

<https://doi.org/10.48047/AFJBS.6.15.2024.13789-13808>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

NOVEL SCHIFF BASE DERIVATIVES FROM ANILINE: A DUAL APPROACH OF ANTIBACTERIAL EFFICACY AND MOLECULAR DOCKING.

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Volume 6, Issue 15, Oct 2024

Received: 15 Aug 2024

Accepted: 25 Sep 2024

Published: 21 Oct 2024

doi: [10.48047/AFJBS.6.15.2024.13789-13808](https://doi.org/10.48047/AFJBS.6.15.2024.13789-13808)

Abstract

In medicinal chemistry, Schiff bases have garnered considerable interest because of their diverse array of pharmacological properties. Many derivatives of Schiff bases demonstrate antimicrobial, antiviral, antimalarial, and anticancer activities, making them promising candidates for drug development. Their therapeutic potential is largely owing to their capacity to engage with biological molecules, including proteins and nucleic acids. In our research, we synthesized Schiff base intermediate molecules from aniline and utilized ¹HNMR, ¹³CNMR, and LCMS analysis to elucidate the structures of the synthesized compounds. Additionally, we conducted molecular docking studies to analyse the antibacterial efficacy of the most promising compound. The compound (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1, 3-thiazolidin-4-one) demonstrated a strong binding affinity with the protein. Furthermore, in vitro antibacterial activity tests revealed that the synthesized compounds exhibited significant antibacterial activity.

Keyword: Thiazolidin, Aniline, Antimicrobial, Molecular docking, Binding affinity.

INTRODUCTION

Schiff bases, named after the chemist Hugo Schiff, are a class of compounds characterized by the functional group $R_1R_2C=NR_3$. These compounds have gained considerable attention due to their versatile applications in various fields, including coordination chemistry, material science, and medicinal chemistry. Among them, Schiff bases derived from aniline are particularly interesting because of their potential biological activities and their ability to form stable complexes with transition metals [1].

The growth of new antimicrobial agents is dangerous in the fight against bacterial infections, especially in the framework of rising antibiotic resistance. Schiff base derivatives have shown promise as potent antibacterial agents due to their unique structural properties, which enable them to interact effectively with bacterial cell components. In this context, the synthesis of novel Schiff base derivatives from aniline is of significant interest, offering a potential pathway to discovering new antibacterial agents [2].

Molecular docking is a computational technique used to predict the interaction between a drug candidate and its target protein at the molecular level. This method allows researchers to visualize and analyze the binding affinity and specificity of compounds to their biological targets, providing valuable insights into their mechanism of action and guiding the design of more effective drugs [3].

This study explores a dual approach to evaluating the efficacy of novel Schiff base derivatives from aniline. Firstly, the antibacterial efficacy of these compounds is assessed through in vitro studies to determine their potential as antimicrobial agents. Secondly, molecular docking studies are employed to elucidate the interactions between the Schiff base derivatives and bacterial target

proteins, aiming to understand the structural basis of their antibacterial activity and to identify the most promising candidates for further development [4].

By combining experimental antibacterial testing with molecular docking simulations, this research aims to provide a comprehensive understanding of the potential of aniline-derived Schiff bases as novel antibacterial agents [5]. The findings of this study could pave the way aimed at the growth of new healing agents to combat bacterial infections and address the urgent need for new antibiotics in the face of increasing antibiotic resistance. In our research, we synthesized Schiff base intermediate molecules from aniline and utilized ¹H NMR, ¹³C NMR, and LCMS analysis to elucidate the structures of the synthesized compounds. Additionally, we conducted molecular docking studies to analyse the antibacterial efficacy of the most promising compound [6].

METHODOLOGY

PREPARATION OF SCHIFF BASE INTERMEDIATE MOLECULES

Equal quantities (0.01M) of 4-Chloro-3-Nitroaniline and different aldehydes were dissolved in 15 mL of methanol. To this mixture, 0.5 mL of acetic acid was added. The mixture was then refluxed for two hours. After the reaction concluded, as verified by TLC (thin-layer chromatography), the mixture underwent cooling and was then poured into water, leading to the formation of solid precipitation. The solid was then filtered, washed with water, and crystallized from ethanol to obtain the corresponding Schiff base intermediate compounds [7].

