Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

Green Synthesis and In Silico Evaluation of a Novel Thiazolidinone Thiourea Scaffold Targeting Orientia tsutsugamushi

Karpagam Ezhilarasan[a] and Satheeshkumar Nagaraj [a] ,Rajalakshmi Ramarajan[*a],

[a]*Department of Chemistry, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu- 608 002.*

*Corresponding author: (Rajalakshmi Ramarajan)

(Received: 10 November 2025 Revised: 23 November 2025 Accepted: 05 December 2025)

KEYWORDS

Thiazolidinone Thiourea Scaffold

ABSTRACT:

A novel series of 1-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)thiourea derivatives was successfully synthesized via a tetrabutylammonium bromide–catalyzed multicomponent condensation involving substituted aldehydes, amines, and thioglycolic acid. The molecular structures of the synthesized 4CTA derivatives were unequivocally confirmed through comprehensive spectroscopic analyses, including FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. In silico studies were conducted against Orientia tsutsugamushi, Candida albicans, and Staphylococcus aureus the etiological agents of scrub typhus, fungal, and bacterial infections, respectively to evaluate the prospective biological efficacy and to correlate computational predictions with experimental findings. The developed synthetic methodology is environmentally benign and operationally efficient, delivering excellent yields (>95%), markedly reduced reaction times, and a broad substrate scope, thereby highlighting its applicability for the rapid construction of biologically relevant thiazolidinone scaffolds. To further elucidate the pharmacological significance, ADMET and ProTox computational assessments were performed, revealing favorable pharmacokinetic profiles and low predicted toxicity. Additionally, Density Functional Theory (DFT) calculations were employed to explore the electronic properties and reactivity descriptors of the active compounds, providing deeper insight into their stability and potential mechanisms of action.

1. Introduction.

Recent research in drug development has focused on organic compounds that possesheteroatoms oxygen, sulphur, and nitrogen due to their critical roles in biological processes[1-3]. One class of these compound is thiazolidine and consisting of a carbonyl group, a nitrogen atom at position 3, and a sulphur atom at position at positions 1, 2, 4, or 5. These compounds and their derivatives are widely studied. The first discovery of thiazolidinones in nature was in penicillin, which contains this structure [4-5]. Many approved medicines have been developed with the 4-thiazolidinone in the core structure.

Thiazolidinone derivatives are known for their many biological effects, including anti-HIV1, antibacterial [6], anti-tuberculosis [7], anti-cancer [8], anti-inflammatory [9], anticonvulsant [10], antioxidant [11], and anti-breast cancer[12], properties. Drugs like thiazolidinone derivatives, which target Streptomyces species, as well as etozoline (used for high blood pressure), pioglitazone (for lowering blood sugar), and ralitoline (an anticonvulsant), have been developed using this structure[13]. There are many ways to produced 4-thiazolidinones, usually involving three main ingredients: A mercapto acid, a carbonyl compound, and an amine [14], Some of the common methods for making these compounds are one-pot, three-component reactions [15–16]. or two-step processes [17–18], each with its own advantages The procedure generally starts with imine formation, followed by its reaction with sulfur, leading to ring closure accompanied by water elimination. Various solvents like diethyl formamide (DMF) [19], 1,4-dioxane ([20].), benzene([21].), and DMSO [22].

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

Recent developments in thiazolidinone derivatives have led to drugs with lower toxicity and better effectiveness. The 4-thiazolidinone ring structure is still very important in drug development. Small changes to the thiazolidinone structure can greatly improve its biological effects. introduce another heterocyclic group to the thiazolidinone structure has been shown to enhance its biological activity [25–30].

The present study focuses on the synthesis of a novel 1-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)thiourea derivative via an eco-friendly multicomponent strategy, followed by comprehensive structural, computational, and pharmacological evaluation. The integration of green chemistry principles with in silico biological and electronic analyses aims to establish a robust framework for the rapid identification of thiazolidinone-based candidates with potential activity against O. tsutsugamushi.

2.Experimental Section

2.1General

A highly pure mixture of Analar-grade compounds was utilized. Metal plates that were previously coated with Merck silica gel 60 F254 were used for thin-layer chromatography (TLC) to analyze all reaction mixtures. A mobile phase consisting of petroleum ether and ethyl acetate was distinctly employed. In (FT-IR) spectra were recorded using a Thermo Nicolet FT-IR Model iS5 Spectrophotometer with KBr discs, and the results were expressed in cm^{-1}. Proton (1H) and carbon Spectra of (13C) NMR were acquired. in $CDCl_3$ using a Bruker 400 MHz NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference for chemical shifts (δ), measured in (ppm). The letters s, d, t, q, and br are used for singlet, doublet, triplet, quartet, and wide, respectively. Multiplets and wide singlets are represented by the letters m and s, respectively.

2.2.Synthesis

2.2.1 (E)-1-(4-chlorobenzylidene)thiosemicarbazide

One mole of thiosemicarbazide and 4-chlorobenzaldehyde were mixed with anhydrous zinc chloride as a catalyst, followed by stirring for twenty minutes at 60°C. Once the reaction was complete, the mixture was removed, and methanol was used to recrystallize the solid product

2.2.2 1-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)thiourea

Equimolar quantities of (E)-1-(4-chlorobenzylidene)thiosemicarbazide (3) and thioglycolic acid (4) were mixed in the presence of a tetrabutylammonium bromide as a catalyst, followed by refluxing for 25 to 35 minutes at 110 °C to synthesize 1-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)thiourea. The reaction mixture was then poured into cold water and maintained at 0 °C for 48 hours. The resulting solid was collected by filtration and purified by recrystallization from benzene."

