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Synthesis and Antimicrobial Evaluation of New Fluorophenyl Hybridized Thiazole and Thiadiazole Derivatives

Yeni Florofenil Hibritleştirilmiş Tiyazol ve Tiyadiazol Türevlerinin Sentezi ve Antimikrobiyal Değerlendirilmesi

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Abstract

In this study, novel hybrid derivatives **(4a–4e)** combining 1,3-thiazole and 1,3,4-thiadiazole rings containing p-fluorophenyl on the same molecule were synthesized and their structures were elucidated using ^{1}H-NMR and ^{13}C-NMR spectroscopic methods. The antimicrobial activities of the compounds were evaluated on Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and yeast species (*Candida albicans*, *Candida krusei*) using agar well diffusion and microdilution methods. In the diffusion test, the inhibition zone diameters ranged from 7.6 ± 0.4 to 12.3 ± 0.3 mm. The most notable activity was produced by compound **4e** which produced inhibition zones of 12.3 ± 0.3 mm against *E. coli*, 12.0 ± 0.3 mm against *P. aeruginosa* and 12.0 ± 0.2 mm against *C. albicans*. Compound **4b** was also highly effective against yeast strains, and its zone diameter against *C. albicans* was 12.0 ± 0.2 mm. Minimum inhibitory concentration (MIC) values were generally determined to be 1250 µg/mL for bacteria, and 625 µg/mL for some species, whereas all compounds exhibited an MIC value of 625 µg/mL against *C. albicans* and *C. krusei*. Therefore, the results obtained indicate that fluorophenyl hybrid thiazole-thiadiazole derivatives generally exhibit weak antimicrobial activity; however, it is predicted that these data will shed light on future modification studies in the context of structure-activity relationship (SAR).

Keywords: Thiazole, 1,3,4-Thiadiazole, Fluorophenyl, Antimicrobial activity.

Öz

Bu çalışmada, *p*-florofenil içeren 1,3-tiyazol ve 1,3,4-tiyadiazol halkalarını aynı molekül üzerinde birleştiren yeni hibrit türevler **(4a–4e)** sentezlenmiş ve yapıları ^{1}H-NMR ve ^{13}C-NMR spektroskopik yöntemleri ile aydınlatılmıştır. Bileşiklerin antimikrobiyal aktiviteleri, Gram-pozitif bakteriler (*Bacillus subtilis, Staphylococcus aureus*), Gram-negatif bakteriler (*Escherichia coli, Pseudomonas aeruginosa*) ve maya türleri (*Candida albicans, Candida krusei*) üzerinde agar kuyucuk difüzyon ve mikrodilüsyon yöntemleri ile değerlendirilmiştir. Difüzyon testinde inhibisyon zon çapları 7.6 ± 0.4 ile 12,3 ± 0,3 mm arasında değişmiştir. En dikkat çekici aktiviteyi, *E. coli*'ye karşı 12,3 ± 0,3 mm, *P. aeruginosa*'ya karşı 12,0 ± 0,3 mm ve C. albicans'a karşı 12,0 ± 0,2 mm inhibisyon bölgeleri oluşturan bileşik **4e** gösterdi. Bileşik **4b** de, maya suşlarına karşı oldukça etkili bulunmuş ve *C. albicans*'a karşı 12,0 ± 0,2 mm zon çapı göstermiştir. Minimum inhibitör konsantrasyon (MİK) değerleri genel olarak bakteriler için 1250 µg/mL ve bazı türler için 625 µg/mL olarak belirlenirken, tüm bileşikler *C. albicans* ve *C. krusei*'ye karşı 625 µg/mL'lik bir MİK değeri sergiledi. Dolayısıyla elde edilen sonuçlar, florofenil hibrit tiyazol-tiyadiazol türevlerinin genel olarak zayıf antimikrobiyal aktivite sergilediğini; ancak bu verilerin, yapı-aktivite ilişkisi (SAR) bağlamında gelecekteki modifikasyon çalışmalarına ışık tutacağı öngörülmektedir.

Anahtar Kelimeler: Tiyazol, 1,3,4-Tiyadiazol, Florofenil, Antimikrobiyal aktivite.

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1. Introduction

The rapid global escalation of antimicrobial resistance has become a major concern for public health systems worldwide. Since the bacteria are developing resistance to the drugs, the common medications are becoming less effective. This is not only a complication to treat but also leads to longer deaths and the spread of drug-resistant infections. Health officials around the world have been warning about this crisis, as with the increase in resistant infections there are very few new antibiotic drugs available (Santos & Ramos, 2018; O'Connell et al., 2013; Theuretzbacher, 2013). For this reason, the discovery of novel types of antibiotics is an extremely important scientific activity.

Heterocyclic compounds play a vital role in medicinal chemistry because they offer different structures and have flexible electronic behavior. The five-membered heterocycles appear in many biological molecules and in various approved medications. The inclusion of these rings in molecular designs leads to substantial changes in the physicochemical properties and pharmacokinetic behavior, and biological effectiveness (Abualnaja et al., 2024). For this reason, such ring systems represent rational templates for the development of novel antibacterial and antifungal agents.