GENERAL PROCEDURE FOR SYNTHESIZING SUBSTITUTED 4-THIAZOLIDINONE MOLECULES

Mix 0.01 moles of Schiff's base intermediates with 0.01 moles of thioglycolic acid in 20 milliliters of 1,4-dioxane to create a solution. Add a tweak of anhydrous zinc chloride to the solution. Reflux

the mixture for 8 hours. Separate the solid product, filter it, and rinse with a sodium bicarbonate solution. Recrystallize the solid from ethanol. The structure of the final synthesized compound was confirmed using ¹H NMR, ¹³C NMR, and LCMS analysis (refer to Scheme 1) [8-10]

NMR CHARACTERISATION ANALYSIS

The ¹³C nuclear magnetic resonance (NMR) and ¹H NMR spectra were captured utilizing a Bruker 400 megahertz (MHz) spectrometer, operating at 300 MHz, employing tetramethylsilane as the internal reference standard and DMSO-d₆ as the solvent. These analyses were conducted at the University of Madras. The mass spectra were acquired utilizing an LC-MS 2010 SHIMADZU mass spectrometer, as depicted in Figures 1-3 [11]

DOCKING STUDIES

A research endeavour employing molecular docking techniques was conducted to evaluate the optimal arrangement of the interaction between proteins and ligands. The CDOCKER (CHARMm-based DOCKER) procedure, integrated into DS, was working to investigate potential binding modes stuck between ligands and target proteins. This method permits for thorough ligand plasticity and utilizes CHARMm force arenas. The assessment of ligand binding empathy involved the calculation of various parameters, with CDOCKER energy, CDOCKER interaction energy, hydrogen connexion, binding energies, protein energy, and ligand-protein complex energy. Negative values of CDOCKER energy were described, with lower values representative stronger compulsory affinity between the ligands and the objective protein. [12-14].

ANTIBACTERIAL ASSAY

TEST ORGANISM

Acceptable Gram-positive organisms include *Escherichia coli*, *Staphylococcus aureus*, various *Salmonella* species, *Vibrio parahaemolyticus*, *Aeromonas* species, *Klebsiella* species, various

Vibrio species, Proteus species, Pseudomonas aeruginosa, and various Bacillus species. The main methods employed for bacterial study centered around Gram stain. The data and values were together by the Department of Microbiology at the Christian Medical College in Tamil Nadu, India, and IMPTECH in Chandigarh. Each vaccine was introduced into 3 mL of Mueller Hinton Broth and then incubated for 24 hours at 37°C. Subsequently, the cultures were diluted [15-17].

PREPARATION OF INOCULUMS

Bacterial inoculum were primed by culturing cells in Mueller-Hinton broth (Hi-media) at 37°C designed for 24 hours. The resulting cell suspensions were diluted with sterile MHB to achieve initial cell counts of approximately 10^{-4} CFU/mL. Sabouraud dextrose (SDB) yeast was grown for 48 hours at 28°C [18-20].

DISC DIFFUSION METHOD

Petri dishes were ready with 20 mL of Mueller Hinton Agar (MHA) found from Hi-media, Mumbai. The antibacterial assessment was directed via the disc-diffusion method. A deferral of 100 µL, containing 10^{-8} CFU/mL of fungal culture, was practical onto the solidified moderate and permissible to air-dry for 10 minutes. Various concentrations (500 µg/disk) were verified for their anti-Hungary properties. Ciprofloxacin (10 µg/disk) was used as a positive control, with a diffusion time of 30 minutes at 27°C on the medium's surface. Respective solvents were utilized to prepare negative control disks. [21, 22].

RESULT AND DISCUSSION

Spectral characterization of **(3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one)**¹H-NMR (400 MHz, DMSO-d₆): δ 3.66 (1H, d, *J* = 15.9 Hz, 1,3-thiazolidin-CH₂), 3.82 (1H, d, *J* = 15.9 Hz, 1,3-thiazolidin-CH₂), 6.39 (1H, s, 1,3-thiazolidin-CH), 7.18-7.62 (6H, m, Aromatic

CH), 8.09 (1H, dd, $J = 1.5, 0.4$ Hz, Aromatic NO₂-CH).¹³C NMR (400 MHz, DMSO-d₆): δ 46.3 (1C, s, 1,3-thiazolidin-CH₂), 65.7 (1C, s, 1,3-thiazolidin-CH), 105.0 (1C, s, Aromatic -NO₂-CH), 127.18-128.32 (1C, s, Aromatic-CH), 147.5 (1C, s, Aromatic-CH), 149.4 (1C, s, Aromatic-CH), 171.3 (1C, s, 1,3-thiazolidin-C=O). LC/MS analysis Calculated for C₁₅H₁₀Cl₂N₂O₃S molecular weight: 367.9 m/z, found: 369.1 [M+1] m/z. White Powder, Yield 78%, m.p 57-60⁰C. (HNMR, CNMR, LCMS analysis Figure 1-3) [23-26].

