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ORIGINAL ARTICLE

Synthesis, Characterization, and Antimicrobial Evaluation of Some Novel Hydrazinecarbothioamides

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ABSTRACT

Objectives: This study focused on synthesizing and characterizing novel thiosemicarbazide derivatives containing a 1,2,4-triazole moiety and evaluating their antimicrobial activity against several bacterial strains. The research aimed to identify key structural features that enhance antimicrobial efficacy through structure-activity relationship analysis and identify the minimum inhibitory concentration (MIC) of the most potent compounds to assess their potential for further development as antimicrobial agents.

Materials and Methods: Nine novel thiosemicarbazide derivatives containing a 1,2,4-triazole moiety were synthesized by reacting 1,2,4-triazole derivatives with thiosemicarbazide precursors, and the products were characterized using infrared spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy, and elemental analysis. The antimicrobial activity of these compounds (**5a-i**) was tested against *Klebsiella pneumoniae (K. pneumoniae), Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa (P. aeruginosa),* using microdilution, disk diffusion, and broth microdilution methods. Dimethyl sulfoxide was used as a negative control, and Vancomycin and Meropenem were used as positive controls, with all results converted to µM for consistent analysis.

Results: The synthesized thiosemicarbazide derivatives (**5a-i**) were confirmed to be structurally correct through Fourier-transform infrared spectroscopy, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Among the tested compounds, **5e** (4-bromophenyl) and **5g** (n-propyl) showed significant antimicrobial activity, with **5g** exhibiting the strongest effects against *S. aureus* and *P. aeruginosa*. Other derivatives, such as **5b** (4-NO_2Ph), **5c** (4-FPh), and **5d** (4-ClPh), showed moderate activity, while no significant activity was observed against *K. pneumoniae* or *E. faecalis*.

Conclusion: The study successfully synthesized a series of novel thiosemicarbazide derivatives with a 1,2,4-triazole moiety and evaluated their antimicrobial potential. Compounds **5e** and **5g** exhibited significant antibacterial activity, particularly against *S. aureus* and *P. aeruginosa*, with MIC values in the low micromolar range. These findings suggest that the compounds hold promise as potential antimicrobial agents, and further studies should focus on optimizing their efficacy and exploring their mechanism of action.

Keywords: Synthesis, hydrazinecarbothioamide, 1,2,4-triazole, antimicrobial activity

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INTRODUCTION

The escalating prevalence of antibiotic-resistant bacteria, fueled by the misuse of antibacterial agents, has become a critical global health crisis.[1,2] As these resistant strains continue to evolve, an increasing number of infections are no longer responding to conventional treatments. Alarmingly, even last-resort antibiotics are losing their efficacy in some cases.[3-5] This surge in antimicrobial resistance underscores the urgent need for new therapeutic agents, making the development of novel treatments a top priority in medicinal chemistry today.[6-8]

Thiosemicarbazides are vital functional groups in medicinal chemistry, known for their role in forming biologically active heterocyclic rings such as 1,3,4-thiadiazoles, 4-thiazolidinones, and 1,2,4-triazole-3-thiones.[9-11] Numerous studies have highlighted the diverse biological activities exhibited by thiosemicarbazides. Triazoles, defined by a five-membered ring containing three nitrogen atoms and two carbon atoms, are particularly noteworthy in this context. Triazole derivatives have garnered significant attention due to their broad spectrum of biological activities.[9,12-16]

Several widely used pharmaceuticals incorporate the 1,2,4-triazole scaffold, including the antifungal agents fluconazole, itraconazole, and voriconazole, the antimigraine drug rizatriptan, and the antiviral drug ribavirin (Figure 1). Additionally, the inclusion of fluorine atoms in drug design has become increasingly popular.[17-20] Fluorine's unique properties, such as its ability to impart diverse physicochemical attributes and its minimal steric impact, make it an attractive choice for enhancing drug interactions with biological systems. Its high electronegativity also significantly alters the physical and chemical properties of molecules, making fluorine incorporation a powerful strategy in medicinal chemistry.[21,22]

Motivated by the urgent need to overcome antibacterial resistance, our primary aim was to design agents based on thiosemicarbazide scaffolds bearing a 1,2,4-triazole heterocycle, selected for their well-established medicinal relevance outlined above.

This study reports the design, synthesis, structural characterization, and antimicrobial evaluation of nine novel thiosemicarbazides (**5a-i**), each incorporating a 1,2,4-triazole moiety (Figure 2). The antimicrobial efficacy of the synthesized compounds was tested against a panel of bacterial strains, including *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (BAA-2146), and *Escherichia coli* (ATCC 25922).

MATERIALS AND METHODS

Materials

Melting points were recorded on a STUART SMP40. Infrared (IR) spectra were recorded on a Shimadzu Fourier-transform infrared (FT-IR) spectrometer using KBr pellets. Affinity-1 FT-IR spectroscopy instrument and an Alpha Bruker FT-IR spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra were measured on a Bruker spectrometer in dimethyl sulfoxide (DMSO)-d_6 solution at 500 Megahertz (MHz) and 125 MHz, respectively. Chemical shifts (δ) were reported in ppm, and coupling constants (J) were recorded in hertz (Hz).

