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Synthesis, and docking studies of novel tetrazole-S-alkyl derivatives as antimicrobial agents

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ABSTRACT

A series of novel tetrazole-S-alkyl-piperazine derivatives were synthesized and evaluated for their antifungal activity against *C. albicans* (ATCC 24433), *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) and antibacterial activity against *E. coli* (ATCC 25922), *S. marcescens* (ATCC 8100), *K. pneumoniae* (ATCC 13883), *P. aeruginosa* (ATCC 27853), *E. faecalis* (ATCC 2942), *B. subtilis* (ATCC), *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228). Among the synthesized compounds, 1-(4-cyclohexylpiperazin-1-yl)-2-((1-methyl-1*H*-tetrazol-5-yl)thio)ethan-1-one (**2b**) (MIC = 7.81 μg/mL) and 1-(4-(4-chlorobenzyl)piperazin-1-yl)-2-((1-methyl-1*H*-tetrazol-5-yl)thio)ethan-1-one (**2f**) (MIC = 3.90 μg/mL) displayed significant antifungal activity and compared to reference drugs voriconazole and fluconazole. Besides, compound **2b** has showed also higher antibacterial activity against *E. faecalis* (ATCC 2942) as a reference drug azithromycin, with a MIC value of 3.90 μg/mL, and compound **2d** was found to be effective against *S. epidermidis* (ATCC 12228) as the same reference drug, with a MIC value of 7.81 μg/mL. All the derivatives were efficiently synthesized by a two-step process. The structure of the newly synthesized compounds was elucidated by their ^{1}H NMR, ^{13}C NMR, LC-MS/MS, and elemental analysis. In this study, the detailed synthesis, spectroscopic and biological evaluation data are reported. Molecular docking studies of all compounds were performed with the sterol 14-alpha demethylase enzyme of *C. albicans*, the target enzyme of azole antifungal drugs.

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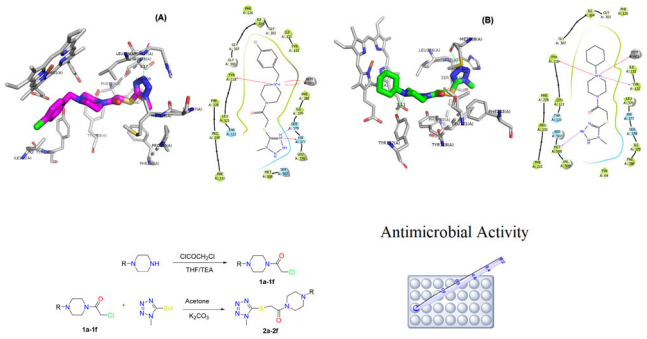
Tetrazole; antimicrobial; candida; molecular docking; 14α-demethylase

GRAPHICAL ABSTRACT



Introduction

Microorganisms can be beneficial or harmful to humans, and antibiotic resistance has emerged through a variety of mechanisms, including intrinsic resistance caused by inherent alterations, inactivation of enzyme synthesis, resistance based on genetic material acquisition, and chromosomal mutations.[1] As a result, there is still an urgent need for medicinal chemists to design and manufacture innovative antimicrobial drugs with lower toxicity and greater potency. Many anti-infectious medications have been synthesized using natural product scaffolds having heterocyclic moieties in the molecular structure.[2] In this context, N-heterocycles are extremely important and have a wide variety of physical and chemical properties that make them particularly useful

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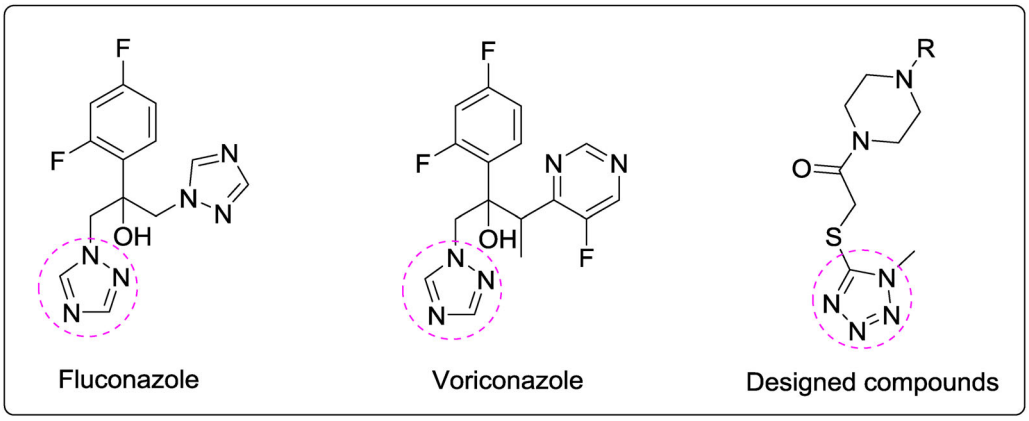