Among sulfur- and nitrogen-containing heterocycles, the 1,3-thiazole scaffold has attracted sustained interest. The aromatic ring containing nitrogen and sulfur atoms is the most excellent combination between electron stability and good binding to biological targets. Thiazole derivatives have been associated with a wide range of pharmacological activities, including antimicrobial, antifungal, anti-inflammatory, and anticancer effects (Gomha et al., 2015; Jaishree et al., 2012; Kalita et al., 2024; Kamat et al., 2020; Kapoor et al., 2025; Kumar & Singh, 2021; Mohanty et al., 2021; Putta et al., 2017; Raveesha et al., 2022; Sargsyan et al., 2025). The clinical medicinal value of thiazole structures is sustained by the fact that they are still being found in therapeutic compounds (Manchare et al., 2025).

Similarly, the 1,3,4-thiadiazole ring represents a privileged pharmacophoric unit with considerable biological relevance. The structure of this ring is aromatic and involves several heteroatoms, which makes it possible to create potent bonds with enzymes and other molecular targets. Thiadiazole is a chemical scaffold, which is studied in the context of its application as an antimicrobial agent, and in the chemistry of stability, and biological activity of different compounds (Anthwal et al., 2025; Gorecki et al., 2025; Indelicato et al., 2025; Kubera et al., 2025). The electronic structure of the thiadiazole ring facilitates its interaction with biological molecules, and particularly heteroatom-centered functional groups (Anthwal et al., 2022).

The modern drug design involves the combination of two heterocyclic motifs that are biologically active, to form one molecular framework. The hybrid molecules can be useful in the addition of complementary pharmacophoric, and this leads to a better lipophilicity, electronic

adjustment and probably a better biological response. This is one of the methods that can be used to produce compounds with varied structures that have a good chance of acting as an antimicrobial.

This research is experimental and produces novel hybrid compounds containing fluorophenyl-substituted thiazole-thiadiazole moieties. Electron distribution and membrane-interaction characteristics would be influenced by the addition of a para-fluorophenyl group, which could contribute to antimicrobial activity. Biological characterization of the synthesized compounds was carried out with the help of a variety of spectroscopic methods. They were subjected to tests for the antibacterial and anti-fungal properties of representative strains of Gram-positive and Gram-negative bacteria, as well as yeast.

2. Materials and Methods

2.1. Synthesis

Commercially available reagents were employed as received. Proton (^{1}H , 400 MHz) and carbon (^{13}C, 100 MHz) NMR spectra were recorded in DMSO-d_6 using a Bruker Fourier-transform NMR instrument. Chemical shift values (δ) are reported in parts per million (ppm), while coupling constants (J) are presented in Hertz (Hz). Resonance patterns are described as singlet (s), doublet (d), triplet (t), or multiplet (m). Elemental analysis was performed using a Leco CHNS-932 analyzer (Leco Corporation, Saint Joseph, MI, USA). Reaction progress was evaluated by TLC analysis performed on silica gel 60 F254 plates.

2.1.1. 2-amino-4-(4-fluorophenyl)thiazole (1)

The thiazole derivative **(1)** was synthesized through the cyclization of 2-bromo-1-(4-fluorophenyl)ethan-1-one with thiourea in ethanol under reflux conditions. Briefly, 2-bromo-1-(4-fluorophenyl)ethan-1-one (1.845 g, 8.50 mmol) and thiourea (0.647 g, 8.50 mmol) were suspended in ethanol (40 mL). The reaction mixture was heated under reflux at approximately 78 °C for 4 h with continuous stirring. The progress of the reaction was monitored by TLC on silica gel 60 F_{254} plates. After completion, the reaction mixture was allowed to cool to room temperature and then diluted with ice-cold distilled water to induce precipitation. The precipitated product was collected by filtration, washed thoroughly with distilled water, and dried. The crude solid was recrystallized from ethanol to afford compound **(1)**.

2.1.2. 2-chloro-N-(4-(4-fluorophenyl)thiazol-2-yl)acetamide (2)

Compound **(1)** (1.360 g, 7.00 mmol) was dissolved in dry THF (35–40 mL), and triethylamine (1.17 mL, 8.40 mmol, 1.20 equiv.) was added as a base. The reaction mixture was cooled to 0–5 °C in an ice bath. Then, 2-chloroacetyl chloride (0.61 mL, 7.70 mmol, 1.10 equiv.) was added dropwise over 15–20 min under stirring. After the addition was complete, the reaction mixture was stirred at 0–5 °C for 30 min and then at room temperature for 4–6 h. Completion of the reaction was monitored by TLC. The precipitated triethylamine hydrochloride was removed by filtration and washed with THF. The filtrate was concentrated under reduced pressure, and the residue was poured into ice-cold distilled water. The resulting solid was filtered, washed thoroughly with water, dried, and recrystallized from ethanol to give compound **(2)**.

