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In-vitro and *In-silico* Studies of a Phenylpropanoid Compound Isolated from *Sterculia quadrifida* Seeds and Its Inhibitory Effect on Matrix Metalloproteinase-9

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ABSTRACT

Previous investigation discovered a phenylpropanoid compound called (2E,4E)-1,5-diphenylpenta-2,4-dien-1-one in the chloroform fraction of *Sterculia quadrifida* seeds. This compound exhibited notable cytotoxic effects, displaying IC_{50} values of 2.29, 9.93, 18.09, and 12.12 µg/mL in 4T1, MCF-7, MDA-MB-435, and T47D breast cancer cell lines respectively. In our current study, we performed an MMP-9 enzyme inhibition test using the fluorescence resonance energy transfer (FRET) assay and utilized molecular docking and molecular dynamics simulations to explore the compound's mechanism of action. The results revealed that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one effectively inhibited the MMP-9 enzyme, achieving a level of 95.78% inhibition with an IC_{50} value of 34.78 µg/mL. Furthermore, the molecular docking and dynamics simulations indicated robust and stable interactions between (2E,4E)-1,5-diphenylpenta-2,4-dien-1-one and the enzyme's catalytic site. In summary, this study offers significant insights into the development of herbal medicines with promising potential as anti-cancer drugs.

Keywords: (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, breast cancer, MMP-9, *Sterculia quadrifida*

Introduction

Breast cancer represents a significant public health concern necessitating early detection and treatment to mitigate mortality rates.[1] Metastasis, the process by which cancer spreads to distant sites, contributes substantially to treatment failure and increased mortality in breast cancer cases.[2] Triple-negative breast cancer, characterized by the absence of estrogen receptors, progesterone receptors, and the HER2/neu protein, exhibits a heightened propensity for metastasis owing to its invasive and highly metastatic nature.[2] Metastasis entails a range of mechanisms, including cancer cells' secretion of protease enzymes. Among these enzymes, matrix metalloproteinases (MMPs) are pivotal in extracellular matrix degradation, facilitating cancer cell invasion and dissemination.[3] MMP-9, a specific member of the matrix metalloproteinase (MMP) enzyme family, has emerged as a prospective biomarker for aggressive subtypes of breast cancer, particularly triple-negative breast cancer. Consequently, the selective inhibition of MMP-9 holds promise as a therapeutic strategy to impede the metastatic progression of breast cancer. However, the availability of specific MMP-9-targeting drugs is currently limited.

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As a result, ongoing research endeavors are actively exploring chemical compounds that exhibit potential as selective MMP-9 inhibitors to uncover viable drug candidates for curtailing the enzymatic activity of MMP-9 in breast cancer.[4]

The compound (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one was previously isolated in our study (Figure 1). It was obtained from the chloroform fraction derived from the seeds *of Sterculia quadrifida*. It has been reported to exhibit cytotoxic activity. In the cytotoxicity assay conducted, (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one displayed IC_{50} values of 2.29, 9.93, 18.09, and 12.12 µg/mL against the 4T1, MCF-7, MDA-MB-435, and T47D breast cancer cell lines, respectively.[5] Furthermore, the selectivity index (SI) values indicated that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one exhibited selectivity towards the cancer cells. Specifically, the SI value was 12.23 for 4T1 cells, 8.92 for MCF-7 cells, and 9.78 for MDA-MB-435 cells. Notably, (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one did not show toxicity towards Vero cells (normal cells), as indicated by an SI value greater than 2.[6] Of significance is the high cytotoxicity observed in 4T1 cells, which represent triple-negative breast cancer. Consequently, the potent cytotoxic activity of (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one in 4T1 cells suggests its potential for further investigation as an inhibitor of MMP-9 enzymes.

In continuation of our previous research, this current study focuses on evaluating the inhibitory effect of (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one on MMP-9 enzyme activity using an in-vitro FRET assay.[7] The rationale for this investigation stems from the cytotoxicity results of aurone compounds obtained from previous tests conducted on 4T1 and MDA-MB-435 cells, which represent triple-negative breast cancer cells. The study's primary objective is to assess the potential of (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one to inhibit the MMP-9 enzyme to impede the metastatic process of breast cancer cells. Subsequently, the study employs molecular docking and molecular dynamics simulation techniques to explore the interaction mechanism between (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one and the active site of the MMP-9 enzyme.