Figure 1. Antifungal drugs containing the triazole structure fluconazole, voriconazole, and the designed tetrazole derivative scaffold as an antifungal.

in medicine. Tetrazole and piperazine form two important members of the N-heterocyclic structure due to their broad-spectrum biological effects.

Tetrazole and its derivatives have generated attention due to their unusual structure and medicinal chemistry applications.[3–7] Tetrazoles exhibit biological activity because of the unique metabolism of the disubstituted tetrazole system and the fact that the tetrazole ring is isosteric with a carboxylic acid group and has a comparable acidity in 5 substituted tetrazole compounds. Tetrazole derivatives outperform carboxylic acids in that they are resistant to a range of biological degradation processes, resulting in enhanced medication bioavailability.[8] These properties are responsible for the bioactive compounds' antibacterial,[9,10] antifungal,[11] anticancer,[12] antitubercular[13] and antioxidant[14] properties, all of which are essential in tetrazole derivatives' potential applications.

Small molecule drug design and discovery continue to be drawn to nitrogen heterocycles. After piperidine and pyridine, piperazines are the third most often utilized N-heterocycle in pharmaceutical small molecule medications.[15,16] It possesses a high polar surface area, structural stiffness, and hydrogen-bond acceptors and donors, all of which help to increase target affinity, specificity, water-solubility, oral bioavailability, and ADME characteristics (absorption, distribution, metabolism, and excretion).[17–20] Piperazine and substituted piperazine compounds were key pharmacophores identified in a variety of marketed medications, including anticancer, antibacterial, antipsychotics, antidepressants, and antibiotics including norfloxacin, ciprofloxacin, and levofloxacin.[21–23] Combining the piperazine moiety with other heterocyclic ring systems, such as tetrazole, has resulted in novel antifungal medicines that frequently exhibit improved antibacterial activity, most likely due to the molecule's increased lipophilicity.[24,25]

Due to its powerful antibacterial qualities, sulfur has been utilized to treat skin conditions since ancient times.[26] The biological activities of organic and inorganic compounds containing sulfur, including their antibacterial, antioxidant, and anticancer characteristics, are well documented.[27,28] The thioether group has a wide range of biological activity in antibacterial aspects and is a very powerful pharmacophore.[29] Thioethers' fungicidal, anti-inflammatory, anticholestemic, hypolipidemic, and neurotropic properties have also been documented.[30,31] In addition, a thioether linker is present in many bioactive compounds to enhance various drug-like properties, particularly by reducing lipophilicity and serving as a hydrogen-bonding acceptor.[32] Herbicide Pyrithione and the ulcer-prevention medication Ufiprazole are examples of commercial agents that contain a thioether linker.

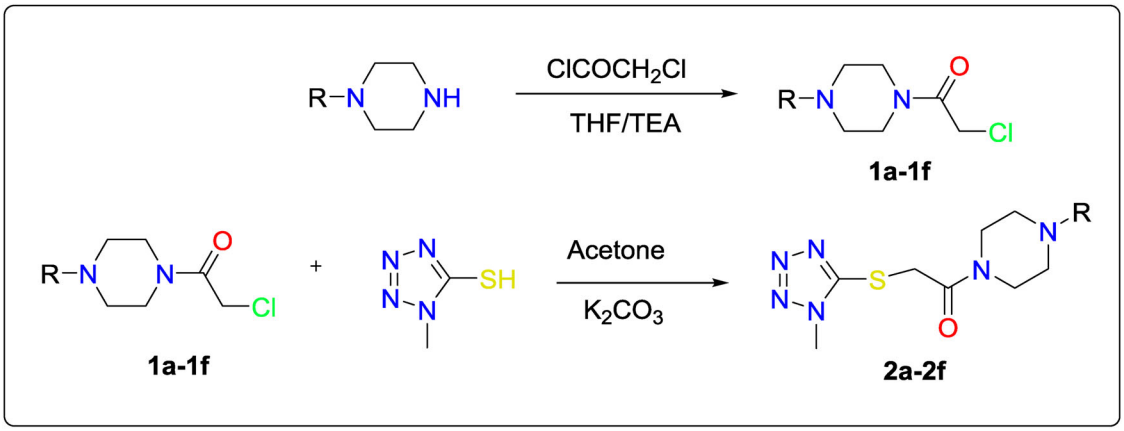
Accordingly, as shown in Figure 1, we envisioned the combination of these attractive functional groups by synthesis of various 1-(4-substituted-piperazin-1-yl)-2-((1-methyl-1*H*-tetrazol-5-yl)thio)ethan-1-one and screened for their antimicrobial activity, inspired by the intense recent research activity in the tetrazole field and pursuit of our continuing interest in pyrazole and tetrazole chemistry. Molecular docking analyzes of compounds **2a**–**2f**, synthesized and evaluated for their antimicrobial effect, were performed against the sterol 14-alpha demethylase enzyme.