2.2.3 1-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)thiourea

The title compound was synthesized, yielding 92% of an orange solid. The melting point was observed between 295 and 312 degrees Celsius. The IR (KBr) spectrum showed characteristic absorptions at 3029 cm^{-1} (aromatic C-H), 1628 cm^{-1} (C=O) of the thiazolidinone, 1595 cm^{-1} (C=C), 1274 cm^{-1} (C=S), 1502 cm^{-1} (C-N), 3440 cm^{-1} NH, 3216-3328 cm^{-1} NH2.

The ^{1}H NMR (DMSO) spectrum displayed signals at δ 5.603 ppm (singlet, 2H, SCH_2 of the thiazolidinone), 3.482 ppm (singlet, 1H, NCH of the thiazolidinone), (singlet, 2H, NH2)11.378, (singlet, 1H, NH)11.105 and 7.61-7.99 ppm (multiplet, 4H, aromatic protons).

The ^{13}C NMR spectrum showed signals at δ 41.57 ppm (C5 of the thiazolidinone), 163.88 ppm (C=O of the thiazolidinone), 61.04 ppm (C2 of the thiazolidinone),175.09 ppm (C=S of the thiazolidinone) and a range of signals from 127.86 to129.95 ppm, consistent with aromatic carbons.

2.3 Computational methodology

The ideal structures of several compounds were determined using density functional theory (DFT) with the software programs Gaussian09 and GaussView5 . Molecular properties such as the molecular electrostatic potential (MEP) and the HOMO–LUMO energy gap were analyzed along with geometric features to find the most stable structure. The optimized geometry meets the criteria for further research. To visualize how electrons are distributed within the molecules, an electron

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

localization function (ELF) diagram was generated using the Multiwfn program, following the atoms in molecules (AIM) theory .

2.4 Molecular Docking

The X-ray crystal structure of the tubulin–combretastatin A-4 complex was obtained from the Protein Data Bank (PDB ID: 6UPU, 5V5Z, 7EL1), with a resolution of 2.40 Å and an observed R-value of 0.192 [42]. For the docking studies, the D-chain—where combretastatin A-4 binds was selected. Molecular docking was carried out following the methods described in earlier studies .

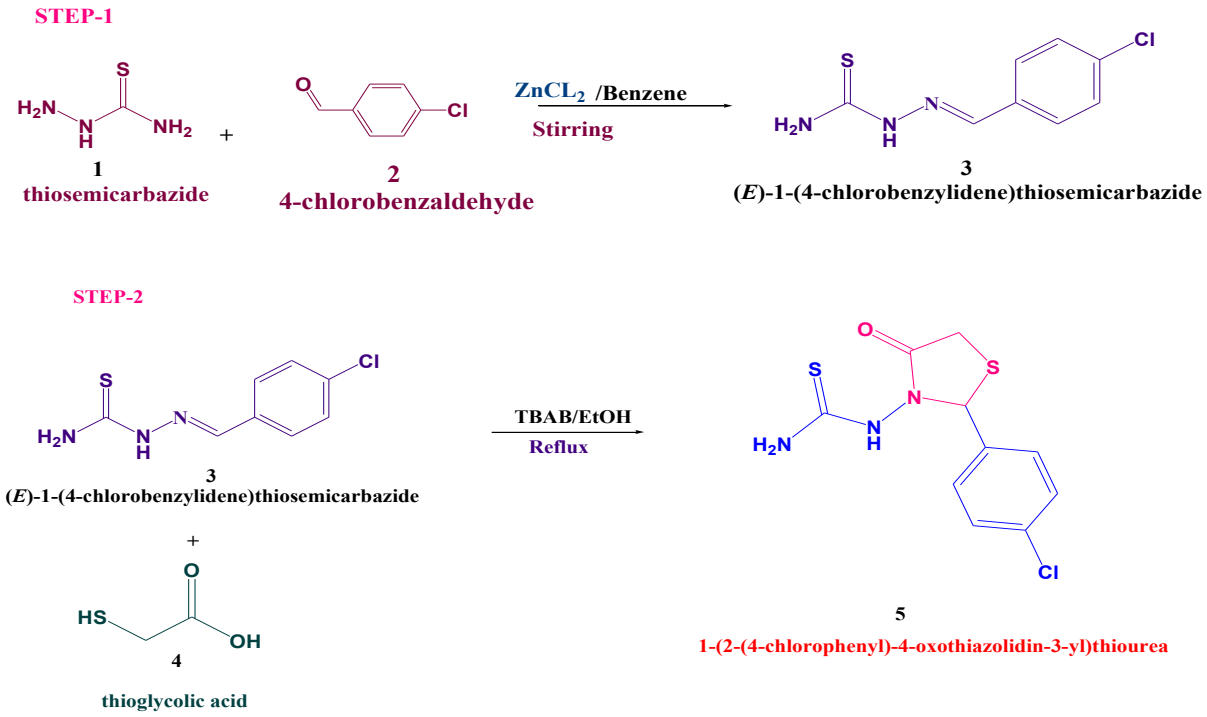
2.5 ADME and Toxicity Prediction

The ADME (absorption, distribution, metabolism, and excretion) properties were predicted using the SwissADME tool available online . Toxicity predictions, including LD50 values and toxicity class, were carried out using the ProTox-II platform .