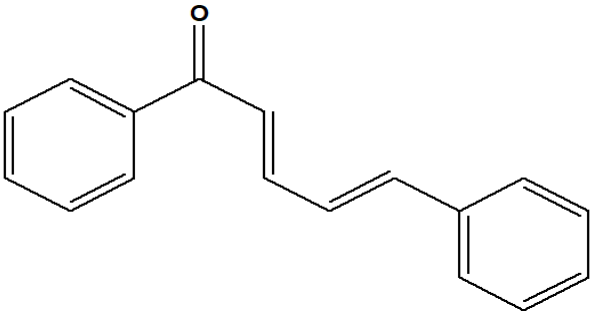

Figure 1: Chemical structure of (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one isolated from *S. quadrifida* seeds

Materials and methods

General

The phenylpropanoid compound, specifically (2E,4E)-1,5-diphenylpenta-2,4-dien-1-one, was obtained from previous scientific investigations. The MMP-9 enzyme kit, sourced from BioVision, consisted of lyophilized MMP-9, a FRET-based MMP-9 substrate (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg), MMP-9 assay buffer, and the NNGH inhibitor (N-isobutyl-N-(4-methoxyphenylsulfonyl)-glycyl hydroxyamic acid) which served as the positive control. The PEX9 1ITV protein structure was acquired from the Protein Data Bank (PDB, www.rcsb.org). Molecular docking was performed using Autodock Vina integrated into Pyrx 0.9.9, and the resulting data were visualized using Biovia Discovery Studio 2016 (www.accelrys.com) and PyMol (www.pymol.org). The computational tasks were carried out on a Lenovo laptop equipped with a Core i5 processor, running on the Windows 10 operating system, with 8 GB of RAM and a 500 GB hard disk drive.

In vitro MMP-9 inhibition assay

The lyophilized MMP-9 enzyme was reconstituted by adding 110 µL of 30% glycerol in deionized water. Subsequently, 550 µL of the enzyme buffer was added to prepare the enzyme for use. The sample was prepared by dissolving it in 1 µL of DMSO, resulting in a microtube's stock concentration of 20,000 µg/mL (ppm). The final concentration of DMSO in the well plate was 1%. Blanks were prepared by adding 100 µL of the MMP-9 test kit buffer to each well of a 96-well plate. Then, 44 µL of the MMP-9 test buffer, 1 µL of the compound solution, and 5 µL of the MMP-9 enzyme were added to the wells. This resulted in a final sample concentration of 200 µg/mL. A solvent control was created by adding 40 µL of the MMP-9 test buffer, 5 µL of the solvent used (ethanol), and 5 µL of the MMP-9 enzyme to the wells. A negative control was prepared by pipetting 45 µL of the MMP-9 test kit buffer and 5 µL of the MMP-9 enzyme into the wells. The positive control consisted of 43 µL of the MMP-9 test buffer, 2 µL of NNGH inhibitor, and 5 µL of the MMP-9 enzyme. All samples, blanks, solvent, negative, and positive control were incubated for 30 minutes at 37°C. After incubation, 1 µL of the FRET-based peptide MMP-9 substrate and 49 µL of buffer was added to the sample, solvent control, negative control, and positive control, followed by another incubation for 60 minutes at 37°C. The fluorescence was then measured using a Microplate Reader at 325/393 nm.[8]

Molecular docking

The crystallographic structure of the PEX9 protein was retrieved from the Protein Data Bank (PDB) under the identification code 1ITV. The native ligand utilized for docking was a sulfate ion, which was initially separated from the PEX9 structure using PyMol.[9] The polar hydrogen atoms were retained in the preparation, and Kollman charges were assigned to the protein. The molecular docking process was carried out employing AutoDock Vina integrated into the PyRx 0.9.9 software, utilizing a grid with a completeness value of 8 and dimensions of 25, 25, and 25 along the x, y, and z axes, respectively. The centre coordinates for the grid was set as x = -43.03, y = -32.12, and z =-7.32. To evaluate the validity of the docking results, the Root-Mean-Square Deviation (RMSD) value was considered, and docking poses with RMSD values less than 2Å were deemed acceptable and reliable.[10]