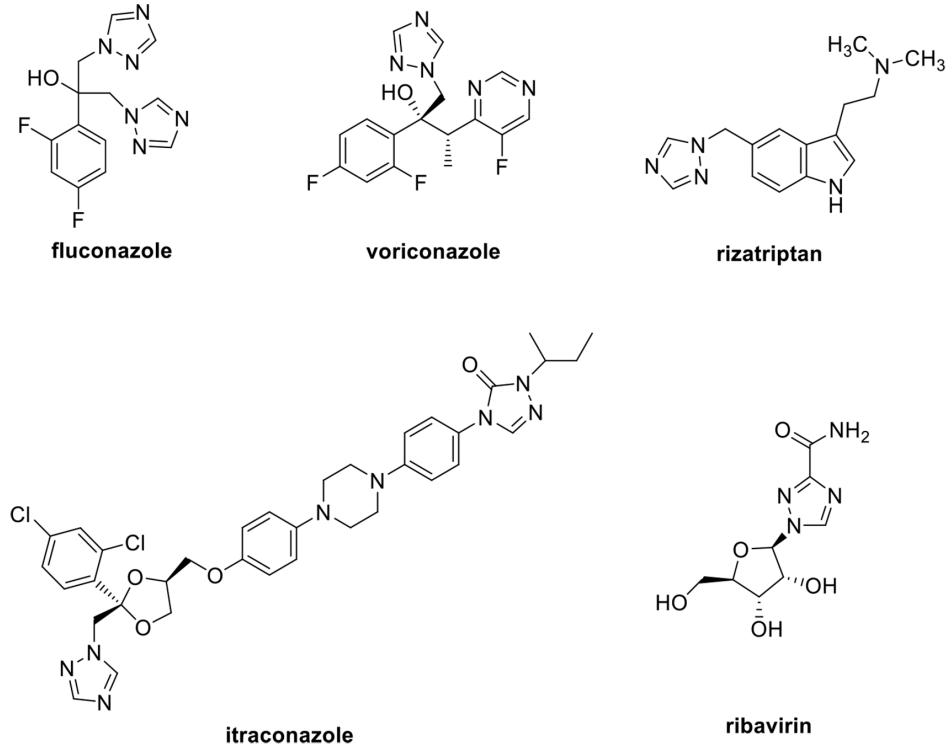


Figure 1. Chemical structures of some drugs containing the 1,2,4-triazole structure

Elemental analysis was recorded using Leco CHNS-932. The reactions were monitored using thin-layer chromatography on aluminum plates, which have a silica gel Kieselgel 60 F 254 layer of thickness 0.25 mm (Merck), using ultraviolet light as a visualizing agent. All reagents and solvents were purchased from Merck, Fluka, and Sigma-Aldrich and were used without further purification.

Chemical synthesis

Synthesis of N-(4-fluorophenyl)-2-(furan-2-carbonyl) hydrazine-1-carbothioamide (1)

N-(4-Fluorophenyl)-2-(furan-2-carbonyl)hydrazine-1-carbothioamide (1) was synthesized with a good yield of 86% through the reaction of equimolar amounts (0.005 mol) of 2-furoic acid hydrazide and 4-fluorophenylisothiocyanate. The reaction proceeded smoothly under reflux in boiling ethanol.[23]

Synthesis of 4-(4-fluorophenyl)-5-(furan-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (2)

A solution of compound 1 (0.005 mol) was prepared in 2N NaOH, and the solution was refluxed for 4-5 hours. Afterward, the solution was neutralized using 12.5% hydrochloric acid (HCl). Lastly, the product was filtered and washed with distilled water (Yield: 93%).[23]

Synthesis of ethyl ((4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazole-3-yl)sulfanyl)acetate (3)

A mixture of 0.005 mol of compound 2, K_2CO_3, and 0.0055 mol of ethylbromoacetate ($BrCH_2COOEt$), in ethanol, was refluxed for 5 hours. After completion, the reaction mixture was cooled and poured onto ice water, yielding ethyl ((4-*fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazole-3-yl)sulfanyl)acetate* (3) with a 75% yield.[24]

Synthesis of 2-[[4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl]sulfanyl]acetohydrazide (4)

A mixture of compound 3 (0.005 mol), ethanol, and $NH_2NH_2.H_2O$ (0.025 mol) was heated under reflux in ethanol for 5-6 hours to yield 2-[[4-(4-fluorophenyl)-5-(furan-2-yl)-4*H*-1,2,4-triazol-3-yl]sulfanyl] acetohydrazide (4), with a 61.1% yield.

The analytical and spectral data were reported in our previous study.[25]

Synthesis of N-(substituted)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamides ***(5a-i)***

0.005 mol of compound 4 is dissolved in ethanol, and 0.005 mol of appropriate isothiocyanate is added to it. It is boiled in a water bath under reflux for 4 hours. At the end of the time, the precipitated product **(5a-i)** is filtered, dried, and the yield is calculated.

N-(Phenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5a)

White powder, mp 178-179 °C, yield: 71.42%, Anal. Calcd. for: $C_{21}H_{17}FN_6O_2S_2$, C, 53.83; H, 3.66; N, 17.94%; Found: C, 53.46; H, 3.785; N, 17.88%. FT-IR υmax (cm^{-1}): 3300, 3252 (N-H), 3146, 3096, 3046 (ar. C-H), 2930, 2834 (al. C-H), 1706 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.40 (s, 1H, CONH), 9.78 (s, 1H, NH), 9.75 (s, 1H, NH), 7.78 (d, *J*: 1.64 Hz, 1H, furan C_5-H), 7.67-7.61 (m, 2H, 4FPh$C_{2,6}$-H), 7.55 (d, *J*: 7.73 Hz, 2H, Ph $C_{2,6}$-H), 7.49 (t, *J*: 8.77 Hz, 2H, 4FPh$C_{3,5}$-H), 7.35 (t, *J*: 7.73 Hz, 2H, Ph $C_{3,5}$-H), 7.18 (t, *J*: 7.73 Hz, 1H, Ph C_4-H), 6.54 (dd, *J*: 3.45; 1.64 Hz, 1H, furan C_4-H), 6.24 (d, *J*: 3.45 Hz, 1H, furan C_3-H), 3.99 (s, 2H, S$\underline{C}H_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.2 (C=S), 167.2 (C=O), 163.2 (d, *J*: 148 Hz; 4FPh C_4), 151.7 (triazole C_2), 147.9 (triazole C_5), 145.4 (furan C_5), 141.2 (furan C_2), 139.4, 130.7 (d, *J*: 9.25 Hz, 4FPh C_2), 130.0 (d, *J*: 2.69 Hz, 4FPh C_1), 128.4, 126.2, 125.6, 117.5 (d, *J*: 23.36 Hz; 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Nitrophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5b)

Light yellow powder, mp 182 °C, yield: 64.93%, Anal. Calcd. for: $C_{21}H_{16}FN_7O_4S_2$ C, 49.12; H, 3.14; N, 19.09%; Found: C, 49.21; H, 3.259; N, 19.04%. FT-IR υmax (cm^{-1}): 3280, 3210 (N-H), 3147, 3101 (ar. C-H), 2928, 2837 (al. C-H), 1721 (C=O), ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.52 (s, 1H, CONH), 10.22 (s, 1H, NH), 10.09 (s, 1H, NH), 8.23 (d, *J*: 8.73 Hz, 2H, 4-NO_2Ph $C_{2,6}$-H), 8.03