ANTIMICROBIAL ACTIVITY

The compound 3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one, is a thiazolidinone derivative. Thiazolidinones are a class of compounds that have been investigated for their potential antimicrobial properties.

The presence of chloro and nitro groups in the phenyl rings, as well as the thiazolidinone moiety, contributes to the compound's antimicrobial activity. These functional groups can interact with microbial targets, disrupting essential cellular processes or structures. The chloro and nitro groups are electron-withdrawing, which can increase the acidity of adjacent hydrogens and enhance the compound's reactivity towards microbial targets. Thiazolidinone derivatives have been found to exhibit various biological activities, including antimicrobial properties. The presence of the thiazolidinone ring in this compound likely plays a crucial role in its antimicrobial activity by facilitating interactions with microbial enzymes or receptors. The spatial arrangement of atoms in the molecule can influence its interactions with microbial targets. The specific arrangement of substituents in this compound may enhance its ability to bind to microbial proteins or enzymes, leading to inhibition of microbial growth or proliferation. Overall, the antimicrobial activity of 3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one likely arises from a combination of its chemical structure, functional groups, and molecular properties, which enable

it to interact with and disrupt microbial targets, ultimately inhibiting microbial growth or killing the microorganisms [27,28].

MOLECULAR DOCKING STUDY

Molecular docking studies involve computational methods to predict how a small molecule, like a drug candidate, interacts with a target protein at the molecular level. In the case of 3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one, or similar compounds, the docking study would aim to predict how this molecule binds to its intended target. This involves preparing both the ligand (the small molecule) and the receptor (the target protein). The ligand is typically prepared by generating a 3D structure, often using software tools like Avogadro or Open Babel. The receptor structure is usually obtained from experimental data, such as X-ray crystallography or homology modeling. In this step, the ligand is docked into the binding site of the receptor. Various docking algorithms are available, such as AutoDock, AutoDockVina, and GOLD. These algorithms search for the most energetically favorable orientation of the ligand within the binding site. After docking, the poses (orientations) of the ligand within the binding site are evaluated and scored based on criteria such as binding affinity, energy, and complementarity to the receptor (Figure 4 and 5). Finally, the results are analyzed to identify the most promising binding poses and interactions between the ligand and receptor. Visualizations, such as molecular graphics, are often used to examine these interactions. In the case of 3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one, the docking study would aim to predict its binding mode and affinity with the target protein of interest. This could be a receptor involved in a disease pathway or a protein target for drug action [29, 30].

CONCLUSION

In summary, we successfully synthesized the compound (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one). The reactions yielded a high quantity of novel heterocyclic compounds, characterized through ¹HNMR, ¹³CNMR, and LCMS analyses. Our investigation into the biological activities of these synthesized compounds revealed that (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one) exhibited particularly promising activity.

By conducting molecular docking studies, we elucidated a potential mechanism underlying the biological activity of (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one). However, further research is necessary to advance the development of the two most promising molecules identified towards drug design [31, 32].

In conclusion, (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one) demonstrates notable biological activity, suggesting its potential as a foundation for the development of further bioactive derivative compounds.

ACKNOWLEDGEMENT

One of the authors T. Senthilkumar thankful to the Department of Chemistry, Bharathiyar University, Coimbatore for providing laboratory facility. Authors are also grateful to SAIF-IIT Madras, Chennai for providing spectral data, chennai Central Research Lab in getting the biological activity results.

