RESEARCH PAPER

Synthesis, Characterization, Molecular Docking And Exploration Of Biological Activities Of 3,5-Diaryl, Diimino, 4-(Aryl Amino)-1,2,4 Dithiazolidine.

Pande P. S.*[1], Ugale M.[2], Shah S.[3]

[1]*G. H. Raisoni University, Amravati, 444701, India*
[2]*G. H. Raisoni University, Amravati, 444701, India*
[3]*Department of Pharmaceutical Chemistry, Priyadarshini J. L. College of Pharmacy, Nagpur, 440016, India.*
Email: pournimapande5@gmail.com

ABSTRACT
Dithiazolidines represent a distinctive class of organic compounds characterized by a heterocyclic ring containing two sulfur and two nitrogen atoms in a defined arrangement. Their chemical behavior and reactivity can be tuned by introducing different substituents or functional groups, making them valuable in diverse fields such as organic synthesis, medicinal chemistry, materials science, and coordination chemistry. Owing to their unique structural features and mechanisms of action, derivatives of dithiazolidines have attracted attention for their potential antimicrobial properties. In the present study, aryl isothiocyanates were reacted with p-phenylenediamine under reflux in chloroform to yield N aryl S chloro isothiocarbamoyl chloride. This intermediate, upon chlorination followed by cyclization, afforded 3,5 diaryl, diimino, 4 (aryl amino) 1,2,4 dithiazolidines. The synthesized compounds were thoroughly characterized using infrared (IR), nuclear magnetic resonance (NMR), and mass spectroscopic techniques. Biological screening revealed that these derivatives exhibited moderate to significant antibacterial and antifungal activity against selected microbial strains. Furthermore, molecular docking studies were performed to investigate the binding interactions of the newly synthesized dithiazolidines with target proteins. The docking results showed good agreement with the experimental findings, thereby reinforcing the potential of these compounds as promising scaffolds for antimicrobial drug development. Overall, this work highlights the relevance of dithiazolidine derivatives in medicinal chemistry and encourages further exploration of their applications..
Keywords: Dithiazolidines, aryl isothiocyanate, cyclization reaction, antimicrobial activity, molecular docking.
How to cite this article: Pande PS, Ugale M, Shah S; Synthesis, Characterization, Molecular Docking And Exploration Of Biological Activities Of 3,5-Diaryl, Diimino, 4-(Aryl Amino)-1,2,4 Dithiazolidine...Int J Drug Deliv Technol. 2026;16(1s): 750-761; DOI: 10.25258/ijddt.16. 750-761
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Heterocyclic compounds remain at the forefront of chemical research because of their remarkable structural versatility and wide-ranging applications in pharmaceuticals, materials science, and synthetic chemistry. Their frameworks play a pivotal role in drug development, offering biologically active scaffolds and functioning as key intermediates in the construction of complex organic molecules. [1–4].

Within the broad spectrum of heterocyclic compounds, thiazolidines stand out as highly adaptable five-membered rings incorporating both nitrogen and sulfur atoms. This unique structural arrangement imparts considerable chemical flexibility, allowing the development of diverse derivatives with significant pharmacological relevance. Reported biological activities of thiazolidine-based molecules include anti-inflammatory, antidepressant, anticancer, anti-tubercular, and antimicrobial effects [5–7]. The presence of multiple heteroatoms within the ring system facilitates structural modifications, thereby enhancing their therapeutic potential and expanding their utility in medicinal chemistry.

Dithiazolidines, a specialized subclass of heterocyclic compounds, have attracted increasing attention in recent years owing to their distinctive ring system composed of two nitrogen and two sulfur atoms. This unusual heteroatomic arrangement imparts structural novelty and offers opportunities for tailoring physicochemical characteristics as well as biological activity. The versatility of the dithiazolidine framework makes it a promising platform for the rational design of new molecules with potential applications in medicinal chemistry, materials science, and synthetic methodology. By enabling diverse functionalization, these compounds provide a valuable scaffold for exploring antimicrobial activity and other therapeutic properties. These moieties were explored as intermediates in organic synthesis [8], pharmacologically active molecules [9], corrosion inhibitors [10–13], and ligands

**Author for Correspondence: Pande P. S.*

in coordination chemistry for stabilizing metal complexes and enabling catalytic applications [14,15].

Recent investigations have focused on the synthesis of both thiazolidine and dithiazolidine derivatives incorporating diverse aryl substituents [16,17]. These structural modifications significantly affect the stability and reactivity of the compounds, thereby expanding their potential applications. Advanced characterization methods, such as nuclear magnetic resonance (NMR) and mass spectrometry, have been employed to elucidate their molecular structures with precision. In parallel, biological assays have demonstrated notable antimicrobial and antifungal activities, highlighting the importance of these heterocycles as promising candidates in the ongoing fight against 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 also done for antimicrobial as well as antifungal activities. Molecular docking studies were conducted to establish structure–activity correlations and support experimental findings.