Molecular dynamic simulation

Molecular Dynamics (MD) simulations were conducted using YASARA Structure version 14.12.2 on a Microsoft Windows 10 operating system. The YAMBER Force field was employed as the force field for the simulations. The calculation of Coulomb distance interactions employed the Ewald particle algorithm, while the Van der Waals force was limited to a range of 8 Å. A cube-shaped simulation box was constructed, encompassing the molecules under investigation, with a distance of 5 nm between the molecules and the box boundaries. The dimensions of the simulation box were set to 50 × 50 × 50 Å, with a value of n = 6 to determine the number of water molecules within the box. The boundaries of the simulation box were treated as periodic, allowing the simulation to account effectively for the system's behaviour. The water density within the simulation was set to 1 g/cc at a temperature of 298 K. The simulations were conducted for 10 ns, with snapshots captured every 100 ps for analysis and observation.[11]

Statistical analysis

Statistical analysis was performed by linear regression method using Microsoft Excel software.

Results and Discussion

MMP-9 in-vitro assay

The experimental findings regarding the MMP-9 enzyme demonstrated notable outcomes. Among the compounds tested, Cisplatin exhibited the most substantial inhibitory effect, with a value of 96.89%. Following closely behind was NNGH, with an inhibitory value of 96.69%, while (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one displayed a slightly lower inhibitory value of 95.78%. The concentrations required to inhibit the MMP-9 enzyme effectively were examined to evaluate the compounds' inhibitory potential further, resulting in the determination of IC_{50} values. The IC_{50} value signifies the concentration at which 50% of the enzyme's activity is hindered. Among the compounds, NNGH, utilized as a control inhibitor for the MMP-9 enzyme, displayed the highest IC_{50} value at 5.98 µg/mL.

Conversely, (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one exhibited a relatively higher IC_{50} value of 34.78 µg/mL, indicating a higher concentration requirement for inhibiting 50% of the MMP-9 enzyme's activity. Statistical analysis was performed to assess the significance of the IC_{50} values, particularly in comparison to NNGH. The results obtained from this analysis, as presented in Table 1, confirmed a significant difference between the IC_{50} value and NNGH.

Molecular docking

Molecular docking is a computational technique used in drug discovery and molecular biology to predict and analyze the binding interactions between a small molecule (ligand) and a target macromolecule (protein or nucleic acid).[12] It plays a crucial role in understanding the molecular mechanisms of ligand-target interactions and aids in the design and optimization of potential drug candidates.[13] The molecular docking results indicated that sulfate ions, employed as a control ligand, displayed an RMSD of 0.7 Å, below the 2 Å threshold. This suggests that the molecular docking parameters were consistent and reliable.[14] The data presented in Table 2 encompassed a range of free energy values (ΔG binding) spanning from -3.5 to -6.2 kcal/mol. The analysis revealed that the NNGH compound, utilized as a positive control, exhibited the highest binding energy of -6.2 kcal/mol, followed by (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one with a value of -5.7 kcal/mol, and sulfate ions with a value of -3.5 kcal/mol. These free energy values indicate that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one exhibits stronger interactions as an inhibitor of the MMP-9 enzyme than sulfate ions (Figure 2). Furthermore, this study demonstrated a positive correlation between the results obtained from molecular docking and in vitro assays, providing evidence that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, which displayed the highest free energy of binding, also exhibited inhibitory activity against the MMP-9 enzyme (Table 1).

The MMP-9 enzyme contains an active site known as the catalytic domain, located on chain A, where specific amino acids such as glutamate14, arginine106, and glutamate157 play a crucial role in generating stimuli.[15] The results obtained from molecular docking reveal that the control ligand, sulfate ions, can form hydrogen bonds with glutamate60, glycine154, and glutamate14 while establishing hydrophobic interactions with phenylalanine59 and alanine13 (Figure 3). Similarly, NNGH exhibits the ability to form hydrogen bonds with glutamate14, glutamate157, glutamine154, glutamate60, and arginine106, and it also engages in hydrophobic interactions with methionine112, phenylalanine59, alanine104, valine158, phenylalanine153, valine152, and glycine16. Furthermore, when analyzing (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, it is observed that it forms hydrogen bonds with glutamate157, serine172, lysine158, asparagine177, phenylalanine142, methionine141, leucine113, serine107, arginine106, and glutamine154 (Figure 4). These findings indicate that the test compounds can establish hydrogen bonds and hydrophobic interactions with the essential amino acids in the catalytic domain of the MMP-9 enzyme.

