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# Synthesis, characterization, molecular docking and exploration of biological activities of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4 dithiazolidine

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## ABSTRACT

Dithiazolidines are a type of organic compound with a dithiazolidine ring that contains two nitrogen atoms and two sulfur atoms arranged in a specific way. These compounds can have various functional groups and substituents attached to the ring, which changes their reactivity and chemical properties. Dithiazolidines may play an important role in several areas, such as coordination chemistry, material science for creating new materials or modifying existing ones, medicinal chemistry, and organic synthesis as building blocks for more complex molecules. Due to their unique structures and ways of functioning, researchers have looked into dithiazolidine derivatives for their possible use as antimicrobial agents. In this study, N-aryl-S-chloro isothiocarbamoyl chloride was formed by refluxing p-phenylene diamine and aryl isothiocyanate with chloroform.

Dithiazolidines are a class of organic compounds with a dithiazolidine ring, consisting of two sulfur atoms and two nitrogen atoms arranged with specific linkage. These compounds can have various substituents and functional groups attached to the ring, which changes their chemical properties and reactivity. Dithiazolidines plays an important role several fields, like organic synthesis, as intermediates to construct more complex molecules; medicinal chemistry; material science for creating new materials or modifying existing ones; and coordination chemistry. Due to unique structures and mechanisms of action, Dithiazolidine derivatives have been explored for their potential as antimicrobial agents. In this research, Aryl isothiocyanate and p-phenylene diamine were refluxed with chloroform to produce N-aryl-S-chloro isothiocarbamoyl chloride. This compound, along with aryl isothiocyanate, underwent chlorination and subsequent cyclization to produce 3,5-diaryl, diimino, 4-(aryl amino) 1,2.4 dithiazolidine. The synthesized products were characterized by spectral and analytical studies like IR, NMR, and mass spectral studies. Their antimicrobial activities was also checked and they showed moderate to good antibacterial as well as antifungal activities. The study highlights the potential of dithiazolidine derivatives as antimicrobial agents, demonstrating their moderate to good activity against selected bacterial and fungal strains. These findings suggest further exploration of dithiazolidines in medicinal chemistry and other applications. Furthermore, the exact binding mode to the selected target proteins for newly synthesized dithiazolidines is determined by performing docking analysis which confirmed well correlation with the experimental observations as per docking results.

**KEYWORDS**: Dithiazolidines, cyclization reaction, aryl isothiocyanate, antimicrobial activity, molecular docking.

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## INTRODUCTION

The dynamic chemistry of heterocyclic compounds continues to attract significant attention owing to their structural diversity and broad applications in pharmaceuticals, material science, and chemical synthesis. These frameworks are indispensable in drug discovery due to their ability to provide biologically active scaffolds and serve as intermediates in complex organic transformations [1–4].

Among them, thiazolidines—five-membered heterocycles containing both nitrogen and sulfur atoms—are particularly versatile. Their derivatives display a various pharmacological activities, including anti-inflammatory [5], antidepressant [5], anticancer [6], anti-tubercular, and antimicrobial [7] activities. The presence of multiple heteroatoms enables chemical modifications that enhance their therapeutic potential.

Diathiazolidines, an advanced subclass, have garnered growing interest due to their unique framework comprising two nitrogen and two sulfur atoms. This structural feature opens avenues for designing compounds with specific physicochemical and biological properties. These moieties have been explored as intermediates in organic synthesis [8], pharmacologically active molecules [9], corrosion inhibitors [10–13], and ligands in coordination chemistry for stabilizing metal complexes and enabling catalytic applications [14,15].

Recent studies have delved into the synthesis of thiazolidine derivatives as well as diathiazolidine derivatives incorporating various aryl groups [16,17]. Such structural variations not only influence the compounds' stability and reactivity but also broaden their applicability. Characterization techniques, including NMR and mass spectrometry, have provided valuable insights into their structures, while biological screening has confirmed their antimicrobial and antifungal potential, underscoring their relevance in

combating microbial resistance.

In the present study, we performed the synthesis of a new series of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4 dithiazolidine (III) was synthesized incorporating different aryl groups: a) o-tolyl, b) p-tolyl, c) phenyl, d) m-chloro phenyl, e) o-anisyl, f) p-anisyl, g) p-chloro phenyl. Spectral and analytical studies, like IR, NMR, and mass spectra studies was done for synthesized compounds. Screening was done for antimicrobial as well as antifungal activities, and molecular docking studies were conducted to establish structure–activity correlations and support experimental findings.

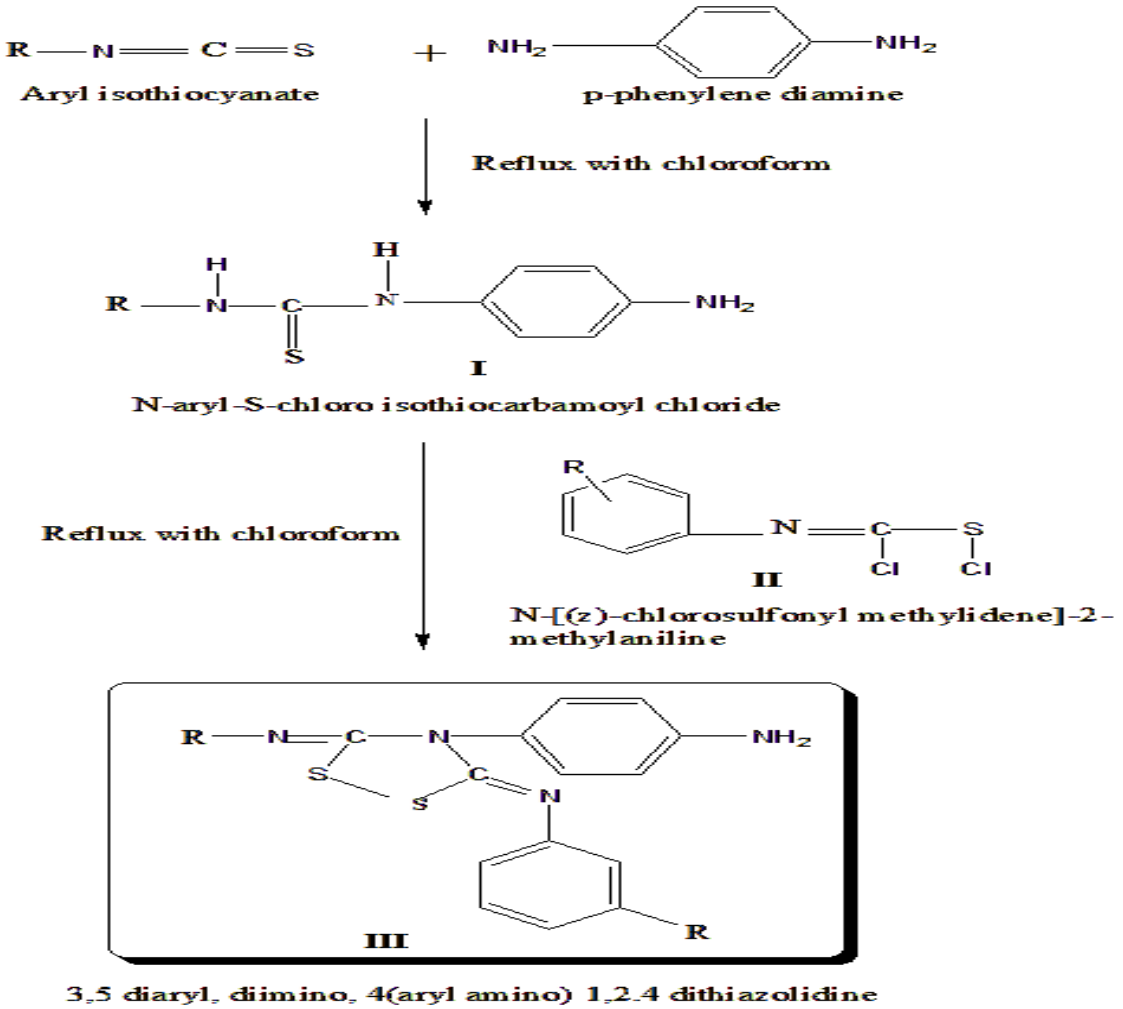
Although thiazolidine and diathiazolidine derivatives have been widely studied, systematic investigations on 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines remain scarce. In particular, the structure–activity relationship of these molecules with diverse aryl substituents has not been adequately explored, and no studies have combined experimental antimicrobial/antifungal screening with molecular docking approaches to rationalize biological activity at the molecular level. To address these gaps, the present work aims to synthesize and characterize a series of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines, evaluate their antimicrobial and antifungal activities, and perform molecular docking studies to establish correlations between structural features and biological potential.