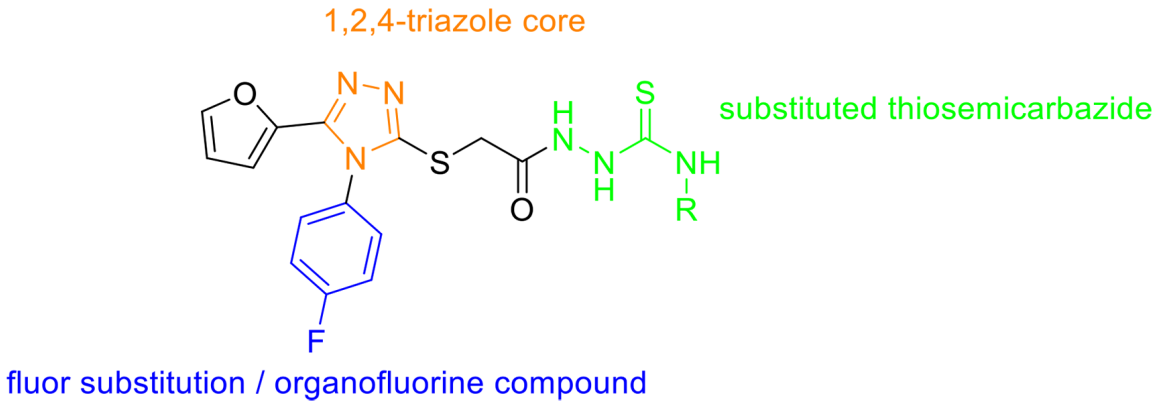


Figure 2. Structure of synthesized compounds

(broad s, 2H, 4-NO_2Ph $C_{3,5}$-H), 7.79 (s, 1H, furan C_5-H), 7.68-7.62 (m, 2H, 4FPh$C_{2,6}$-H), 7.49 (t, *J*: 8.54 Hz, 2H, 4FPh$C_{3,5}$-H), 6.54 (m, 1H, furan C_4-H), 6.26 (d, *J*: 3.56 Hz, 1H, furan C_3-H); 3.98 (s, 2H, S$C\underline{H}_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 180.9 (C=S), 167.5 (C=O), 163.2 (d, *J*: 248.64 Hz, 4FPh C_4), 151.7 (triazole C_2), 147.9 (triazole C_5), 145.8 (4-NO_2Ph C_4), 145.5 (furan C_5), 144.0 (4-NO_2Ph C_1), 141.2 (furan C_2), 130.7 (d, *J*: 9.26 Hz, 4FPh C_2), 130.3 (d, *J*: 2.75 Hz, 4FPh C_1), 125.2 (4-NO_2Ph C_3), 124.1 (4-NO_2Ph C_2), 117.5 (d, *J*: 23.31 Hz, 4FPh C_3), 112.2 (furan C_3), 112.1 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Fluorophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5c)

White powder, mp 178 °C, yield: 77.24%, Anal. Calcd. for: $C_{21}H_{16}F_2N_6O_2S_2$, C, 51.84; H, 3.31; N, 17.27%; Found: C, 51.48; H, 3.444; N, 17.17%. FT-IR υmax (cm^{-1}): 3298 (N-H), 3122, 3095 (ar. C-H), 2929, 2833 (al. C-H), 1715 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.33 (s, 1H, CONH), 9.74 (s, 1H, NH), 9.69 (s, 1H, NH), 7.69 (d, *J*: 1.82 Hz, 1H, furan C_5-H), 7.58-7.52 (m, 2H, 4FPh$C_{2,6}$-H), 7.46-7.37 (m, 4H, 4FPh$C_{2,3,5,6}$-H), 7.09 (t, *J*: 8.75 Hz, 2H, 4FPh$C_{3,5}$-H), 6.45 (dd, *J*: 3.46, 1.82 Hz, 1H, furan C_4-H), 6.15 (d, *J*: 3.46 Hz, 1H, furan C_3-H), 3.89 (s, 2H, S$C\underline{H}_2$).^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.5 (C=S), 167.2 (C=O), 163.2 (d, *J*: 248 Hz, 4FPh C_4), 160.0 (d, *J*: 248 Hz, 4FPh C_4), 151.8 (triazole C_2), 147.9 (triazole C_5), 145.4 (furan C_5), 141.2 (furan C_2), 135.7 (d, *J*: 2.77 Hz, 4FPh C_1), 130.6 (d, *J*: 9.27 Hz, 4FPh C_2), 130.0 (d, *J*: 2.77 Hz, 4FPh C_1), 117.5 (d, *J*: 23.05 Hz, 4FPh C_3), 115.1 (d, *J*: 23.05 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Chlorophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5d)

White powder, mp 214 °C, yield: 82.66%, Anal. Calcd. for: $C_{21}H_{16}ClFN_6O_2S_2$, C, 50.15; H, 3.21; N, 16.71%; Found: C, 50.04; H, 3.268; N, 16.75%. FT-IR υmax (cm^{-1}): 3295 (N-H), 3153, 3097 (ar. C-H), 2957 (al. C-H), 1709 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.42 (s, 1H, CONH), 9.89 (s, 1H, NH), 9.81 (s, 1H, NH), 7.78 (d, *J*: 1.24 Hz, 1H, furan C_5-H), 7.68-7.60 (m, 2H, 4FPh$C_{2,6}$-H), 7.59 (d, *J*: 8.7 Hz, 2H, 4-ClPh $C_{2,6}$-H), 7.49 (t, *J*: 8.60 Hz, 2H, 4FPh$C_{3,5}$-H), 7.40 (d, *J*: 8.7 Hz, 2H, 4-ClPh $C_{3,5}$-H), 6.54 (dd, *J*: 3.56; 1.78 Hz, 1H, furan C_4-H), 6.25 (d, *J*: 3.56 Hz, furan C_3-H), 3.97 (s, 2H, S$C\underline{H}_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.2 (C=S), 167.3 (C=O), 163.2 (d, *J*: 248 Hz, 4FPh C_4), 151.8 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.2 (furan C_2), 138.4 (4-ClPh C_1), 130.7 (d, *J*: 9.27 Hz, 4FPh C_2), 130.0 (d, *J*: 2.77 Hz, 4FPh C_1), 129.6 (4-ClPh C_4), 128.3 (4-ClPh $C_{3,5}$), 127.8 (4-ClPh $C_{3,5}$), 117.5 (d, *J*: 23.05 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(4-Bromophenyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5e)

White powder, mp 210 °C, yield: 61.66%, Anal. Calcd. for: $C_{21}H_{16}BrFN_6O_2S_2.2H_2O$ C, 43.23; H, 3.46; N, 14.40%; Found: C, 43.71; H, 3.017; N, 14.77%. FT-IR υmax (cm^{-1}): 3293 (N-H), 3157, 3098 (ar. C-H), 2943 (al. C-H), 1708 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.34 (s, 1H, CONH), 9.82 (s, 1H, NH), 9.71 (s, 1H, NH), 7.70 (d, *J*: 1.79 Hz, 1H, furan C_5-H), 7.58-7.53 (m, 2H, 4FPh$C_{2,6}$-H), 7.47-7.41 (m, 6H, 4FPh$C_{2,6}$-H and 4BrPh$C_{2,3,5,6}$-H), 6.46 (dd, *J*: 3.5; 1.79 Hz, 1H, furan C_4-H), 6.17 (d, *J*: 3.5 Hz, 1H, furan C_3-H), 3.89 (s, 2H, S$C\underline{H}_2$).^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.2 (C=S), 167.3 (C=O), 163.2 (d, *J*: 248.64 Hz, 4FPh C_4), 151.8 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.2 (furan C_2), 138.8, 131.3, 130.6 (d, *J*: 9.26 Hz, 4FPh C_2), 130.0 (d, *J*: 2.75 Hz, 4FPh C_1), 117.5 (d, *J*: 23.31 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 35.2 (-S$\underline{C}H_2$).