Molecular dynamic simulation

Molecular dynamics (MD) simulations are computational methods used to study the movement and behavior of atoms and molecules over time. MD simulations simulate the physical interactions and dynamics of a system by numerically solving the equations of motion for each atom or molecule, allowing researchers to observe the system's behavior at the atomic level.[16] Molecular dynamics simulations further analyzed the interactions between the control ligand and the isolate as an MMP-9 enzyme inhibitor.[17] The RMSD analysis of the protein-native ligand complex (SO_4) revealed deviations ranging from 0.414 to 1.693 Å (Figure 5), indicating stable interaction stability. Similarly, the RMSF analysis of the protein-native ligand complex showed deviations ranging from 0.43 to 2.68 Å (Figure 6), suggesting a stable interaction. Furthermore, the RMSD analysis of NNGH indicated deviations ranging from 0.383 to 1.641 Å (Figure 5). The RMSF analysis of the protein-NNGH complex displayed deviations ranging from 0.44 to 3.13 Å (Figure 6), with the largest deviation observed at residue 62 during the 10 ns simulation. These findings provide valuable insights into the stability of the protein-ligand interactions. The RMSD and RMSF analyses enable us to observe the dynamic behavior of the complexes and identify significant fluctuations or deviations.[17]

The analysis of the RMSD values for the protein-(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one complex revealed deviations ranging from 0.417 to 1.693 Å (Figure 5). Initially, the RMSD values remained stable during the 4.3 ns of simulation, but they increased afterward. Additionally, the analysis of the RMSF values for the protein-(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one complex showed fluctuations ranging from 0.32 to 3.89 Å throughout the 10 ns simulation (Figure 6). Notably, residues 30 to 34 fluctuated at 2.54 Å, while residue 18 fluctuated at 3.89 Å. The binding energy analysis indicated that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one (-243.832 kJ/mol) displayed a higher energy value, suggesting stronger binding and interactions. These findings align with the stability values, indicating that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one exhibits superior binding stability.

(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, a phenylpropanoid compound, has demonstrated inhibitory effects on breast cancer cell migration in various studies. Tian *et al.*[18] discovered valtrate, derived from the *Valeriana jatamansi* plant, which effectively inhibits the migration of MDA-MB-231 breast cancer cells by suppressing the expression of MMP-2 and MMP-9. Valtrate also modulates the levels of p-Akt (Ser 473), cyclin B1, and caspase 8, while increasing the expression of p21, p-cdc2, cleaved-caspase 3, cleaved-caspase 7, and poly (ADP-ribose) polymerase (PARP). Moreover, Vo *et al.*[19] isolated phenylpropanoid glycosides, baicalin and isoverbascoside, from the Staurogyne concinnula plant, revealing their ability to inhibit the expression of MMP-2 and MMP-9. Additionally, novel phenylpropanoid compounds, namely (7S,8R)-1-(1-ethoxy-2-hydroxypropyl)-2-methoxy-3,4-(methylenedioxy)benzene, (7S,8S)-1-(1-ethoxy-2-hydroxypropyl)-2-methoxy-3,4-(methylenedioxy)benzene, and (7S,8R)-1-(1-methoxy-2-hydroxypropyl)-2-methoxy-3,4-(methylenedioxy)benzene, were isolated from the *Chloranthus anhuiensis* plant, which have been proven to inhibit the expression of MMP-9. These findings underscore the potential of phenylpropanoid compounds as inhibitors of MMP-2 and MMP-9, shedding light on their mechanisms of action in suppressing breast cancer cell migration.[20]

Phenylpropanoid compounds have been identified as potential inhibitors of MMP-9 (matrix metalloproteinase-9).[21] MMP-9 is an enzyme involved in the degradation of extracellular matrix components and plays a crucial role in various physiological and pathological processes, including tissue remodelling, inflammation, and cancer metastasis. Studies have shown that certain phenylpropanoid compounds possess inhibitory effects on MMP-9 activity. These compounds are characterized by their chemical structure, which includes a phenylpropane backbone with various functional groups or substituents.[22] The specific mechanism of inhibition may involve binding to the active site of MMP-9 or modulating its activity through other molecular interactions.

