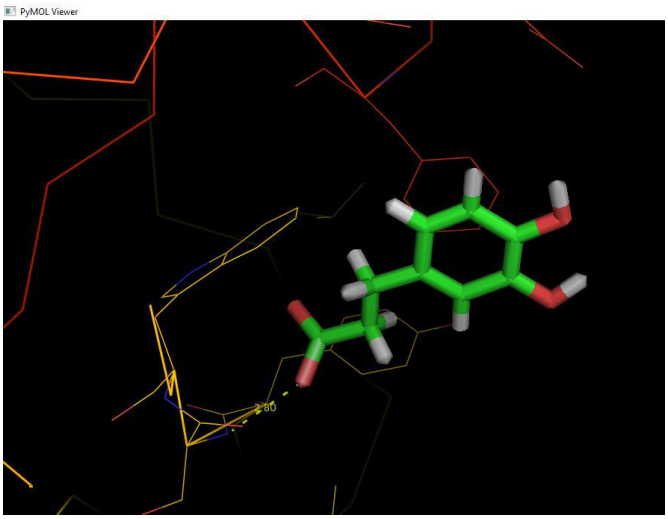

Fig. 20: Visualization of docked complex in Pymol Tool (Caspase-3 docked with Caffeic acid)

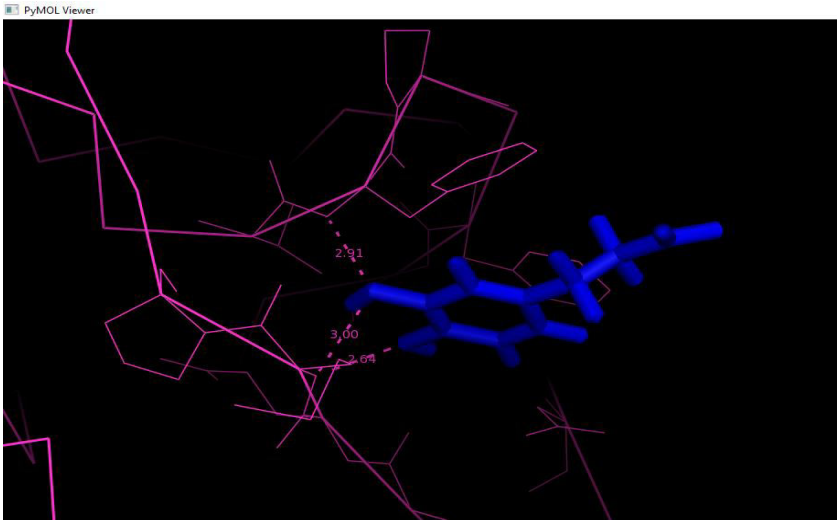

Fig. 21: Visualization of docked complex in Pymol Tool (Caspase-9 docked with Caffeic acid).