To the best of our knowledge, this is first systematic report on the synthesis and biological evaluation of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines, combining both experimental and computational approaches. This integrated strategy highlights their potential as promising candidates for future drug discovery method.

## MATERIALS AND METHODS:

### 1.1. Synthesis:

Aryl Isothiocyanates were prepared by already known procedures [18]. Aryl isothiocyanate (0.01M) and p-phenylene diamine (0.01M) were refluxed with chloroform to produce N-aryl-S-chloro isothiocarbamoyl chloride (I). Aryl isothiocyanate on chlorination (1:1) produced N-[(z)-chlorosulfonyl methylidene]-2-methylaniline (II). N-aryl-S-chloro isothiocarbamoyl chloride (I) and N-[(z)-chlorosulfonyl methylidene]-2-methylaniline (II) in solvent $CHCl_3$ is refluxed with subsequent cyclization reaction and produced 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4 dithiazolidine (III). Aryl groups here are a) o-tolyl b) p-tolyl c) phenyl d) 3-chloroaniline e) o-anisyl f) 2-chloroaniline.



Where R in (III) 3,5 diaryl, diimino, 4(aryl amino) 1,2,4 dithiazolidine are a) o-tolyl (III-a) b) p-tolyl(III-b) c) phenyl (III-c) d) 3-chloroaniline (III-d) e) o-anisyl (III-e) f) 2-chloroaniline (III-f)

**2.2 Characterization:**

Structural confirmation of the newly synthesized compounds was achieved through a combination of spectroscopic techniques, including IR, $^{1}$H-NMR, $^{13}$C-NMR as well as mass spectral study.

**2.2.1 Spectral Data of Synthesized Compounds**

**(III-a)** 3,5-di-2-tolyl, diimino, 4-(2-tolyl amino)-1,2,4 dithiazolidine

IR (KBr) ν max cm$^{-1}$: 3209.55(N-H), 3030.17(C-H aromatic), 1687.71(C=N), 1512.19(C=C Aromatic ring stretch), 1332.81(C=N), 715.59(C-S), 493.78(S-S). $^{1}$H NMR ($CDCl_3$) $\delta_H$ ppm: 1.25 (s, 2H, R-$NH_2$), 6.8 to 7.2 (m,16H, Ar-H), 2.35(s, 6H, Ar-$CH_3$). $C^{13}$NMR ($CDCl_3$) $\delta_H$ ppm: 137.613-120.972 (24C, Aromatic ring carbons), 21.413-21.049 (2C, Ar-$CH_3$ carbon). MS(m/z): 478.20 (M-2H Deprotonated)+, 373.15(M-N-Ar-$NH_2$), 298.05(M-2(Ar-$CH_3$)). HRMS((m/z): 480.19 (M observed), 481.18 (M+ observed), 464.21 (M-$NH_2$), 259.08 (M-Ar-$CH_3N_3C_2S_2$), 373.15(M-Ar-$NNH_2$). Molecular formula of III-a: $C_{28}H_{24}N_4S_2$. Molecular weight 480. Colour: Greyish black.

**(III-b)** 3,5-di-4-tolyl, diimino, 4-(4-tolyl amino)-1,2,4 dithiazolidine

IR (KBr) ν max cm$^{-1}$: 3215.34(N-H), 3018.6(C-H aromatic), 1610.56(C=N), 1510.26(C=C Aromatic ring stretch), 1334.74(C-N), 723.21(C-S), 449.41(S-S). $^{1}$H NMR (DMSO)$\delta_H$ ppm: 1.25 (s, 2H, R-$NH_2$), 7.45-7.1 (m,16H, Ar-H), 2.38(s, 6H, Ar-$CH_3$). $C^{13}$ NMR (DMSO) $\delta_H$ ppm: 180 (2C, C=N dithiazolidine ring carbon), 130.786-122.437 (24C, Aromatic ring carbons), 18.225-17.765 (2C, Ar-$CH_3$ carbon). MS(m/z): 479.21(M-H Deprotonated)+, 298.05(M-2(Ar-$CH_3$)), 374.15(M-N-Ar-$NH_2$). Molecular formula of III-b: $C_{28}H_{24}N_4S_2$. Molecular weight 480. Colour: Black.

**(III-c)** 3,5-diphenyl, diimino, 4-(phenyl amino)-1,2,4 dithiazolidine

IR (KBr) ν max cm$^{-1}$: 3211.48(N-H), 2956.87(C-H aromatic), 1625.99(C=N), 1510.26(C=C Aromatic ring stretch), 1290.38(C-N), 746.45(C-S), 472.56(S-S). $^{1}$H NMR ($CDCl_3$)$\delta_H$ ppm: 3.5 (s, 2H, R-$NH_2$), 7.5-7.1 (m, 18H, Ar-H). $C^{13}$ NMR ($CDCl_3$) $\delta_H$ ppm: 179 (2C, C=N dithiazolidine ring carbon), 143.827-115.064 (24C, Aromatic ring carbons). MS(m/z): M+ not observed. 360.13(M-Ar-$NH_2$). Molecular formula of III-c: $C_{26}H_{20}N_4S_2$. Molecular weight 452. Colour: Brown.

