



Share Your Innovations through JACS Directory

Journal of Advanced Chemical Sciences

Visit Journal at <https://www.jacsdirectory.com/jacs>

Design, Synthesis and Biological Evaluation of Thiazole Derivatives: An Integrated Molecular Docking and Density Functional Theory Investigation

Abhijit Gaikwad^{1,*}, Dattatray Ghotekar¹, Archana Pangavhane², Sandip Pathade², Vishnu Adole³, Rahul Shinde³, Akash Gaikwad⁴¹Department of Chemistry, Centre for Nutan Vidya Prasarak Mandal's Arts, Commerce and Science College, Lasalgaon – 422 306, Maharashtra, India.²Department of Chemistry, Medicinal SNJB's KKHA Arts, SMGL Commerce and SPHJ Science College, Neminagar, Nashik – 423 101, Maharashtra, India.³Department of Chemistry, MGV's LVH Arts, Science and Commerce College, Panchavati (Autonomous), Nashik – 422 003, Maharashtra, India.⁴Department of Physics, MVP Samaj's K. K. Wagh Arts, Science and Commerce College, Pimpalgaon (B) – 422 209, Maharashtra, India.

ARTICLE DETAILS

Article history:

Received 23 September 2026

Accepted 04 October 2026

Available online 14 October 2026

Keywords:

Thiazole

Pyrazole

Antimicrobial

Molecular Docking

Density Functional Theory

ABSTRACT

A series of nine thiazole–hydrazone hybrids (**D1–D9**) was prepared in two steps: 5-chloro-1,3-dimethyl-1H-pyrazole-4-carbaldehyde was condensed with thiosemicarbazide, and the resulting thiosemicarbazone was cyclized with substituted phenacyl bromides. The products were characterized by Fourier-transform infrared (FT-IR) spectroscopy, ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. Antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*, and antifungal activity against *Candida albicans*, were screened by the Kirby–Bauer disc diffusion method. Compounds **D1**, **D2** and **D7** were the most promising, mainly against the Gram-negative strains and *C. albicans*. Docking of the most active compounds into four microbial target proteins (Protein Data Bank IDs 4URM, 1KZN, 5ECX and 5TZ1) gave binding energies of –6.7 to –8.7 kcal/mol, with hydrogen-bonding and hydrophobic contacts in the active sites. Density functional theory (DFT) calculations supplied frontier orbital energies, reactivity descriptors and electrostatic potential maps, and in silico absorption, distribution, metabolism and excretion (ADME) screening suggested drug-like behavior. These results point to thiazole–hydrazone hybrids as useful starting points for antimicrobial lead development.

1. Introduction

Heterocyclic hybrids have drawn steady interest in drug discovery. Joining two pharmacophores in one molecule can produce activity that neither part shows alone, and in some reports the hybrids outperform drugs already in clinical use [1–3]. Combining two biologically active heterocycles is a common way to improve therapeutic effect [4,5]. Nitrogen-rich frameworks are especially attractive because they occur widely in natural products and underpin many synthetic bioactive molecules [6,7]. Thiazole is one of the best-studied of these cores. It is generally regarded as having low toxicity and good biocompatibility, and thiazole derivatives have been reported as anti-inflammatory [8], antibacterial [9], anticancer [10], antifungal [11] and antiviral agents [12].

Pyrazoles form another important family of nitrogen heterocycles, valued for their biological profile and for their usefulness in synthesis [13]. Molecules that carry both a thiazole and a pyrazole ring display a broad range of biological effects [13–15], and many thiazole-based hybrids have been assembled by attaching other heterocyclic units to the thiazole core [14,16]. Thiazole–hydrazone conjugates in particular have been studied against cancer, tuberculosis, fungi and bacteria, and as anti-inflammatory agents [17–22]. Because the hydrazone unit and neighboring groups can be exchanged for bio isosteres, linking a hydrazone to a thiazole gives a convenient handle for tuning activity.

We therefore chose to build new thiazole–hydrazone hybrids in two steps. A thiosemicarbazone is prepared first and is then cyclized with phenacyl bromides to form the 1,3-thiazole ring [17,23]. Computational tools now sit next to bench work in most early-stage drug studies because they shorten screening and lower its cost. Molecular docking describes how a ligand sits in its receptor, DFT reveals structural and electronic features, and ADME prediction gives a first view of pharmacokinetics and drug-likeness. Given the importance of the thiazole and pyrazole units,

rational design of thiazole–hydrazone hybrids look like a sound route to antimicrobial agents with better properties [23–25].

In this paper we describe a set of nine functionalized thiazole derivatives (**D1–D9**) and characterize them by FT-IR, ¹H NMR, ¹³C NMR and mass spectrometry. Their antibacterial and antifungal activities were screened against selected microbial strains. Docking was used to study ligand–target interactions, and DFT calculations to examine electronic properties. Putting these experimental and computational results side by side should help identify candidates for future antimicrobial work.

2. Experimental Methods

2.1 Materials

5-Chloro-1,3-dimethyl-1H-pyrazole-4-carbaldehyde, thiosemicarbazide and the substituted phenacyl bromides were purchased from Sigma-Aldrich and Merck and used without further purification. Reactions were followed by thin-layer chromatography (TLC) on silica gel plates. The reference strains *Bacillus subtilis* (ATCC 11774), *Escherichia coli* (NCBI 0157), *Klebsiella pneumoniae* (ATCC 4352) and *Staphylococcus aureus* (MTCC 1430), and the fungal strain *Candida albicans* SC5314, were obtained from the Microbiology Department. ofloxacin – confirm which was used served as the antibacterial positive control and fluconazole as the antifungal control.

2.2 General Procedure for the Synthesis of Thiazole–Hydrazone Hybrids

2.2.1 Step 1: Thiosemicarbazone Intermediate (I)

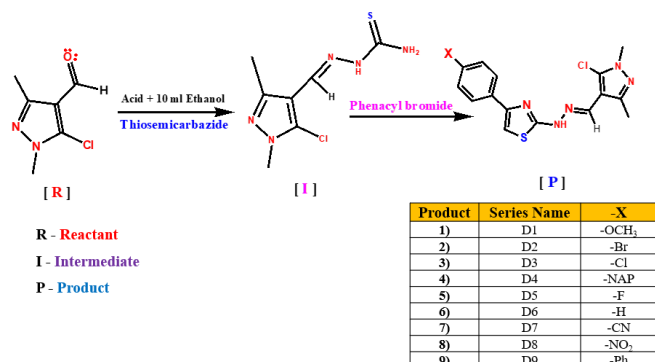
5-Chloro-1,3-dimethyl-1H-pyrazole-4-carbaldehyde (1.0 mmol) and thiosemicarbazide (1.0 mmol) were dissolved in 15 mL of absolute ethanol, a few drops of glacial acetic acid were added, and the mixture was refluxed for 4–6 h with TLC monitoring. After cooling to room temperature, the precipitate was filtered off, washed with cold ethanol and dried to give the thiosemicarbazone (**I**).