N-(Ethyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5f)

White powder, mp 180 °C, yield: 79.36%, Anal. Calcd. for: $C_{17}H_{17}FN_6O_2S_2$, C, 48.56; H, 4.08; N, 19.99%; Found: C, 48.34; H, 4.218; N, 19.93%. FT-IR υmax (cm^{-1}): 3311 (N-H), 3050 (ar. C-H), 2972, 2934, 2869 (al. C-H), 1709 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.21 (s, 1H, CONH), 9.33 (s, 1H, NH), 8.24 (s, 1H, NH), 7.79 (d, *J*: 1.8 Hz, 1H, furan C_5-H), 7.67-7.57 (m, 2H, 4FPh$C_{2,6}$-H); 7.49 (t, *J*: 8.8 Hz, 2H, 4FPh$C_{3,5}$-H), 6.55 (dd, *J*: 3.5; 1.8 Hz, 1H, furan C_4-H), 6.24 (d, *J*: 3.5 Hz, 1H, furan C_3-H), 3.90 (s, 2H, S$C\underline{H}_2$), 3.56 (p, *J*: 7 Hz, 2H, -$C\underline{H}_2$-CH_3), 1.12 (t, *J*: 7 Hz, 3H, -CH_2-$C\underline{H}_3$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.5 (C=S), 167.0 (C=O), 163.3 (d, *J*: 248.5 Hz, 4FPh C_4), 152.1 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.1 (furan C_2), 130.6 (d, *J*: 9.16 Hz, 4FPh C_2), 129.9 (d, *J*: 2.92 Hz, 4FPh C_1), 117.5 (d, *J*: 23.05 Hz, 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 39.0 (N-$\underline{C}H_2$-CH_3), 34.6 (-S$\underline{C}H_2$), 14.9 (N-CH_2-$\underline{C}H_3$).

N-(Propyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5g)

White powder, mp 168-169 °C, yield: 80.19%, Anal. Calcd. for: $C_{18}H_{19}FN_6O_2S_2$, C, 49.76; H, 4.41; N, 19.34%; Found: C, 49.39; H, 4.540; N, 19.26%. FT-IR υmax (cm^{-1}): 3317 (N-H), 3149, 3086 (ar. C-H), 2962, 2930, 2862 (al. C-H), 1704 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.22 (s, 1H, CONH), 9.32 (s, 1H, NH), 8.21 (s, 1H, NH), 7,80 (d, *J*: 1.7 Hz, 1H, furan C_5-H), 7.65-7.58 (m, 2H, 4FPh$C_{2,6}$-H), 7.50 (t, *J*: 8.69 Hz, 2H, 4FPh$C_{3,5}$-H), 6.55 (dd, *J*: 3.5 ; 1.7 Hz, 1H, furan C_4-H), 6,24 (d, *J*: 3.5 Hz, 1H, furan C_3-H), 3.90 (s, 2H, S$C\underline{H}_2$), 3.46 (m, 2H, N-$C\underline{H}_2$-CH_2-CH_3), 1.56 (h, *J*: 7.4 Hz, 2H, -CH_2-$C\underline{H}_2$-CH_3), 0.83 (t, *J*: 7.4 Hz, 3H, -CH_2-CH_2-$C\underline{H}_3$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.7 (C=S), 167.0 (C=O), 163.3 (d, *J*: 148.95 Hz; 4FPh C_4), 152.1 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.1 (furan C_2), 130.6 (d, *J*: 9.25 Hz, 4FPh C_2), 129,8 (d, *J*: 2.69 Hz, 4FPh C_1), 117.5 (d, *J*: 23.36 Hz; 4FPh C_3), 112.2 (furan C_3), 112.0 (furan C_4), 45.7 (N-$\underline{C}H_2$-CH_2-CH_3), 34.5 (-S$\underline{C}H_2$), 22.4 (N-CH_2-$\underline{C}H_2$-CH_3), 11.5 (N-CH_2-CH_2-$\underline{C}H_3$).

N-(Butyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5h)

White powder, mp 197 °C, yield: 74.46%, Anal. Calcd. for: $C_{19}H_{21}FN_6O_2S_2$, C, 50.88; H, 4.72; N, 18.74%; Found: C, 50.71; H, 4.942; N, 18.70%. FT-IR υmax (cm^{-1}): 3340 (N-H), 3163, 3095 (ar. C-H), 2955, 2933, 2872 (al. C-H), 1712 (C=O). 1H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.22 (s, 1H, NH); 9.33 (s, 1H, NH); 8.18

(s, 1H, NH); 7.79 (d, *J*: 1.15 Hz, 1H, furan C_5-H); 7.66-7.59 (m, 2H, 4FPh$C_{2,6}$-H); 7.49 (t, *J*: 8.7 Hz, 2H, 4FPh$C_{3,5}$-H); 6.54 (dd, *J*: 3.5; 1.8 Hz, 1H, furan C_4-H); 6.25 (d, *J*: 3.5 Hz, 1H, furan C_3-H); 3.91 (s, 2H, SC$\underline{H}_2$); 3.51 (q, *J*: 7.4 Hz, 2H, N-C$\underline{H}_2$-CH_2-CH_2-CH_3); 1.54 (p, *J*: 7.4 Hz, 2H, N-CH_2-C$\underline{H}_2$-CH_2-CH_3); 1.27 (h, *J*: 7.4 Hz, 2H, N-CH_2-CH_2-C$\underline{H}_2$-CH_3); 0.87 (t, *J*: 7.4 Hz, 3H, N-CH_2-CH_2-CH_2-C$\underline{H}_3$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 181.8 (C=S); 167.0 (C=O); 163.3 (d, *J*: 248.62 Hz, 4FPh C_4); 152.1 (triazole C_2); 147.9 (triazole C_5); 145.5 (furan C_5); 141.1 (furan C_2); 130.6 (d, *J*: 9.19 Hz, 4FPh C_2); 129.8 (d, *J*: 2.93 Hz, 4FPh C_1); 117.5 (d, *J*: 23.28 Hz; 4FPh C_3); 112.2 (furan C_3); 112.0 (furan C_4); 43.8 (N-C$\underline{H}_2$-CH_2-CH_2-CH_3), 34.5 (-SC$\underline{H}_2$), 31.3 (N-CH_2-C$\underline{H}_2$-CH_2-CH_3), 19.8 (N-CH_2-CH_2-C$\underline{H}_2$-CH_3), 14.2 (N-CH_2-CH_2-CH_2-C$\underline{H}_3$).