2.1.3. 5-(substituted-amino)-1,3,4-thiadiazole-2-thiol derivatives (3a-e)

The corresponding 5-(substituted-amino)-1,3,4-thiadiazole-2-thiol derivatives **(3a–e)** were prepared in two steps. In the first step, the corresponding substituted isothiocyanate (2.00 mmol) was dissolved in ethanol (10–15 mL) and cooled in an ice bath to 0–5 °C. Hydrazine hydrate (80%, 0.12 mL, approximately 2.00 mmol) was added dropwise under continuous stirring while maintaining the temperature at 0–5 °C. After the addition was complete, the reaction mixture was stirred in the ice bath for 30 min and then allowed to reach room temperature. Stirring was continued at room temperature for 1–2 h. The precipitated N-substituted hydrazine carbothioamide intermediate was collected by filtration, washed with cold ethanol, and dried. In the second step, the obtained N-substituted hydrazinecarbothioamide (2.00 mmol) was dissolved or suspended in ethanol (15 mL) containing sodium hydroxide (0.08 g, 2.00 mmol). Carbon disulfide (0.145 mL, 2.40 mmol, 1.20 equiv.) was added dropwise, and the mixture was heated under reflux for 6–8 h. After completion of the reaction, as monitored by TLC, the reaction mixture was cooled to room temperature and poured into ice-cold distilled water. The solution was acidified slowly with dilute hydrochloric acid to approximately pH 5–6. The precipitated product was filtered, washed thoroughly with water until neutral, dried, and recrystallized from ethanol to obtain the corresponding thiadiazole-2-thiol derivatives **(3a–e)**.

2.1.4. General Procedure for the Synthesis of Target Compounds (4a-e)

The final thiazole–thiadiazole hybrid derivatives **(4a–e)** were synthesized by nucleophilic substitution between compound **(2)** and the corresponding thiadiazole-2-thiol derivatives **(3a–e)** in

the presence of potassium carbonate. Compound **(2)** (0.2707 g, 1.00 mmol), the appropriate thiadiazole-2-thiol derivative (1.00 mmol), and anhydrous potassium carbonate (0.1658 g, 1.20 mmol) were suspended in dry acetone (15–20 mL). The reaction mixture was heated under reflux at approximately 56 °C for 6–8 h with continuous stirring. The reaction progress was monitored by TLC. After completion, the mixture was cooled to room temperature, and the inorganic salts were removed by filtration and washed with acetone. The filtrate was concentrated under reduced pressure, and the residue was poured into distilled water. The precipitated solid was collected by filtration, washed thoroughly with water, dried under vacuum, and recrystallized from ethanol to afford the target compounds **(4a–4e)**.

N-(4-(4-fluorophenyl)thiazol-2-yl)-2-((5-(methylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (**4a**): Yield 75%. ^{1}H NMR: δ 2.85 (3H, s, CH_3), 4.12 (2H, s, CO-CH_2), 7.24-7.28 (2H, m, 1,4-disubstituted benzene), 7.66 (1H, s, NH), 7.94 (2H, s, 1,4-disubstituted benzene), 8.53 (1H, s, thiazole CH), 12.42 (1H, s, NH). ^{13}C NMR: δ 31.51, 37.85, 108.71, 116.06 (d, J(CF)= 21.36 Hz), 128.21 (d, J(CF)= 8.07 Hz), 131.19 (d, J(CF)= 3.08 Hz), 148.37, 156.84, 160.23, 162.28 (br.d, J(CF)= 243.23 Hz), 166.99, 171.20. Anal. Calcd for: $C_{14}H_{12}FN_5OS_3$, C, 44.08; H, 3.17; N, 18.36; S, 25.21 Found: C, 43.98; H, 3.16; N, 18.32; S, 25.52.

N-(4-(4-fluorophenyl)thiazol-2-yl)-2-((5-(ethylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (**4b**): Yield 78%. ^{1}H NMR: δ 1.14 (3H, t, J=6.92 Hz, CH_3), 3.26 (2H, br.s, CH_2), 4.12 (2H, s, CO-CH_2), 7.25-7.29 (2H, m, 1,4-disubstituted benzene), 7.66 (1H, s, NH), 7.94 (2H, s, 1,4-disubstituted benzene), 8.52 (1H, s, thiazole CH), 12.41-12.54 (1H, br.s, NH). ^{13}C NMR: δ 14.64, 37.82, 108.67, 116.07 (d, J(CF)= 21.32 Hz), 128.23 (d, J(CF)= 8.00 Hz), 131.24 (d, J(CF)= 5.56 Hz), 148.39, 148.67, 156.83, 160.23, 162.29 (br.d, J(CF)= 242.37 Hz), 166.99, 170.27. Anal. Calcd for: $C_{15}H_{14}FN_5OS_3$, C, 45.55; H, 3.57; N, 17.71; S, 24.32 Found: C, 45.39; H, 3.55; N, 17.73; S, 24.48.

N-(4-(4-fluorophenyl)thiazol-2-yl)-2-((5-(isobutylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (**4c**): Yield 72%. ^{1}H NMR: δ 0.87 (6H, d, J=3.04 Hz, $CH(CH_3)_2$), 1.83 (1H, br s, CH), 3.05 (2H, br s, CH_2), 4.11 (2H, s, CO-CH_2), 7.25-7.28 (2H, m, 1,4-disubstituted benzene), 7.65 (1H, s, NH), 7.94 (2H, s, 1,4-disubstituted benzene), 8.53 (1H, s, thiazole CH), 12.41-12.54 (1H, br.s, NH). ^{13}C NMR: δ 20.48, 27.95, 37.83, 52.68, 108.65, 116.05 (d, J(CF)= 22.10 Hz), 128.21 (d, J(CF)= 8.2 Hz), 131.21 (dd , J(CF)= 2.9 Hz), 148.42, 156.83, 160.22, 162.28 (d, J(CF)= 244.5 Hz), 167.01, 170.68. Anal. Calcd for: $C_{17}H_{18}FN_5OS_3$, C, 48.21; H, 4.28; N, 16.54; S, 22.71 Found: C, 48.38; H, 4.27; N, 16.62; S, 22.79.