*Corresponding Author: abhisnjb84@gmail.com (Abhijit Gaikwad)



2.2.2 Step 2: Target Thiazole Derivatives (D1–D9)

The thiosemicarbazone (I, 1.0 mmol) was dissolved in 15 mL of ethanol, and an equimolar amount (1.0 mmol) of the appropriate substituted 2-bromo-1-arylethanone (phenacyl bromide) was added. The mixture was refluxed for 6–8 h until TLC showed complete conversion. It was then concentrated and allowed to cool to room temperature. The precipitate was filtered, washed with cold ethanol and recrystallized from a suitable solvent to give the pure products (Scheme 1).



Scheme 1 Synthesis of the thiazole–hydrazone derivatives **D1–D9**. (i) Condensation of 5-chloro-1,3-dimethyl-1H-pyrazol-4-carbaldehyde with thiosemicarbazide in ethanol under acid catalysis gives the thiosemicarbazone (I); (ii) cyclocondensation of (I) with substituted phenacyl bromides forms the 1,3-thiazole ring. Yields were 55–75%.

2.3 Characterization and Spectral Data

FT-IR spectra were recorded using a Shimadzu IRAffinity-1/-1S FT-IR spectrometer (S/N: A22135500805). The ¹H NMR spectrum was recorded at 400.30 MHz in CDCl₃ at 298.1 K. Mass spectrometric analysis was performed using a Spark Analytical mass spectrometer (Instrument ID: SAB/AD/INS/002). The experimental FT-IR and NMR records were used for spectral characterization of the synthesized compound. IR bands are given in cm⁻¹, chemical shifts (δ) in ppm and coupling constants (J) in Hz. Mass values are m/z for [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-(4-methoxyphenyl)thiazol-2-yl)hydrazine (D1)

Yield 72%; solid. IR (ν, cm⁻¹): 3126, 2980, 2744, 1618 (C=N), 1454, 1240, 432. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.12 (d, 2H, *J* = 8), 7.68 (d, 2H, *J* = 8), 7.26 (s, 1H), 7.01 (d, 2H, *J* = 8), 6.56 (s, 1H), 3.85 (s, 3H), 2.43 (s, 3H), 1.25 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 168.7, 148.3, 142.1, 133.1, 129.7, 128.8, 122.4, 114.9, 101.8, 55.4, 36.1, 14.7. MS (*m/z*): 348.85 [M + H].

(E)-1-(4-(4-bromophenyl)thiazol-2-yl)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)hydrazine (D2)

Yield 68%; solid. IR (ν, cm⁻¹): 3126, 2980, 1618 (C=N), 1489, 1454, 1240, 1072, 819. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.15 (d, 2H, *J* = 8), 7.63 (d, 2H, *J* = 8), 7.26 (s, 1H), 6.73 (s, 1H), 3.82 (s, 3H), 2.43 (s, 3H), 1.58 (s, 3H). MS (*m/z*): 397.68 [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-(4-nitrophenyl)thiazol-2-yl)hydrazine (D3)

Yield 65%; yellow solid. IR (ν, cm⁻¹): 3126, 2980, 1618 (C=N), 1520, 1454, 1340. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.01 (s, 1H), 7.78 (d, 2H, *J* = 8), 7.62 (d, 2H, *J* = 8), 7.26 (s, 1H), 7.12 (d, 2H, *J* = 8), 6.75 (s, 1H), 2.44 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 169.1, 164.2, 161.8, 147.9, 147.0, 140.1, 138.0, 127.7, 115.9, 115.0, 110.8, 101.8, 77.3, 76.9, 36.0, 14.7. MS (*m/z*): 350.15 [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-(naphthalen-2-yl)thiazol-2-yl)hydrazine (D4)

Yield 70%; brown solid. IR (ν, cm⁻¹): 3057 (Ar C–H), 1612 (C=N), 1454, 1257. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.18–8.10 (m, 3H), 7.90–7.70 (m, 4H), 7.51 (s, 1H), 7.30 (s, 1H), 6.60 (s, 1H), 3.81 (s, 3H), 2.43 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 168.7, 148.3, 142.4, 133.6, 133.1, 129.7, 128.8, 127.6, 127.5, 125.6, 125.0, 122.4, 110.3, 101.3, 36.1, 14.7. MS (*m/z*): 382.14 [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-(4-fluorophenyl)thiazol-2-yl)hydrazine (D5)

Yield 63%; off-white solid. IR (ν, cm⁻¹): 3126, 2980, 1618 (C=N), 1508, 1454, 1226, 1157, 825. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.16 (d, 2H, *J* = 8), 7.71 (d, 2H, *J* = 8), 7.26 (s, 1H), 6.75 (s, 1H), 3.84 (s, 3H), 2.45 (s, 3H). MS (*m/z*): 366.10 [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-phenylthiazol-2-yl)hydrazine (D6)

Yield 75%; white solid. IR (ν, cm⁻¹): 3126, 2980, 1618 (C=N), 1454, 1315, 1240. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.14 (d, 2H, *J* = 8), 7.88 (t, 2H, *J* = 8), 7.58 (d, 2H, *J* = 8), 7.26 (s, 1H), 6.95 (s, 1H), 3.82 (s, 3H), 2.45 (s, 3H). MS (*m/z*): 318.78 [M + H].