N-(Allyl)-2-{[(4-(4-fluorophenyl)-5-(furan-2-yl)-4H-1,2,4-triazol-3-yl)sulfanyl]acetyl}hydrazine-1-carbothioamide (5i)

White powder, mp 151-152 °C, yield: 69.76%, Anal. Calcd. for: $C_{18}H_{17}FN_6O_2S_2$, C, 49.99; H, 3.96; N, 19.43%; Found: C, 49.50; H, 4.063; N, 19.31%. FT-IR υmax (cm^{-1}): 3316 (N-H), 3156, 3083, 3012 (ar. C-H), 2921, 2855 (al. C-H), 1705 (C=O). ^{1}H-NMR (500 MHz) (DMSO-d_6) δ (ppm): 10.28 (s, 1H, CONH), 9.44 (s, 1H, NH), 8.37 (s, 1H, NH), 7.79 (d, *J*: 1.80 Hz, 1H, furan C_5-H), 7.65-7.58 (m, 2H, 4FPh$C_{2,6}$-H), 7.48 (t, *J*: 8.7 Hz, 2H, 4FPh$C_{3,5}$-H), 6.54 (dd, *J*: 3.53; 1.80 Hz, 1H, furan C_4-H), 6.24 (d, *J*: 3.53 Hz, 1H, furan C_3-H), 5.85 (ddt, *J*: 17.20; 10.30; 5.3 Hz, 1H, -NCH_2C$\underline{H}$=CH_2), 5.13 (dd, *J*: 17.20; 1.70 Hz, 1H, -NCH_2CH=C$\underline{H}_2$), 5.02 (dd, J: 10.30; 1.7 Hz, 1H, -NCH_2CH=C$\underline{H}_2$), 4.17 (s, 2H,-NC$\underline{H}_2$CH=CH_2), 3.95 (s, 2H, SC$\underline{H}_2$). ^{13}C-NMR (APT) (125 MHz) (DMSO-d_6) δ (ppm): 182.1 (C=S), 167.1 (C=O), 163.3 (d, *J*: 148 Hz; 4FPh C_4), 152.1 (triazole C_2), 147.9 (triazole C_5), 145.5 (furan C_5), 141.1 (furan C_2), 135.2 (-NCH_2CH=CH_2), 130.6 (d, *J*: 9.25 Hz, 4FPh C_2), 129,8 (d, *J*: 2.69 Hz, 4FPh C_1), 117.5 (d, *J*: 23.36 Hz; 4FPh C_3), 115.7 (-NCH_2CH=CH_2), 112.2 (furan C_3), 112.0 (furan C_4), 46.3 (-NCH_2CH=CH_2), 34.6 (-SC$\underline{H}_2$).

Antibacterial activity studies

The antimicrobial activity of a series of thiosemicarbazides bearing 1,2,4-triazole **(5a-i)** was investigated against *K. pneumoniae* (BAA-2146), *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *E. faecalis* (ATCC 29212), and *P. aeruginosa* (ATCC 27853). Both disk diffusion and broth microdilution methods were employed to assess activity, ensuring consistency across experiments.[26,27]

For the broth microdilution method, a stock solution of each compound was prepared at a concentration of 100 micromolar (μM), followed by serial dilutions in sterile 96-well microplates. Muller, Muller-Hinton broth was added to each well, and bacterial suspensions were adjusted to a 0.5 McFarland standard. The plates were incubated at 35-37 °C for 18-24 hours under appropriate conditions, and the minimum inhibitory concentrations (MICs) were determined manually.

In the disk diffusion assay, blank antibiotic disks were impregnated with the compounds, and the zone diameters of inhibition were measured after incubation. The tests were standardized with DMSO as a negative control and specific antibiotics as positive controls: Vancomycin (20 μM) for *S. aureus* and *E. faecalis*, and Meropenem (78 μM) for *P. aeruginosa*. All experiments were conducted following European Committee on Antimicrobial Susceptibility Testing guidelines, and bacterial strains were maintained at -80 °C until use.

Statistical analysis

The antimicrobial assay results were expressed in μM units to enable reliable comparison among the synthesized derivatives and reference compounds. The obtained data were descriptively evaluated, with MIC values serving as the primary measure of antimicrobial potency. This ensured a consistent and reproducible framework for interpreting the biological activity across all tested compounds. No further statistical testing was applied.

RESULTS

Chemistry

All designed novel thiosemicarbazide compounds (**5a-i**) were obtained according to the methods depicted in the experimental section. The chemical pathway shown in Scheme 1 outlines a synthesis process that involves five steps. Furan-2-carbohydrazide and 4-fluorophenylisothiocyanate were boiled under reflux in ethanol to yield N-(4-Fluorophenyl)-2-(furan-2-carbonyl)hydrazine-1-carbothioamide (**1**). Solution of (**1**) in NaOH (2N) was heated under reflux for 4 h, then it was neutralized with 12.5% HCl to yield 4-(4-Fluorophenyl)-5-(furan-2-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (**2**). A mixture of (**2**), K_2CO_3 and $BrCH_2COOEt$ was refluxed in acetone to obtain Ethyl ((4-Fluorophenyl)-5-(furan-2-yl)-*4H*-1,2,4-triazole-3-yl) sulfanyl)acetate (**3**). Compound 3 and $NH_2NH_2.H_2O$ were heated under reflux in ethanol to yield 2-[[4-(4-Fluorophenyl)-5-(furan-2-yl)-*4H*-1,2,4-triazol-3-yl]sulfanyl]acetohydrazide (**4**). A solution of 4 and the appropriate isothiocyanate in absolute ethanol was heated under reflux to yield **5a-i**. The designed compounds (**5a-i**) were synthesized with good yields (61.10-82.66%).

