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Design, synthesis, spectral analysis, and biological evaluation of Schiff bases with a 1,3,4-thiadiazole moiety as an effective inhibitor against bacterial and fungal strains

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ABSTRACT

Many distinct natural and pharmaceutical items include the well-known heterocyclic nucleus 1,3,4-thiadiazole. Ten Schiff bases of 1,3,4-thiadiazole derivatives have been synthesized using equimolar amounts of 5-styryl-1,3,4-thiadiazol-2-amine and substituted acetophenones in the catalytic amount of ethanol. The synthesized derivatives of Schiff's bases were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. The 1,3,4-thiadiazole Schiff's bases (RM-1 to RM-10) were tested for their *in vitro* antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* using the disc diffusion method. The 1,3,4-thiadiazole Schiff bases showed strong antibacterial activity against bacterial and fungal species, however, their activity was noticeably less effective than that of the evaluated conventional antibiotics.

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1. Introduction

Heterocyclic compounds are among the organic substances that have biological activity and are used as medicines in both veterinary and human medicine or as pesticides [1]. Many commercially available drugs contain chemical rings, which could have pharmacological effects or act as a base for pharmacophoric groups to interact with receptors (Figure 1) [2].

Due to the lack of effective agents needed to eradicate newly emerging bacterial strains, millions of people per year perished. To solve this problem and stop it from getting worse, an efficient antibacterial agent is needed [3]. More than any other area of medical therapy that has developed to this point, the use of antimicrobial agents has historically been associated with saving human lives. However, this area of medicine has had to deal with the issue of resistance of microorganisms to common antibacterial drugs.

A new strain of resistant bacteria emerges as a result of the excessive and unreasonable use of these products. To combat the newly emerging resistant bacteria, new treatment drugs must be introduced and demonstrated to have good activity [4].

One sulfur and two nitrogen atoms make up 1,3,4-thiadiazole, a well-known heterocyclic compound with a five-membered ring [5]. It has been thoroughly studied to determine how effective they are against bacteria [6]. Thiadiazole can be found in a variety of isomers, including 1,2,3-thiadiazole, 1,2,4-thiadiazole, 1,2,5-thiadiazole, and 1,3,4-thiadiazole (Figure 2) [7].

Numerous studies have demonstrated that substances containing 1,3,4-thiadiazole are a promising class of chemicals that can be used in the field of antibacterial treatment [8-10]. They function as antibacterial, antitubercular, and anticancer drugs [11]. Both the 1,3,4-thiadiazole and imine moieties have well-established antibacterial properties and, as a result, products containing the two groups may have increased antibacterial action, therefore, we are creating novel products with the moieties and testing their biological and antibacterial activity is a wise investment [12].

Many molecules with the thiadiazole moiety exhibit a variety of biological properties, including antimicrobial [13,14], antiproliferative [15], antitumor [16], antituberculous [17], anti-infammatory [18], anticonvulsant [19], antioxidant [20], antileishmanial [21], antibacterial [22,23], antiviral [24], anal-

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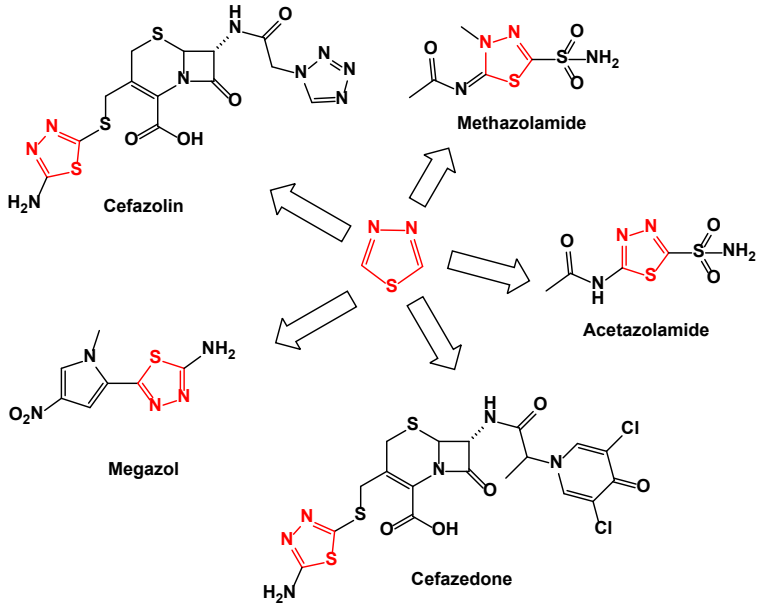


Figure 1. Drugs available on the market that contain the 1,3,4-thiadiazole ring.