(E)-4-(2-(2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)hydrazinyl)thiazol-4-yl)benzonitrile (D7)

Yield 66%; yellowish solid. IR (ν, cm⁻¹): 3126, 2980, 2223 (C≡N), 1618 (C=N), 1454. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.23 (d, 2H, *J* = 8), 7.80 (d, 2H, *J* = 8), 7.61 (d, 2H, *J* = 8), 7.26 (s, 1H), 6.58 (s, 1H), 3.82 (s, 3H), 2.44 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 168.7, 148.3, 142.4, 133.1, 130.1, 129.4, 125.7, 125.0, 122.4, 118.7, 110.3, 101.8, 36.1, 14.7. MS (*m/z*): 332.16 [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-(4-nitrophenyl)thiazol-2-yl)hydrazine (D8)

Yield 55%; yellow solid. IR (ν, cm⁻¹): 3126, 2980, 1618 (C=N), 1520, 1340, 855, 745. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.26 (d, 2H, *J* = 8), 8.10 (d, 2H, *J* = 8), 7.84 (s, 1H), 7.26 (s, 1H), 6.56 (s, 1H), 3.80 (s, 3H), 2.43 (s, 3H). ¹³C NMR (125 MHz, DMSO-*d*₆) δ: 168.7, 147.1, 145.7, 141.2, 136.0, 129.1, 123.7, 122.5, 121.7, 120.9, 101.6, 36.1, 14.7. MS (*m/z*): 363.79 [M + H].

(E)-2-((5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)methylene)-1-(4-([1,1'-biphenyl]-4-yl)thiazol-2-yl)hydrazine (D9)

Yield 60%; yellow solid. IR (ν, cm⁻¹): 3126, 2980, 1618, 1454, 1240. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.15 (d, 2H, *J* = 8), 7.85 (d, 2H, *J* = 8), 7.58 (d, 2H, *J* = 8), 7.26 (s, 1H), 6.53 (s, 1H), 3.83 (s, 3H), 2.44 (s, 3H). MS (*m/z*): 408.10 [M + H].

2.4 Antimicrobial Assays

Antibacterial and antifungal activities of **D1–D9** were tested in vitro by the Kirby–Bauer disc diffusion method [26]. Antimicrobial activity was expressed as the zone of inhibition (mm). The supplied observation data report individual zone diameters for each compound against the tested microorganisms; replicate number and replicate-wise measurements were not recorded in the provided table. Consequently, the values are presented as individual measurements rather than mean ± SD.

2.4.1 Antibacterial Activity

Bacterial responses were assessed by disc diffusion as described previously [27]. Cultures were adjusted to a 0.5 McFarland standard (about 1.5 × 10⁸ CFU/mL), and 100 μL of the suspension was spread over Mueller–Hinton agar (MHA) plates. Sterile discs loaded with the test compounds (**D1–D9**, 0–1000 μg/disc in DMSO) or with the positive control (20 μg/disc, ofloxacin) were placed on the surface. After 24 h at 37 °C, the diameter of each clear zone was measured in millimeters.

2.4.2 Antifungal Activity

C. albicans was grown in Sabouraud dextrose broth (SDB) and adjusted to a 0.5 McFarland standard (about 1.5 × 10⁸ CFU/mL). Whatman No. 1 discs were loaded with the thiazole compounds **D1–D9** (0–1000 μg/disc in DMSO), with DMSO alone as the solvent control, or with 25 μg of fluconazole as the reference drug. A 100 μL portion of the fungal suspension was spread on Sabouraud dextrose agar (SDA) plates, the discs were placed on top, and the plates were incubated at 37 °C for 24 h. Zones of inhibition (ZOI) were then measured in millimeters.

2.5 Molecular Docking

Docking was used to examine how the new compounds might interact with selected target proteins. Protein and ligand files were prepared, the grid box was defined and the docking runs were carried out with AutoDock Tools 1.5.7 and AutoDock Vina [28,29]. Crystal structures were taken from the Protein Data Bank (PDB): the *B. subtilis* protein 4URM (described in

the Methods as DNA gyrase), the *E. coli* protein 1KZN, the *K. pneumoniae* protein 5ECX and *C. albicans* CYP51 (5TZ1). Water molecules and co-crystallized ligands were deleted, and polar hydrogens and Gasteiger charges were added. Ligand geometries were minimized with the MM2 force field in Chem3D Pro before docking [30,31]. The grid box was centered on the active site residues. Poses were ranked by binding energy (kcal/mol) and inspected visually, and the ligand–protein complexes were analyzed in Discovery Studio Visualizer (DSV) [32,33].

2.6 Density Functional Theory Calculations

All DFT calculations were run in the gas phase with Gaussian 03 [27,34], using Becke's three-parameter hybrid exchange functional (B3LYP) and the All DFT calculations were performed in the gas phase using Gaussian 03 [27,34] at the B3LYP/[basis set to be confirmed] level of theory. basis set. Structures were visualized with GaussView 4.1.2. Global reactivity descriptors were obtained from the frontier orbital energies through Koopmans' theorem: ionization energy $I = -E_{\text{HOMO}}$, electron affinity $A = -E_{\text{LUMO}}$, electronegativity $\chi = (I + A)/2$, chemical hardness $\eta = (I - A)/2$, softness $S = 1/\eta$, chemical potential $\mu = -\chi$, electrophilicity index $\omega = \mu^2/2\eta$ and maximum electron transfer $\Delta N_{\text{max}} = -\mu/\eta$.

2.7 ADME Prediction

Absorption, distribution, metabolism and excretion (ADME) properties were predicted with the free SwissADME web tool.

3. Results and Discussion

3.1 Chemistry

The thiazole–hydrazone derivatives were made in two steps (Scheme 1). In the first, 5-chloro-1,3-dimethyl-1H-pyrazole-4-carbaldehyde reacted with thiosemicarbazide under acidic conditions to give the thiosemicarbazone (**1**) by nucleophilic addition followed by loss of water. Refluxing (**1**) with the corresponding 2-bromo-1-arylethanone in ethanol then closed the thiazole ring. The thioamide sulfur attacks the α -carbon of the phenacyl bromide, and intramolecular cyclization and dehydration follow. The isolated yields of **D1–D9** were between 55% and 75%.

The structures were assigned from the data in Section 2.3, and the full spectra are given in the supplementary file. In the IR spectra we assign the bands at 3050–3150 cm^{-1} to aromatic C–H stretching, those at 1500–1620 cm^{-1} to C=N and C=C vibrations of the thiazole and pyrazole rings, and the hydrazone N–H stretch. The ^1H NMR spectra show the azomethine ($-\text{N}=\text{CH}-$) singlet, aromatic signals in the expected region and methyl singlets from the pyrazole ring. The $[\text{M} + \text{H}]$ ions in the mass spectra were used to confirm the molecular weights.