3. Results and discussion

3.1 Chemistry

Thiosemicarbazide (1), when condensed with 4-chlorobenzaldehyde (2) in the presence of anhydrous zinc chloride, forms the corresponding Schiff's bases (E)-1-(4-chlorobenzylidene)thiosemicarbazide (3). An Efficient Synthesis of Thiazolidinone Derivatives via Cyclo-Condensation of Schiff Bases with Thioglycolic Acid in the Presence of Tetrabutylammonium hydroxide. An efficient method has been developed for synthesizing thiazolidinone derivatives through the cyclo-condensation of Schiff bases with thioglycolic acid in a water-ethanol medium, using tetrabutylammonium hydroxide (Bu4NBr) as a base. Initially, we evaluated the reaction between thioglycolic acid and the Schiff base under these conditions. The reaction proceeds via nucleophilic attack by the sulfur atom of thioglycolic acid on the electrophilic carbon of the imine (C=N) group in the Schiff base, followed by ring closure with the elimination of water molecule. This annulation was carried out using 1 mL of 50% aqueous Bu_4NBr at 70°C for 2 hours, affording the desired thiazolidinone derivative(5) in high yields.



Scheme-1

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

3.2 Spectral analysis for compound 5

All the products are characterized by FT-IR, 1H NMR , 13C NMR and Mass spectral analysis. In the IR spectrum of the representative compound 15, the carbonyl (>C=O) stretching frequency of the thiazolidinone ring is observed at 1632 cm-1. The absorption band at 1107 cm-1 was attributed to the >C-S- in the thiazolidinone ring. The (C-N) stretching frequency of the thiazolidinone ring is observed at 1252 cm1. The absorption band at 1364cm-1 was attributed to the >C=S- in the thiazolidinone. The (NH) stretching frequency of the thiazolidinone is observed at 3205 cm-1.

The 1H spectrum of compound 13 is taken as the representative spectrum, the singlet with a two-proton integral at 5.959 ppm is assigned to the methine proton of the thiazolidinone ring. The singlet at 3.550 ppm, integrating for one proton, corresponds to the methylene protons of the thiazolidinone ring. The singlet with a two-proton integral at 10.320 ppm is assigned to the amine (NH2) protons. A singlet at 10.512 ppm, integrating for one proton, is attributed to the amine (NH) proton. The multiplet appears in the range of 7.08–7.86 ppm is due to the aromatic protons.

In 13C NMR spectrum of compound 15, the downfield carbon signal at 165.46 ppm is attributed to the carbonyl carbon (>C=O) of the thiazolidinone ring. The methylene carbon of the thiazolidinone ring appears at 41.57 ppm. The methine carbon of the thiazolidinone ring appears at 58.85 ppm. The downfield carbon signal at 178.42 ppm is assigned to the thio carbonyl (>C=S). The signal ranging from 122.73 and 131.75 ppm unambiguously assigned to aromatic proton.

3.3 Molecular docking analysis thaizolidinones against protein (6UPU)

The computational assessment commenced with molecular docking simulations performed using AutoDock 4.2 and BIOVIA Discovery Studio to elucidate the binding orientations and predict the binding affinities of the synthesized compound (5) toward the deubiquitinase enzyme of *Orientia tsutsugamushi* (OtDUB). The crystallographic structure of the target protein (PDB ID: 6UPU) was employed as the docking template. Prior to docking, the protein structure was prepared by removing crystallographic water molecules, co-crystallized ligands, and other heteroatoms to ensure an unobstructed and reliable binding site.

O. tsutsugamushi is an obligate intracellular Gram-negative bacterium transmitted by infected chigger mites of the *Leptotrombidium* genus and is the etiological agent of scrub typhus. This febrile illness is prevalent in the Asia–Pacific region and is characterized by nonspecific clinical manifestations, including fever, headache, myalgia, rash, and the presence of an eschar at the site of the mite bite. If left untreated, scrub typhus may progress to severe complications such as pneumonitis, meningoencephalitis, multi-organ dysfunction, and even death. Furthermore, the extensive antigenic diversity of the pathogen poses significant challenges to accurate diagnosis and effective vaccine development.

Molecular docking studies were conducted to evaluate the interaction profile of synthesized compound 5 within the active site of the target protein (6UPU). The docking results revealed that compound 5 exhibits a binding affinity of −7.89 kcal/mol, indicating a strong and thermodynamically favorable interaction with the protein target.

Detailed interaction analysis demonstrated that compound 5 forms multiple stabilizing contacts with key amino acid residues, including Arg74(L), Leu73(L), Arg72(L), Leu71(L), Thr9(L), Leu8(L), Leu115(I), and His52(I). The coexistence of charged residues (Arg72 and Arg74) and hydrophobic residues (Leu8, Leu71, Leu73, and Leu115) within the binding pocket suggests that the ligand is well accommodated in the active site through a synergistic combination of electrostatic interactions, hydrogen bonding, and hydrophobic stabilization.

The involvement of arginine residues, particularly Arg72 and Arg74, is noteworthy, as their positively charged guanidinium groups are known to enhance ligand anchoring through strong hydrogen bonds or electrostatic interactions. Additionally, interactions with hydrophobic leucine residues contribute to the structural stability of the ligand–protein complex by promoting favorable van der Waals interactions. The interaction with His52(I) further reinforces binding, potentially through hydrogen bonding or π–π interactions.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

Overall, the docking study demonstrates that compound 5 possesses a favorable binding affinity toward the OtDUB enzyme (6UPU), highlighting its potential as a promising lead molecule for further structural optimization. The diverse interaction profile underscores the critical role of hydrophobic and electrostatic contributions in stabilizing the ligand within the active site, thereby supporting its suitability for subsequent biological and pharmacological investigations.