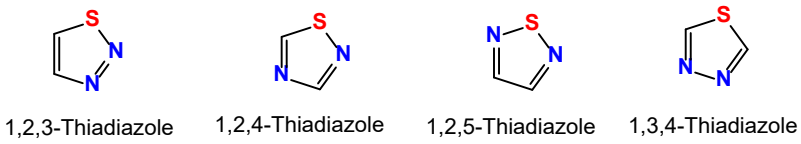


Figure 2. Isomers of thiadiazole.

gesic [25], antipsychotic [26], antihistamine [27], anti-depressive [28] and antihypertensive [29].

Thiadiazol and imidazol derivatives were synthesized by combining benzyl and benzaldehyde with ammonium acetate to produce intermediate I. Subsequently, intermediate I was used to synthesize intermediate II through a reaction with methyl chloroacetylchloride, thiosemicarbazide, and NaOH. Intermediate II was then further treated with chloroacetyl chloride, and two Gram positive bacteria (*S. aureus* and *E. facaels*) and two Gram negative bacteria (*E. coli* and *K. pneumonia*) were used to test the antibacterial effects of cefixime and metronidazole, as well as anaerobic bacteria (*S. pyogen*) [30]. The reaction of 2-amino-5-mercapto-1,3,4-thiadiazole with aromatic aldehydes was carried out under phase transfer catalyst (PTC) conditions to produce several new derivatives of Schiff bases with a 1,3,4-thiadiazole moiety [31]. These substances showed antibacterial action against *S. aureus* (RTCC 1885) and *E. coli* bacteria (ATCC 35922) [32]. Its antimicrobial activity was then tested using the agar well diffusion method at concentrations of 250 g/mL and 500 g/mL, with excellent to moderate inhibition activity against the three bacterial strains *E. coli*, *S. aureus*, and *K. pneumonia* [33].

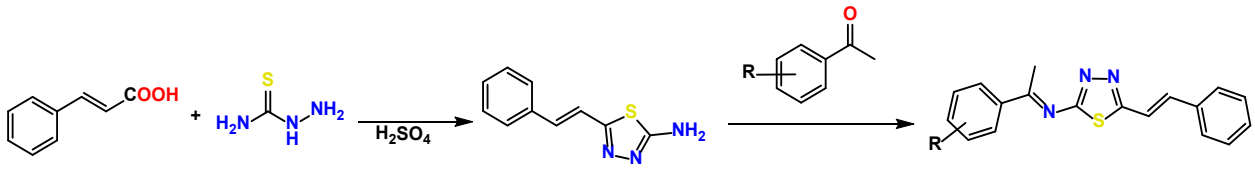
Several aromatic aldehydes were combined with 1,2,4,5-tetra-(5-amino-1,3,4-thiadiazole-2-yl)benzene to form a new tetra Schiff base of thiadiazole derivatives. When tested against the bacterial strains *S. aureus*, *S. epidermidis*, *M. luteus*, *B. cereus*, *E. coli*, and *P. aeruginosa*, 1,2,4,5-tetra-[5-(4-nitrobenzylideneamino)-1,3,4-thiadiazole-2-yl]benzene was shown to have the strongest antibacterial activity [34]. All strains, including *E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *E. faecalis*, *S. aureus* and *B. cereus*, were effectively eliminated through the integration of thiadiazole compounds with Schiff base structures [35].

Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria were significantly resistant to 1,3,4-thiadiazole derivatives of Schiff base when tested *in vitro* [36]. Different levels of antibacterial, antifungal, and anthelmintic activities were found when Schiff bases of 1,3-thiazoles were tested against Gram positive and Gram negative bacterial species [37]. Two Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) and three Gram-negative bacterial strains (*E. coli*, *P. aeruginosa*, and *S. typhi*) were examined. The results of a series of compounds produced by combining ferrocene with thiadiazole showed superior antibacterial activity [38]. An *in vitro* test conducted with pathogenic *E. coli* and *S. typhi* bacterial strains revealed that a thiadiazole derivative of substituted formazans, produced from the initial Mannich base, exhibited significant antimicrobial activity [32].

The *micrococcus letus* (ATCC 9341) bacterial strains were compared with the *Pseudomonas auroginosa* (ATCC 27853) strains using the reference cephalosporin (cephalexin), a special combination of a Schiff base of thiadiazole groups that linked the sulfide or disulfide bonds has shown better antimicrobial activities against *Staphylococcus aureus* (ATCC 25923) and *E. coli* (ATCC 25922) than *P. auroginosa* (ATCC 27853), *M. letus* (ATCC 9341) [39].

In vitro antimicrobial testing of a new series of Schiff bases made from 1,3,4-thiadiazole and 1,2,4-triazole derivatives using the broth microdilution method revealed the highest to moderate antibacterial activity against a total of 19 bacterial strains, including Gram-positive bacteria, Gram-negative bacteria, and Candida yeasts [40]. Compared to ciprofloxacin in a broth microdilution technique, the majority of newly synthesized 2,5-disubstituted-1,3,4-thiadiazoles demonstrated high to exceptional antibacterial efficacy against Gram-positive and Gram-negative bacteria, according to the data (MIC Assay) [41].

The 1,3,4-thiadiazole derivatives containing Schiff base moieties were produced with strong tyrosinase inhibitory characteristics, as shown by analysis of structure-activity



Scheme 1. Synthesis of Schiff bases with a moiety of 1,3,4-thiadiazoles.

relationships (SAR) and docking findings [42]. Six, 1,3,4-thiadiazole and Schiff base derivatives were synthesized and tested against four bacterial strains, including the popular antibiotic cefuroxime, two Gram-positive strains (*S. aureus* and *B. cereus*) and two Gram-negative strains (*E. coli* and *P. aeroginosa*) [43]. When 1,3,4-thiadiazole containing 1,3,4-oxadiazole derivatives was synthesized, many pathogenic bacterial strains were isolated from patients, including *Streptococcus*, *Acinetobacter*, *E. coli*, *Klebsiella*, *Staphylococcus*, and *Aeromonas*, showed increased to moderate antibacterial activity [44]. The antibacterial activity of the complexes (Cu, Fe, Co and Zn) was investigated against *S. aureus* and *S. epidermidis* as Gram-positive bacteria, and *E. coli*, *P. mirabilis*, *C. freundii* and *P. aeruginosa* as Gram-negative bacteria to determine the activity of the synthesized complexes, and the results exhibited higher activity [45].

The minimum inhibitory concentrations (MICs) of the compounds were also established using the agar streak dilution method. Schiff's base of 2-amino-1,3,4-thiadiazole demonstrated moderate antibacterial activity against (*S. aureus*, *S. epidermidis*, *E. coli* and *P. aeruginosa*) using the disc diffusion method [46]. Synthesized derivatives of 5-amino-1,3,4-thiadiazole-2-thiol Schiff bases with electron-withdrawing fluorine and nitro groups, with a MIC of 8 g/mL, showed excellent inhibitory efficacy against *S. aureus*, *A. niger*, and *C. tropicalis* [47].

The 5-substituted-1,3,4-thiadiazole-2-amines produced a variety of Schiff base compounds. When evaluated using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) techniques, these compounds exhibited strong antibacterial activity against *S. epidermidis* [48]. The antimicrobial activity of newly functionalized bis-1,3,4-thiadiazoles was evaluated using the agar diffusion well method against Gram-negative bacteria (*P. vulgaris*), and one of the compounds demonstrated potency as an antibacterial drug by forming three π-hydrogen interactions with Leu 144, Tyr 156, and Phe 203, as well as one hydrogen acceptor interaction with Ser 19 with a binding energy of -1.8 (Kcal/mol). This information was obtained from the screening of their molecular docking results [49]. Metronidazole derivatives were designed by introducing pharmacologically active 1,3,4-thiadiazole and Schiff base compounds were also tested for anthelmintic activity at a concentration of 2 mg/ml against two species of worms, *P. posthuma* and *P. excavatus* showed comparable antibacterial activity [50].