N-(4-(4-fluorophenyl)thiazol-2-yl)-2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (**4d**): Yield 69%. ^{1}H NMR: δ 4.25 (2H, s, CO-CH_2), 7.25-7.29 (2H, m, 1,4-disubstituted benzene), 7.56 (1H, br.dd J: 7.00 Hz), 7.67 (4H, s, 1,4-disubstituted benzene), 7.94 (2H, s, 1,4-disubstituted benzene), 8.53 (1H, s, thiazole CH), 10.40 (1H, s, NH), 12.41 (1H, s, NH). ^{13}C NMR: δ 37.42, 108.73,

116.07 (d, J (CF)=21.32 Hz), 117.89, 122.52, 125.68, 128.22 (d, J(CF)= 8.10 Hz), 129.57, 131.18, 135.99, 148.37, 156.83, 160.23, 162.29 (br d, J(CF)= 244.49 Hz), 166.83. Anal. Calcd for: $C_{19}H_{14}FN_5OS_3$, C, 51.45; H, 3.18; N, 15.79; S, 21.68 Found: C, 51.25; H, 3.19; N, 15.73; S, 21.62.

N-(4-(4-fluorophenyl)thiazol-2-yl)-2-((5-p-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetamide (**4e**): Yield 66%. ^{1}H NMR: δ 2.50 (3H, s, CH_3), 4.21 (2H, s, CO-CH_2), 7.20 (2H, s, 1,4-disubstituted benzene), 7.25-7.29 (2H, m, 1,4-disubstituted benzene), 7.27 (2H, s, 1,4-disubstituted benzene), 7.94 (2 H, s, 1,4-disubstituted benzene), 8.53 (1H, s, thiazole CH), 12.41 (1H, s, NH), 12.58 (1H, s, NH). ^{13}C NMR: δ 18.39, 37.50, 108.69, 116.07 (d, J(CF)= 21.43 Hz), 124.45, 127.10, 128.17 (d, J(CF)=8.10 Hz), 129.38, 131.17 (dd , J(CF)=3.02 Hz), 131.30, 148.40, 156.82, 160.23, 162.28 (br d, J(CF)= 245.50 Hz), 166.92. Anal. Calcd for: $C_{20}H_{16}FN_5OS_3$, C, 52.50; H, 3.52; N, 15.31; S, 21.02 Found: C, 52.71; H, 3.54; N, 15.37; S, 21.09.

2.2. Antimicrobial Assay

The biological evaluation of the prepared derivatives was performed using representative Gram-positive, Gram-negative, and yeast reference strains: *Bacillus subtilis* NRRL B478, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 3521, *Candida albicans* ATCC 90028, and *Candida krusei* ATCC 6258.

2.2.1. Agar Well Diffusion Method

Fresh microbial cultures were prepared and adjusted to a turbidity equivalent to the 0.5 McFarland standard. The standardized microbial suspensions were uniformly spread onto Mueller–Hinton Agar (MHA) for bacterial strains and Sabouraud Dextrose Agar (SDA) for yeast strains. Wells approximately 6 mm in diameter were aseptically prepared in the agar, and each well was filled with 100 µL of the test compound solution prepared in DMSO at a concentration of 5000 µg/mL. DMSO at the corresponding volume was included as a solvent control and showed no detectable antimicrobial activity. Ciprofloxacin at 50 µg/mL (5 µg/well) and amphotericin B at 200 µg/mL (20 µg/well) were used as reference antibacterial and antifungal agents, respectively. A volume of 100 µL of each reference antimicrobial solution was applied to the corresponding wells. The plates were maintained at 4 °C for a short equilibration period to facilitate diffusion of the compounds into the agar and were subsequently incubated at 37 °C for 24 h. Following incubation, antimicrobial activity was evaluated by measuring the inhibition zone diameters in millimeters. (CLSI, 2025). All assays were performed in triplicate.

2.2.2. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations (MICs) of the synthesized compounds were determined using the broth microdilution method in sterile 96-well microplates in accordance with CLSI recommendations (CLSI, 2002; 2007; 2012). Mueller–Hinton Broth (MHB) was used for bacterial strains, whereas RPMI 1640 medium was used for Candida strains. The test compounds were dissolved in DMSO and subjected to twofold serial dilutions to obtain final concentrations ranging from 1250 to 39.06 µg/mL. Fresh bacterial suspensions were prepared to provide a final inoculum concentration of approximately 5×10^5 CFU/mL in each well. For Candida strains, the inoculum was prepared according to the CLSI M27 procedure to obtain a final concentration of approximately 0.5–2.5×10^3 CFU/mL. The final assay volume was 100 µL per well. Ciprofloxacin and amphotericin B were used as positive antimicrobial controls for bacterial and yeast strains, respectively. Growth controls containing inoculated medium without the test compound, sterility controls containing uninoculated medium, and DMSO solvent controls were included in each experiment. The DMSO control showed no detectable inhibition of microbial growth at the concentration used in the assay. Microplates were incubated at 37 °C for 24 h. MIC was determined visually and defined as the lowest concentration of the tested compound at which no visible microbial growth was observed compared with the untreated growth control. All MIC determinations were performed in triplicate.