**(III-d)** 3,5-di-3-chloro phenyl, diimino, 4-(3-chloro phenyl amino)-1,2,4 dithiazolidine

IR (KBr) ν max cm$^{-1}$: 3176.76(N-H), 3068.75(C-H aromatic), 1625.99(C=N), 1512.19(C=C Aromatic ring stretch), 1323.17(C-N), 785.03(C-S), 698.23(C-Cl) 447.49(S-S). $^{1}$H NMR (DMSO)$\delta_H$ ppm: 3.4 (s, 2H, R-$NH_2$), 7.7-6.6 (m, 16H, Ar-H). $C^{13}$ NMR (DMSO) $\delta_H$ ppm: 180 (2C, C=N dithiazolidine ring carbon), 144.382-114.355 (24C, Aromatic ring carbons). MS(m/z): M+ not observed. 151.03(M-$C_2S_2N_3ArArArNH_2Cl_2$), 413.5(M-N-Ar-$NH_2$), 92.05(M-$C_2S_2N_3ArArArCl_2$). Molecular formula of III-d: $C_{26}H_{18}N_4S_2Cl_2$. Molecular weight 520. Colour: Greyish black.

**(III-e)** 3,5-di-2-anisyl, diimino, 4-(2-anisyl amino)-1,2,4 dithiazolidine

IR (KBr) ν max cm$^{-1}$: 3213.41(N-H), 3024.38(C-H aromatic), 1610.56(C=N), 1512.19(C=C Aromatic ring stretch), 1338.60(C-N), 1033.85(C-O), 723.31(C-S), 451.34(S-S). $^{1}$H NMR (DMSO)$\delta_H$ ppm: 3.8 (s, 2H, R-$NH_2$), 7.5-6.8 (m, 16H, Ar-H), 3.8(Ar-$OCH_3$). $C^{13}$ NMR (DMSO) $\delta_H$ ppm: 180 (2C, C=N dithiazolidine ring carbon), 136.799-113.609 (24C, Aromatic ring carbons), 156.465(Ar-$OCH_3$). MS(m/z): 511.20 (M-H Deprotonated)+, 406.15(M-N-Ar-$NH_2$), 405.14 (M-Ar-$OCH_3$), 390.15(M-N-Ar-$OCH_3$). Molecular formula of III-e: $C_{28}H_{24}N_4S_2O_2$. Molecular weight 512. Colour: Grey.

**(III-f)** 3,5-di-2-chloro phenyl, diimino, 4-(2-chloro phenyl amino)-1,2,4 dithiazolidine

IR (KBr) ν max cm$^{-1}$: 3169.04(N-H), 3003.17(C-H aromatic), 1687.71(C=N), 1510.26(C=C Aromatic ring stretch), 1300.02(C-N)725.23(C-S), 623.01(C-Cl), 451.34(S-S). $^{1}$H NMR (DMSO) $\delta_H$ ppm: 3.8 (s, 2H, R-$NH_2$), 7.45-6.8 (m, 16H, Ar-H). $C^{13}$ NMR (DMSO) $\delta_H$ ppm: 180 (2C, C=N dithiazolidine ring carbon), 137.382-114.113 (24C, Aromatic ring carbons). MS(m/z): M+ not observed, 151.03(M-$C_2S_2N_3ArArArNH_2Cl_2$), 317.99(M-ArArNCl), 413.03(M-N-Ar-$NH_2$). Molecular formula of III-f: $C_{26}H_{18}N_4S_2Cl_2$. Molecular weight 520. Colour: Black.

**2.3 Molecular Docking:**

**2.3.1 Platform for molecular docking**

Using AutoDock Vina, molecular docking simulations were done to assess the binding of selected ligand to the trimeric protein target [19,20].

**2.3.2 Protein preparation**

Molecular docking process of selected synthesized compounds was executed on proteins which are fetched from protein data bank (https://www.rcsb.org). The list of proteins and their descriptions is given below in Table 1. The selected chain alongwith a native ligand bound to it is selected for protein preparation. However, co-crystallized water molecules and non-essential molecular fragments were removed. The structure was then subjected to geometry optimization and energy minimization using the Dock Prep module in UCSF Chimera (version 1.17 [21]. To mimic the protonation behavior of phytocompounds at physiological pH, polar hydrogen atoms were incorporated, and corresponding partial atomic charges were systematically assigned.. [22].

**Table 1: Molecular targets selected for docking studies with their corresponding PDB IDs and source organisms**

| Sr. No. | Target Details | PDB ID | Organisms |
|---|---|---|---|
| 1. | Dihydrofolate reductase (DHFR) | 3QLS | Candida albicans (Antifungal target) |
| 2. | Secreted aspartic protease | 3Q70 | |
| 3. | N-myristoyl transferase | 1IYL | |
| 4. | Dihydrofolate reductase | 3FYV | Staphylococcus aureus (Antibacterial target) |
| 5. | Gyrase B | 3G7B | |
| 6. | Sortase A | 2MLM | |
| 7. | Rhomboid protease | 3UBB | Escherichia coli (Antibacterial target) |

### 2.3.3 Ligand preparations

The synthesized compounds' structures were sketched using the ChemDraw Ultra tool v14.0. Further, UFF force field in Avogadro software v1.2.0 was used to have energy minimization of all the ligands. The ligands were then retrieved into UCSF Chimera tools and prepared using the Dockprep tool of the software for the addition of hydrogens and partial charges.

### 2.3.4 Molecular Docking Procedure

Docking studies were performed and predicted the binding energy based on its complex geometry, and the binding interaction between ligands against selected target proteins was explored. AutoDock Vina [23] tool integrated with UCSF Chimera software v1.17[21] was utilized, applying default values for the parameters, a grid box centered at native ligands with 0.375 Å of grid spacing. The details of the grid box co-ordinates, locations, and grid dimensions are given in Table 2 below. The binding affinity of ligands was explored using the View Dock tool. Using the 'View Dock' tab docking results were seen. Docked conformation visualzations obtained were studied using Discovery Studio 2020 Client [24] and PyMol software [25], respectively.

**Table 2: Grid box center coordinates (x, y, z) and dimensions (Å) defined for docking simulations of protein targets**

| Sr. No. | PDB ID | Co-ordinates locations (x, y and z) | Grid dimensions (Å) |
|---|---|---|---|
| 1. | 3QLS | -0.079, 4.940, 32.034 | 25 x 25 x 25 |
| 2. | 3Q70 | -24.144, -13.387, 21.695 | 28 x 28 x 28 |
| 3. | 1IYL | 13.415, 47.742, -1.041 | 26 x 26 x 26 |
| 4. | 3FYV | 30.705, 11.534, 42.122 | 26 x 25 x 25 |
| 5. | 3G7B | 50.354, -2.964, 19.129 | 25 x 25 x 25 |
| 6. | 2MLM | 25.189, 17.840, 10.687 | 27 x 18 x 25 |
| 7. | 3UBB | -0.001, 51.460, 32.646 | 25 x 25 x 25 |

### 2.4 Antimicrobial and Antifungal Studies:

The screening of synthesized compounds was performed to assess their antimicrobial and antifungal activity. Antibacterial screening was done as per standard procedure [26]. Antifungal screening is done as per standard procedure [27].