In this study, heterocyclic bis-Schiff bases of 2,5-disubstituted-1,3,4-thiadiazole are synthesized (Scheme 1) and screened against one Gram-positive bacteria (*B. subtilis*) and two strains of Gram-negative bacteria (*P. aeruginosa*, *E. coli*) for an antibacterial activity study compared to the standard strong antibiotic drug (tetracycline), and report their antifungal studies against *A. niger, A. fumigatus,* and *A. flavus* compared to the standard drug (Amphotericin B). All synthesized compounds showed noticeable antibacterial and antifungal properties. We are producing unique products containing 1,3,4-thiadiazoles with remarkable antimicrobial activities.

2. Experimental

2.1. Instrumentation

The melting points were determined by an open capillary method. Infrared spectra were measured on a Shimadzu FT-IR spectrometer using KBr pellets. ^{1}H NMR recorded on a Bruker AM 400 MHz spectrometer at room temperature in DMSO-d_6 solution using tetramethyl silane (TMS) as an internal reference and mass spectra recorded on a Q TOF MS ES (LCMS) instrument at 70 eV.

2.2. Material

The chemicals and reagents were obtained from Merck and Sigma-Aldrich. TLC was used to keep an eye on chemical reactions. A UV light chamber was used to visualize TLC. The purity of the synthesized compounds was checked by thin-layer chromatography (TLC).

2.3. General procedure for the synthesis of Schiff Bases (RM-1 to RM-10)

Equimolar quantities of 5-styryl-1,3,4-thiadiazol-2-amine and substituted acetophenones were reacted to prepare Schiff bases. Subsequently, a small amount of ethanol and two to three drops of lemon juice were added, and the entire reaction mixture was stirred at room temperature for two hours. In ice-cold water, a mass of reaction was poured. The resulting solid product was collected by filtration and dried at 70 °C in a drying oven. The product was re-crystallized from ethanol, and drying produced a pure product.

1-Phenyl-N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)ethan-1-imine (**RM-1**): Color: Red. Yield: 88.12%. M.p.: 100-102 °C. FT-IR (KBr, ν, cm^{-1}): 2962 (Aliph. C-H), 1612 (Ar. C-H), 1612 (Ar-C=N), 1446 (Aliph. C=N), 1246 (N-N), 648 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.24 (s, 3H, CH_3), 6.43 (s, 1H, =CH-Phenyl), 7.39-7.78 (m, 10H, Ar-H), 8.42, (s, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.0, 78.4, 78.4, 78.7, 78.0, 115.3, 127.8, 128.0, 128.7, 128.9, 128.4, 132.0, 133.9, 136.2, 141.1, 158.7, 160.8, 163.9, 165.7. MS (*m/z*): 305.50.

1-(4-Fluorophenyl)-N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl) ethan-1-imine (**RM-2**): Color: Brown. Yield: 90.55%. M.p.: 94-96 °C. FT-IR (KBr, ν, cm^{-1}): 2845 (Aliph. C-H), 1612 (Ar. C-H), 1629 (Ar-C=N), 1471 (Aliph. C=N), 1255 (N-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.84 (s, 3H, CH_3), 6.84 (s, 1H, =CH-Phenyl), 7.36-7.32 (m, 2H, Ar-H), 7.58-7.50 (m, 5H, Ar-H), 7.89-7.85 (m, 2H, Ar-H), 8.49 (s, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.5, 116.21, 127.98, 128.8, 128.9, 129.4, 132.1, 133.9, 136.7, 141.6, 158.8, 160.9, 163.7, 165.9. MS (*m/z*): 323.22.