Results and discussion

Chemistry

In this study, we synthesized tetrazole piperazine derivatives (**2a**–**2f**) in two steps. The synthetic path of the target compounds was illustrated in Scheme 1. Initially, 4-substituted piperazine derivatives were reacted with chloroacetyl chloride in the presence of THF and TEA (Triethylamine) to obtain 2-chloro-1-(4-substituted piperazin-1-yl)ethan-1-one derivatives (**1a**–**1f**). In the second reaction step, 1-(4-substitued)piperazin-1-yl)-2-((1-methyl-1*H*-tetrazol-5-yl)thio)ethan-1-one (**2a**–**2f**) derivatives were treated with 5-mercapto-1-methyl-1*H*-tetrazole and 2-chloro-1-(4-substituted)piperazin-1-yl)-ethan-1-one (**1a**–**1f**) in the presence of potassium carbonate in acetone by heating for 6 h. Then recrystallized from ethanol to afford final compounds (**2a**–**2f**).

The structures of the synthesized compounds **2a**–**2f** were determined by interpretation of ^{1}H NMR, ^{13}C NMR, and MS spectral methods. ^{1}H NMR spectral analysis of the compounds **2a**–**2f** demonstrated that the CH_3 (tetrazole-methylene) protons were observed as singlet between 3.97 and 3.99 ppm. The protons of piperazine moiety are seen as multiplet at 2.24–3.99 ppm. In the ^{1}H NMR spectrum of the compounds **2d** and **2f** carrying the 4-substituted group in the fourth position of the piperazine ring, the signals



Comp.	R
2a	Ethyl
2b	Cyclohexyl
2c	Pyridine
2d	4-Fluorophenyl
2e	Pyrimidine
2f	4-Chlorobenzyl

Scheme 1. General synthesis pathways of target tetrazole derivative compounds **2a**–**2f**

belonging to aromatic protons were found at 6.96–7.38 ppm. In the $-OC_2H_5$ group of compounds **2a** on the phenyl ring, $-OCH_2$ protons were observed at 4.47 ppm and CH_3 protons were observed at 1.07 ppm. The –CH group of compounds **2b** on the phenyl ring was observed at 1.18–1.79 ppm. The aromatic protons of the compounds **2c** and **2e** were found at around 6.67–8.38 ppm. When the ^{13}C NMR spectra of the compounds were examined, the carbon belonging to the alkyl group resonated at 25.15–37.89 ppm. The peak in the range of 41.22–63.42 ppm was assigned to the piperazine ring and the peaks of many aromatic carbon atoms were observed at the range of 116.35–131.14 ppm. In the spectra of 1,4-disubstituted phenyl-containing derivatives (**2d** and **2f**), signals originating from equivalent carbons in the aromatic region were determined. Carbonyl carbon gave a peak of 165.17–169.63 ppm. All masses of the compounds were in accordance with the estimated M + H values.

Antimicrobial activity

The antimicrobial activity of the new compounds was tested *in vitro* and the results were presented in **Tables S1** and **S2** (Supplemental Materials). *In vitro* antifungal screening of all synthesized compounds was evaluated against *C. albicans*, *C. krusei* and *C. parapsilosis*, using the twofold serial dilution technique recommended by CLSI. Voriconazole and fluconazole were used as reference drugs. The minimum inhibitory concentration (MIC) of the compounds and the control drug are summarized in **Table S 1**. According to the antifungal screening, it was concluded that most of the newly synthesized compounds effectively inhibited the growth of *C. parapsilosis*-with MIC values ranging from 31.25 to 15.625 μg/mL. All of the final compounds exhibited moderate antifungal activities against *C. krusei*, with MIC values ranging from 62.5 to 3.90 μg/mL. Especially, compounds **2b** and **2f** were found effective against *C. krusei* as the reference drug, with a MIC value of 7.81 and 3.90 μg/mL.All compounds were found to have less active against *C. albicans* strains.