2.2.3. Statistical Analysis

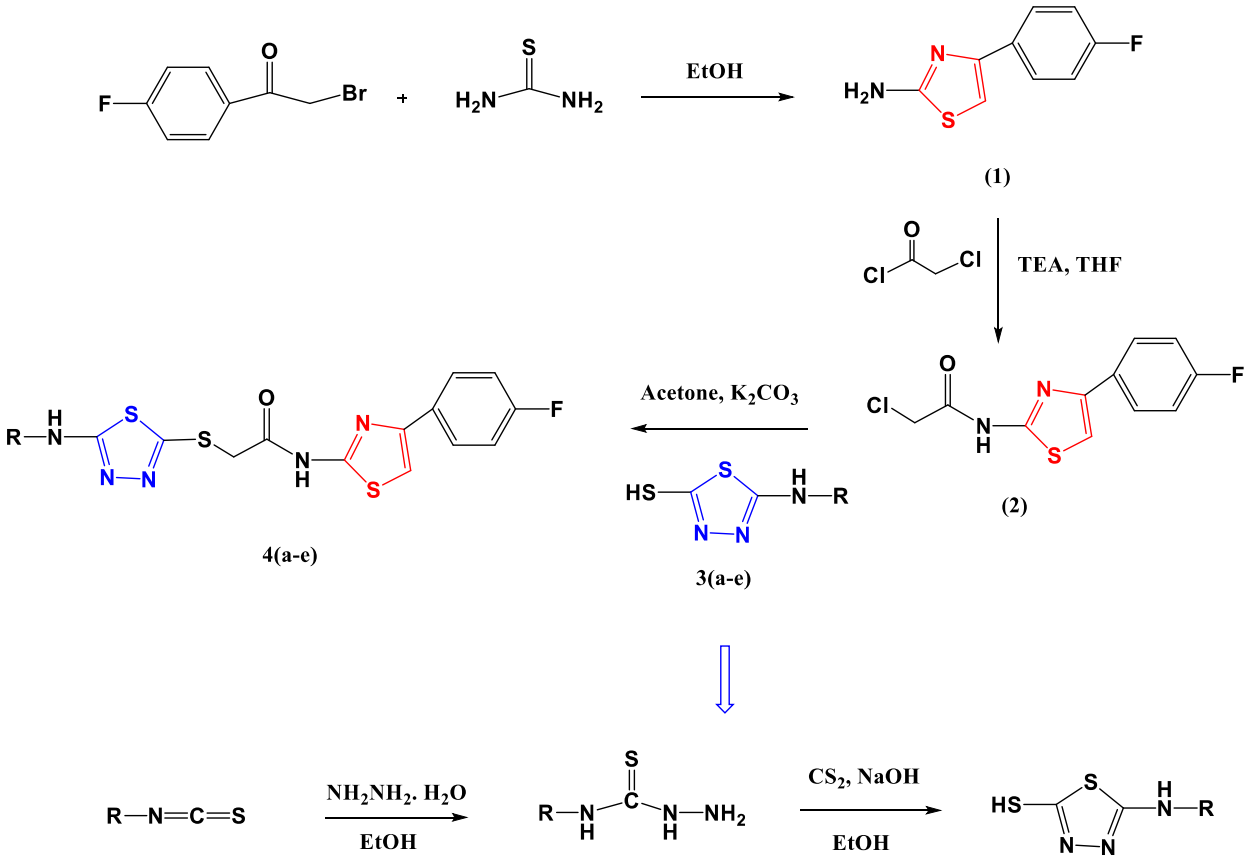
The agar well diffusion assay was performed using three independent biological replicates, and the results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA). For each microorganism, differences in inhibition zone diameters among the synthesized compounds (4a–4e) were analyzed separately using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to identify specific pairwise differences between the compounds. A value of $p < 0.05$ was considered statistically significant. Significant differences identified by the post-hoc analysis are indicated in Figure 1 using different superscript letters.

3. Findings and Discussion

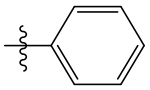
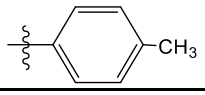
The synthetic pathway employed for the preparation of the new fluorophenyl-hybridized thiazole–thiadiazole derivatives **(4a–4e)** is outlined in **Scheme 1**. The synthesis was accomplished

through a multistep strategy starting from commercially available reagents and proceeded in overall good yields.

Scheme 1. Synthetic strategy for compounds **4a** to **4e**.



Compound	R
4a	-CH_3
4b	-CH_2CH_3
4c	-CH_2-$CH(CH_3)_2$

Compound	R
4d	
4e	

Abbreviations: EtOH = ethanol; TEA = triethylamine; THF = tetrahydrofuran; K_2CO_3 = potassium carbonate; CS_2 = carbon disulfide; NaOH = sodium hydroxide; $NH_2NH_2.H_2O$= hydrazine hydrate.

Initially, 2-amino-4-(4-fluorophenyl)thiazol **(1)** was obtained via the cyclization reaction of 2-bromo-1-(4-fluorophenyl)ethan-1-one with thiourea under reflux conditions in ethanol. Successful cyclization to the thiazole framework was verified by the presence of a diagnostic singlet signal for the thiazole CH proton observed in the downfield region of the ^{1}H-NMR spectrum.