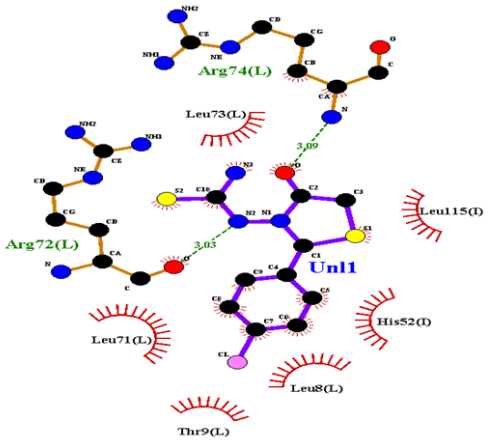


Figure 1. Docking images of compound 5 against 6UPU

Table 1. Binding affinity of the synthesized compound 5 against 6UPU

COMPOUNDS	BINDING AFFINITY	AMINO ACID RESIDUES
5	-7.89	Agr74(L), Leu73(L), Agr72(L), Leu71(L), Thr9(L), Leu8(L), Leu115(I), His52(I).

3.4 Molecular docking analysis thaizolidinones against protein (5V5Z)

The computational investigation commenced with molecular docking simulations to elucidate the binding orientations and predict the binding affinities of the synthesized compound (5) against *Candida albicans* lanosterol 14-α-demethylase (CYP51). The crystallographic structure of the target protein (PDB ID: 5V5Z) was employed as the docking template, and all simulations were carried out using AutoDock 4.2 in conjunction with BIOVIA Discovery Studio. Prior to docking, the protein structure was prepared by removing crystallographic water molecules, co-crystallized ligands, and other heteroatoms to ensure unobstructed ligand accommodation within the active site.

Candida albicans is a yeast-like fungal organism that normally exists as a commensal within the human microbiota, predominantly colonizing the skin and mucosal surfaces. However, disturbances in microbial homeostasis arising from antibiotic exposure, hormonal fluctuations, immunosuppression, physiological stress, or underlying diseases can promote fungal overgrowth, leading to infections collectively referred to as candidiasis. These infections range from superficial mucocutaneous manifestations to severe, potentially life-threatening systemic conditions.

The binding potential of synthesized compound 5 toward the target protein (5V5Z) was assessed through molecular docking analysis, which revealed a binding affinity of −6.89 kcal/mol, indicative of a moderately strong and energetically favorable interaction within the catalytic pocket of the receptor. Interaction analysis demonstrated that compound 5 is stabilized by several key amino acid residues, including Thr79(A), Thr41(A), Leu50(A), Pro49(A), Ser42(A), and Gln38(A), which collectively contribute to ligand orientation and binding stability through hydrogen bonding and hydrophobic interactions.

Notably, the presence of polar residues such as threonine and serine (Thr79, Thr41, and Ser42) suggests the formation of hydrogen-bonding interactions via their hydroxyl groups, facilitating effective ligand anchoring. In parallel, hydrophobic contacts with Leu50(A) and Pro49(A) enhance the structural stabilization of compound 5 within the binding pocket, while the involvement of Gln38(A) further supports polar interactions that may improve binding specificity.

Overall, the docking results indicate that compound 5 exhibits a favorable binding profile toward CYP51 (5V5Z), driven by a complementary balance of polar and hydrophobic interactions. Although the

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

binding affinity is slightly lower than that observed for its interaction with 6UPU, the interaction pattern supports its potential relevance for further biological evaluation and structural optimization.

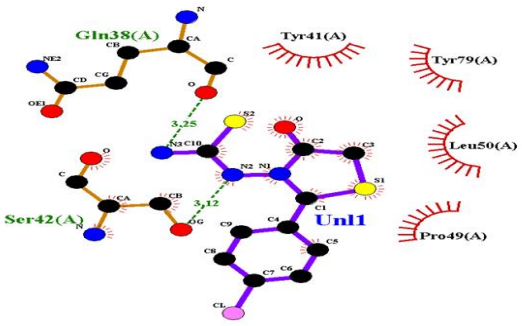


Figure 2. Docking images of compound 5 against 5V5Z

Table 2. Binding affinity of the synthesized compounds 13-17 against 5V5Z

COMPOUNDS	BINDING AFFINITY	AMINO ACID RESIDUES
5	-6.89	Thr79(A), Thr41(A), Leu50(A), Pro49(A), Ser42(A), Gln38(A),

3.5 Molecular docking analysis thaizolidinones against protein (7EL1)

The computational assessment was initiated with molecular docking simulations to elucidate the binding orientations and predict the binding affinities of the synthesized compounds (13–16) toward Staphylococcus aureus signal peptidase I (SPase I; SPAS). The crystallographic structure of the target protein (PDB ID: 7EL1) was employed as the docking template, and all simulations were carried out using AutoDock 4.2 in combination with BIOVIA Discovery Studio. Prior to docking, the protein structure was prepared by removing crystallographic water molecules, co-crystallized ligands, and other heteroatoms to ensure unhindered ligand accommodation within the active site.

Staphylococcus aureus is a Gram-positive coccus that commonly colonizes the skin and nasal cavities of healthy individuals as part of the normal microbiota; however, it is also a highly adaptable opportunistic pathogen capable of causing a broad spectrum of infections. These range from mild cutaneous conditions to severe and life-threatening diseases, including pneumonia, septicemia, and toxin-mediated food poisoning. The clinical relevance of S. aureus is further exacerbated by the emergence of multidrug-resistant strains, particularly methicillin-resistant S. aureus (MRSA), which present significant therapeutic challenges in both hospital and community settings. Molecular docking analysis of compound 5 with the target protein (7EL1) revealed a binding affinity of −5.42 kcal/mol, indicative of a moderate yet energetically favorable interaction within the active site. Although this affinity is comparatively lower than that observed for its interactions with 6UPU and 5V5Z, the binding mode suggests that compound 5 is capable of forming a stable ligand protein complex.