The potential of phenylpropanoid compounds as MMP-9 inhibitors has attracted attention in drug discovery and development. Researchers aim to harness the inhibitory properties of these compounds to develop novel therapeutic agents for conditions associated with excessive MMP-9 activity, such as cancer, cardiovascular diseases, and inflammatory disorders.[21] Further studies are ongoing to investigate the structure-activity relationships of different phenylpropanoid compounds, optimize their inhibitory potency, and assess their potential as effective and selective MMP-9 inhibitors. Such research holds promise for developing new drugs targeting MMP-9 and may contribute to advancing therapeutic strategies for various diseases involving MMP-9 dysregulation.

Conclusion

The phenylpropanoid compound, (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one, displays inhibitory effects against MMP-9, as evidenced by its IC_{50} value of 34.78 µg/mL. Examination of the binding energy indicates that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one exhibits a higher energy value, suggesting stronger binding interactions. These findings align with the stability analysis, which suggests that (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one demonstrates enhanced binding stability.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 1: MMP-9 *in-vitro* test results

Compound	Inhibition (%)	IC_{50} (µg/mL)
Cisplatin	96.89	15.22
N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH)	96.65	5.98
(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one	95.78	34.78

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Table 2: Compound bonding affinity, interactions, and amino acid residues

Ligand	Free energy bond (kcal/mol)	Hydrogen bond	Hydrophobic interaction
Sulfate ion (*native ligand*)	-3,5	Glutamic acid60, glycine154, glutamic acid14	Phenylalanine59, alanine13
N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH)	-6,2	Glutamic acid14, glutamic acid157, glutamine154, glutamic acid60, arginine106	Methionine112, phenylalanine59, alanine104, valine158, phenylalanine153, valine152, glycine16
(2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one	-5,7	-	Glutamic acid157, serine172, lysine158, aspargine177, phenylalanine142, methionine141, leucine 113, serine107, arginine 106, glutamine154

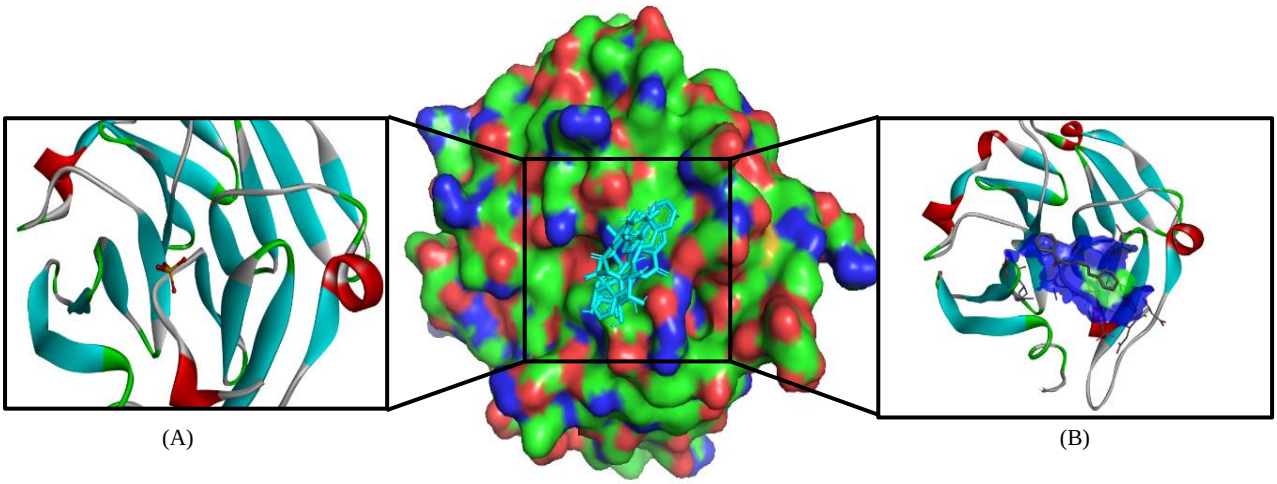

Figure 2: The docking pose of (A) SO_4 ion, (B) (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one