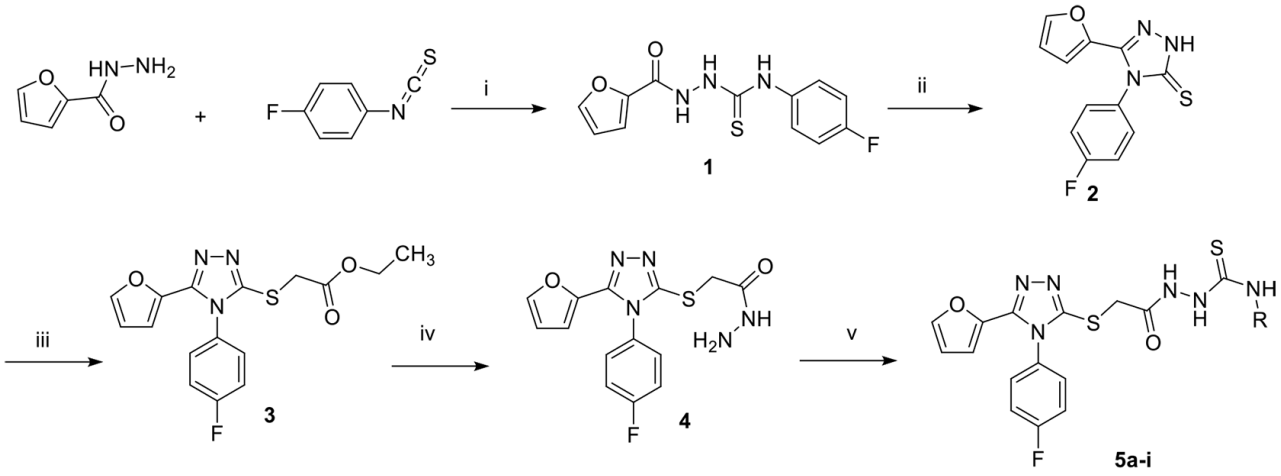
Antimicrobial activity

According to the *in vitro* assay results, against *P. aeruginosa* compound **5g** showed the lowest MIC (<0.78 μM), at least 100-fold more potent than Meropenem (MIC = 78 μM), while **5i** (MIC = 1.56 μM) was ~50-fold more potent. For *S. aureus*, **5e** (MIC = 12.5 μM) outperformed Vancomycin (MIC = 20 μM), and **5b** and **5h** (MIC = 25 μM) were comparable to Vancomycin. No compound was active (MIC >100 μM) against *E. coli*, *K. pneumoniae*, or *E. faecalis*, as can be seen in Table 1.

DISCUSSION

IR spectra provide a straightforward diagnostic of the hydrazide-to-thiosemicarbazide transformation: in compound **4**, the terminal -NH_2 group exhibits the characteristic pair of N-H stretching bands, whereas in **5a-i** these bands disappear and are replaced by a single N-H stretch consistent with conversion of -NH_2 to a secondary -NH- within the thiosemicarbazide framework.[28]

Scheme 1. Synthesis pathway of the titled compounds (5a-i) Reagent and conditions: *i:* EtOH, *ii:* 2N NaOH, 12.5% HCl, *iii:* α-BrCH2COOC2H5/K2CO3/acetone, *iv:* NH2NH2.H2O/EtOH, *v:* RNCS/EtOH



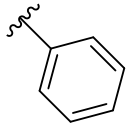
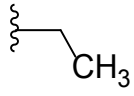
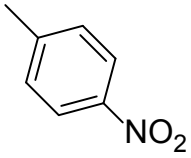
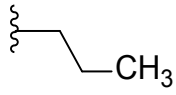
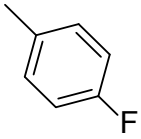
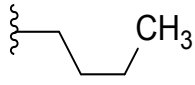
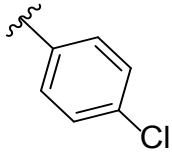
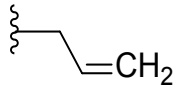
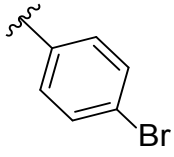
Compound	R	Compound	R
5a		5f	
5b		5g	
5c		5h	
5d		5i	
5e		-	-

Table 1. MICs of thiosemicarbazide derivatives (5a-i) against various microorganisms

Compound	Microorganisms				
	Escherichia coli	Pseudomonas aeruginosa	Klebsiella pneumoniae	Staphylococcus aureus	Enterococcus faecalis
Dimethyl sulfoxide	>100	>100	>100	>100	>100
5a	>100	>100	>100	>100	>100
5b	>100	>100	>100	**25**	>100
5c	>100	>100	>100	>100	>100
5d	>100	>100	>100	>100	>100
5e	>100	>100	>100	**12.5**	>100
5f	>100	>100	>100	>100	>100
5g	>100	**<0.78**	>100	>100	>100
5h	>100	>100	>100	**25**	>100
5i	>100	**1.56**	>100	>100	>100
Vancomycin	-	-	-	20	20
Meropenem	-	**78**	-	-	-

MICs: Minimum inhibitory concentrations

In the ^{1}H-NMR spectra, the terminal NH_2 protons of compound 4, initially observed at 4.32 ppm, disappeared in the thiosemicarbazide derivatives (**5a-i**). However, the NH_2 protons of the newly formed thiosemicarbazide group in **5a-i** appeared in the range of 10.22-8.18 ppm, confirming their formation.[29] Additionally, the aryl protons of the isothiocyanate groups in **5a-i** further supported the confirmation of the structure.

In the ^{13}C (APT) NMR spectra, the carbonyl carbons (-C=O) of compounds **4** and **5a-i** were detected between 166.4 and 167.0 ppm. Carbons associated with the -C=S group in **5a-i**, indicative of the thiosemicarbazide functionality, appeared at 182.1-180.9 ppm.[30] The aryl carbons of the isothiocyanate groups also reinforced the structural integrity of the compounds.