## RESULTS AND DISCUSSION

### 3.1 Synthesis, characterization of molecules

The synthesis of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4 dithiazolidine derivatives (IIIa–IIIf) was carried out in two steps utilizing a mixture of substituted aryl isothiocyanate and p-phenylene diamine. $^{13}$C-NMR, $^{1}$H-NMR, and FT-IR spectroscopic studies and elemental analysis were performed to assess the structure of the synthesized molecules.

FTIR Shimadzu (Affinity) Elmer spectrum RXI (8300 to 350 $cm^{-1}$) FT IR spectrometer was used to record IR spectra. JOEL ECZR Series 600 MHz NMR spectrometer was used for $^{1}$H NMR spectra. The sample was prepared in DMSO/$CDCl_3$ solution with TMS as an internal reference. Maldi-TOF Synapt XS HD Mass Spectrometer was used to record the mass spectra.

The IR spectra displayed characteristic absorptions corresponding to functional groups present in the dithiazolidine framework (Table 3). A strong absorption in the range of **3169–3215 $cm^{-1}$** was attributed to the N–H stretching vibration, while peaks between **3003–3068 $cm^{-1}$** indicated aromatic C–H stretching. The C=N stretching appeared in the **1610–1687 $cm^{-1}$** region, confirming the imino functionality. Absorptions in the ranges of **715–785 $cm^{-1}$** and **447–493 $cm^{-1}$** were assigned to C–S and S–S bonds, respectively, verifying the dithiazolidine core. Additionally, compounds III-d and III-f exhibited bands at **698–623 $cm^{-1}$** corresponding to C–Cl stretching, while III-e showed a prominent peak at **1033 $cm^{-1}$** due to C–O stretching of the methoxy group.

**Table 3: FT-IR absorption bands of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives (IIIa–IIIf) with corresponding functional group assignments.**

| Compound | Absorption Observed ($cm^{-1}$) | Assignment | Absorbance expected ($cm^{-1}$) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **(III-a)** (R = o-toluidine) | 3209.55 | N-H | 3450-3100 |
| | 3030.17 | C-H Aromatic | 3050-2850 |
| | 1687.71 | C=N | 1689-1470 |
| | 1512.19 | C=C Aromatic Ring Stretch | 1680-1400 |
| | 1332.81 | C-N | 1350-1270 |
| | 715.59 | C-S | 800-600 |
| | 493.78 | S-S | 520-400 |
| **(III-b)** (R = P-toluidine) | 3215.34 | N-H | 3450-3100 |
| | 3018.6 | C-H Aromatic | 3050-2850 |
| | 1610.56 | C=N | 1689-1470 |
| | 1510.26 | C=C Aromatic Ring Stretch | 1680-1400 |
| | 1334.74 | C-N | 1350-1270 |
| | 723.21 | C-S | 800-600 |
| | 449.41 | S-S | 520-400 |
| **(III-c)** (R = aniline) | 3211.48 | N-H | 3450-3100 |
| | 2956.87 | C-H Aromatic | 3050-2850 |
| | 1625.99 | C=N | 1689-1470 |
| | 1510.26 | C=C Aromatic Ring Stretch | 1680-1400 |
| | 1290.38 | C-N | 1350-1270 |
| | 746.45 | C-S | 800-600 |
| | 472.56 | S-S | 520-400 |
| **(III-d)** R = 3 chloroaniline (m) | 3176.76 | N-H | 3450-3100 |
| | 3068.75 | C-H Aromatic | 3050-2850 |
| | 1625.99 | C=N | 1689-1470 |
| | 1512.19 | C=C Aromatic Ring Stretch | 1680-1400 |
| | 1323.17 | C-N | 1350-1270 |
| | 785.03 | C-S | 800-600 |
| | 698.23 | C-Cl | 850-550 |
| | 447.49 | S-S | 520-400 |
| **(III-e)** R = o-anisidine) | 3213.41 | N-H | 3450-3100 |
| | 3024.38 | C-H Aromatic | 3050-2850 |
| | 1610.56 | C=N | 1689-1470 |
| | 1512.19 | C=C Aromatic Ring Stretch | 1680-1400 |
| | 1338.6 | C-N | 1350-1270 |
| | 1033.85 | C-O | 1320-1000 |
| | 723.31 | C-S | 800-600 |
| | 451.34 | S-S | 520-400 |
| **(III-f)** R = 2 chloroaniline (o) | 3169.04 | N-H | 3450-3100 |
| | 3003.17 | C-H Aromatic | 3050-2850 |
| | 1687.71 | C=N | 1689-1470 |

| | | |
|---|---|---|
| 1510.26 | C=C Aromatic Ring Stretch | 1680-1400 |
| 1300.02 | C-N | 1350-1270 |
| 725.23 | C-S | 800-600 |
| 623.01 | C-Cl | 850-550 |
| 451.34 | S-S | 520-400 |

The $^{1}$H-NMR spectra further substantiated the structures (Table 4). All compounds displayed singlets in the region of **1.2–3.8 ppm** attributable to the amine protons (–$NH_2$). Multiplets between **6.6–7.7 ppm** corresponded to aromatic protons, consistent with the aryl substituents. Characteristic signals for substituents were also observed, such as the methyl protons in III-a and III-b at **δ 2.35–2.38 ppm**, and the methoxy protons in III-e at **δ 3.8 ppm**.

**Table 4: $^{1}$H-NMR spectral data of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives (IIIa–IIIf).**

| Compound | Peaks observed in $^{1}$H NMR study | | | |
|---|---|---|---|---|
| **(III-a)** (R = o-toluidine) | 1.25 (s,2H, R-$NH_2$) | 6.8-7.2 (m,16H, Ar-H) | 2.35(s,6H,Ar-$CH_3$) | |
| **(III-b)** (R = P-toluidine) | 1.25 (s,2H, R-$NH_2$) | 7.45-7.1 (m,16H, Ar-H) | 2.38(s,6H,Ar-$CH_3$) | |
| **(III-c)** (R = aniline) | 3.5 (s,2H, R-$NH_2$) | 7.5-7.1 (m,18H, Ar-H) | | |
| **(III-d)** R = 3 chloroaniline (m) | 3.4 (s,2H, R-$NH_2$) | 7.7-6.6 (m,16H, Ar-H) | | |
| **(III-e)** (R = o-anisidine) | 3.8 (s,2H, R-$NH_2$) | 7.5-6.8 (m,16H, Ar-H) | | 3.8 (Ar-OCH3) |
| **(III-f)** R = 2 chloroaniline (o) | 3.8 (s,2H, R-$NH_2$) | 7.45-6.8 (m,16H, Ar-H) | | |