3.2 Antimicrobial Activity

The strains were chosen to cover the main pathogen groups: Gram-positive bacteria (*S. aureus*, *B. subtilis*), Gram-negative bacteria (*E. coli*, *K. pneumoniae*) and a fungus (*C. albicans*). Activity was judged from the zones of inhibition measured in the disc diffusion assay (Table 1).

Table 1 Zones of inhibition (mm) of compounds **D1–D9** against bacteria and fungus

Compound	Gram-positive		Gram-negative		Fungus
	<i>S. aureus</i> MTCC 1430	<i>B. subtilis</i> ATCC 11774	<i>E. coli</i> NCBI 0157	<i>K. pneumoniae</i> ATCC 4352	<i>C. albicans</i> SC5314
D1	–	–	21	19	18
D2	–	–	–	21	19
D3	–	–	17	14	18
D4	–	–	20	–	18
D5	–	–	15	–	16
D6	–	13	18	–	16
D7	–	–	–	23	17
D8	–	14	19	16	16
D9	–	13	16	–	12
Reference ^a	14	18	15	26	18 ^b

–, no inhibition. ^a Antibacterial reference: ofloxacin, 20 $\mu\text{g}/\text{disc}$. ^b Antifungal reference: fluconazole, 25 $\mu\text{g}/\text{disc}$. DMSO alone gave no zone

<https://doi.org/10.30799/jacs.285.26120505>

3.2.1 Gram-Positive Bacteria

None of the nine compounds inhibited *S. aureus* under the test conditions. Against *B. subtilis*, only **D6** (13 mm), **D8** (14 mm) and **D9** (13 mm) produced measurable zones, which we regard as moderate activity; **D1–D5** and **D7** were inactive. The three active compounds differ in the group on the phenyl ring, so that substituent clearly matters here. Three actives and no dose–response data are not enough, however, for a firm structure–activity conclusion.

3.2.2 Gram-Negative Bacteria

The Gram-negative strains responded better. Against *E. coli*, **D1** gave the largest zone (21 mm), followed by **D4** (20 mm), **D8** (19 mm), **D6** (18 mm), **D3** (17 mm), **D9** (16 mm) and **D5** (15 mm), while **D2** and **D7** were inactive. Against *K. pneumoniae*, **D7** was the most active compound of the whole series (23 mm), followed by **D2** (21 mm), **D1** (19 mm), **D8** (16 mm) and **D3** (14 mm); **D4**, **D5**, **D6** and **D9** showed no zone. No single electronic trend explains these results. The electron-donating methoxy group of **D1** gave the biggest zone for *E. coli*, the electron-withdrawing cyano (**D7**) and bromo (**D2**) groups were best against *K. pneumoniae*, and the nitro compound **D8** was active against both.

3.2.3 Antifungal Activity

All nine compounds inhibited *C. albicans*, with zones between 12 and 19 mm. **D2** was the most active (19 mm), **D1**, **D3** and **D4** came close behind (18 mm each), and **D9** was the weakest (12 mm). The DMSO control gave no zone, so the inhibition can be attributed to the compounds and not to the solvent.

3.2.4 Comparison with the Reference Drugs

For *B. subtilis* (18 mm) and *K. pneumoniae* (26 mm), no compound reached the reference zone, and against *S. aureus* the comparison is moot because no compound was active. Against *E. coli*, on the other hand, six compounds (**D1**, **D3**, **D4**, **D6**, **D8** and **D9**) gave larger zones than the reference (15 mm), and **D2** (19 mm) slightly exceeded fluconazole (18 mm) against *C. albicans*. The reference and test discs differ in drug load, so these comparisons are only qualitative. Overall, we consider the series moderately active. Determination of minimum inhibitory concentrations, replicate measurements and tests on drug-resistant isolates are the obvious next steps.

Table 2 Docking scores and key interactions of the antimicrobial-active compounds with the selected protein targets

Compound	Target (PDB ID)	Docking score (kcal/mol)	Key interactions
D8	4URM	–6.7	Conventional hydrogen bond with GLY109; carbon–hydrogen bond with PRO87; alkyl interactions with ALA98 and PRO87; π -alkyl interaction with ILE86; hydrophobic stabilization in the binding pocket.
D1	1KZN	–7.4	Attractive charge and π -anion interactions with GLU50; amide- π stacking with ASN46 and ALA47; alkyl interactions with VAL43, VAL120 and VAL167; π -alkyl interaction with ILE78; hydrophobic stabilization in the receptor cavity.
D7	5ECX	–8.7	Carbon–hydrogen bond with TRP22; π - π stacking with PHE31; π -alkyl interactions with MET50, NAP201, ALA7 and ILE20; extensive hydrophobic contacts in the active site.
D2	5TZ1	–7.9	Conventional hydrogen bonds with TYR505 and SER507; π -sigma interactions with LEU87 and HIS377; π - π stacking with PHE233; π - π T-shaped interaction with HIS377; alkyl interactions with LEU376, LEU87, LYS90 and MET92; π -alkyl interactions with TYR118, PRO230 and LEU376.

3.3 Molecular Docking

The docking targets were chosen on the assumption that the compounds act by inhibiting essential microbial enzymes. For each organism we docked the compound that gave the largest zone into a protein with a published crystal structure: **D8** into the *B. subtilis* protein (4URM), **D1** into the *E. coli* protein (1KZN), **D7** into the *K. pneumoniae*

protein (5ECX) and **D2** into *C. albicans* CYP51 (5TZ1). Binding energies and key interactions are listed in Table 2, and the poses are shown in Figs. 1–4.