These spectroscopic results (^{1}H NMR, ^{13}C NMR, and FT-IR), along with elemental analysis, unequivocally confirmed the successful transformation of compound four into the thiosemicarbazide derivatives **5a-i**. No significant side reactions were observed, and the target compounds were obtained in moderate to good yields, with all data aligning with the assigned structures and literature.[23,24]

Based on our MIC data, we can describe the structure-activity relationship (SAR) in more detail - see Table 1 and Figure 3. Against *P. aeruginosa*, the three-carbon **n-propyl** group on the thiosemicarbazide nitrogen gave the best result (compound **5g**, MIC <0.78 µM), which is more than 100-fold better than Meropenem (MIC 78 µM). When **n-propyl** was changed to **allyl**, the activity dropped about two-fold (compound **5i**, 1.56 µM). Shorter (**ethyl**) or longer (**n-butyl**) chains were inactive (MIC >100 µM), and replacing the aliphatic chain with an **aryl** (phenyl) group also removed the activity. These trends suggest that *P. aeruginosa* needs a simple, three-carbon saturated chain at this position, probably because it gives the right size and shape for the target and also helps the compound pass the outer membrane; adding an aromatic ring may be too bulky or too rigid, and changing to an allyl group may change the geometry or polarity in a way that weakens binding.

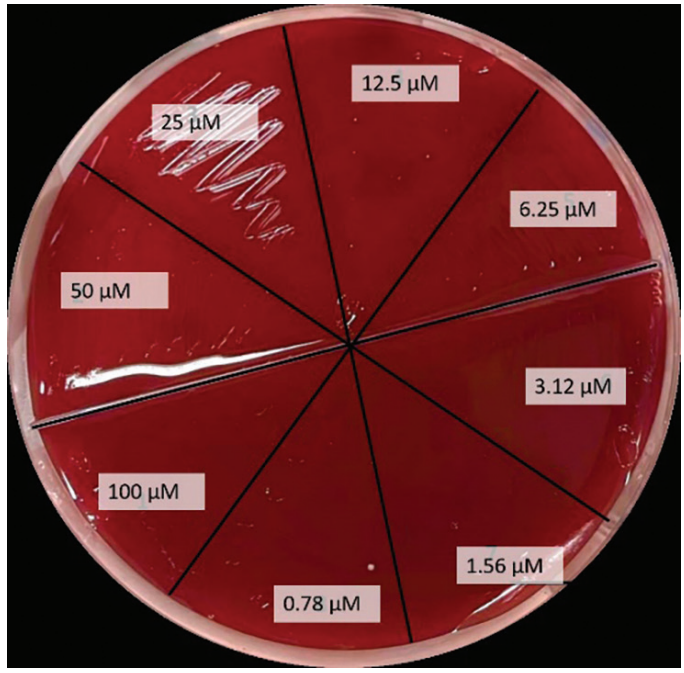


Figure 3. No inhibition toward the growth of *Pseudomonas aeruginosa* (ATCC 27853) at different concentrations of the compound 5 g

Against *S. aureus*, activity increased when the para substituent on the phenyl ring was **less electronegative**: ***p*-Br** (5e, 12.5 µM) was stronger than ***p*-NO_2** (5b, 25 µM), while ***p*-F** and ***p*-Cl** were inactive (MIC >100 µM). One simple explanation is that

bromine is less electronegative and more polarizable, so it may improve hydrophobic contacts in the binding site, while very electron-withdrawing or very small substituents reduce those contacts. In the alkyl series for *S. aureus*, only **n-butyl** (5h, 25 μM) was active; **ethyl**, **n-propyl**, and **allyl** analogs were inactive, which may indicate that a slightly longer hydrophobic tail helps interaction with the Gram-positive cell envelope or with the target pocket.

None of the compounds were active against *E. coli, K. pneumoniae*, or *E. faecalis* (MIC >100 μM). The reason is not clear at this stage; common possibilities include poor permeability, strong efflux, or a different/absent target in these organisms. In future work we plan to check simple permeability and efflux effects and do basic target-engagement tests to understand these findings and to guide the design of more active analogs (for example, small changes around **n-propyl** for *P. aeruginosa* and around **p-Br** for *S. aureus*).

Upon evaluating all data thoroughly, to clarify the SAR key observations and insights are given as follows, see in Table 1 and Figure 3.

1. The three-carbon alkyl chain (n-propyl, compound **3g**) is optimal for activity against *Pseudomonas aeruginosa*. Shorter (ethyl, compound **3f**) or longer (n-butyl, compound **3h**) chains are inactive
2. Unsaturation and aryl substitution reduce activity against *P. aeruginosa*.
   - n-propyl > allyl (≈2× loss in potency when changing to allyl)
   - n-propyl > allyl > phenyl (aryl) (phenyl = inactive)
3. Less-electronegative para substituents improve activity against *Staphylococcus aureus*.
   - *p*-Br > *p*-NO_2, while *p*-F and *p*-Cl are inactive
   - Among alkyls, only n-butyl shows activity

CONCLUSION

There is still a significant danger arising from various bacteria today. Because many bacterial types, such as *S. aureus* and *P. aeruginosa*, are quickly resistant to the current antibiotics, the design and evaluation of novel potent antimicrobial molecules are vital. In this study, nine novel thiosemicarbazide-bearing 1,2,4-triazole compounds were synthesized using simple and practical methods, and their structure was characterized using methods such as FT-IR, ^{1}H-NMR, ^{13}C-NMR, elemental analysis, and mass spectroscopy. All synthesized compounds were investigated for their antibacterial activities against diverse bacteria including *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*. Especially the compounds carrying 4-bromophenyl substitution at the N position of the thiosemicarbazide nucleus ring showed high antibacterial activities against *S. aureus* with 12.5 μM, whereas the n-propyl substituted derivative displayed the most activity against *P. aeruginosa* with 0.78 μM. Consequently, we believe that the biological assay data and SAR evaluation obtained from this study may assist in the future discovery of new and more potent antimicrobial compounds.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Design: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Data Collection or Processing: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Analysis or Interpretation: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Literature Search: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G., Writing: E.D.D., E.D.K., H.Ö.D., F.B., E.G., N.U.G.

Conflict of Interest: The authors declare no conflicts of interest.