Although thiazolidine and diathiazolidine scaffolds have been widely investigated for their chemical versatility and biological relevance, systematic studies on 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines are still lacking. In particular, the influence of different aryl substituents on their physicochemical behavior and antimicrobial potential has not been thoroughly mapped. Furthermore, earlier reports have generally focused on either synthetic or biological aspects in isolation, without integrating experimental screening with molecular docking analyses to explain activity at the molecular level.

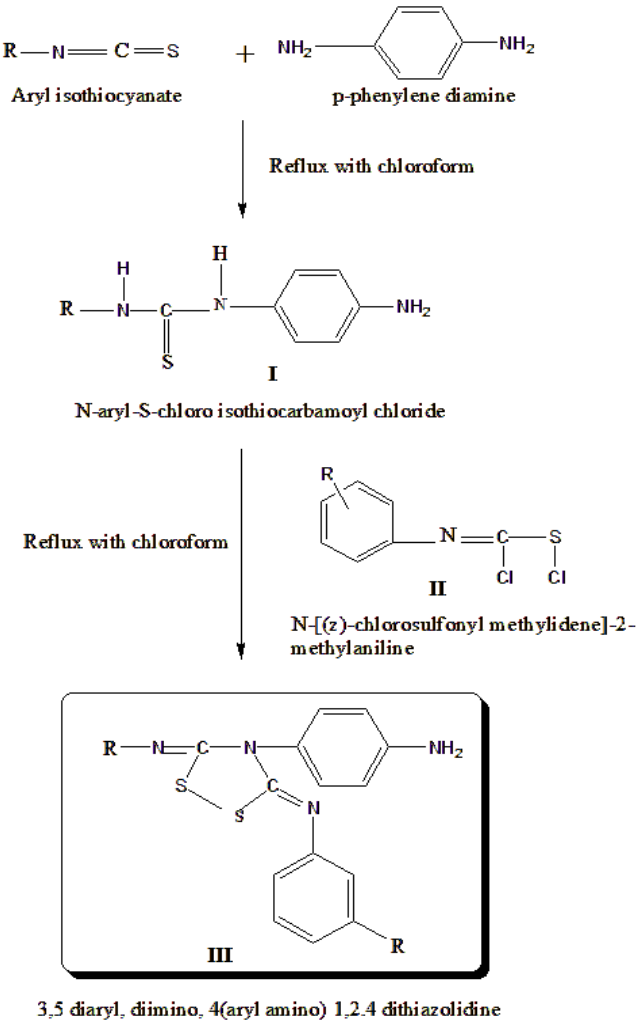
This research bridges the existing gap by designing and synthesizing a new series of dithiazolidine derivatives with varied aryl groups, followed by comprehensive characterization using IR, NMR, and mass spectrometry to establish their structural integrity. The biological potential of these compounds was evaluated through standardized antibacterial and antifungal assays, while molecular docking studies were employed to correlate structural features with binding interactions against key microbial protein targets. Together, this integrated approach provides both experimental validation and computational insight, highlighting the promise of these novel dithiazolidine derivatives as scaffolds for future antimicrobial drug developmentBy combining synthetic chemistry, biological evaluation, and computational modeling, this work provides the first comprehensive account of this subclass of dithiazolidines. The findings not only highlight their promise as antimicrobial candidates but also establish a framework for rational drug design based on structure–activity relationships.

To the best of our knowledge, this work represents the first systematic investigation into the synthesis and biological evaluation of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines, integrating both experimental assays and computational docking analyses. By combining chemical synthesis, spectral characterization, antimicrobial screening, and molecular modeling, the study establishes a comprehensive framework that underscores the potential of these novel derivatives as promising scaffolds for future drug discovery

MATERIALS AND METHODS:

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 ($CDCl_3$) δ_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 ($CDCl_3$) δ_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)δ_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) δ_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 ($CDCl_3$)δ_H ppm: 3.5 (s, 2H, R-NH_2), 7.5-7.1 (m, 18H, Ar-H). C^{13} NMR ($CDCl_3$) δ_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)δ_H ppm: 3.4 (s, 2H, R-NH_2), 7.7-6.6 (m, 16H, Ar-H). C^{13} NMR (DMSO) δ_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)δ_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) δ_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) δ_H ppm: 3.8 (s, 2H, R-NH_2), 7.45-6.8 (m, 16H, Ar-H). C^{13} NMR (DMSO) δ_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
	Dihydrofolate reductase (DHFR)	3QLS	Candida albicans (Antifungal target)
	Secreted aspartic protease	3Q70	
	N-myristoyl transferase	1IYL	
	Dihydrofolate reductase	3FYV	Staphylococcus aureus (Antibacterial target)
	Gyrase B	3G7B	
	Sortase A	2MLM	
	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)
	3QLS	-0.079, 4.940, 32.034	25 x 25 x 25
	3Q70	-24.144, -13.387, 21.695	28 x 28 x 28
	1IYL	13.415, 47.742, -1.041	26 x 26 x 26
	3FYV	30.705, 11.534, 42.122	26 x 25 x 25
	3G7B	50.354, -2.964, 19.129	25 x 25 x 25
	2MLM	25.189, 17.840, 10.687	27 x 18 x 25
	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})
	3209.55	N-H	3450-3100

(III-a) (R = o-toluidine)	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
	3169.04	N-H	3450-3100

(III-f) R = 2 chloroaniline (o)	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, C=N dithiazolidine ring carbon),	143.827-115.064 (24C, Aromatic ring carbons)	
(III-d) R = 3 chloroaniline (m)	180 (2C, C=N dithiazolidine ring carbon),	144.382-114.355 (24C, Aromatic ring carbons),	
(III-e) (R = o-anisidine)	180 (2C, C=N dithiazolidine ring carbon),	136.799-113.609 (24C, Aromatic ring carbons),	156.465(Ar-OCH_3)
(III-f) R = 2 chloroaniline (o)	180 (2C, C=N dithiazolidine ring carbon),	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.

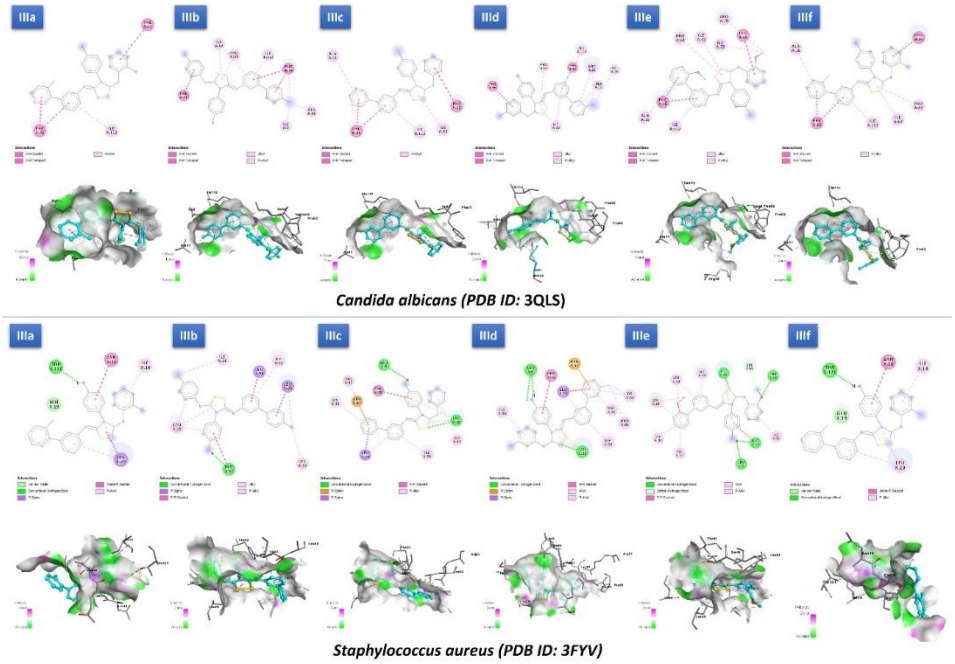


Figure 1. Molecular docking interactions of synthesized dithiazolidine derivatives (IIIa–IIIf) with *Candida albicans* DHFR (PDB ID: 3QLS) and *Staphylococcus aureus* DHFR (PDB ID: 3FYV). The top panels show 2D ligand–residue interaction maps, and the bottom panels depict 3D binding poses within the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