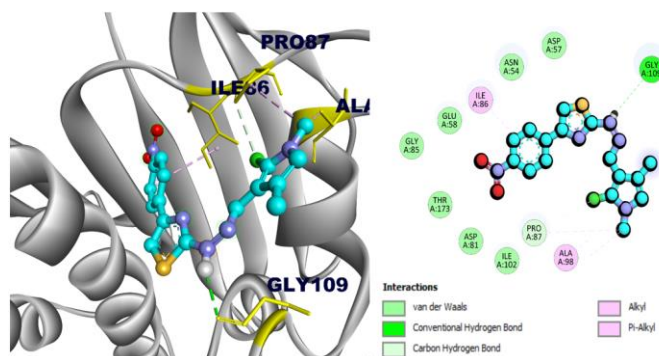


Fig. 1 Three-dimensional and two-dimensional views of compound **D8** docked into the *B. subtilis* target protein (PDB ID: 4URM)

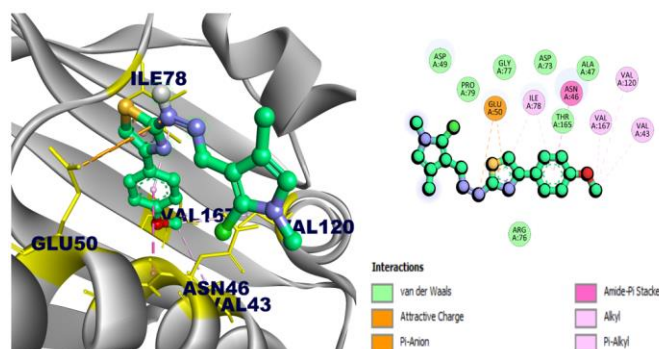


Fig. 2 Three-dimensional and two-dimensional views of compound **D1** docked into the *E. coli* target protein (PDB ID: 1KZN)

D8 docked into 4URM with a score of -6.7 kcal/mol (Fig. 1) For *Bacillus subtilis*, molecular docking was performed against the protein target represented by PDB ID 4URM. The pose is held by a conventional hydrogen bond with GLY109 and a carbon-hydrogen bond with PRO87, together with alkyl contacts to ALA98 and PRO87 and a π -alkyl contact to ILE86. **D1** bound to 1KZN at -7.4 kcal/mol (Fig. 2). In that complex the ligand is stabilized by attractive charge and π -anion interactions with GLU50, amide- π stacking with ASN46 and ALA47, and several alkyl and π -alkyl contacts.

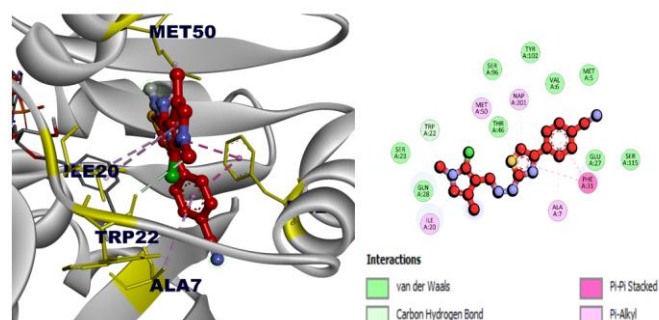


Fig. 3 Three-dimensional and two-dimensional views of compound **D7** docked into the *K. pneumoniae* target protein (PDB ID: 5ECX)

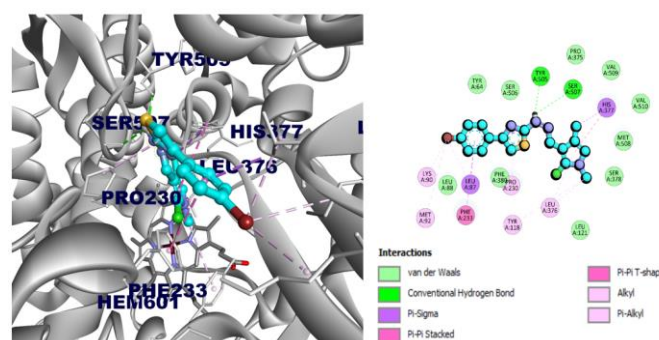


Fig. 4 Three-dimensional and two-dimensional views of compound **D2** docked into *C. albicans* CYP51 (PDB ID: 5TZ1)

<https://doi.org/10.30799/jacs.285.26120505>

The best score in the series was obtained for **D7** in 5ECX (-8.7 kcal/mol; Fig. 3). A carbon-hydrogen bond with TRP22 and π - π stacking with PHE31 anchor the ligand, and π -alkyl contacts with MET50, NAP201, ALA7 and ILE20 add hydrophobic stabilization. **D2** docked into *C. albicans* CYP51 (5TZ1) with a score of -7.9 kcal/mol (Fig. 4). It forms conventional hydrogen bonds with TYR505 and SER507, π -sigma contacts with LEU87 and HIS377, π - π stacking with PHE233 and a T-shaped π - π contact with HIS377, and it is packed against LEU376, LYS90, MET92, TYR118 and PRO230 through alkyl and π -alkyl contacts.

Across the four complexes the scores range from -6.7 to -8.7 kcal/mol, and each pose combines polar anchoring contacts with extensive hydrophobic packing. These scores are predictions from a single docking program, so they are best read alongside the disc assay data and not as proof of the mode of action.

3.4 Density Functional Theory Study

To look at the electronic structure and reactivity of the new compounds, **D1–D9** were optimized in the gas phase without symmetry constraints (basis set as given in Section 2.6). The optimized structures are shown in Fig. 5. The thiazole ring stays close to planar in all nine molecules, which allows π -electron delocalization along the conjugated backbone. Changing the substituent on the aryl ring alters a few bond lengths and angles around that ring, but the thiazole core is essentially unaffected. Such delocalization may help the molecules interact with biological targets.