Detailed interaction analysis showed that compound 5 engages several key amino acid residues within the binding pocket, including Lys770(A), Tyr769(A), Lys767(A), Asp998(A), Tyr771(A), and Ile997(A). These residues collectively provide a complementary network of charged, polar, and hydrophobic interactions that contribute to binding stabilization. In particular, the involvement of lysine residues (Lys770 and Lys767) implies the formation of electrostatic and hydrogen-bonding interactions via their positively charged side chains, while the negatively charged Asp998 may further anchor the ligand through ionic or polar contacts. Additionally, interactions with tyrosine residues (Tyr769 and Tyr771) suggest possible π–π stacking or hydrogen-bonding interactions, enhancing binding specificity, whereas Ile997 contributes hydrophobic stabilization through favorable van der Waals interactions that help maintain ligand orientation within the active site. Overall, the docking study indicates that compound 5 exhibits a stable but moderate binding affinity toward SPase I (7EL1), governed by a balanced interplay of electrostatic, hydrogen-bonding, and hydrophobic interactions. These findings support its potential biological relevance while also highlighting the need for further structural

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

optimization to enhance binding efficiency against this particular target.

Table 3. Binding affinity of the synthesized compounds 5 against 7EL1

COMPOUNDS	BINDING AFFINITY	AMINO ACID RESIDUES
5	-5.42	Lys770(A), Tyr769(A), Lys767(A), Asp998(A), Tyr771(A), Ile997(A).

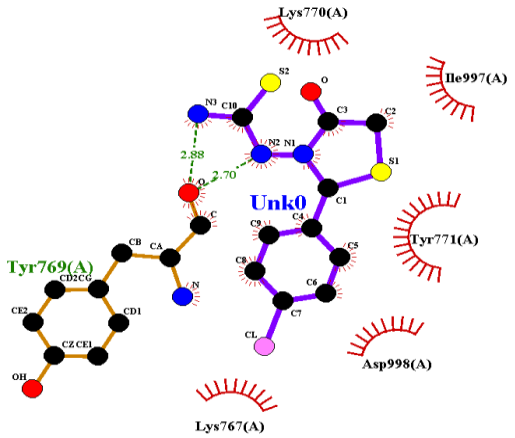


Figure 3. Docking images of compound 5 against 7EL1

3.6. *In silico* ADMET study

The ADMET evaluation of compound 5 provides important insights into its drug-likeness and pharmacokinetic behavior. The compound, with a molecular formula $C_{10}H_{10}ClN_3OS_2$ and a molar mass of 287.79 g/mol, falls within the acceptable molecular weight range recommended for orally active drug candidates, indicating favorable molecular size for biological interactions and permeability.

Compound 5 contains one hydrogen bond acceptor and two hydrogen bond donors, satisfying Lipinski's rule of five criteria. This suggests good potential for membrane permeability and oral bioavailability. The iLogP value of 1.76 indicates moderate lipophilicity, reflecting an optimal balance between hydrophobicity and hydrophilicity. Such a profile is desirable because it supports adequate absorption while minimizing the risk of nonspecific binding or poor solubility.

The compound exhibits moderate water solubility (2.02 mg/mL), which is acceptable for orally administered drug molecules. Furthermore, compound 5 shows high gastrointestinal (GI) absorption, reinforcing its suitability for oral delivery. The topological polar surface area (TPSA) of 115.75 Å² falls near the upper limit typically associated with good permeability; however, the molecule still maintains high predicted GI uptake, indicating that its structural features support efficient absorption despite a relatively high polarity.

Importantly, SwissADME analysis confirms that compound 5 meets drug-likeness criteria, indicating no major violations of commonly used medicinal chemistry filters. This suggests that the compound possesses an overall balanced physicochemical profile suitable for further optimization and biological evaluation.

Collectively, the ADMET data indicate that compound 5 demonstrates favorable pharmacokinetic characteristics, including acceptable lipophilicity, good GI absorption, compliance with drug-likeness rules, and adequate solubility. These properties support its potential as a promising lead molecule for further preclinical development. The Bioavailability Radar for compounds 5 is shown in Figure 5.

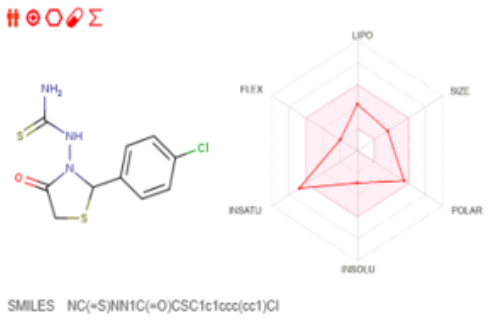


Figure 4: The bioavailability radar of the compounds (5). The optimum range for each property is shown by the pink area.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

Table 4: Complete ADMET profile of (5), including pharmacokinetics, physicochemical properties, lipophilicity, water-solubility, pharmacokinetics, drug-likeness and medicinal chemistry.

Ligand	Chemical Formula	Swiss ADME Filters							
		Molar mass (g/mol)	H bond acceptor	H bond donor	iLogP	Water Solubility mg/ml	GI absorption	TPSA (Å2)	Drug-like ness
5	$C_{10}H_{10}ClN_3OS_2$	287.79	1	2	1.76	2.02(Moderate)	High	115.75	Yes

3.2.7. *In silico* toxicological assessment

The ProTox-III toxicity assessment of compound 5 provides valuable insights into its predicted safety profile, including LD_{50}, toxicity class, organ toxicity, and endpoint toxicity predictions. The model estimates an oral LD_{50} of 1500 mg/kg, placing the compound in toxicity class 4, which corresponds to "harmful if swallowed" but still represents a relatively low level of acute toxicity compared to more hazardous classes. This LD_{50} value suggests that compound 5 has an acceptable safety margin for further drug development when administered at therapeutic doses.