1-(4-Chlorophenyl)-N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl) ethan-1-imine (**RM-3**): Color: Yellow. Yield: 88.20%. M.p.: 82-84 °C. FT-IR (KBr, ν, cm^{-1}): 2960 (Aliph. C-H), 1604 (Ar. C-H), 1440 (Ar-C=N), 1471 (Aliph. C=N), 1228 (N-N), 773 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.84 (s, 3H, CH_3), 6.81 (s, 1H, =CH-Phenyl), 7.49-7.45 (m, 7H, Ar-H), 7.77-7.76 (m, 2H, Ar-H), 8.40 (s, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.29, 78.3, 78.6, 78.9, 115.2, 127.8, 128.8, 128.9, 129.4,

132.1, 133.9, 136.1, 141.2, 158.7, 160.8, 163.9, 165.7. MS (*m/z*): 340.55.

1-(3-Bromophenyl)-N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl) ethan-1-imine (**RM-4**): Color: Yellow. Yield: 84.22%. M.p.: 90-92 °C. FT-IR (KBr, ν, cm^{-1}): 3186 (Ar-C-H), 1591 (Ar-C=N), 1442 (Aliph. C=N), 1381 (N-N), 671 (C-S), 758. ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.84 (s, 3H, CH_3), 6.86 (s, 1H, =CH-Phenyl), 7.45-7.58 (m, 6H, Ar-H), 7.72-7.70 (m, 1H, Ar-H), 7.82 (d, 1H, Ar-H), 7.95 (d, 1H, Ar-H), 8.47 (s, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.6, 115.2, 122.1, 126.7, 128.1, 129.1, 131.1, 133.9, 134.1, 141.3, 158.8, 162.6, 164.1, 166.0. MS (*m/z*): 383.42.

4-((E)-1-((5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)imino)ethyl) benzonitrile (**RM-5**): Color: Brown. Yield: 90.08%. M.p.: 102-104 °C. FT-IR (KBr, ν, cm^{-1}): 3080 (Ar-C-H), 2846-2956 (Aliph. C-H), 2351 (C≡N), 1595 (C=C), 1450 (Ar-C=N), 677 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.84 (s, 3H, CH_3), 6.86 (s, 1H, =CH-Phenyl), 7.58-7.51 (m, 5H, Ar-H), 7.94-7.97 (m, 4H, Ar-H), 8.57 (s, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.6, 115.8, 118.5, 128.1, 128.5, 129.1, 132.8, 134.0, 137.7, 141.4, 158.4, 162.7, 164.1, 165.9. MS (*m/z*): 331.26.

1-(3-Nitrophenyl)-N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl) ethan-1-imine (**RM-6**): Color: Yellow. Yield: 88.10%. M.p.: 88-90 °C. FT-IR (KBr, ν, cm^{-1}): 3074 (Ar-C-H), 2852-2922 (Aliph.C-H), 1631 (Ar-C=N), 1471 (Aliph. C=N), 1340 (NO_2), 1597 (C=C), 1267 (N-N), 680 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.86 (s, 3H, CH_3), 6.87 (s, 1H, =CH-Phenyl), 7.58-7.49 (m, 5H, Ar-H), 7.76 (t, 1H, Ar-H), 8.22 (d, 1H, Ar-H), 8.32-8.30 (m, 1H, Ar-H), 8.60 (s, 1H, Ar-H), 8.60-8.62 (m, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.4, 78.4, 78.7, 79.0, 115.4, 122.2, 125.3, 126.2, 127.9, 129.0, 130.3, 133.7, 133.9, 135.2, 141.1, 148.1, 158.0, 162.1, 164.0, 165.8. MS (*m/z*): 349.12.

N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)-1-(p-tolyl)ethan-1-imine (**RM-7**): Color: Red. Yield: 94.70%. M.p.: 86-88 °C. FT-IR (KBr, ν, cm^{-1}): 3043 (Ar-C-H), 2953-2873 (Aliph.C-H), 1438 (Aliph. C=N), 1604 (C=C), 1246 (N-N), 680 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.36 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 6.82 (s, 1H, =CH-Phenyl), 7.31 (d, 2H, Ar-H, *J* = 8.0 Hz), 7.56-7.50 (m, 5H, Ar-H), 7.70 (d, 2H, Ar-H, *J* = 8.0 Hz), 8.42 (s, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.5, 114.8, 1282, 128.2, 129.2, 129.5, 130.8, 134.2, 141.9, 141.7, 159.9, 160.3, 164.1, 166.0. MS (*m/z*): 318.35.