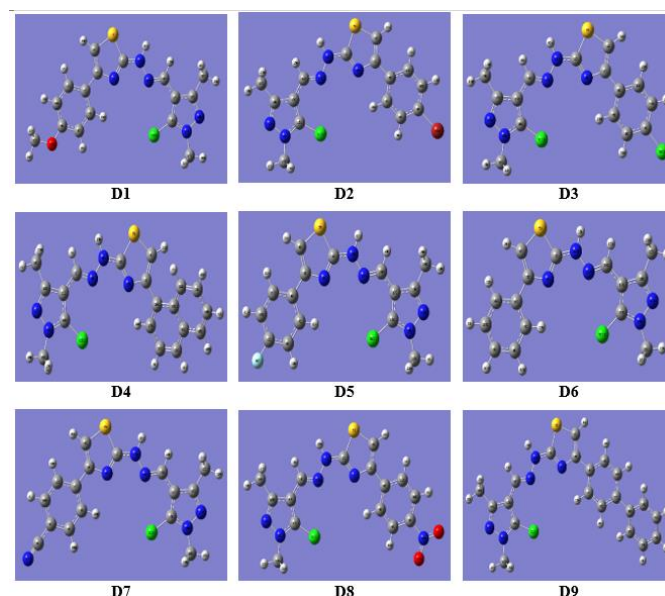


Fig. 5 Optimized structures of compounds **D1–D9**

Table 3 Frontier orbital energies and related parameters of compounds **D1–D9**

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	Ionization energy, I (eV)	Electron affinity, A (eV)
D1	-4.9617	-1.0204	3.9412	4.9617	1.0204
D2	-5.2701	-1.1975	4.0724	5.2701	1.1975
D3	-5.2711	-1.1992	4.0719	5.2711	1.1992
D4	-5.1192	-1.1132	4.0060	5.1192	1.1132
D5	-5.1930	-1.1333	4.0596	5.1930	1.1333
D6	-5.1445	-1.0778	4.0667	5.1445	1.0778
D7	-5.4738	-1.6272	3.8466	5.4738	1.6272
D8	-5.5339	-2.3039	3.2299	5.5339	2.3039
D9	-5.1195	-1.1238	3.9957	5.1195	1.1238

$\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$; $I = -E_{\text{HOMO}}$; $A = -E_{\text{LUMO}}$

3.4.1 Frontier Molecular Orbitals

Frontier molecular orbital (FMO) analysis links electronic transitions to chemical reactivity. The HOMO reflects the ability to donate electrons and the LUMO the ability to accept them. In these compounds the HOMO density sits mainly on the thiazole sulfur and the substituted aryl ring, which are therefore the likely sites of electron donation. The LUMO density lies mostly over the thiazole ring and the adjacent conjugated system, marking the likely sites of electron acceptance (Fig. 6). The HOMO energies

range from -5.5339 to -4.9617 eV and the LUMO energies from -2.3039 to -1.0204 eV (Table 3).

The HOMO–LUMO gap is a compact indicator of stability. A small gap means easier electron redistribution and higher reactivity, while a large gap points to a harder, less reactive molecule. **D8** has the smallest gap in the series (3.2299 eV), so it is the softest and most reactive compound and is the best candidate for charge-transfer processes. **D2** has the largest gap (4.0724 eV) and is the hardest and most stable.

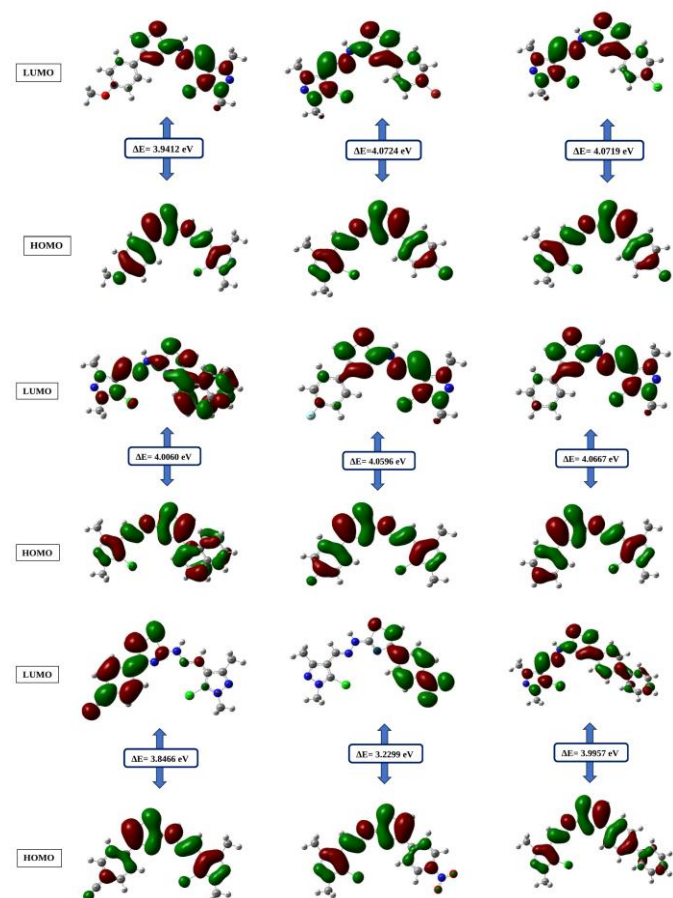


Fig. 6 HOMO and LUMO orbital plots of compounds **D1–D9**, with the HOMO–LUMO gap (ΔE) of each compound

3.4.2 Global Reactivity Descriptors

The descriptors defined in Section 2.6 were calculated for all compounds (Table 4). **D8** has the lowest chemical hardness (1.6149 eV), the highest softness (0.6191 eV $^{-1}$), the highest electrophilicity index (4.7549 eV) and the largest $\Delta N_{\max}$ (2.4266), which fits its small gap and suggests a flexible molecule with a strong tendency to accept electrons. **D7** comes second in electrophilicity (3.2772 eV). All chemical potentials are negative (-2.9912 to -3.9189 eV), as expected for stable molecules.

Table 4 Global reactivity descriptors of compounds **D1–D9**

Compound	Electro-negativity, χ , η (eV)	Hardness S (eV $^{-1}$)	Softness, S (eV $^{-1}$)	Chemical potential, μ (eV)	Electro-philicity index, ω (eV)	Max. electron transfer, $\Delta N_{\max}$ (dimensionless)
D1	2.9912	1.9706	0.5074	-2.9912	2.2699	1.5178
D2	3.2338	2.0362	0.4911	-3.2338	2.5678	1.5881
D3	3.2351	2.0359	0.4911	-3.2351	2.5703	1.5891
D4	3.1162	2.0030	0.4992	-3.1162	2.4241	1.5557
D5	3.1631	2.0298	0.4926	-3.1631	2.4646	1.5583
D6	3.1112	2.0333	0.4917	-3.1112	2.3801	1.5301
D7	3.5505	1.9233	0.5199	-3.5505	3.2772	1.8461
D8	3.9189	1.6149	0.6191	-3.9189	4.7549	2.4266
D9	3.1216	1.9978	0.5005	-3.1216	2.4388	1.5625

Comparing these trends with the disc assays gives a partial match. The three compounds with the smallest gaps (**D8**, **D7** and **D1**) are also among the most active in the assays: **D8** was the most active compound against *B. subtilis*, **D7** against *K. pneumoniae* and **D1** against *E. coli*. **D2**, however, has the widest gap and was still the most active compound against *C. albicans*. Electronic descriptors alone therefore cannot account for the activity, and steric and lipophilic factors are probably involved as well.