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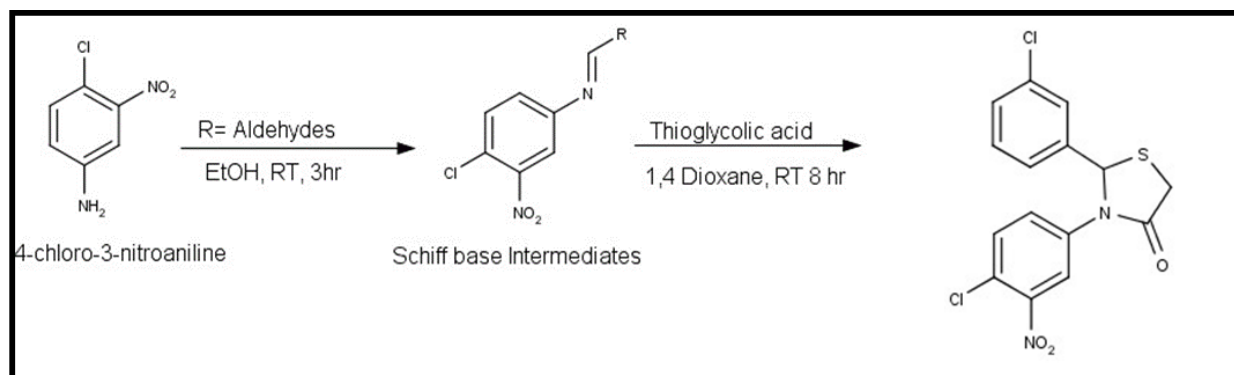
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SCHEME. 1

Novel compound of (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one)

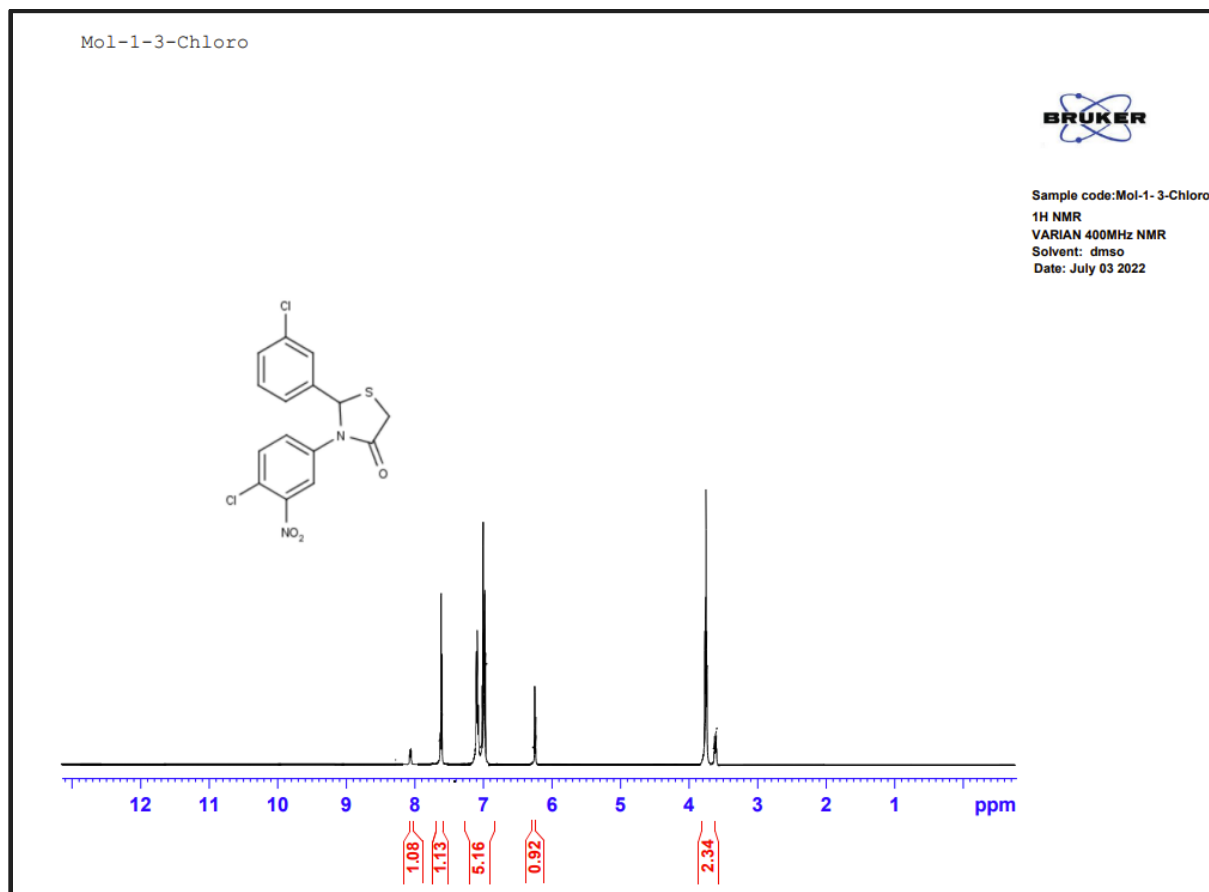


Figure 1. ¹H NMR analysis of (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one)