The $^{13}$C-NMR spectra (Table 5) showed characteristic signals for the **C=N carbon of the dithiazolidine ring at δ ~179–180 ppm**, confirming the presence of the diimino group. Aromatic carbons resonated between **113–144 ppm**, while substituent-specific peaks were also evident, such as the methoxy carbon in III-e at **δ 156.46 ppm** and the methyl carbons in III-a and III-b at **δ 17–21 ppm.**

**Table 5: $^{13}$C NMR chemical shifts of synthesized 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives (IIIa–IIIf).**

| Compound | Peaks observed in $^{13}$C NMR study | | | |
|---|---|---|---|---|
| **(III-a)** (R = o-toluidine) | | 137.613-120.972 (24C, Aromatic ring carbons), | 21.413-21.049 (2C, Ar-$CH_3$ carbon) | |
| **(III-b)** (R = p-toluidine) | 180 (2C, C=N dithiazolidine ring carbon), | 130.786-122.437 (24C, Aromatic ring carbons), | 18.225-17.765 (2C, Ar-$CH_3$ carbon) | |

| | | | | |
|---|---|---|---|---|
| **(III-c)** (R = aniline) | 179 (2C, dithiazolidine carbon), | C=N ring | 143.827-115.064 (24C, Aromatic ring carbons) | |
| **(III-d)** R = 3 chloroaniline (m) | 180 (2C, dithiazolidine carbon), | C=N ring | 144.382-114.355 (24C, Aromatic ring carbons), | |
| **(III-e)** (R = o-anisidine) | 180 (2C, dithiazolidine carbon), | C=N ring | 136.799-113.609 (24C, Aromatic ring carbons), | 156.465(Ar-$OCH_3$) |
| **(III-f)** R = 2 chloroaniline (o) | 180 (2C, dithiazolidine carbon), | C=N ring | 137.382-114.113 (24C, Aromatic ring carbons), | |

Mass spectrometric data provided molecular ion peaks or major fragment ions consistent with the proposed structures. For instance, compound III-a showed a deprotonated molecular ion at **m/z 478.20,** along with diagnostic fragments corresponding to the loss of aryl substituents. Similarly, compound III-e displayed a molecular ion at **m/z 511.20**, confirming its methoxy substitution. Compounds III-c, III-d, and III-f did not show molecular ion peaks but exhibited characteristic fragment ions supporting their assigned structures. The elemental composition as well as physical properties of the synthesized compounds are summarized in Table 6. The molecular weights ranged from **452 to 520 g/mol**, consistent with the calculated values, and the compounds were obtained as crystalline solids varying from brown to black and greyish-black in color.

**Table 6: Molecular weight, molecular formula, and physical appearance of synthesized 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives (IIIa–IIIf).**

| Name of the synthesized compounds | | Molecular Weight | Molecular Formula | Colour |
|---|---|---|---|---|
| **(III-a)** | R = o-toluidine | 480 | $C_{28}H_{24}N_4S_2$ | Greyish black |
| **(III-b)** | R = p-toluidine | 480 | $C_{28}H_{24}N_4S_2$ | Black |
| **(III-c)** | R = aniline | 452 | $C_{26}H_{20}N_4S_2$ | Brown |
| **(III-d)** | R = 3 Chloroaniline (m) | 520 | $C_{26}H_{18}N_4S_2Cl_2$ | Greyish black |
| **(III-e)** | R = o-anisidine | 512 | $C_{28}H_{24}N_4S_2O_2$ | Grey |
| **(III-f)** | R = 2 chloroaniline (o) | 520 | $C_{26}H_{18}N_4S_2Cl_2$ | Black |

Collectively, these spectral as well as analytical data obtained confirm successful synthesis of the desired 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives.

### 3.2 Molecular Docking Studies

Molecular docking analyses were conducted to elucidate the binding mechanisms and interaction profiles of the synthesized dithiazolidine derivatives with the fungal and bacterial protein targets and to compare their affinities with those of the respective native ligands. The docking scores (Table 7) revealed that all compounds showed stronger binding affinities than the native ligands across most targets, highlighting their potential as effective antimicrobial agents.

**Table 7: Docking scores (binding affinities in kcal/mol) of synthesized compounds (IIIa to IIIf) compared with native ligands.**

| Compound no. | Docking Scores against 3QLS | 3Q70 | 1IYL | 3FYV | 3G7B | 2MLM | 3UBB |
|---|---|---|---|---|---|---|---|
| **Native Ligands** | -7.9 | -8.9 | -9.9 | -8.9 | -8.1 | -6.6 | -7.8 |
| III-a | -10.2 | -9.0 | -10.2 | -9.8 | -8.0 | -8.1 | -8.3 |
| III-b | -9.6 | -9.0 | -9.5 | -9.8 | -7.8 | -7.5 | -7.7 |
| III-c | -9.6 | -8.7 | -10.1 | -10.3 | -8.1 | -7.3 | -8.6 |
| III-d | -9.5 | -8.4 | -9.9 | -10.8 | -8.2 | -7.2 | -8.4 |
| III-e | -9.3 | -8.3 | -9.8 | -9.8 | -8.0 | -7.4 | -7.6 |
| III-f | -10.0 | -8.8 | -9.7 | -9.9 | -8.1 | -8.2 | -8.0 |

Among the antifungal proteins, **compound IIIa exhibited the highest binding affinity towards *Candida albicans* DHFR (3QLS, –10.2 kcal/mol),** outperforming the native ligand (–7.9 kcal/mol). As illustrated in **Figure 1,** IIIa formed multiple hydrogen bonds with active site residues along with stabilizing hydrophobic contacts, which explain its superior binding affinity.