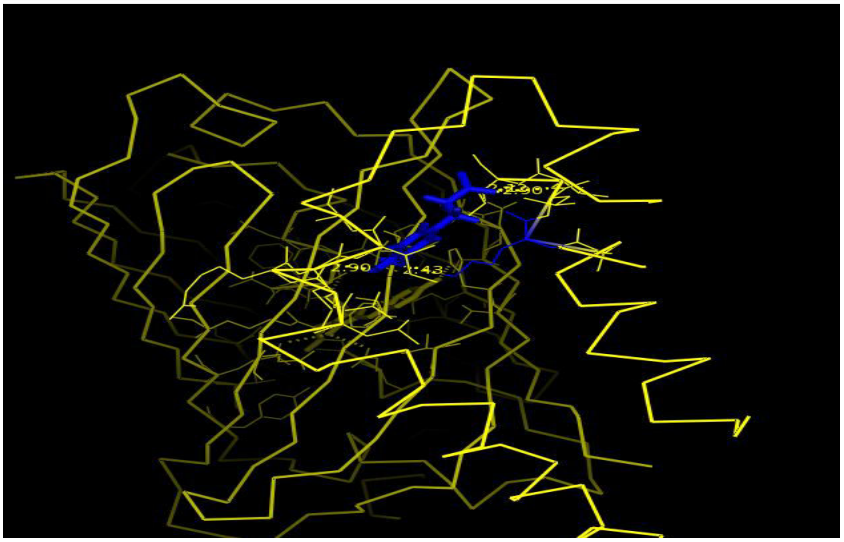

Fig. 22: Visualization of docked complex in Pymol Tool (Bax docked with Caffeic acid).

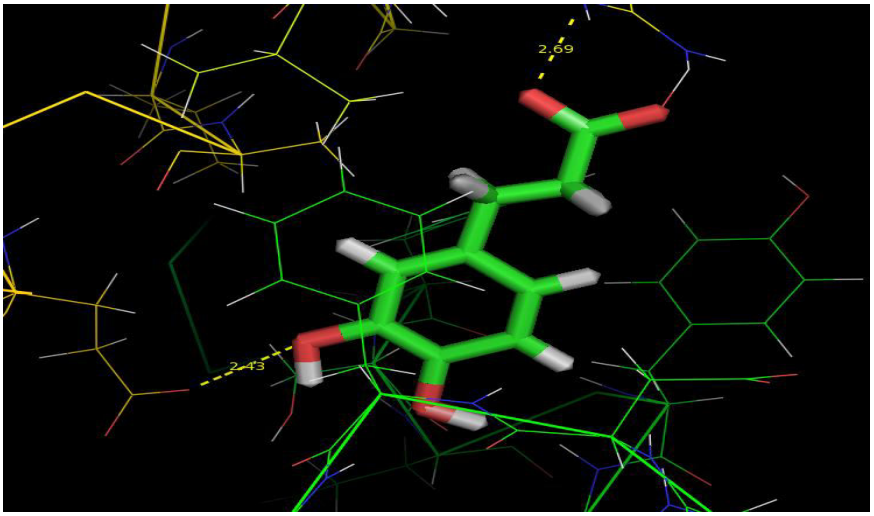

Fig. 23: Visualization of docked complex in Pymol Tool (Bcl-2 docked with Caffeic acid).

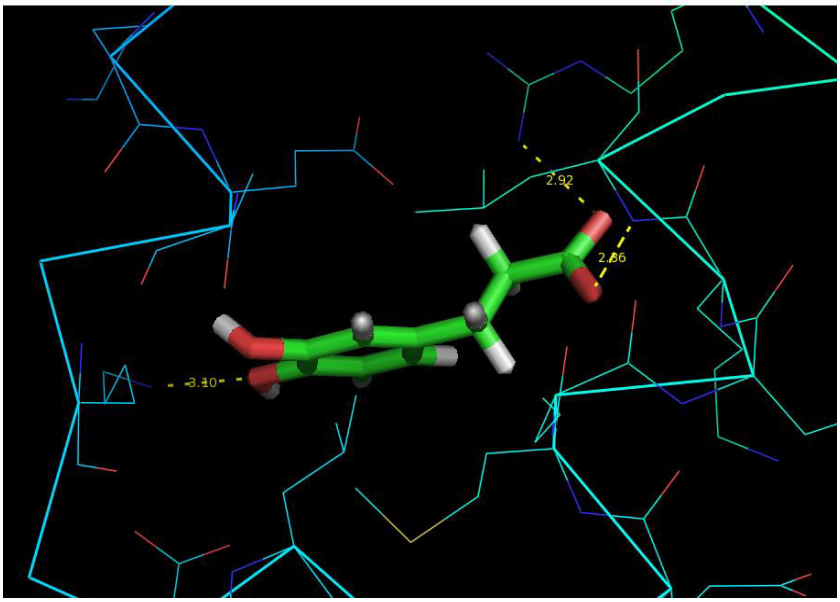

Fig. 24: Visualization of docked complex in Pymol Tool (Bcl-XL docked with Caffeic acid).