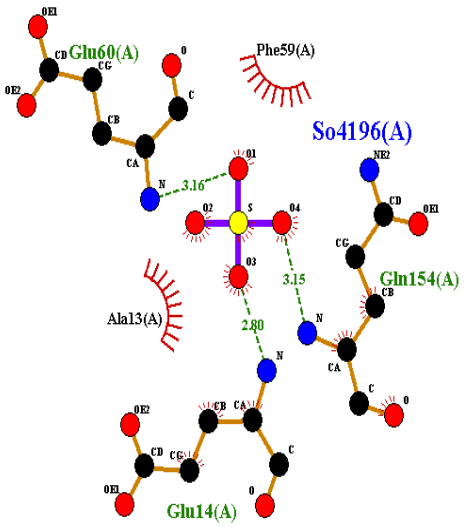

Figure 3: The molecular interactions of sulfate ions with amino acids in the MMP-9 enzyme

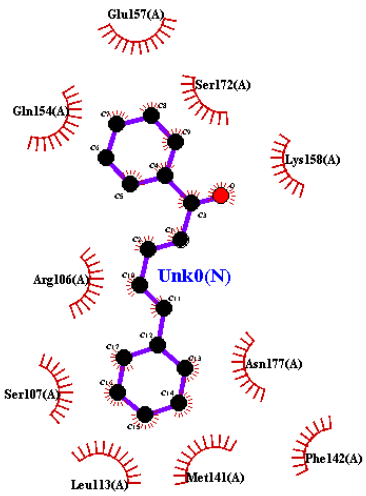

Figure 4: The molecular interactions of (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one with amino acids in the MMP-9 enzyme.

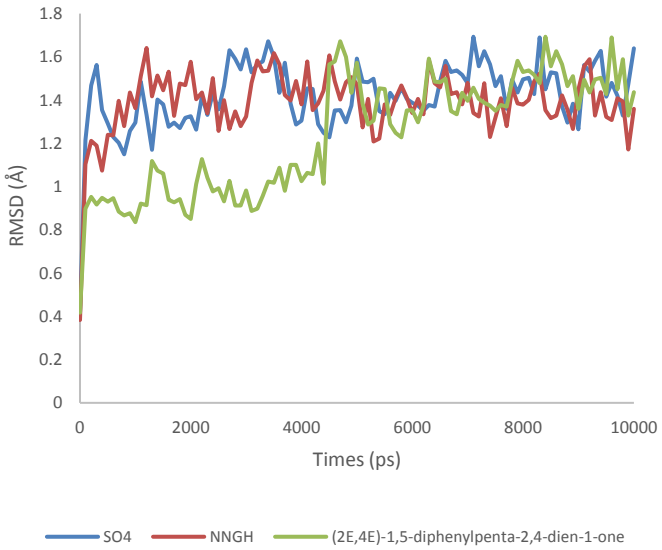

Figure 5: RMSD (Root Mean Square Deviation) of sulfate ion (blue), NNGH (orange), and (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one (gray) during a 10 ns molecular dynamics simulation,100 frames.

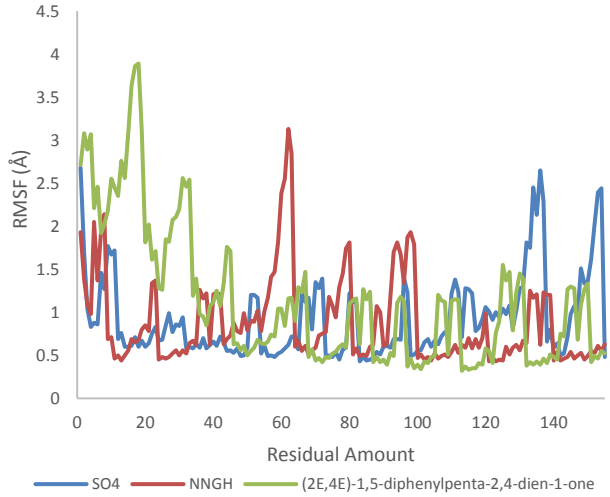

Figure 6: RMSF (Root Mean Square Fluctuation) of sulfate ion (blue), NNGH (orange), and (2*E*,4*E*)-1,5-diphenylpenta-2,4-dien-1-one (gray) during a 10 ns molecular dynamics simulation,100 frames.

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