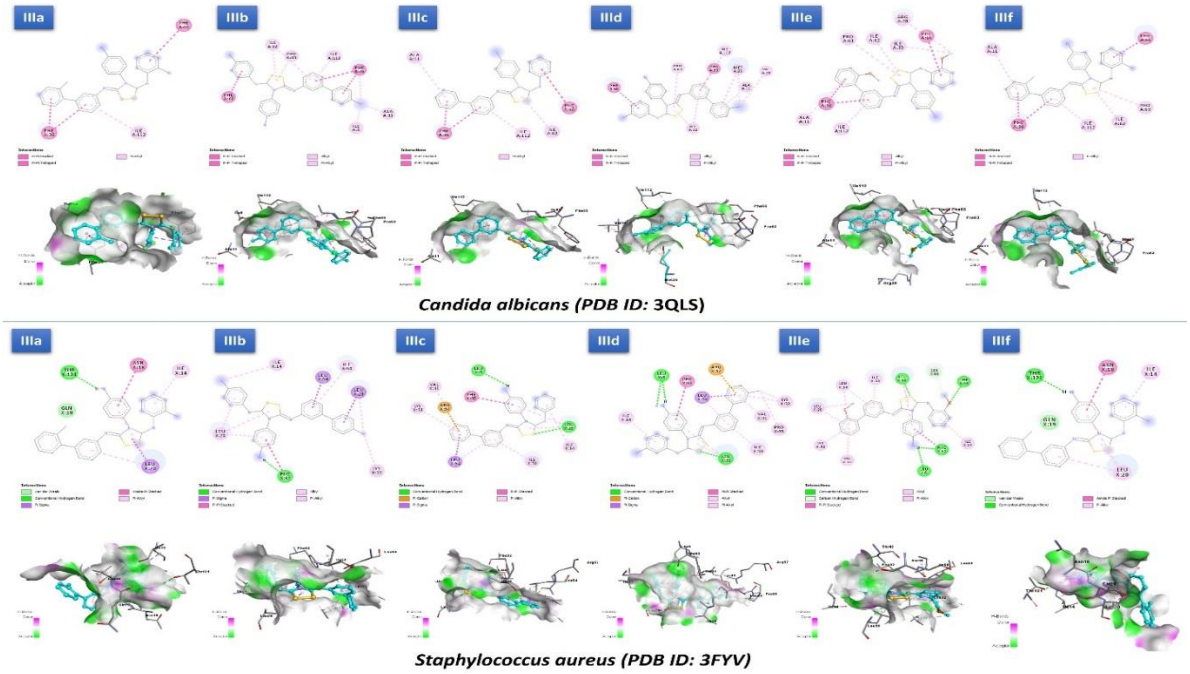



**Figure 1. Molecular docking interactions of synthesized dithiazolidine derivatives (IIIa–IIIf) with *Candida albicans* DHFR (PDB ID: 3QLS) and *Staphylococcus aureus* DHFR (PDB ID: 3FYV). The top panels show 2D ligand–residue interaction maps, and the bottom panels depict 3D binding poses within the active site pocket.**

Similarly, **IIIc and IIId demonstrated stronger binding to *Staphylococcus aureus* DHFR (3FYV, –10.3 and –10.8 kcal/mol, respectively)** compared with the native ligand (–8.9 kcal/mol). The docking pose of IIId (Figure 2) revealed stable hydrogen bonding interactions and van der Waals contacts, rationalizing its higher docking score and supporting its potent antibacterial potential.

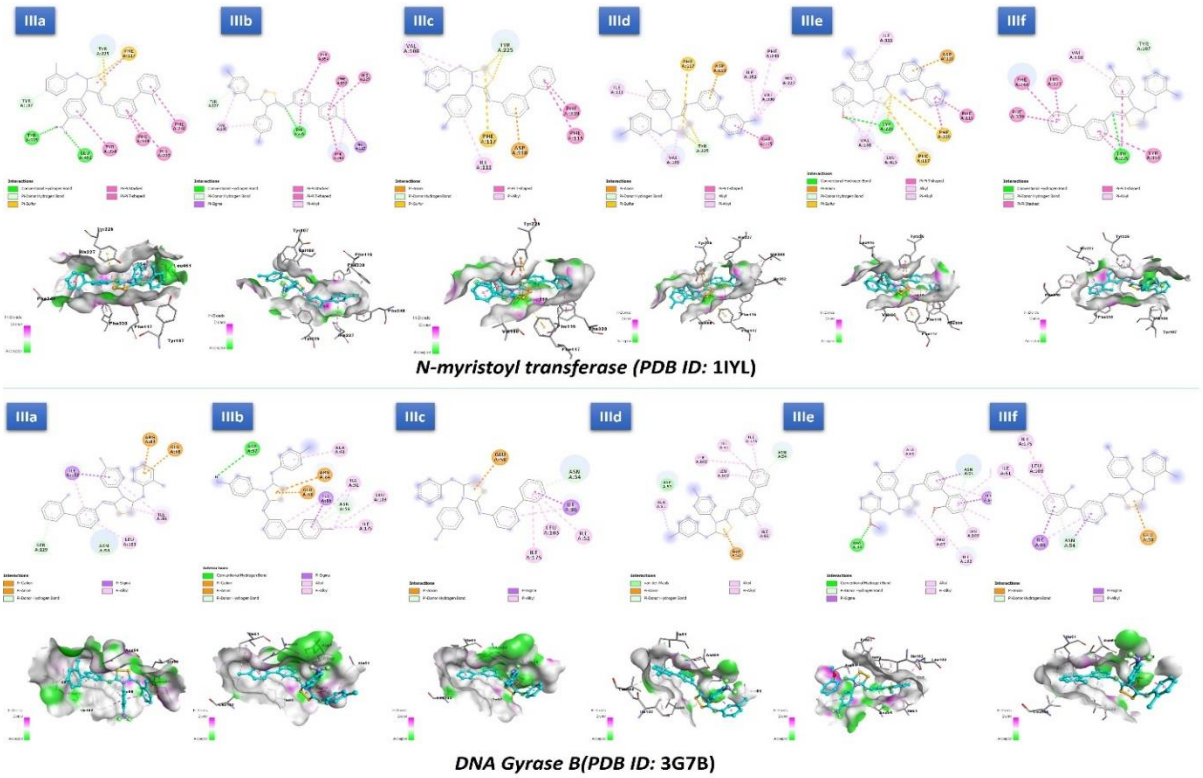



**Figure 2. Molecular docking interactions of synthesized dithiazolidine derivatives (IIIa–IIIf) with *Candida albicans* N-myristoyl transferase (PDB ID: 1IYL) and bacterial DNA Gyrase B (PDB ID: 3G7B). The top panels represent 2D interaction profiles, while the bottom panels show 3D binding conformations within the enzyme cavity.**

For N-myristoyl transferase (1IYL), compounds IIIa and IIIc displayed remarkable affinities (–10.2 and –10.1 kcal/mol, respectively), suggesting that methyl and chloro substituents enhanced binding stability. In the case of bacterial proteins such as DNA gyrase B (3G7B) and Sortase A (2MLM), the binding affinities of the synthesized compounds (–7.2 to –8.2 kcal/mol) were comparable to those of the native ligands. Against rhomboid protease (3UBB), however, **compound IIIc (–8.6 kcal/mol)** demonstrated improved binding relative to the native ligand (–7.8 kcal/mol), indicating favorable interactions (Figure 3).