The endpoint toxicity analysis reveals that compound 5 is predicted to be active only in hepatotoxicity, with a probability score of 0.50, indicating a moderate likelihood of liver-related effects at higher concentrations. All other major toxicological endpoints—including cardiotoxicity (0.77), immunotoxicity (0.99), mutagenicity (0.57), and cytotoxicity (0.73)—are predicted to be inactive, demonstrating a favorable toxicity profile in these categories. The high inactivity probabilities for immunotoxicity and mutagenicity (0.99 and 0.57, respectively) suggest low risk of immune disruption or genetic damage.

The ProTox-III model also reports an average structural similarity score of 84.33%, indicating good alignment of compound 5 with known non-toxic or moderately toxic compounds in the database. Furthermore, the prediction accuracy of 70.97% reflects a reliable confidence level in the algorithm's toxicity classification.

Overall, the ProTox-III results suggest that compound 5 displays a generally safe toxicological profile, with low predicted risks for cardiotoxic, mutagenic, immunotoxic, and cytotoxic effects. Although moderate hepatotoxic potential is indicated, it remains within a manageable range for early-stage drug candidates and can be further assessed through in vitro and in vivo studies. These findings support the continued evaluation of compound 5 as a promising lead molecule with acceptable toxicity characteristics for future preclinical development. The overall toxicity radar profiles of compounds 5 are presented in Figure 4.

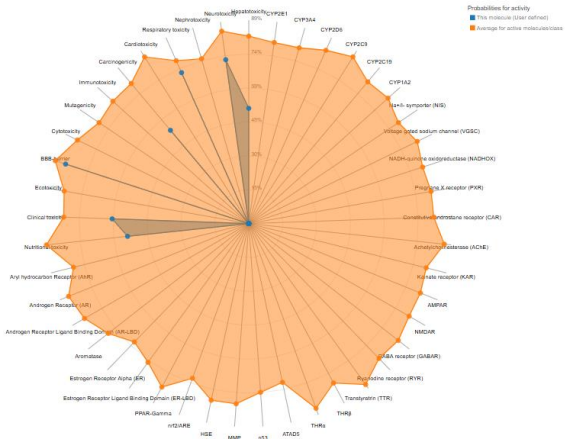


Figure 5. Overall toxicity radar chart of compound 5

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

Table 5: ProTox-III predicts bioactive chemical oral organ and endpoint toxicity of compound 5

Compound No	Const.LD50 dos (mg/kg)	Prediction toxicity The toxicity prediction criteria and % probability value of bioactive molecules						Average similarity	Prediction Accuracy
	prediction	class	Hepato	Cardio	Immuno.	Mutag.	Cyto.	%	%
5	1500	4	Active (0.50)	Inactive (0.77)	Inactive (0.99)	Inactive (0.57)	Inactive (0.73)	84.33	70.97

3.8 Frontier Molecular Orbitals

The Frontier Molecular Orbital (FMO) analysis of compound 5 provides important insights into its electronic behavior, chemical stability, and potential biological reactivity. The HOMO energy level of –6.979 eV reflects the molecule's ability to donate electrons, whereas the LUMO energy level of –2.286 eV represents its capacity to accept electrons. The calculated HOMO–LUMO energy gap (ΔE) of 4.694 eV indicates moderate chemical stability. A gap of this magnitude suggests that compound 5 possesses balanced reactivity—sufficiently stable under physiological conditions while still reactive enough to interact with biological targets.

The compound demonstrates a chemical potential (μ) of 4.633 eV, indicating a moderate energetic tendency to migrate electrons during interactions. The corresponding electronegativity (χ = –4.633 eV) reflects the molecule's overall electron-attracting ability, highlighting its potential to engage in electronic interactions within enzyme active sites.

The hardness value (η = 2.347 eV) suggests that compound 5 has a moderate resistance to charge transfer, aligning with its observed stability. Conversely, the softness value (S = 0.213) indicates that the molecule can undergo polarizability and electron redistribution when interacting with biomolecular targets. This characteristic is particularly important in ligand–receptor interactions, as softer molecules often adapt more efficiently within diverse binding environments.

The electrophilicity index (ω = 4.572) shows that compound 5 exhibits a strong electrophilic character. This high electrophilicity suggests a high tendency to accept electrons, making the molecule reactive toward nucleophilic sites in biological macromolecules. Such an electrophilic profile can contribute to effective molecular docking interactions, complementing the compound's previously reported binding affinity values.

Overall, the FMO and global reactivity descriptor analysis indicates that compound 5 possesses a well-balanced combination of chemical stability, electrophilicity, and reactivity, supporting its ability to interact efficiently with protein targets. These electronic properties reinforce the compound's potential for further drug development and bioactivity exploration. The HOMO–LUMO distribution of compound 5 is depicted in Figure 6.

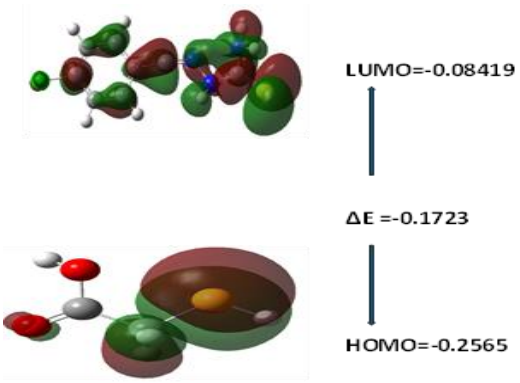


Figure 6 HOMO–LUMO of Compound 5

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

Table 6 Frontier Molecular Orbitals values of compound 5

Compound	Homo	Lumo	Energy Gap (Δ)(eV)	Chemical potential (μ)	Electro-negativity (χ)	Hardness (η)	Softness (S)	Elecro-philicity (ω)
5	-6.979	-2.286	4.694	4.633	-4.633	2.347	0.213	4.572