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REFERENCES

1. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat MKF, Baloch Z. Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist. 2018;11:1645-1658.
2. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MAA. Antimicrobial resistance: a growing serious threat for global public health. Healthcare (Basel). 2023;11:1946.
3. Corrêa RCG, Heleno SA, Alves MJ, Ferreira ICFR. Bacterial resistance: antibiotics of last generation used in clinical practice and the arise of natural products as new therapeutic alternatives. Curr Pharm Des. 2020;26:815-837.
4. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MTS, Chinedu Ikem J, Victor Chigozie U, Saki M. Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal. 2022;36:e24655.
5. Chandra P, Mk U, Ke V, Mukhopadhyay C, U DA, M SR, V R. Antimicrobial resistance and the post antibiotic era: better late than never effort. Expert Opin Drug Saf. 2021;20:1375-1390.
6. Mishra VR, Ghanavatkar CW, Mali SN, Qureshi SI, Chaudhari HK, Sekar N. Design, synthesis, antimicrobial activity and computational studies of novel azo linked substituted benzimidazole, benzoxazole and benzothiazole derivatives. Comput Biol Chem. 2019;78:330-337.
7. Jorge SD, Masunari A, Rangel-Yagui CO, Pasqualoto KF, Tavares LC. Design, synthesis, antimicrobial activity and molecular modeling studies of novel benzofuroxan derivatives against *Staphylococcus aureus*. Bioorg Med Chem. 2009;17:3028-3036.
8. Ammar YA, Farag AA, Ali AM, Ragab A, Askar AA, Elsisi DM, Belal A. Design, synthesis, antimicrobial activity and molecular docking studies of some novel di-substituted sulfonylquinoxaline derivatives. Bioorg Chem. 2020;104:104164.
9. Dincel ED, Hasbal-Celikok G, Yilmaz-Ozden T, Ulusoy-Güzeldemirci N. Design, biological evaluation, molecular docking study and in silico ADME prediction of novel imidazo[2,1-*b*]thiazole derivatives as a novel class of α-glucosidase inhibitors. J Mol Struct. 2021;1245:131260.
10. Cihan-Üstündağ G, Gürsoy E, Naesens L, Ulusoy-Güzeldemirci N, Çapan G. Synthesis and antiviral properties of novel indole-based

thiosemicarbazides and 4-thiazolidinones. Bioorg Med Chem. 2016;24:240-246.

11. Güzeldemirci NU, Küçükbasmaci O. Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazo[2,1-b]thiazole moiety. Eur J Med Chem. 2010;45:63-68.
12. Lin J, Zhou S, Xu JX, Yao WQ, Hao GF, Li YT. Design, synthesis, and structure-activity relationship of economical triazole sulfonamide aryl derivatives with high fungicidal activity. J Agric Food Chem. 2020;68:6792-6801.
13. Ammazzalorso A, Gallorini M, Fantacuzzi M, Gambacorta N, De Filippis B, Giampietro L, Maccallini C, Nicolotti O, Cataldi A, Amoroso R. Design, synthesis and biological evaluation of imidazole and triazole-based carbamates as novel aromatase inhibitors. Eur J Med Chem. 2021;211:113115.
14. Anil DA, Aydin BO, Demir Y, Turkmenoglu B. Design, synthesis, biological evaluation and molecular docking studies of novel 1H-1,2,3-Triazole derivatives as potent inhibitors of carbonic anhydrase, acetylcholinesterase and aldose reductase. J Mol Struct. 2022;1257:132613.
15. Ali AAS, Khan D, Naqvi A, Al-Blewi FF, Rezki N, Aouad MR, Hagar M. Design, synthesis, molecular modeling, anticancer studies, and density functional theory calculations of 4-(1,2,4-Triazol-3-ylsulfanylmethyl)-1,2,3-triazole derivatives. ACS Omega. 2021;6:301-316.
16. Amin NH, El-Saadi MT, Ibrahim AA, Abdel-Rahman HM. Design, synthesis and mechanistic study of new 1,2,4-triazole derivatives as antimicrobial agents. Bioorg Chem. 2021;111:104841.
17. Abula A, Xu Z, Zhu Z, Peng C, Chen Z, Zhu W, Aisa HA. Substitution effect of the trifluoromethyl group on the bioactivity in medicinal chemistry: statistical analysis and energy calculations. J Chem Inf Model. 2020;60:6242-6250.
18. He J, Li Z, Dhawan G, Zhang W, Sorochinsky AE, Butler G, Soloshonok VA, Han J. Fluorine-containing drugs approved by the FDA in 2021. Chinese Chemical Letters. 2023;34:107578.
19. Dehnen S, Schafer LL, Lectka T, Togni A. Fluorine: a very special element and its very special impacts on chemistry. Inorg Chem. 2021;60:17419-17425.
20. Yu Y, Liu A, Dhawan G, Mei H, Zhang W, Izawa K, Soloshonok VA, Han J. Fluorine-containing pharmaceuticals approved by the FDA in 2020: synthesis and biological activity. Chinese Chemical Letters. 2021;32:3342-3354.
21. Henary E, Casa S, Dost TL, Sloop JC, Henary M. The role of small molecules containing fluorine atoms in medicine and imaging applications. Pharmaceuticals (Basel). 2024;17:281.
22. Richardson P. Applications of fluorine to the construction of bioisosteric elements for the purposes of novel drug discovery. Expert Opin Drug Discov. 2021;16:1261-1286.
23. Çapan G, Ergenç N, Ötük G. Synthesis and biological evaluation of 4-alkyl/aryl-1-(2-furoyl)-3-thiosemicarbazides and 4-alkyl/aryl-2,4-dihydro-5-(2-furyl)-3H-1,2,4-triazole-3-thiones. J. Fac. Pharm. Istanbul. 1992;26-28.
24. Dincel ED, Ulusoy-Güzeldemirci N, Şatana D, Küçükbasmacı Ö. Design, synthesis, characterization and antimicrobial evaluation of some novel hydrazinecarbothioamide, 4-thiazolidinone and 1,2,4-triazole-3-thione derivatives. J Heterocycl Chem. 2021;58:195-205.
25. Kuran ED, Uner B, Cam ME, Ulusoy-Guzeldemirci N. Novel hydrazide-hydrazone containing 1,2,4-triazole as potent inhibitors of antiapoptotic protein Bcl-xL: synthesis, biological evaluation, and docking studies. Arch Pharm (Weinheim). 2024;357:e2400562.
26. Sumengen Ozdenefe M, Tanoglu F, Suer K, Guler E, Mercımek Takcı HA. Antioxidant and antimicrobial activity of Ficus sycomorus fruit and leaf extracts. Marmara Med J. 2020;33:133-140.
27. Şenol H, Şahin RB, Mercümek B, Kapucu HB, Hacıosmanoğlu E, Dinç HÖ, Yüksel Mayda P. Synthesis of ursolic acid arylidene-hydrazide hybrid compounds and investigation of their cytotoxic and antimicrobial effects. Nat Prod Res. 2023;37:2500-2507.
28. Stuart BH. Infrared spectroscopy: fundamentals and applications. Chichester: John Wiley & Sons. 2004.
29. Erdik E. Organik kimyada spektroskopik yöntemler. 7th ed. Ankara: Gazi Kitabevi; 2020.
30. Balcı M. Nükleer manyetik rezonans spektroskopisi [Nuclear magnetic resonance (NMR) spectroscopy]. Ankara: Türkiye Bilimler Akademisi (TÜBA); 2025.