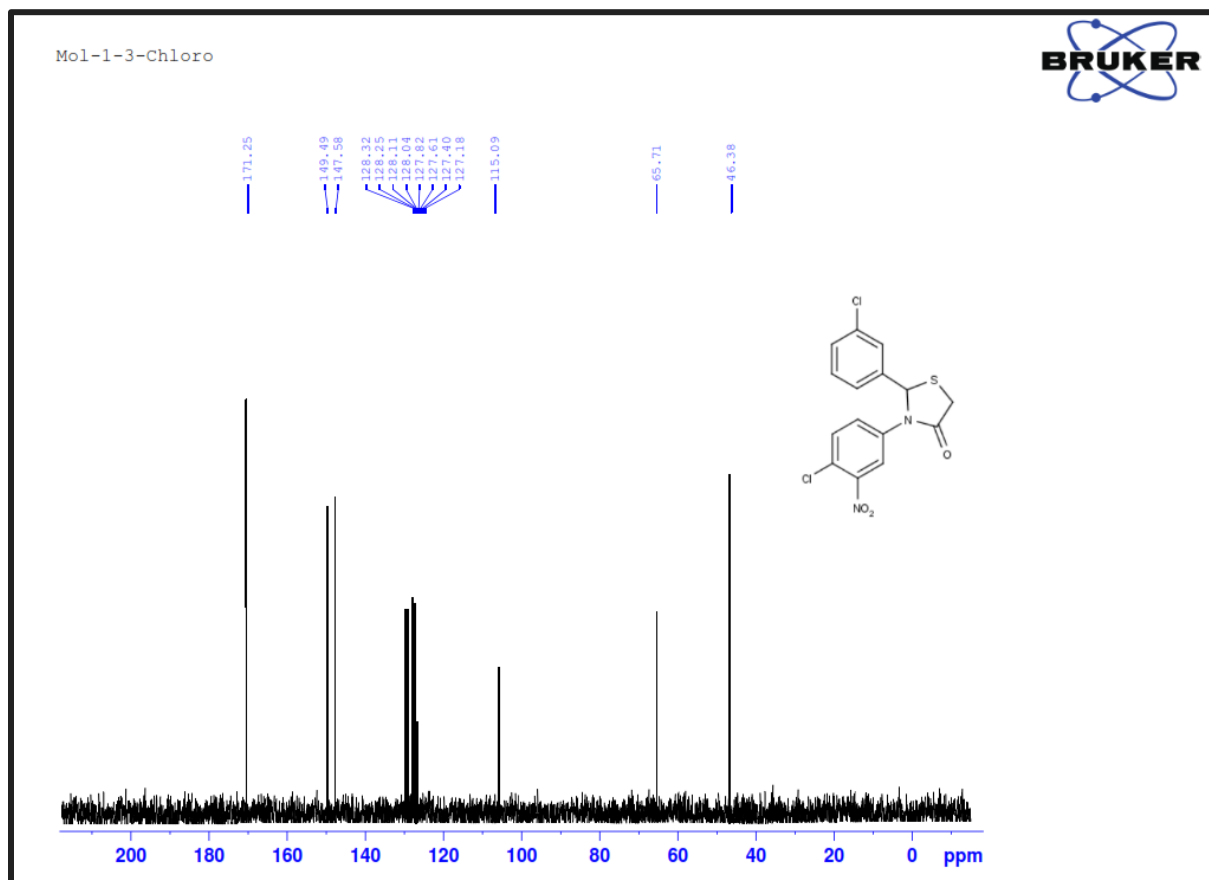


Figure 2. ¹³CNMR analysis of (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one)