Subsequently, acylation of compound **(1)** with 2-chloroacetyl chloride in the presence of triethylamine afforded the key intermediate 2-chloro-N-(4-(4-fluorophenyl)thiazol-2-yl)acetamide

(2). The successful formation of the acetamide moiety was evidenced by the emergence of a methylene signal (–CO–CH_2–) around 4.1–4.3 ppm in the ^{1}H-NMR spectra and a corresponding carbon signal near 36–38 ppm in the ^{13}C NMR spectra.

In parallel, 5-(substituted-amino)-1,3,4-thiadiazole-2-thiol derivatives **(3a–e)** were synthesized via the cyclization of the corresponding N-substituted hydrazinecarbothioamides with carbon disulfide under basic conditions. The formation of the 1,3,4-thiadiazole ring was supported by characteristic downfield ^{13}C-NMR signals above 150 ppm, attributable to the C=N carbons of the thiadiazole ring.

A nucleophilic substitution reaction between compound (2) and thiadiazole-2-thiol derivatives 3a–e afforded the target compounds **4a–4e**. This formed a thioether bond between the thiazole and thiadiazole moieties to form hybrid heterocyclic systems. The structures of the final compounds were confirmed by ^{1}H and ^{13}C-NMR analysis. The ^{1}H- NMR spectra of compounds **4a-4e** showed that the methylene protons between the carbonyl and sulfur atoms (-CO-CH_2-S-) appeared as singlets in the region of 4.11-4.25 ppm, indicating the formation of thioether. The thiazole CH proton was a singlet in all derivatives at around 8.52-8.53 ppm, which was a characteristic peak of the thiazole ring.

The aromatic protons of the para-fluorophenyl group showed the expected resonance pattern in the regions of 7.20–7.30 and 7.90–7.95 ppm. The evidence of fluorine substitution was further supported by the presence of carbon-fluorine coupling peaks in the NMR spectra and in the case of aromatic carbons bound to fluorine, the double peaks were characteristic.

The substituted amino group signals on the thiadiazole ring were different and were dependent on the type of the substituent. The expected upfield signals in the areas of aliphatic substituents, i.e., methyl, ethyl, and isobutyl were observed and aromatic substituents gave further aromatic signals. The NH protons were always detected as downfield singlets at the value of 7.65-12.58 ppm, which indicated hydrogen bonded amino and amide groups.

In the ^{13}C NMR spectra, the amide carbonyl carbon resonances were observed at approximately 166.8–167.0 ppm, supporting the presence of the acetamide moiety. The carbons of the thiadiazole and thiazole rings appeared in the expected downfield regions and were consistent with the proposed structures. In addition, the characteristic carbon–fluorine coupling patterns observed for the *p*-fluorophenyl group further supported the structural assignments. Overall, the spectroscopic data were consistent with the proposed molecular structures of compounds **4a–4e**.

Antimicrobial evaluation of compounds **4a–4e** was performed by agar well diffusion at 5000 µg/mL, followed by broth microdilution assays for determination of minimum inhibitory concentrations (MICs). The activity profile was assessed against representative Gram-positive species (*Bacillus subtilis, Staphylococcus aureus*), Gram-negative organisms (*Escherichia coli, Pseudomonas aeruginosa*), and yeast strains including *Candida albicans* and *Candida krusei*.

In the agar well diffusion test, each compound generated distinct and quantifiable growth inhibition areas. Diameters ranging from 7.6 ± 0.4 to 12.3 ± 0.3 mm. Among Gram-positive bacteria, *S. aureus* showed inhibition zones of 9.4 ± 0.5–10.2 ± 0.2 mm, with the highest activity observed for **4b** (10.2 ± 0.2 mm) and **4d** (10.1 ± 0.1 mm). For Gram-negative bacteria, inhibition zones against *E. coli* ranged from 8.0 ± 0.3 to 12.3 ± 0.3 mm, with **4e** showing the largest zone (12.3 ± 0.3 mm). In contrast, *P. aeruginosa* demonstrated slightly lower sensitivity overall, with zone diameters between 7.6 ± 0.3 and 12.0 ± 0.3 mm, although **4a**, **4d**, and **4e** produced zones ≥10 mm, suggesting relatively enhanced activity against this intrinsically resistant species. The antifungal activity of the compounds was notable, with inhibition zones against *C. albicans* ranging from 9.6 ± 0.3 to 12.0 ± 0.2 mm and against *C. krusei* from 7.6 ± 0.4 to 10.4 ± 0.4 mm. In particular, **4b** and **4e** demonstrated the strongest antifungal effects, yielding inhibition zones comparable across both yeast species (Table 1, Figure 1). Statistical analysis demonstrated significant differences among the synthesized compounds for the inhibition zone diameters against the tested microorganisms (one-way ANOVA, $p < 0.0001$).