Some of these compounds **2a**–**2f** exhibited moderate antimicrobial activity against *E. coli*, *S. marcescens*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, *B. subtilis*, *S. aureus*, and *S. epidermidis*. Compounds **2b** revealed also higher antibacterial activity against *E. faecalis* as the reference drug azithromycin, with a MICvalue of 3.90 μg/mL. Besides, compound **2d** was found to be twice as effective against *S. epidermidis* as the reference drug, with a MIC value of 7.81 μg/mL.

When the chemical structure of the compounds is examined, it is seen that the cyclohexyl structure attached to the piperazine ring increases the antifungal activity. When the relationship between the structure and activity of compounds **2d** (4-fluorophenyl) and **2f** (4-chlorobenzyl) is examined, it is seen that the presence of the phenyl ring attached to the piperazine ring reduces the antifungal activity. However, the presence of the methyl group between the piperazine ring and the phenyl ring seems to increase the activity significantly.

Cytotoxicity assay

The cytotoxicity effect of compounds **2a**–**2f** was evaluated against the L929 cell line. For preliminary screening, the

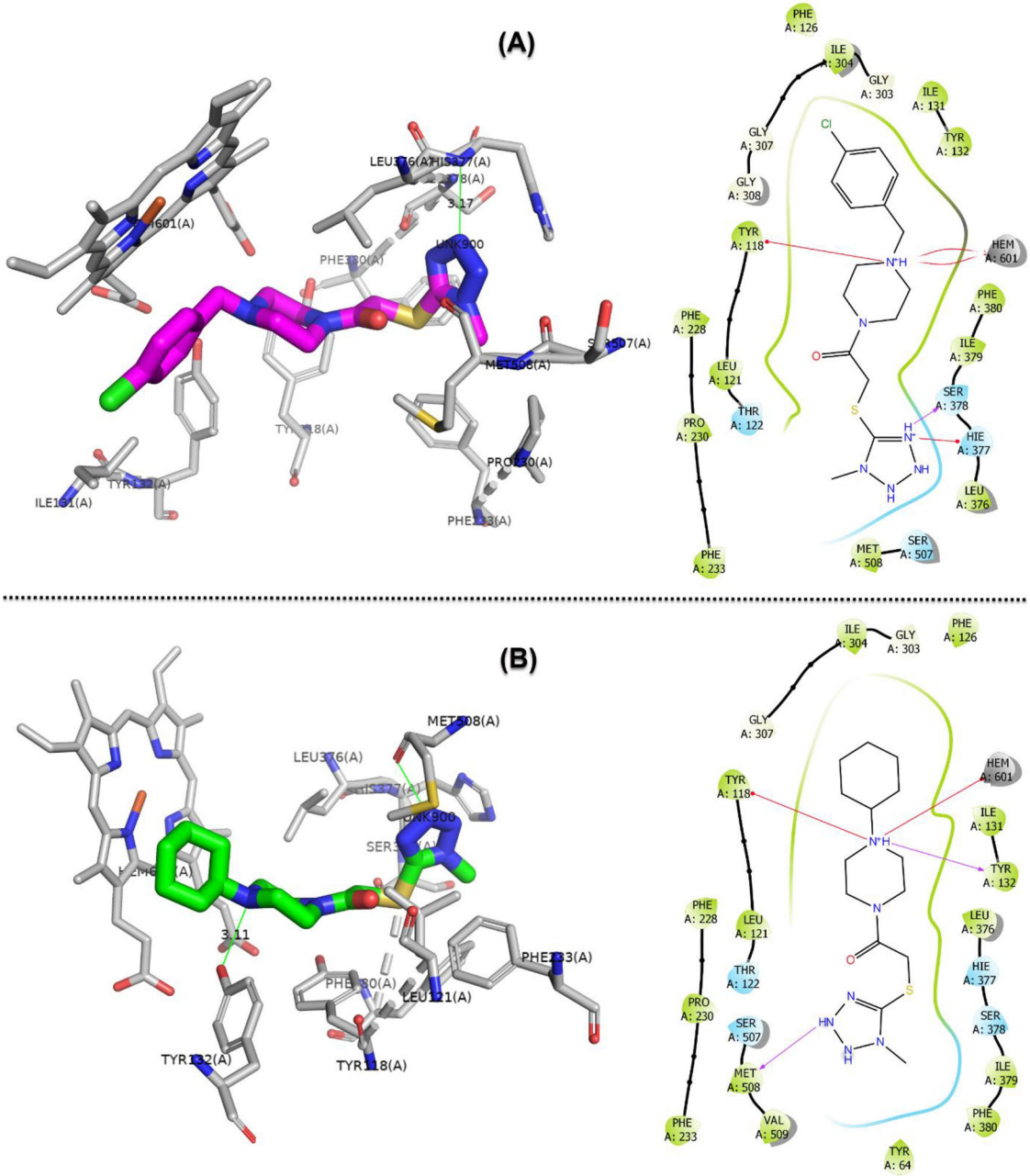


Figure 2. Binding poses and schematic protein-ligand interaction diagrams of most anticandidal two compound (**A**) **2f** and (**B**) compound **2b** in the active site of *C. albicans'* sterol 14-alpha demethylase enzyme (PDB ID:5TZ1).

cytotoxic bioactivity of synthesized compounds was evaluated *in vitro* against the L929 cell line with the MTT assay. To evaluate the cytotoxic potency of target compounds, the fibroblast cells were treated with the compounds at 100 μM constant concentration. Cell viability percentages were calculated after the treatment of cells for 48 h. Preliminary cytotoxic effect results of compounds **2a–2f** against L929 fibroblast are presented in **Table S3** (Supplemental Materials). As a result of the maximum dose applied, all compounds except compounds **2d** and **2e** showed 80% and more viability.

Molecular docking