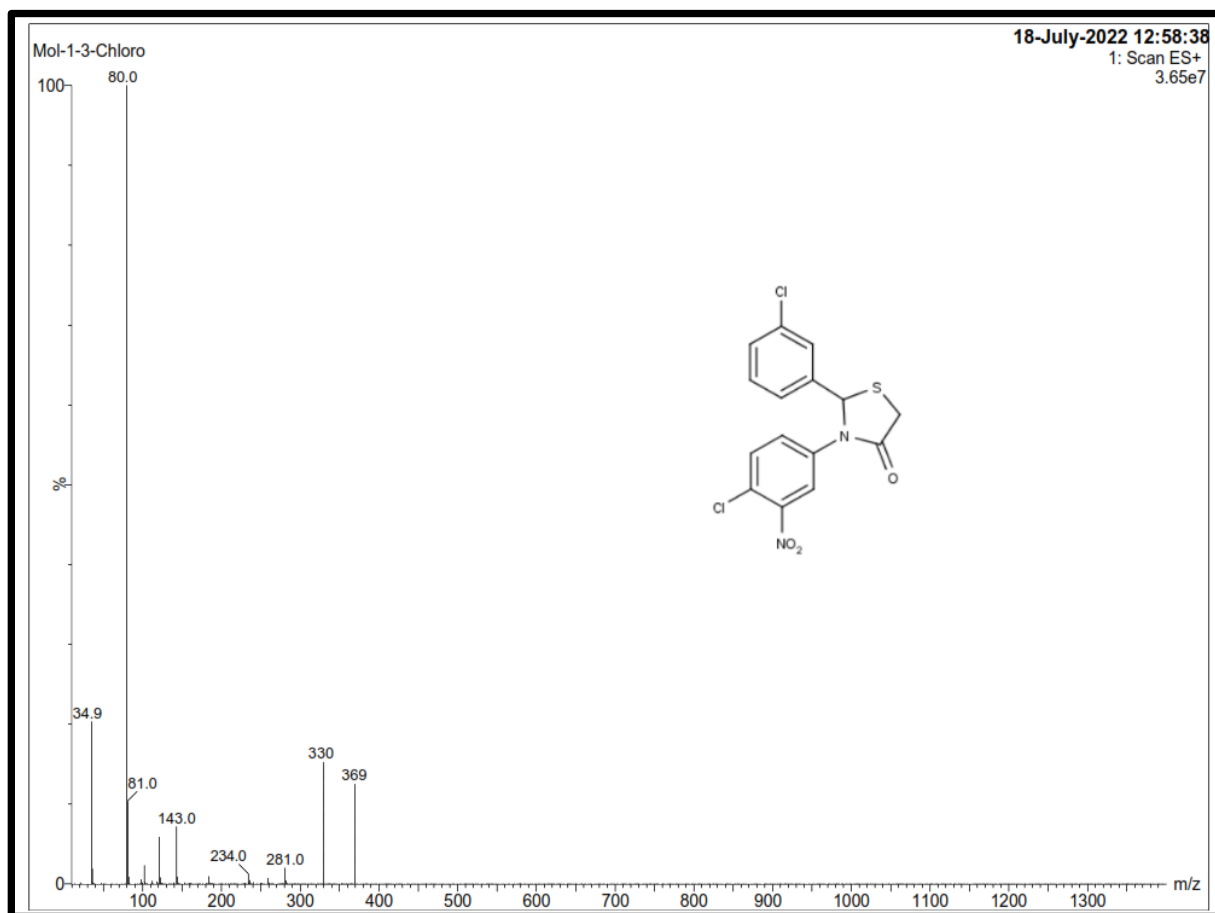


Figure 3. LCMS analysis of (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one)