Table 1: Ligand and protein interaction with docking score

Proteins	Ligands	Docking Score (Kcal/Mol)	Distance (Å)
Caspase-3		-9.06	4
Caspase-9		**NO INTERACTION**	
Bax		-8.80	1
Bcl-2		-8.52	3
Bcl XL		**NO INTERACTION**	
Caspase-3	**Caffeic acid**	-8.56	1
Caspase-9		-8.42	3
Bax		-7.80	4
Bcl-2		-10.68	2
Bcl XL		-8.31	3

analyzed compounds are potential therapeutic drugs. The docking study and *in silico* toxicity results demonstrate the potential application of the compounds (Adhatodine and Caffeic acid) as natural therapeutic agents for disease treatment.

To explore the potential interactions between certain proteins (Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL) and two ligands (Adhatodine and Caffeic acid). Here is an explanation of the possible reasons behind the results mentioned:

Downloading protein structures:

The three-dimensional structures of the proteins (Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL) were obtained from the Protein Data Bank (PDB) database. This step ensures that the experiment utilizes accurate structural information of the proteins for further analysis.

Drawing ligand structures:

The structures of the ligands (Adhatodine and Caffeic acid) were created using the ACD ChemSketch software. Drawing the ligands allows for their representation in three-dimensional space, which is essential for subsequent docking studies.

Docking:

The protein structures were docked with the ligands using the ArgusLab tool. Docking is a computational technique that predicts the possible binding interactions between a protein

and a ligand. It explores different orientations and conformations of the ligand within the protein's

binding site to calculate a docking score that represents the strength of the protein-ligand interaction.

Docking analysis:

The results of the docking study were analyzed using the PyMOL visualization tool. This step allows the visualization and examination of the docked protein-ligand complexes, enabling a better understanding of the potential binding interactions and the overall binding mode.

Binding site identification:

The docking results indicated the presence of a binding site between the proteins (Caspase-3, Caspase-9, Bax, Bcl-2, and Bcl XL) and the ligands (Adhatodine and Caffeic acid). This suggests that the ligands can potentially interact with the proteins at specific regions, which may have implications for their biological activity.

Hydrogen bond formation:

The docking results also revealed the formation of hydrogen bonds between the proteins and ligands. Hydrogen bonds are important intermolecular interactions that contribute to the stability and specificity of protein-ligand complexes. The presence of hydrogen bonds suggests that the ligands can form favorable interactions with the proteins.

Lipinski rule analysis:

The Lipinski rule is a set of guidelines used to assess the drug-likeness and pharmacokinetic properties of small molecules. The results of the Lipinski rule suggest that the analyzed compounds (Adhatodine and Caffeic acid) have properties that make them potential therapeutic drugs. This implies that the compounds possess favorable characteristics for further development as drugs.

In silico toxicity analysis:

The docking study was complemented by an *in silico* toxicity analysis, which likely involved predicting the compounds' potential toxicity using computational methods. The passage mentions that the results demonstrate the potential application of Adhatodine and Caffeic acid as natural therapeutic agents for disease treatment, suggesting that the compounds exhibited low toxicity in the computational analysis.

The results provided are from a docking study where various proteins (Caspase 3, Caspase 9, Bax, Bcl-2, and Bcl-XL) were docked with two ligands (Adhatodine and Caffeic acid). Docking is a computational method used to predict the binding interactions between a protein and a small molecule (ligand). The docking score represents the strength of the binding between the protein and ligand, and a lower docking score generally indicates a stronger binding interaction.

Following is the interpretation of the results for each protein-ligand pair:

Caspase 3 docked with Adhatodine:

Docking score: -9.06 kcal/mol

Distance: 4 Å

Caspase 3 showed a strong binding interaction with Adhatodine, as indicated by the low docking score. The distance of 4 Å suggests that the ligand is positioned relatively close to the protein.