3.4.3 Molecular Electrostatic Potential

Molecular electrostatic potential (MEP) surfaces show how charge is distributed and where electrophilic and nucleophilic attack is most likely (Fig. 7).

Negative potential is concentrated around the thiazole nitrogen and the oxygen atoms of the substituents. These regions are favorable for electrophilic attack and for accepting hydrogen bonds. Positive potential surrounds hydrogens bonded to heteroatoms, which mark possible hydrogen-bond donor sites. Most of the aromatic surface is nearly neutral (green), pointing to an even electron distribution over the conjugated system. Negative potential around the heteroatoms could support hydrogen bonds with residues in a protein active site, which is consistent with the docking results.

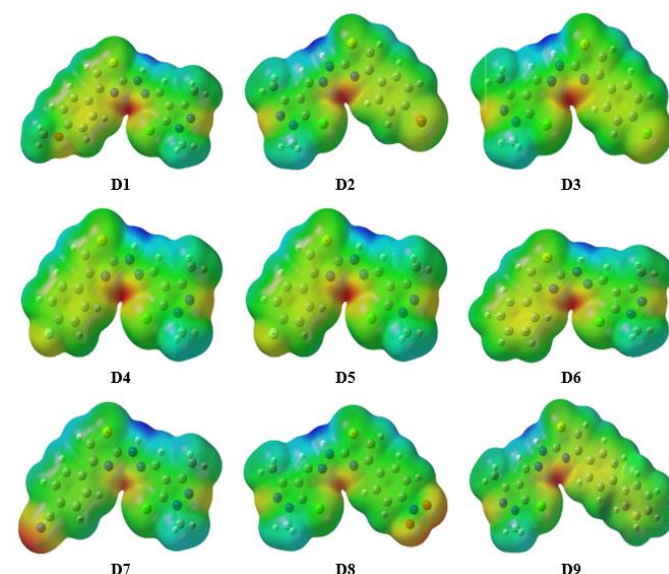


Fig. 7 Molecular electrostatic potential surfaces of compounds **D1–D9**. Red and yellow regions are negative, blue regions positive and green regions close to neutral

3.5 ADME Screening

SwissADME is a widely used web tool for estimating pharmacokinetic behavior, drug-likeness and medicinal chemistry friendliness. For **D1–D9** it returned physicochemical profiles that differ in polarity, hydrogen-bonding capacity and molecular flexibility, in line with the structural variation across the series; polar surface area and lipophilicity vary accordingly. Most compounds satisfy Lipinski's rule of five, with a few exceptions, and are predicted to be highly bioavailable orally with moderate solubility. The synthetic accessibility scores indicate that the compounds should be easy to make. On this *in silico* evidence the compounds look drug-like, which justifies further biological testing.

4. Conclusion

Nine thiazole–hydrazone hybrids (**D1–D9**) were prepared by a two-step condensation and cyclization route and characterized by FT-IR, NMR and mass spectrometry. In the disc diffusion assays **D1**, **D2** and **D7** were the most promising compounds, acting mainly on the Gram-negative strains and *C. albicans*. None of the compounds inhibited *S. aureus*, and overall potency was moderate next to the reference drugs. Docking of the most active compounds into four microbial target proteins (4URM, 1KZN, 5ECX and 5TZ1) gave binding energies of -6.7 to -8.7 kcal/mol with plausible hydrogen-bonding and hydrophobic contacts.

DFT calculations provided frontier orbital energies and reactivity descriptors, with **D8** showing the smallest HOMO–LUMO gap, and SwissADME predicted drug-like profiles. Together, these findings mark thiazole–hydrazone hybrids as reasonable starting points for further optimization, ideally supported by minimum inhibitory concentration data and tests on resistant strains.