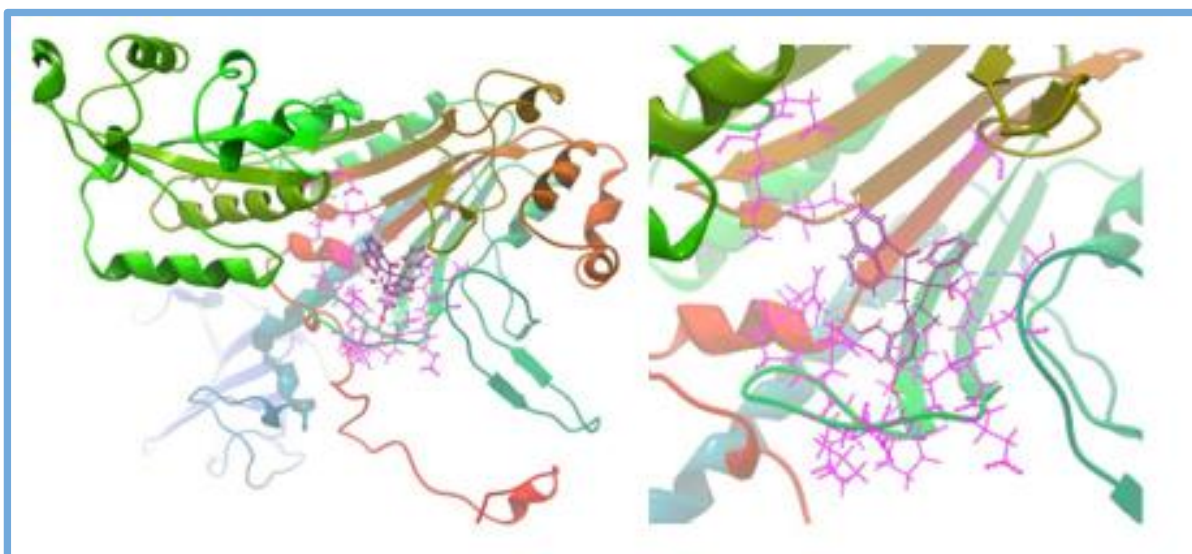


Figure 4. Binding interactions of Aspartyl-tRNA synthetases of *E. coli* and the compound (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1, 3-thiazolidin-4-one). The binding of the (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1, 3-thiazolidin-4-one) in active site of Aspartyl-tRNA synthetases

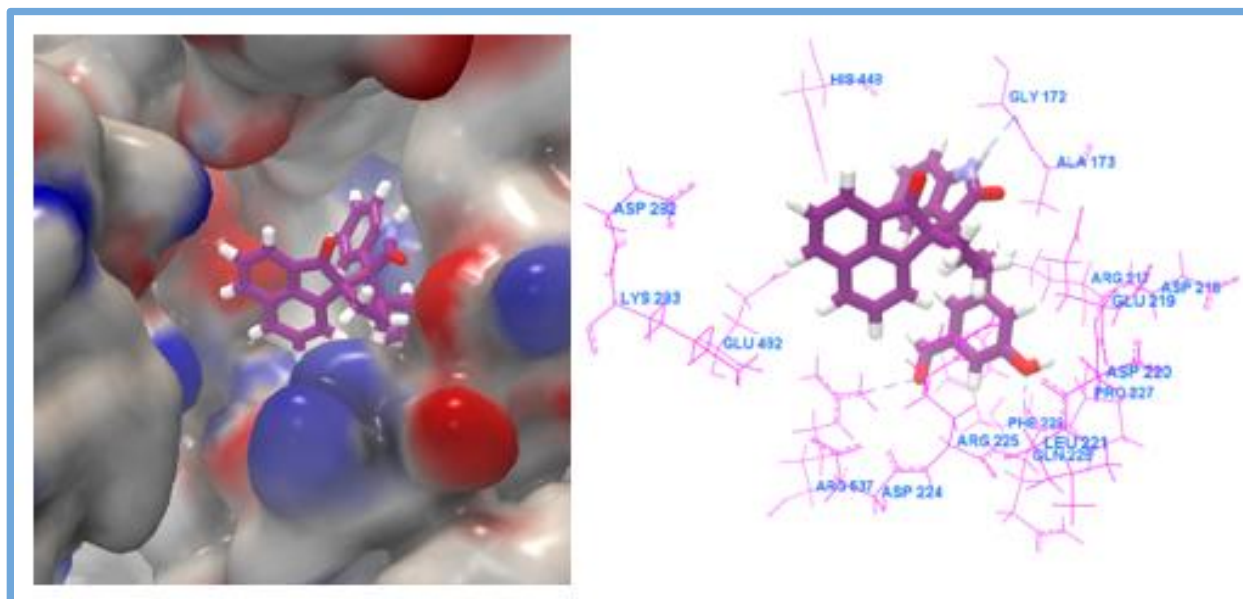


Figure 5. Binding interactions of Aspartyl-tRNA synthetases of *E. coli* and spiro compound (3-(4-chloro-3-nitrophenyl)-2-(3-chlorophenyl)-1,3-thiazolidin-4-one).