Caspase 9 docked with Adhatodine:

Docking score: No docking interaction

Caspase 9 did not show any significant binding interaction with Adhatodine. The docking score indicates that there was no favorable binding between the protein and the ligand.

Bax docked with Adhatodine:

Docking score: -8.80 kcal/mol

Distance: 1 Å

Bax demonstrated a relatively strong binding interaction with Adhatodine, as indicated by the low docking score. The ligand is positioned very close to the protein, with a distance of 1 Å.

Bcl-2 docked with Adhatodine:

Docking score: -8.52 kcal/mol

Distance: 3 Å

Bcl-2 exhibited a moderate binding interaction with Adhatodine. The docking score suggests a favorable binding affinity, and the distance of 3 Å indicates a moderate proximity between the ligand and the protein.

Bcl-XL docked with Adhatodine:

Docking score: No docking interaction

Bcl-XL did not show any significant binding interaction with Adhatodine. The docking score suggests that there was no favorable binding between the protein and the ligand.

Caspase 3 docked with Caffeic acid:

Docking score: -8.56 kcal/mol

Distance: 1 Å

Caspase 3 demonstrated a relatively strong binding interaction with Caffeic acid, as indicated by the low docking score. The ligand is positioned very close to the protein, with a distance of 1 Å.

Caspase 9 docked with Caffeic acid:

Docking score: -8.42 kcal/mol

Distance: 3 Å

Interpretation: Caspase 9 showed a favorable binding interaction with Caffeic acid. The docking score suggests a reasonably strong binding affinity, and the distance of 3 Å indicates a moderate proximity between the ligand and the protein.

Bax docked with Caffeic acid:

Docking score: -7.80 kcal/mol

Distance: 4 Å

Bax exhibited a moderate binding interaction with Caffeic acid. The docking score suggests a relatively favorable binding affinity, and the ligand is positioned at a distance of 4 Å from the protein.

Bcl-2 docked with Caffeic acid:

Docking score: -10.68 kcal/mol

Distance: 2 Å

Bcl-2 showed a very strong binding interaction with Caffeic acid, as indicated by the low docking score. The ligand is positioned relatively close to the protein, with a distance of 2 Å.

Bcl-XL docked with Caffeic acid:

Docking score: -8.31 kcal/mol

Distance: 3 Å

Bcl-XL exhibited a moderate binding interaction with Caffeic acid. The docking score suggests a relatively favorable binding affinity, and the distance of 3 Å indicates a moderate proximity between the ligand and the protein.

The differences in docking results could be due to the specific molecular properties of the ligands and the proteins. Each protein has a unique binding site and structural characteristics, which can influence the binding affinity with different ligands. Additionally, ligands can have different chemical properties and conformations, which can affect their interactions with proteins.

It is important to note that docking is a computational method and the results are predictions based on algorithms and scoring functions. While docking can provide valuable insights into potential binding interactions, experimental validation is necessary to confirm the actual binding affinities and interactions between proteins and ligands.

In summary, the docking results indicate the predicted binding strengths and distances between the proteins and the ligands. A lower docking score generally indicates a stronger binding interaction, and the distances provide insights into the spatial arrangement of the ligand and the protein.

References

Shi J, Cao HF, Wang CF, Gao S, Wang JY, Zhao L, Fei Y and Fu, Y. (2023). In silico approach of novel HPPD/PDS dual target inhibitors by pharmacophore, AILDE and molecular docking. J Taiwan Institute Chem Engineers 143: 104711.

Yeşilkaynak T, Özkömeç FN, Çeşme M, Demirdöğen RE, Sezer CV, Kutlu HM and Emen FM. (2023) Novel thiourea derivative compounds: Thermal behavior, biological evaluation, Hirshfeld surfaces and frontier orbitals analyses, in silico ADMET profiling and molecular docking studies. J Molec Structure 1280: 135086.