4-((E)-1-((5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)imino)ethyl) phenol (**RM-8**): Color: Yellow. Yield: 90.08%. M.p.: 82-84 °C. FT-IR (KBr, ν, cm^{-1}): 3037 (Ar-C-H), 2964 (Aliph.C-H), 3201 (Phenolic O-H), 1450 (Aliph. C=N), 1606 (C=C), 1247 (N-N), 663 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.72 (s, 3H, CH_3), 6.78 (s, 1H, =CH-Phenyl), 6.87 (d, 2H, Ar-H, *J* = 8.6 Hz), 7.56-7.41 (m, 5H, Ar-H), 7.65 (d, 2H, Ar-H, *J* = 8.6 Hz), 8.32 (s, 1H, =CH-Thiadiazole ring), 10.17 (s, 1H, phenolic-OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.4, 114.6, 115.8, 123.8, 124.5, 128.1, 128.7, 128.9, 129.0, 129.0, 130.1, 134.2, 141.8, 158.9, 159.7, 1606, 164.0, 165.9. MS (*m/z*): 320.35.

2-((E)-1-((5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)imino)ethyl) phenol (**RM-9**): Color: Red. Yield: 88.30%. M.p.: 90-92 °C. FT-IR (KBr, ν, cm^{-1}): 2991 (Ar-C-H), 2929 (Aliph. C-H), 3192 (Phenolic O-H), 1591 (C=C), 1467 (N-N), 657 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.84 (s, 3H, CH_3), 6.87-6.37 (m, 4H, Ar-H, 1H, =CH-Phenyl), 7.55-7.45 (m, 5H, Ar-H), 8.32 (s, 1H, =CH-Thiadiazole ring), 9.63 (s, 1H, phenolic-OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.2, 78.2, 78.6, 78.9, 113.0, 115.1, 118.8, 120.1, 127.8, 128.8, 128.9, 129.6, 133.9, 134.5, 142.4, 157.5, 160.0, 160.2, 163.9, 163.8. MS (*m/z*): 320.35.

1-(Pyridin-4-yl)-N-(5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)et han-1-imine (**RM-10**): Color: Colorless. Yield: 82.30%. M.p.: 102-104 °C. FT-IR (KBr, ν, cm^{-1}): 3034 (Ar-C-H), 2962 (Aliph.C-H), 1639 (C=C), 1460 (C=N), 746 (C-S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.85 (s, 3H, CH_3), 6.87 (s, 1H, =CH-Phenyl), 7.53-7.75 (m, 4H, Ar-H), 8.56-8.77 (m, 5H, Ar-H, 1H, =CH-Thiadiazole ring). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 52.8, 115.1, 121.5, 128.1, 129.1, 134.1, 134.3, 142.4, 150.5, 157.9, 160.9, 163.1, 165.0. MS (*m/z*): 305.50.

2.4. Antimicrobial activity

The *in vitro* antibacterial activity properties of the compounds were evaluated using various microorganisms using a microbroth dilution assay [51]. The following microbial strains were acquired from the National Chemical Laboratory, Pune, India: *Pseudomonas aeruginosa* (NCIM 5031), *Escherichia coli* (NCIM 2065), *Bacillus subtilis* (NCIM 2699), *Aspergillus niger* (NCIM 620), *Aspergillus fumigatus* (NCIM 902), and *Aspergillus flavus* (NCIM 549). At 37 °C, bacterial strains were maintained in nutrient broth (NB), while fungal strains were cultured in Sabouraud dextrose broth.

2.4.1. Preparation of inoculums

For bacteria: At a temperature of 37 °C, the bacterial strains used as inoculums were grown to an optical density of 0.6 at 600 nm. Using the serial plate dilution technique, colony forming units (CFU) were enumerated and bacterial counts were adjusted to 1×10^5 - 1×10^6 CFU/mL for susceptibility test [52].