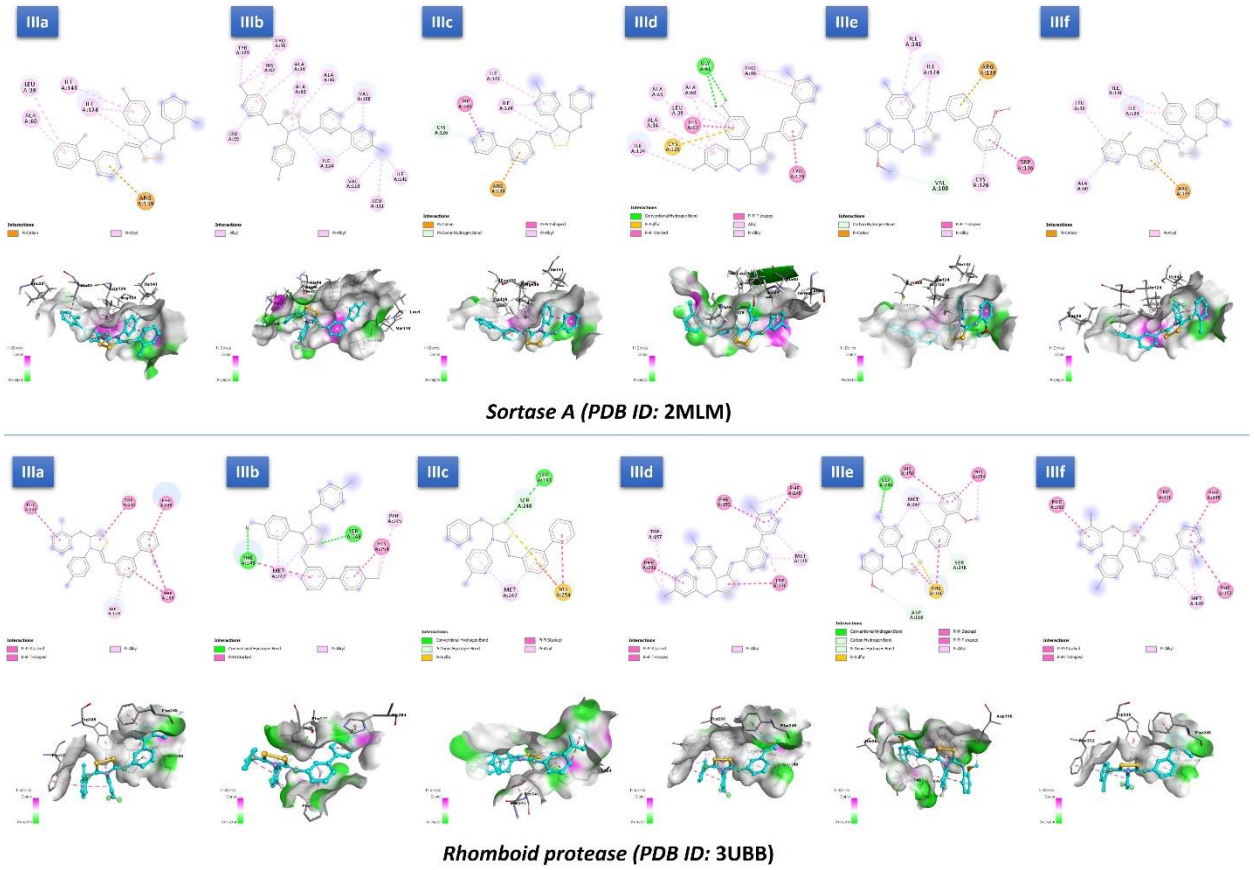



**Figure 3. Molecular docking interactions of synthesized dithiazolidine derivatives (IIIa–IIIf) with *Staphylococcus aureus* Sortase A (PDB ID: 2MLM) and *Escherichia coli* Rhomboid protease (PDB ID: 3UBB). The upper panels illustrate 2D ligand–residue interaction diagrams, and the lower panels highlight 3D docking poses inside the binding pocket.**

Overall, compounds **IIIa to IIId consistently exhibited favorable docking scores across both fungal and bacterial proteins**, correlating with their promising antimicrobial and antifungal activities. The docking interaction profiles underscore the importance of aryl substituents, particularly chloro and methyl groups, in enhancing binding affinity. These findings provide a molecular basis for the observed structure–activity relationship (SAR) and support the role of **3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines** as promising scaffolds for antimicrobial drug discovery.

### 3.3 Antimicrobial and Antifungal Activity.

The antibacterial efficacy of the newly synthesized dithiazolidine compounds was assessed against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacterial strains. Additionally, their antifungal potential was evaluated against Candida albicans. The inhibition zones observed at a concentration of 500 µg/mL and are are summarized in Table 8.

**Table 8: Antibacterial and antifungal activity of synthesized 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives expressed as zone of inhibition (mm) at 500 µg/mL**

| Compounds | Zone of inhibition (mm) at 500 µg/mL | | |
|---|---|---|---|
| | S. aureus | E.coli | C. albicans |
| III-a | 13 | 0 | 0 |
| III-b | 12 | 0 | 0 |

| | | | |
|---|---|---|---|
| III-c | 30 | 20 | 20 |
| III-d | 13 | 14 | 0 |
| III-e | 0 | 0 | 15 |
| III-f | 15 | 0 | 18 |

(Inhibition zone diameter in mm) (500 µ g/ml is the concentration)

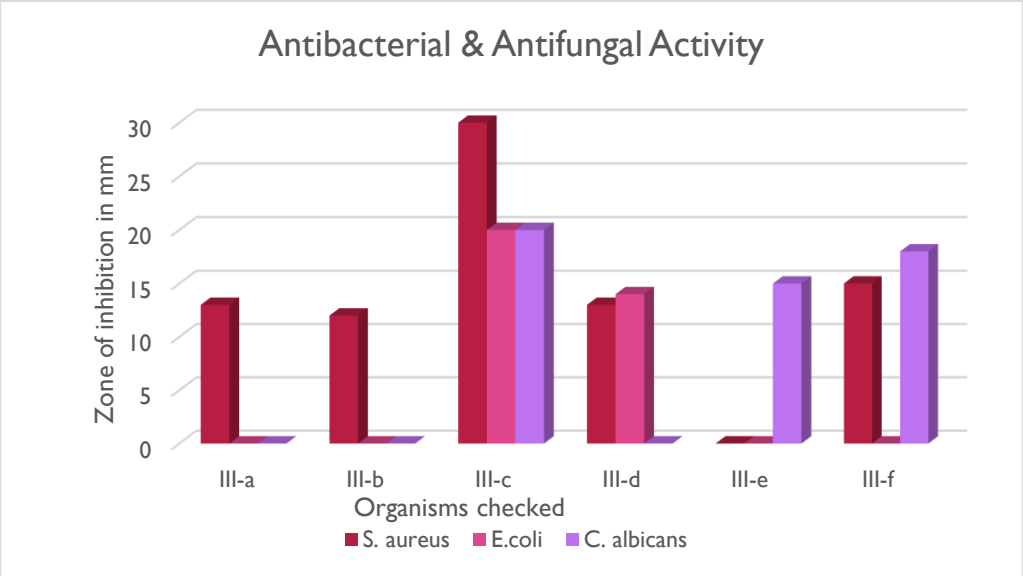



**Figure 4. Antibacterial and antifungal activity of synthesized dithiazolidine derivatives (IIIa–IIIf) against *Staphylococcus aureus, Escherichia coli, Candida albicans*, expressed as zone of inhibition in mm at 500 µg/mL.**

Moderate to good antibacterial as well as antifungal activities were observed for the newly synthesized compounds. Among the series, compound III-c (aniline substituent) exhibited the highest antibacterial activity, producing an inhibition zone of 30 mm against S. aureus, and also showed significant activity against E. coli (20 mm) and C. albicans (20 mm). Compounds III-a (o-tolyl), III-b (p-tolyl), III-d (3-chloroaniline), and III-f (2-chloroaniline) demonstrated moderate activity against S. aureus (12–15 mm zones). Compound III-d showed moderate inhibition of E. coli (14 mm), while compounds III-e (o-anisidine) and III-f were active against C. albicans with inhibition zones of 15 mm and 18 mm, respectively.