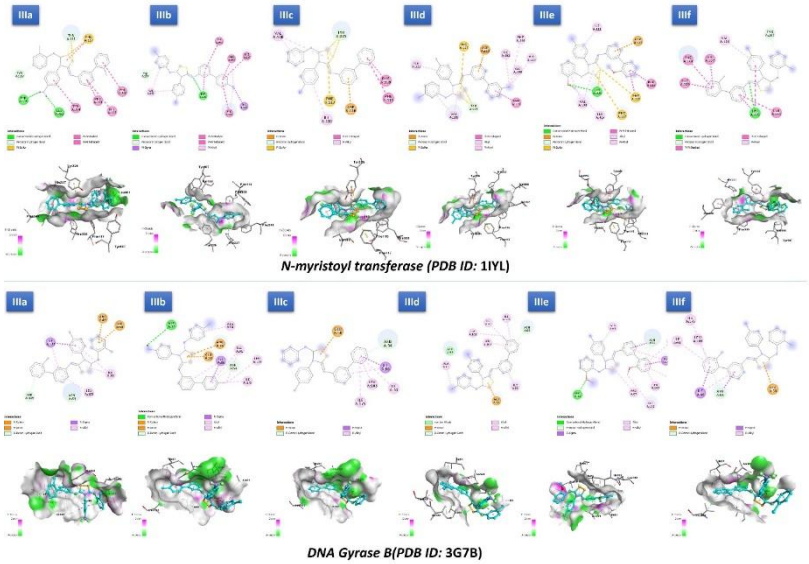


Figure 2. **Molecular docking interactions of synthesized dithiazolidine derivatives (IIIa–IIIf) with *Candida albicans* N-myristoyl transferase (PDB ID: 1IYL) and bacterial DNA Gyrase B (PDB ID: 3G7B). The top panels represent 2D interaction profiles, while the bottom panels show 3D binding conformations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

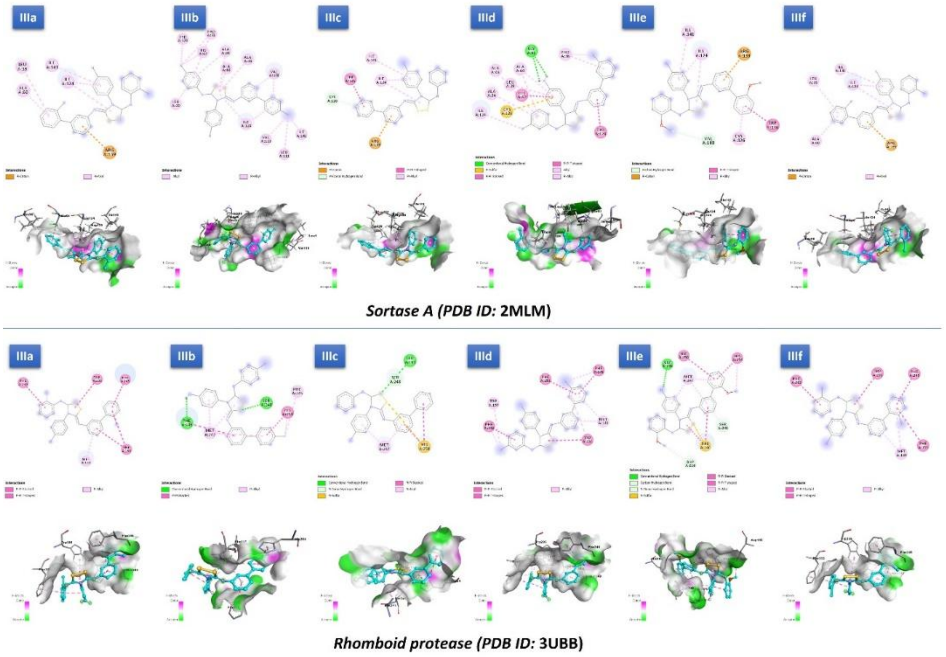


Figure 3. **Molecular docking interactions of synthesized dithiazolidine derivatives (IIIa–IIIf) with *Staphylococcus aureus* Sortase A (PDB ID: 2MLM) and *Escherichia coli* Rhomboid protease (PDB ID: 3UBB). The upper panels illustrate 2D ligand–residue interaction diagrams, and the lower panels highlight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