For fungus: Cultures grown on potato dextrose agar medium that were used to make the fungal inoculums were 10 days old. Using a sterile spatula, the conidia were scraped from the Petri dishes after being inundated with 8 to 10 mL of distilled water. With the use of a spectrophotometer (A595 nm), the spore density of each fungus was adjusted to produce a final concentration of around 1×10^5 spores/mL [53].

2.4.2. Micro broth dilution assay

According to the NCCLS guidelines, the minimum inhibitory concentration (MIC) was determined using the micro broth dilution technique 20 [54]. Eight different concentrations of compounds (20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 mg/mL) were made in DMSO using the two-fold dilution method in the wells [55]. Tetracycline at the same quantities for bacteria and Amphotericin B at the same concentrations for fungi were used as positive and negative controls, respectively. For the incubation of bacteria and fungi, 96-well plates were incubated at 37 °C for 24 and 48 hours, respectively.

3. Results and discussion

3.1. Synthesis

The synthesis of the final product was performed according to the reactions described in Scheme 1. Initially, the Schiff base was prepared by the reaction of 5-styryl-1,3,4-thiadiazol-2-amine and substituted acetophenones in the presence of 2-3 drops of lemon juice [56]. The physicochemical qualities given in the experimental section and the spectral features were used to identify the produced compounds. Using FT-IR, ^{1}H NMR, ^{13}C-NMR, and mass spectrometry, the chemical structures of the synthesised 1-phenyl-*N*-(5-((E)-styryl)-1,3,4-thiadiazol-2-yl) ethan-1-imine molecules (RM-1 to RM-10) were identified.

The IR spectrum of 1-phenyl-*N*-(5-((E)-styryl)-1,3,4-thiadiazol-2-yl)ethan-1-imine derivatives (RM-1 to RM-10) showed the characteristic IR band of 2964-2845 cm^{-1} which indicated the presence of the aliphatic C-H stretching frequency and the characteristic IR band at 3080-3034 cm^{-1} (stretching) and 1606-1639 cm^{-1} (bending) showed that the aromatic ring had the C-H and C=C groups, respectively. The presence of an IR absorption band at 3201 and 3192 cm^{-1} confirms the presence of a phenolic group (Ar-OH) in compounds RM-8 and RM-9.

Table 1. Antimicrobial activity of 1,3,4-thiadiazole Schiff bases in µg/mL.

Compounds	*P. aeruginosa*	*E. coli*	*B. subtilis*	*A. niger*	*A. fumigatus*	*A. flavus*
RM-1	10.0	2.5	5.0	2	2.5	2
RM-2	2.5	2.5	0.156	1.25	2.5	2.5
RM-3	5.0	5.0	0.312	1.25	5.0	2.5
RM-4	5.0	5.0	0.156	1.00	5.0	2.5
RM-5	10.5	5.0	5.0	1.25	2	2.5
RM-6	10.5	5.0	5.0	5.0	10.5	2.5
RM-7	5.0	5.0	5.0	5.0	5.0	2.5
RM-8	10.0	10.0	2.5	2.5	5.0	2.5
RM-9	2.5	5.0	5.0	5.0	5.0	2
RM-10	1.25	2	2.5	2.5	0.156	2.5
Tetracycline	0.00125	0.01	0.00125	-	-	-
Amphotericin B	-	-	-	0.00125	0.000156	0.000156

The nitrile group (CN) was present because 4-((E)-1-(((5-((E)-styryl)-1, 3, 4-thiadiazol-2-yl)imino)ethyl)benzonitrile (RM-5) had the distinctive band at 2351 cm^{-1}. The IR stretching vibrations between 812-731 cm^{-1} in the spectral data of the synthesised derivatives (RM-2 to RM-9) displayed the presence of various substituted functional groups in the aromatic nucleus substituted at the ortho, meta and para position, and another IR stretching vibration at 773-648 and 1438-1473 cm^{-1} in the spectral data of all synthesised derivatives (RM-1 to RM-10) due to the existence of C-S and C=N group in the thiadiazole ring, respectively.