Interestingly, these experimental findings correlate well with the molecular docking results. The strong antibacterial activity of **III-c and III-d** corresponds to their high docking affinities against *S. aureus* DHFR (3FYV, –10.3 and –10.8 kcal/mol, respectively). Likewise, the antifungal activity observed for **III-c, III-e, and III-f** aligns with their favorable docking scores against *Candida albicans* DHFR (3QLS) and N-myristoyl transferase (1IYL). In particular, the broad-spectrum activity of **III-c** is consistent with its consistently strong binding affinities across both fungal and bacterial targets, confirming that **docking predictions rationalize the observed antimicrobial profiles.**

Overall, the results highlight that **aryl substituents strongly influence biological activity.** Chloro and aniline substitutions enhance antibacterial potential, while methoxy and chloro derivatives contribute to antifungal activity. The combined biological and computational findings establish **3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines** as promising scaffolds for antimicrobial drug discovery.

| **Compound** | **Substituent (R)** | **Best Experimental Activity** | **Observed Target Sensitivity** | **Key Docking Correlation** |
|---|---|---|---|---|
| III-a | o-Tolyl | Moderate vs *S. aureus* (13 mm) | Gram-positive bacteria | Moderate affinity for DHFR (*S. aureus*) |
| III-b | p-Tolyl | Moderate vs *S. aureus* (12 mm) | Gram-positive bacteria | Moderate affinity for DHFR (*S. aureus*) |
| III-c | Aniline | Strong vs *S. aureus* (30 mm); Moderate vs *E. coli* (20 mm) & *C. albicans* (20 mm) | Broad-spectrum | High docking scores with DHFR (*S. aureus*, *C. albicans*) and N-myristoyl transferase (1IYL) |
| III-d | 3-Chloroaniline | Moderate vs *S. aureus* (13 mm), *E. coli* (14 mm) | Gram-positive & Gram-negative | Strong affinity for bacterial DHFR (3FYV) |

| Compound | Substituent (R) | Best Experimental Activity | Observed Target Sensitivity | Key Docking Correlation |
|---|---|---|---|---|
| | (m) | | bacteria | |
| III-e | o-Anisidine | Antifungal vs *C. albicans* (15 mm) | Fungus only | Docking support with *C. albicans* DHFR and NMT (1IYL) |
| III-f | 2-Chloroaniline (o) | Moderate vs *S. aureus* (15 mm), Antifungal vs *C. albicans* (18 mm) | Mixed activity (bacteria + fungi) | Strong binding with *C. albicans* DHFR and Rhomboid protease (3UBB) |

From this SAR analysis, it is evident that **compound III-c (aniline substituent) is** the most potent broad-spectrum candidate, active against both bacterial and fungal strains, which is in strong agreement with its consistently high docking affinities across multiple targets. **Chloro-substituted derivatives (III-d, III-f)** contribute to antibacterial activity, particularly against *S. aureus* and *E. coli*, while **methoxy substitution (III-e)** favors antifungal activity. These results clearly demonstrate that **the electronic and steric effects of aryl substituents strongly dictate biological performance**, and molecular docking provides supportive mechanistic insights into their observed activity profiles.

## CONCLUSION

The synthesis and characterization of diathiazolidine derivatives presented in this study build upon earlier works focusing on heterocyclic compounds. While previous studies have explored the biological activities and synthetic routes of thiazolidines and dithiazolidines (References 15–19), this research expands on the scope by introducing a variety of aryl-substituted derivatives, including groups such as o-tolyl, p-tolyl, phenyl, m-chloro phenyl, o-anisyl, p-anisyl, and p-chloro phenyl. The findings on antimicrobial and antifungal activities align with previous observations, reinforcing the pharmacological potential of these compounds. However, subtle variations in activity may be attributed to the introduction of specific substituents, demonstrating the significant impact of structural modifications.

The synthesized diathiazolidine derivatives offer versatile applications, showcasing potential as antimicrobial agents due to their distinct mechanisms of action. Their spectral and analytical characterization using IR, NMR, and mass spectrometry not only confirmed their structural integrity but also provided insights into their reactivity patterns. The diverse biological activities observed in this study underscore the relevance of diathiazolidines in addressing global challenges such as antimicrobial resistance, while their chemical versatility positions them as valuable intermediates in organic synthesis.

A notable observation in this study is the influence of aryl substituents on the bioactivity of the diathiazolidine derivatives. Electron-donating substituents, such as those in o-anisyl and p-anisyl derivatives, appear to enhance antimicrobial efficacy, possibly through increased lipophilicity, facilitating better interaction with microbial targets. Conversely, electron-withdrawing groups like m-chlorophenyl and p-chlorophenyl might contribute to altered reactivity profiles. This correlation between substituents and biological activity provides crucial insights for future design strategies aimed at optimizing pharmacological properties.

The findings of this study pave the way for numerous applications. Diathiazolidine derivatives can be further explored for their therapeutic potential, particularly in the development of drugs targeting resistant microbial strains. Their structural features also make them candidates for advanced material synthesis, where the modification of existing materials or the creation of novel materials is required. In coordination chemistry, these derivatives can serve as ligands for designing metal-based catalysts, offering opportunities for innovation in catalytic processes.

While the study successfully synthesized and characterized the diathiazolidine derivatives, certain limitations must be acknowledged. The scope of antimicrobial and antifungal screening was limited to specific strains, leaving room for broader biological evaluations. Additionally, detailed mechanistic studies exploring the exact mode of action of these compounds were not within the current scope. Future research should aim to address these gaps, including structural refinements and expanded biological testing.

This study contributes to the growing field of heterocyclic chemistry by providing novel insights into the synthesis as well as applications of diathiazolidine derivatives. The structural versatility and biological significance of these compounds highlight their interdisciplinary importance, with potential impacts on medicinal chemistry, material science, and industrial applications. The research underscores the need for continued exploration of heterocyclic scaffolds in addressing emerging challenges across various fields.

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**CRediT authorship contribution statement**

Author contributions: Concept, Design and Materials – Pournima Pande and Manjusha Aware (Ugale); Supervision – Manjusha Aware (Ugale); Data Collection &/or Processing – Pournima Pande; Analysis &/or Interpretation – Pournima Pande and S. K. Shah; Literature Search and Writing – Pournima Pande; Critical Reviews – Manjusha Aware (Ugale) and S. K. Shah.

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**CONFLICT OF INTEREST STATEMENT**

The authors affirm that there are no conflicts of interest associated with this research.

**Declaration of competing interest**

The authors affirm that there are no financial interests, personal relationships, or affiliations that could have influenced the research findings or their interpretation in this manuscript.

**Declaration of usage of AI tool**

No generative AI tools or AI-assisted technologies were utilized during the writing of this manuscript.

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