Table 1. Antimicrobial inhibition diameters (mm) of the compounds **(4a-4e)**

Samples	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. krusei*
4a	8.0 ± 0.2	9.4 ± 0.5	8.0 ± 0.3	10.2 ± 0.2	10.0 ± 0.3	9.6 ± 0.2
4b	9.9 ± 0.2	10.2 ± 0.2	10.3 ± 0.3	9.7 ± 0.2	12.0 ± 0.2	10.2 ± 0.2
4c	9.7 ± 0.3	9.6 ± 0.4	8.3 ± 0.3	8.3 ± 0.2	9.6 ± 0.3	7.6 ± 0.4
4d	10.2 ± 0.3	10.1 ± 0.1	8.3 ± 0.3	10.0 ± 0.2	11.9 ± 0.2	10.3 ± 0.3
4e	9.4 ± 0.4	9.6 ± 0.3	12.3 ± 0.3	12.0 ± 0.3	12.0 ± 0.2	10.4 ± 0.4
Ciprofloxacin	20	21	18	15	-	-
Amphotericin B	-	-	-	-	14	15

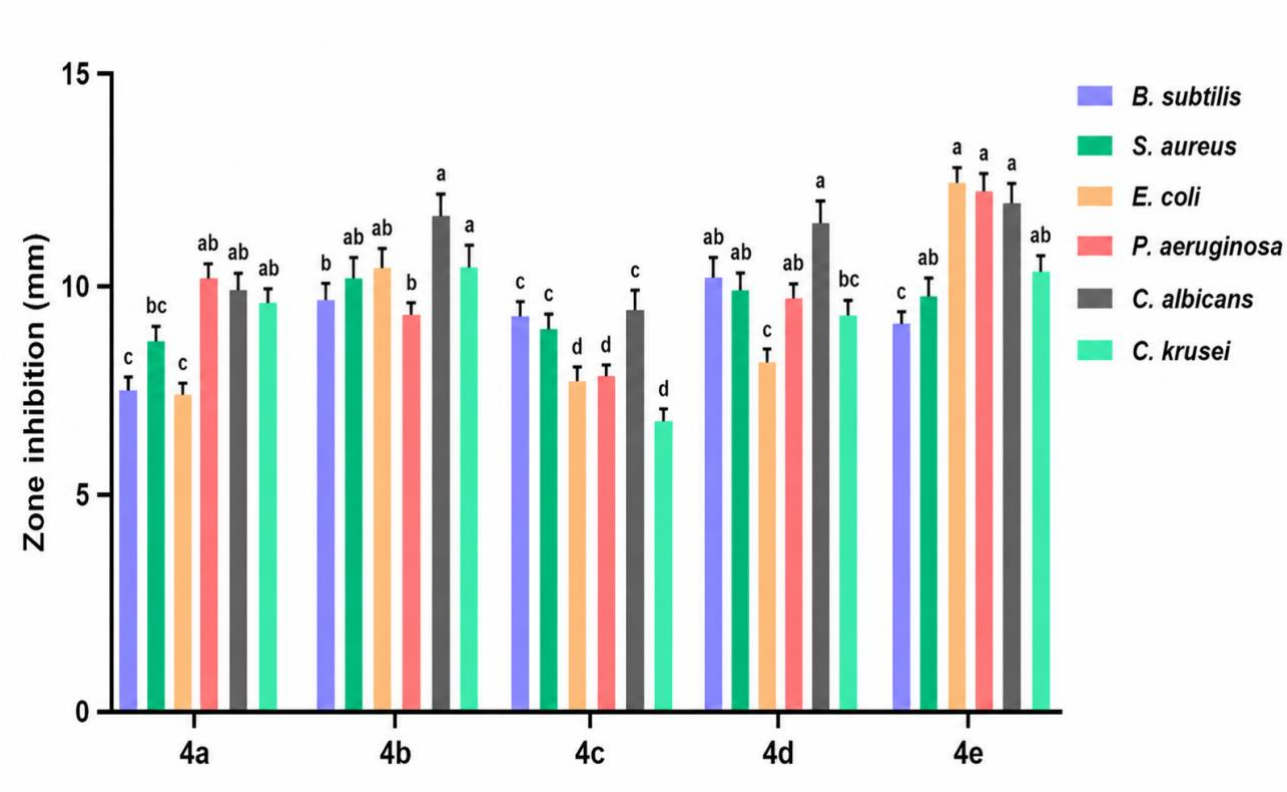


Figure 1. Inhibition zone diameters of the synthesized compounds (4a–4e) against the tested microorganisms. Data are presented as mean ± SD of three independent biological replicates. For each microorganism, differences among the compounds were analyzed separately using one-way ANOVA followed by Tukey's multiple comparison test. Different lowercase letters above the bars indicate statistically significant differences among compounds for the same microorganism ($p < 0.05$), whereas bars sharing the same letter are not significantly different.

For the majority of the tested microorganisms, MIC values were observed at 1250 µg/mL, although some of the experimental compounds were found to have lower values of 625 µg/ml when tested against selected organisms, particularly when used against *P. aeruginosa* and the two types of *Candida*. The entire compound set was found to exhibit similar antifungal MIC values of 625 µg/ml in opposition to both *C. albicans* and *C. krusei*. The findings based on the diffusion of agar were reinforced by the MIC analysis (**Table 2**).

When evaluated in terms of structure-activity relationship, it is observed that the amino substituent attached to the thiadiazole ring in the synthesized **4a–4e** derivatives has a certain but limited effect on the antimicrobial response. Since all compounds share the common p-fluorophenyl-thiazole/thiadiazole hybrid skeleton, the activity differences can be primarily attributed to the electronic, steric, and lipophilic properties of the methyl, ethyl, isobutyl, phenyl, and p-tolyl groups. However, the significantly higher MIC values obtained compared to the reference drugs indicate that these substituent changes only modulate the activity to a limited extent, rather than significantly enhancing it.