Conclusion

In conclusion, the present study demonstrates an efficient and environmentally sustainable strategy for the synthesis of novel 1-(2-(4-chlorophenyl)-4-oxothiazolidin-3-yl)thiourea derivatives with excellent yields and broad substrate tolerance. Comprehensive spectroscopic characterization confirmed the successful formation of the target compounds. In silico biological evaluations against Orientia tsutsugamushi, Candida albicans, and Staphylococcus aureus, together with ADMET and ProTox predictions, indicate promising antimicrobial potential, favorable pharmacokinetic behavior, and low predicted toxicity. Furthermore, DFT analyses provided valuable insights into the electronic properties, reactivity, and stability of the active molecules. Collectively, these findings highlight the synthesized thiazolidinone derivatives as promising lead candidates for further experimental validation and rational drug development.

Referance

[1] Maruthanila VL., Elancheran R, Kunnumakkara A.B., Kabilan .S., Kotoky.J., Recent development of targeted approaches for the treatment of breast cancer. Breast Cancer. **2017** Mar;24:191-219.DOI 10.1007/s12282-016-0732-1

[2] Gomez-Monterrey., Isabel., Giovanni Santelli., Pietro Campiglia., Daniela Califano, Fabiano Falasconi, Claudio Pisano, Loredana Vesci, Teresa Lama, Paolo Grieco, and Ettore Novellino. "Synthesis and cytotoxic evaluation of novel spirohydantoin derivatives of the dihydrothieno [2, 3-b] naphtho-4, 9-dione system." *Journal of medicinal chemistry* 48, no. 4 (2005): 1152-1157.

[3] Rajalakshmi., Ramarajan., Rajavel Santhi., and Thangaraj Elakkiya., "Synthesis, Characterization, Docking studies, Evaluation of Thiazinyl-thiazolidinone derivatives as potential in vitro antidiabetic and antioxidant agents." *Synthesis* 29, no. 08 (**2020**): 6091-6103.

[4] Singh, Shiva P., Surendra S. Parmar, Krishna Raman, and Virgil I. Stenberg. "Chemistry and biological activity of thiazolidinones." *Chemical Reviews* 81, no. 2 (**1981**): 175-203.

[5].Brown, Frances C. "4-Thiazolidinones." *Chemical Reviews* 61, no. 5 (**1961**): 463-521.

[6]. Andres, Charles J., Joanne J. Bronson, Stanley V. D'Andrea, Milind S. Deshpande, Paul J. Falk, Katharine A. Grant-Young, William E. Harte et al. "4-Thiazolidinones: novel inhibitors of the bacterial enzyme MurB." *Bioorganic & medicinal chemistry letters* 10, no. 8 (**2000**): 715-717.

[8]. Grasso, Silvana, A. Chimirri, P. Monforte, G. Fenech, Maria Zappalà, and Anna Maria Monforte. "Compounds with potential antitumor activity. VI--2-Alkyl-3-[2-(1, 3, 4-thiadiazolyl)]-4-thiazolidinones." *Il Farmaco; Edizione Scientifica* 43, no. 10 (**1988**): 851-856.

[9]. Diurno, M. Vittoria, Orazio Mazzoni, Eugenio Piscopo, Antonio Calignano, Federico Giordano, and Adele Bolognese. "Synthesis and antihistaminic activity of some thiazolidin-4-ones." *Journal of medicinal chemistry* 35, no. 15 (**1992**): 2910-2912.

[10]. Look, Gary C., John R. Schullek, Christopher P. Holmes, Jason P. Chinn, Eric M. Gordon, and Mark A. Gallop. "The identification of cyclooxygenase-1 inhibitors from 4-thiazolidinone combinatorial libraries." *Bioorganic & medicinal chemistry letters* 6, no. 6 (**1996**): 707-712.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 3456-3467 | ISSN:2251-6727

[11].Chimirri, Alba, Silvana Grasso, Anna Maria Monforte, Maria Zappala, Angelina De Sarro, and G. B. De Sarro. "Synthesis and anticonvulsant properties of 3-(1, 3, 4-thiadiazol-2-yl) thiazolidin-4-ones." *Farmaco (Societa Chimica Italiana: 1989)* 46, no. 7-8 (**1991**): 935-943.

[12]. Hamdi, Naceur, Abdullah Sulaiman Al-Ayed, Ridha Ben Said, and Alary Fabienne. "Synthesis and characterization of new thiazolidinones containing coumarin moieties and their antibacterial and antioxidant activities." *Molecules* 17, no. 8 (**2012**): 9321-9334.

[13]. Tahmasvand, Raheleh, Peyman Bayat, Seyyed Mahmood Vahdaniparast, Soudeh Dehghani, Zahra Kooshafar, Sepideh Khaleghi, Ali Almasirad, and Mona Salimi. "Design and synthesis of novel 4-thiazolidinone derivatives with promising anti-breast cancer activity: Synthesis, characterization, in vitro and in vivo results." *Bioorganic chemistry* 104 (**2020**): 104276.

[14]. Zhou, Hongyu, Shuhong Wu, Shumei Zhai, Aifeng Liu, Ying Sun, Rongshi Li, Ying Zhang et al. "Design, synthesis, cytoselective toxicity, structure–activity relationships, and pharmacophore of thiazolidinone derivatives targeting drug-resistant lung cancer cells." *Journal of Medicinal Chemistry* 51, no. 5 (**2008**): 1242-1251.