References

- [1] M. Mallappa, M. Chahar, N. Choudhary, K.K. Yadav, M.T. Qasim, et al., Recent advances in the synthesis of nitrogen-containing heterocyclic compounds via multicomponent reaction and their emerging biological applications: a review, *J. Iran. Chem. Soc.* 22 (2025) 1–33.
- [2] M.R. Elmorsy, S.H. Yousef, E. Abdel-Latif, S.A. Badawy, Molecular structures and in silico molecular docking of new pyrazine-based heterocycles as antibacterial agents, *BMC Chem.* 19 (2025) 164.
- [3] A.I. Hassaballah, A.K. El-Ziaty, M.M. Gado, H.A.E. Sayed, M. Kamal, R.S. Ali, Design synthesis, characterization, molecular docking and antimicrobial evaluation of novel heterocycles with acrylonitrile and anthracene moieties, *Sci. Rep.* 15 (2025) 19370.
- [4] M.I. Ali, M.M. Naseer, Recent biological applications of heterocyclic hybrids containing s-triazine scaffold, *RSC Adv.* 13 (2023) 30462–30490.
- [5] A.K. Singh, A. Kumar, H. Singh, P. Sonawane, H. Paliwal, et al., Concept of hybrid drugs and recent advancements in anticancer hybrids, *Pharmaceuticals* 15 (2022) 1071.
- [6] N. Kerru, L. Gummidu, S. Maddila, K.K. Gangu, S.B. Jonnalagadda, A review on recent advances in nitrogen-containing molecules and their biological applications, *Molecules* 25 (2020) 1909.
- [7] R.F.A. Gomes, B.M.F. Gonçalves, K.H.S. Andrade, B.B. Sousa, N. Maulide, et al., Unlocking the potential of bio-based nitrogen-rich furanic platforms as biomass synthons, *Angew. Chem.* 135 (2023) e202304449.
- [8] B.P. Bandgar, S.A. Patil, R.N. Gacche, B.L. Korbad, B.S. Hote, et al., Synthesis and biological evaluation of nitrogen-containing chalcones as possible anti-inflammatory and antioxidant agents, *Bioorg. Med. Chem. Lett.* 20 (2010) 730–733.
- [9] M. Aatif, M.A. Raza, K. Javed, S. Mohd. Nashre-ul-Islam, M. Farhan, M.W. Alam, Potential nitrogen-based heterocyclic compounds for treating infectious diseases: a literature review, *Antibiotics* 11 (2022) 1750.
- [10] D.K. Lang, R. Kaur, R. Arora, B. Saini, S. Arora, Nitrogen-containing heterocycles as anticancer agents: an overview, *Anticancer Agents Med. Chem.* 20 (2020) 2150–2168.
- [11] S. Chandu, A. Manupathi, S. Gundekari, M. Varkolu, A review on the synthesis and biological evaluation of N-containing heterocyclic derivatives, *Curr. Org. Chem.* 30 (2026) 691–726.
- [12] T.N. Tran, M. Henary, Synthesis and applications of nitrogen-containing heterocycles as antiviral agents, *Molecules* 27 (2022) 2700.
- [13] K. Karrouchi, S. Radi, Y. Ramli, J. Taoufik, Y.N. Mabkhot, et al., Synthesis and pharmacological activities of pyrazole derivatives: a review, *Molecules* 23 (2018) 134.
- [14] J.R. Pollard, M. Mortimore, Discovery and development of Aurora kinase inhibitors as anticancer agents, *J. Med. Chem.* 52 (2009) 2629–2651.
- [15] N.Y. Guerrero-Pepinosa, M.C. Cardona-Trujillo, S. Cantón-Castaño, L.A. Veloza, J.C. Sepúlveda-Arias, Antiproliferative activity of thiazole and oxazole derivatives: a systematic review of in vitro and in vivo studies, *Biomed. Pharmacother.* 138 (2021) 111495.
- [16] M. Gümüş, M. Yakan, İ. Koca, Recent advances of thiazole hybrids in biological applications, *Future Med. Chem.* 11 (2019) 1979–1998.
- [17] N. TH, S.S. Nair, A. Kumar, V. B, D. P A, A review on synthesis and biological activity of thiazole and its derivatives, *Int. J. Pharm. Sci. Rev. Res.* 70 (2021) 189–193.
- [18] A.R. Patil, B.C. Yallur, S. Chinnam, G.S. Ananthnag, C.R. Santhosh, et al., Pharmaceutical perspectives of thiazole analogues: an overview, *Results Chem.* 12 (2024) 101820.
- [19] R. Rana, N. Kumar, H.K. Gulati, A. Sharma, A. Khanna, et al., A comprehensive review on thiazole-based conjugates as anti-cancer agents, *J. Mol. Struct.* 1292 (2023) 136194.
- [20] Y.R. Sable, R.A. Shinde, H.K.A. Yasin, N. Ghanwate, S.N. Mali, et al., Furan-thiazole hydrazone scaffolds as promising antitubercular and antibacterial agents: synthesis, characterization, bioevaluation and computational analysis, *RSC Adv.* 15 (2025) 30001–30025.
- [21] S. Ramos-Inza, C. Aydllo, C. Sanmartín, D. Plano, Thiazole moiety: an interesting scaffold for developing new antitumoral compounds, in: *Heterocycles – synthesis and biological activities*, IntechOpen, Rijeka, Croatia, Spain, 2020.
- [22] G. Kumari, S. Dhillon, P. Rani, M. Chahal, D.K. Aneja, M. Kinger, Development in the synthesis of bioactive thiazole-based heterocyclic hybrids utilizing phenacyl bromide, *ACS Omega* 9 (2024) 18709–18746.
- [23] N. Kaur, M. Yadav, P. Grewal, A review on the synthesis of biologically active thiazoles, *Curr. Top. Med. Chem.* 26 (2026).
- [24] Sahil, K. Kaur, V. Jaitak, Thiazole and related heterocyclic systems as anticancer agents: a review on synthetic strategies, mechanisms of action and SAR studies, *Curr. Med. Chem.* 29 (2022) 4958–5009.
- [25] P. Bhukta, A. Verma, R. Kumar, A.B. Marianesan, S. Ballal, et al., A perspective on the synthetic methodologies and biological attributes of thiazole-hydrazone compounds: a medicinal chemistry-based investigation, *Mol. Divers.* 30 (2025) 2979–3008.
- [26] F.D. Schoenknecht, The Kirby–Bauer technique in clinical medicine and its application to carbenicillin, *J. Infect. Dis.* 127(Suppl. 2) (1973) S111–S115.
- [27] S. Pardeshi, V. Jadhav, S. Disale, H. Suryavanshi, V.A. Adole, B. Sapkal, Indole-functionalized 1,2,3-triazole derivatives: synthesis, antimicrobial activity, molecular docking, DFT and ADME studies, *J. Mol. Struct.* 1369 (2026) 146282.
- [28] M.S. Valdés-Tresanco, M.E. Valdés-Tresanco, P.A. Valiente, E. Moreno, AMDock: a versatile graphical tool for assisting molecular docking with Autodock Vina and Autodock4, *Biol. Direct* 15 (2020) 12.
- [29] D. Seeliger, B.L. de Groot, Ligand docking and binding site analysis with PyMOL and Autodock/Vina, *J. Comput. Aided Mol. Des.* 24 (2010) 417–422.
- [30] G. Sandeep, K.P. Nagasree, M. Hanisha, M.M.K. Kumar, AUDocker LE: a GUI for virtual screening with AUTODOCK Vina, *BMC Res. Notes* 4 (2011) 445.
- [31] T.F. Vieira, S.F. Sousa, Comparing AutoDock and Vina in ligand/decoy discrimination for virtual screening, *Appl. Sci.* 9 (2019) 4538.
- [32] S.M. Gomha, H.A. Abdelhady, D.Z.H. Hassain, A.H. Abdelmonsef, M. El-Naggar, et al., Thiazole-based thiosemicarbazones: synthesis, cytotoxicity evaluation and molecular docking study, *Drug Des. Devel. Ther.* 15 (2021) 659–677.
- [33] A. Balupuri, C.G. Gadhe, P.K. Balasubramanian, G. Kothandan, S.J. Cho, In silico study on indole derivatives as anti-HIV-1 agents: a combined docking, molecular dynamics and 3D-QSAR study, *Arch. Pharm. Res.* 37 (2014) 1001–1015.
- [34] H.A.K. Kyhoiesh, H.M. Hassan, Synthesis, characterization, in silico DFT, molecular docking, ADMET profiling studies and toxicity predictions of Ag(I) complex derived from 4-aminoacetophenone, *ChemistrySelect* 9 (2024) e202304429.