Table 2. MIC values (µg/mL) of the synthesized compounds

Compounds	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*C. krusei*
4a	1250	1250	625	1250	625	625
4b	1250	1250	1250	1250	625	625
4c	1250	1250	1250	1250	625	625
4d	1250	1250	625	1250	625	625
4e	1250	1250	1250	1250	625	625
Ciprofloxacin	1	2	1	0.5	-	-
Amphotericin B	-	-	-	-	1	1

Compound **4a**, carrying the methylamino substituent, can be considered the least sterically hindered derivative due to its small-volume alkyl structure. This compound showed a 10.2 ± 0.2 mm inhibition zone and a 625 µg/mL MIC value, particularly against *P. aeruginosa*. However, the formation of smaller zone diameters against *E. coli* and Gram-positive bacteria indicates that the small alkyl group alone does not provide a broad-spectrum activity advantage. This observation may possibly be associated with the relatively limited hydrophobic contribution of the methyl substituent; however, effects on membrane penetration or target interactions were not directly investigated in the present study.

The ethylamino derivative **4b**, showed a more stable activity profile compared to the methyl derivative. The higher inhibition zones obtained, particularly against *S. aureus*, *E. coli*, and *C. albicans*, may be associated with differences in the physicochemical properties introduced by the ethyl substituent, including its potentially greater hydrophobic character compared with the methyl group. However, the fact that MIC values generally remained at around 1250 µg/mL for bacteria indicates that this increase in zones does not correspond to a strong improvement in true inhibitory potency. Therefore, the ethyl substitution may have a modest influence on the observed activity, although the underlying physicochemical basis was not directly investigated.

Contrary to expectations, compound **4c**, which carries the isobutylamino substituent, did not significantly increase activity. Although the more voluminous and branched isobutyl substituent may confer greater hydrophobic character, the lower zone values, especially against *P. aeruginosa*, *E. coli*, and *C. krusei*, could possibly be related to differences in properties such as solubility, agar diffusion, or microbial cell penetration. These possibilities were not directly investigated in the present study. These results suggest that, within the limited compound series investigated, the branched isobutyl substituent did not provide an apparent activity advantage.

Compounds **4d** and **4e**, containing aromatic substituents, showed a different trend. The phenylamino derivative **4d** formed relatively high inhibition zones, especially against Gram-positive bacteria and *Candida* species, and also showed an MIC value of 625 µg/mL against *P. aeruginosa*. The observed activity profile could possibly be associated with the physicochemical characteristics of the phenyl substituent, including potential hydrophobic or π-related interactions. However, the limited activity against *E. coli* and the high overall MIC values indicate that the phenyl group does not provide broad-spectrum superiority.

The *p*-Tolylamino derivative **4e**, stood out as the most remarkable compound in the series in the agar diffusion test. Specifically, the formation of inhibition zones of 12.3 ± 0.3 mm against *E. coli*, 12.0 ± 0.3 mm against *P. aeruginosa*, and 12.0 ± 0.2 mm against *C. albicans* suggests that the addition of a methyl group in the para-position to the phenyl ring can increase apparent activity against some microorganisms. This trend may possibly be associated with the electronic and hydrophobic characteristics introduced by the *p*-methyl substituent. However, the fact that the MIC values of **4e** against bacteria remained at 1250 µg/mL indicates that the observed high zone diameters did not translate into strong inhibitory potency. Therefore, although **4e** is the most remarkable derivative in terms of agar diffusion results, it should be considered not as a strong antimicrobial candidate in terms of MIC data, but rather as a precursor structure for further structural modifications.

In conclusion, agar diffusion and MIC findings indicate that the compounds in the series have a weak but measurable antimicrobial activity that is repeatable and wide spectrum. The difference in size of inhibition zones and MIC results has shown how diffusion and physical-chemical properties affect the antimicrobial action. The compounds that were synthesized were less active than the standard antibiotics but their reliable action against *Candida* species and certain Gram-negative bacteria makes them useful compounds that can be modified in the future or as primary compounds in the development of new antimicrobial drugs.

4. Conclusions and Recommendations

In this study, five novel hybrid compounds **(4a–4e)** combining 1,3-thiazole and 1,3,4-thiadiazole rings containing 4-fluorophenyl on the same molecule were successfully synthesized, and their structures were confirmed by ^{1}H-NMR, ^{13}C-NMR and elemental analyses. According to the antimicrobial evaluation results, the compounds showed MIC values of 1250 µg/mL against Gram-positive bacteria (*B. subtilis, S. aureus*). Against the Gram-negative bacterium *P. aeruginosa*, compounds **4a** and **4d** provided inhibition at a relatively lower concentration with a MIC value of 625 µg/mL, while this value was determined as 1250 µg/mL in the other derivatives. All compounds showed a MIC value of 625 µg/mL against *C. albicans* and *C. krusei*. In the agar diffusion test,

inhibition zone diameters ranged from 7.6 ± 0.4 mm to 12.3 ± 0.3 mm; the highest zone value was measured as 12.3 ± 0.3 mm in *E. coli* for compound **4e**. When compared with the MIC values of reference drugs (ciprofloxacin: 0.5–2 µg/mL; amphotericin B: 1 µg/mL), the antimicrobial potency of the synthesized derivatives is found to be lower. The obtained results demonstrate limited biological effectiveness of the hybrid thiazole–thiadiazole framework against selected microbial strains. The activity of the compound can be enhanced by structural diversification and by implementing suitable substitution methods.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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