The ^{1}H NMR spectrum of the synthesised RM-1 to RM-10 compounds showed a singlet between δ 3.24-3.84 ppm due to the existence of -CH_3 attached to the electron withdrawing group (imine). Compounds RM-8 and RM-9 showed singlet at δ 10.17 and 9.63 ppm due to the existence of a phenolic group (Ar-OH) at para and ortho positions, respectively. One of the compounds, RM-7 showed a singlet at δ 2.36 ppm due to the existence of -CH_3 at the para position. The aromatic proton of synthetic derivatives can be identified by multiplet signals in proton-NMR spectra between δ 6.87 and 8.62 ppm. The multiplet signals for four aromatic hydrogens between δ 7.36 and 7.96 ppm are observed in the RM-2, RM-3, and RM-5 proton-NMR spectra. Compound RM-7 showed two doublets at δ 7.70 (2H, *J* = 8 Hz) and 7.31 ppm (2H, *J* = 8 Hz) while RM-8 showed two doublets at δ 7.65 (2H, *J* = 8.6 Hz) and 6.87 ppm (2H, *J* = 8.6 Hz) indicating substitution at para positions. All synthesised derivatives showed a singlet between δ 8.32-8.60 and 6.43-8.87 ppm due to the HC = CH group, which confirmed the highly δ ppm value, that is, the highly deshielded, HC=CH group attached to the thiadiazole ring.

3.2. Antimicrobial activity

The antimicrobial activity of Schiff bases derived from 1,3,4-thiadiazole was evaluated using the broth microdilution method. Three bacterial strains, namely, *P. aeruginosa*, *E. coli*, and *B. subtilis*, were used as test microorganisms, along with three fungal species: *A. niger*, *A. fumigatus* and *A. flavus*. The results obtained from the microdilution method revealed that, among the compounds tested, RM-2 and RM-10 exhibited potent inhibitory effects on the growth of both bacterial and fungal species. The minimum inhibitory concentration (MIC) ranged from 0.156 to 2.500 µg/mL.

Significantly, the evaluated compounds showed selective activity. For example, RM-2, RM-3, and RM-4 demonstrated excellent activity against Gram-positive bacteria and *A. niger*, while showing moderate activity against Gram-negative bacteria and other tested *Aspergillus* species. On the other hand, compounds RM-5 to RM-9 exhibited moderate antibacterial and antifungal activity against the pathogens tested (Table 1). The observed selectivity of the 1,3,4-thiadiazole Schiff bases may arise from variances in cellular permeation or potentially through the selective inhibition of specific targets. Further investigations are required to elucidate the mechanisms of action for each compound.

Despite the potent antimicrobial activity displayed by the 1,3,4-thiadiazole Schiff bases against bacterial and fungal species, their activity was significantly inferior to that of the standard antibiotics tested (Table 1). However, the Schiff bases synthesised within this study hold potential as a foundational structure for subsequent enhancements aimed at achieving more potent antimicrobial effects.

4. Conclusion

It has been possible to synthesize and get in a good yield 1,3,4-thiadiazole Schiff bases. It has been purified, and the table contains a report and summary of the physical properties. The use of FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy to confirm the compounds' structures. The antimicrobial activity of the synthesized compounds was assessed. Tetracycline and amphotericin B, two common standard drugs, were used to compare the outcomes. Six different antimicrobial strains were used: *P. aeruginosa*, *E. coli*, *B. subtilis*, *A. niger*, *A. fumigatus* and *A. flavus*. When the prepared compound was compared to a standard drug, the prepared compound demonstrated a pronounced antimicrobial activity.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
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CRediT authorship contribution statement

Conceptualization: Mustakim Sharif; Methodology: Sajid Ajit Malak; Validation: Sajid Ajit Malak; Formal Analysis: Jamatsing Darbarsing Rajput; Data Curation: Sajid Ajit Malak; Writing - Original Draft: Sajid Ajit Malak; Writing - Review and Editing: Sajid Ajit Malak; Supervision: Mustakim Sharif.

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