[15]. D'Andrea, Stan, Zhizhen Barbara Zheng, Kenneth DenBleyker, Joan C. Fung-Tomc, Hyekyung Yang, Junius Clark, Dennis Taylor, and Joanne Bronson. "Synthesis and antibacterial activity of dihydro-1, 2-oxazine and 2-pyrazoline oxazolidinones: novel analogs of linezolid." *Bioorganic & medicinal chemistry letters* 15, no. 11 (**2005**): 2834-2839.

[16]. Chanu, Leimajam V., Khumukcham Nongalleima, Salam P. Singh, Wahengbam K. Chanu, Chingakham B. Singh, and Okram M. Singh. "Synthesis, anti-inflammatory evaluation and in silico studies of naphtho [1, 2-e][1, 3] oxazine derivatives as potential non-steroidal anti-inflammatory agents." *Medicinal Chemistry Research* 29 (**2020**): 229-242.

[15] P. Ramachandra Reddy, P. Adivireddy, V. Padmavathi, *Journal of Heterocyclic Chemistry, **2015**,* 52, 1474-1482.

[17]. Preetam, Amreeta, and Mahendra Nath. "Ambient temperature synthesis of spiro [indoline-3, 2′-thiazolidinones] by a DBSA-catalyzed sequential reaction in water." *Tetrahedron Letters* 57, no. 13 (**2016**): 1502-1506.

[18]. Fraga-Dubreuil, Joan, and Jean Pierre Bazureau. "Efficient combination of task-specific ionic liquid and microwave dielectric heating applied to one-pot three component synthesis of a small library of 4-thiazolidinones." *Tetrahedron* 59, no. 32 (2003): 6121-6130. [18] P. Eleftheriou, A. Geronikaki, D. Hadjipavlou-Litina, P. Vicini, O. Filz, D. Filimonov, V. Poroikov, S. S. Chaudhaery, K. K. Roy, A. K. Saxena, *European journal of medicinal chemistry*, **2012**, 47, 111-24.

[19]. Ehsanifar, Mojtaba, and Zeinab Montazeri. "Neuroprotective Eff ects of Thiazolidine-4-Carboxylic Acid Derivatives on Memory Impairment and Neurodegeneration." *Journal ISSN* 2766 (2022): 2276. [20] J. N. Chandra, M. Malviya, C. T. Sadashiva, M. N. Subhash, K. S. Rangappa, *Neurochemistry international,* **2008**, 52, 376-83.

[21] Carpenter, Erica L., Barbara A. Vance, Rachel S. Klein, Alfredo Voloschin, Josep Dalmau, and Robert H. Vonderheide. "Functional analysis of CD8+ T cell responses to the onconeural self protein cdr2 in patients with paraneoplastic cerebellar degeneration." *Journal of neuroimmunology* 193, no. 1-2 (**2008**): 173-182.

[22] Subramaniyan, Ramkumar, Rajalakshmi Ramarajan, Arulraj Ramalingam, Sivakumar Sambandam, Amalraj Petersamy, Ahlam Roufieda Guerroudj, Nourdine Boukabcha, and Abdelkader Chouaih. "Microwave assisted synthesis, vibrational spectra, Hirshfeld surface and interaction energy, DFT, topology, in silico ADMET and molecular docking studies of 1, 2-bis (4-methoxybenzylidene) hydrazine." *Journal of Molecular Structure* 1278 (**2023**): 134946.

[23] Meiselbach, Heike, and Heinrich Sticht. "Effect of the SH3-SH2 domain linker sequence on the

structure of Hck kinase." *Journal of molecular modeling* 17 (**2011**): 1927-1934.

[24] Henot, Faustine, Elisa Rioual, Adrien Favier, Pavel Macek, Elodie Crublet, Pierre Josso, Bernhard Brutscher et al. "Visualizing the transiently populated closed-state of human HSP90 ATP binding domain." *Nature Communications* 13, no. 1 (**2022**): 7601..

[25] Ramkumar, Subramaniyan, and Rajalakshmi Ramarajan. "Design, Synthesis, Spectral Characterization, Antioxidant Activity, Molecular Docking and in silico ADMET Studies of 1, 3 Oxazepines." *ChemistrySelect* 8, no. 9 (**2023**): e202204818.

[26] Ramkumar, Subramaniyan, and Rajalakshmi Ramarajan. "Green synthesis, Single-Crystal X-RD, Hirshfeld Analysis and Anti-Covid-19 Molecular Docking Investigation of Symmetrical Azines." *ChemistrySelect* 8, no.6 (**2023**): e202204494.

[27] S. J. Gilani, K. Nagarajan, S.P. Dixit, M. Taleuzzaman, S.A. Khan. *Arabian Journal of Chemistry*. **2016**, 1, 9, S1523-31.

[28] Bonde, Chandrakant G., and Naresh J. Gaikwad. "Synthesis and preliminary evaluation of some pyrazine containing thiazolines and thiazolidinones as antimicrobial agents." *Bioorganic & medicinal chemistry* 12, no. 9 (**2004**): 2151-2161.

[29] Singh, Bhawani, A. Maheshwari, G. Dak, K. Sharma, and G. L. Talesara. "Studies of antimicrobial activities of some 4-thiazolidinone fused pyrimidines,[1, 5]-benzodiazepines and their oxygen substituted hydroxylamine derivatives." *Indian journal of pharmaceutical sciences* 72, no. 5 (**2010**): 607.

[30] Belwal, Chandra Kant, and Kaushik A. Joshi. "Synthesis and antifungal activity of some novel thiazolidinone derivatives of 4-(4-oxo-2-phenylthiazolidin-3-yl) benzoic acid." *Int. J. Chem. Tech. Res* 4, no. 4 (2012): 1758-1764.