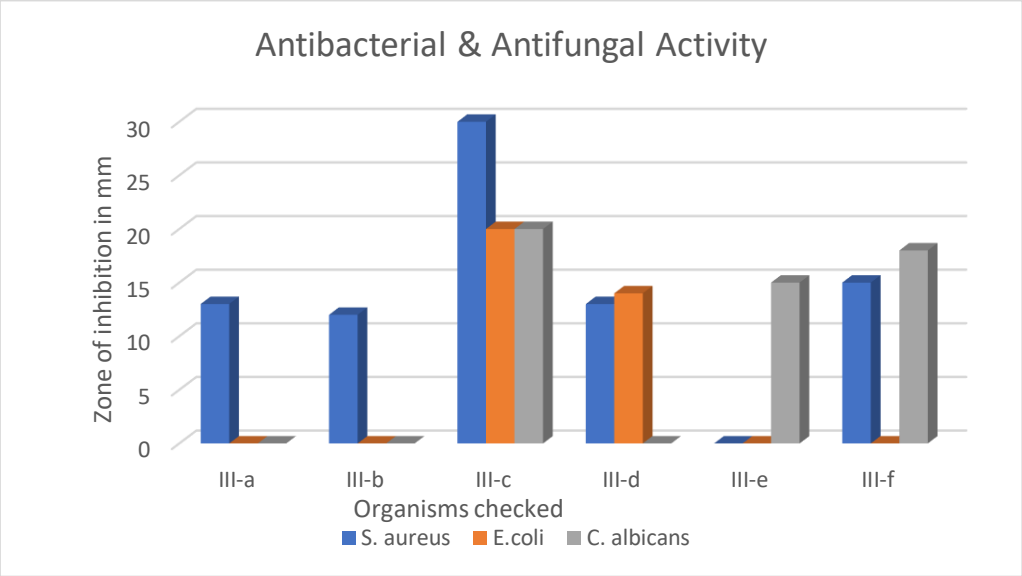


Figure 4. **Antibacterial and antifungal activity of synthesized dithiazolidine derivatives (IIIa–IIIf) against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, expressed as zone of inhibition in mm at 500 µg/mL.**

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)

Compound	Substituent (R)	Best Experimental Activity	Observed Target Sensitivity	Key Docking Correlation
III-d	3-Chloroaniline (m)	Moderate vs *S. aureus* (13 mm), *E. coli* (14 mm)	Gram-positive & Gram-negative bacteria	Strong affinity for bacterial DHFR (3FYV)
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 present investigation provides a comprehensive account of the synthesis, characterization, and biological evaluation of a new series of 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidine derivatives. By introducing diverse aryl substituents such as o-tolyl, p-tolyl, phenyl, m-chlorophenyl, o-anisyl, p-anisyl, and p-chlorophenyl, the study expands the chemical diversity of dithiazolidines and demonstrates how subtle structural modifications can significantly influence biological activity. Spectral analyses using IR, NMR, and mass spectrometry confirmed the structural integrity of the synthesized compounds and provided valuable insights into their functional group arrangements and reactivity patterns.

Biological screening revealed moderate to strong antimicrobial and antifungal activities, with variations in potency attributable to the electronic nature of the substituents. Electron-donating groups, particularly anisyl derivatives, enhanced antimicrobial efficacy, likely due to increased lipophilicity and improved microbial target interactions, whereas electron-withdrawing substituents such as chlorophenyl groups altered reactivity and binding behavior. These findings underscore the importance of substituent effects in guiding structure–activity relationships and highlight the potential of rational design strategies for optimizing pharmacological properties.

Molecular docking studies further strengthened the experimental observations by revealing favorable binding affinities of the synthesized compounds toward key microbial protein targets, including dihydrofolate reductase, N-myristoyl transferase, and DNA gyrase B. Several derivatives exhibited stronger binding than native ligands, supported by hydrogen bonding, hydrophobic contacts, and van der Waals interactions within active site pockets. This integrated experimental–computational approach provides mechanistic insights into the antimicrobial potential of dithiazolidines and validates their promise as scaffolds for drug discovery.

Beyond medicinal chemistry, the structural versatility of these compounds positions them as valuable intermediates in organic synthesis, candidates for advanced material development, and potential ligands in coordination chemistry for designing metal-based catalysts. While the current study was limited to selected microbial strains and did not explore detailed mechanistic pathways, it establishes a strong foundation for future research. Expanded biological evaluations, structural refinements, and mechanistic studies will be essential to fully realize the therapeutic and industrial potential of this class.

In conclusion, this work represents the first systematic report on aryl-substituted 3,5-diaryl, diimino, 4-(aryl amino)-1,2,4-dithiazolidines, integrating synthesis, spectral characterization, antimicrobial screening, and molecular docking. The findings highlight their interdisciplinary significance, bridging chemistry, biology, and materials science, and point toward exciting opportunities for innovation in drug discovery and beyond.

ACKNOWLEDGEMENTS

The authors hereby acknowledge the help of SAIF IIT Bombay, SAIF Chandigarh, and Dadasaheb Balpande College of Pharmacy, Nagpur, for providing the spectral data. The authors acknowledge the help of G H Raisoni College of Engineering & Management for providing laboratory facilities.

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.

Funding
No dedicated financial support was provided for this research by public institutions, commercial enterprises, or non-profit organizations

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

REFERENCE
N\A.