Since the synthesized compounds are azole derivatives, molecular docking was performed with the sterol 14-alpha demethylase enzyme of *C. albicans*, the target enzyme of azole-derived antifungal drugs.[33] For the validation of molecular docking, cocrystal VT1 redocking in the PDB ID: 5TZ1 structure selected was performed and the root mean square deviation (RMSD) value of 0.4278 Å was measured between the docking pose and the natural pose. RMSD value of less than 3 Å may indicate that the molecular docking study is estimating the correct pose.[34] After the molecular docking validation process, azole synthesized compounds **2a**–**2f**, reference drugs fluconazole, and voriconazole was docked with Glide SP. The glide gscore and emodel interaction energies obtained from the molecular docking study were between −5.743 and −8.390, and −61.919 kcal/mol and −80.492 kcal/mol, as given in **Table S4** (Supplemental Materials). The most active compound, **2f**, was the

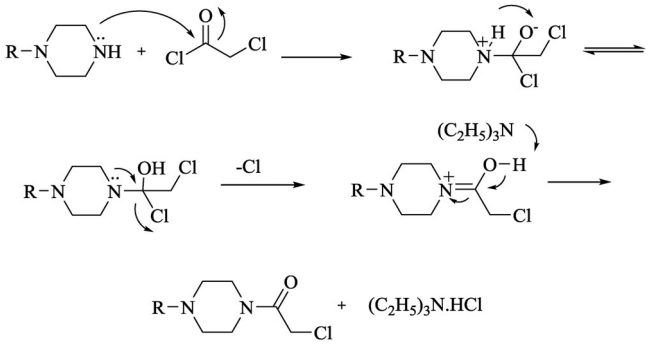


Figure 3. The reaction mechanism of compounds **1a–1f**.

compound that gave the highest molecular docking interaction energy with a value of −8.390 kcal/mol. For the inhibition of sterol 14-alpha demethylase enzyme, π-cation interactions with Hem in its structure are prominent. Except for compound **2e**, which gave the lowest docking score, all compounds formed π-cation interactions with Hem. In Figure 2, the binding poses and schematic protein-ligand interactions of compounds **2f** and **2b** are shown. Compound **2f** gave a hydrogen bond with Ser378 (1.70 Å) and π-cation interactions with Hem601 (5.29 and 5.48 Å), Tyr118 (5.36 Å), and His377 (4.50 Å). The second active compound, **2b**, gave two hydrogen bonds with Thr132 (2.12 Å) and Met508 (1.75 Å) and π-cation interactions with Hem601 (5.72 Å) and Tyr118 (5.16 Å). Details of protein-ligand interactions of other synthesized compounds, cocrystal ligand VT1, standard fluconazole, and voriconazole are given in **Table S4**.

Material and methods

All chemicals employed in the synthetic procedure were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the obtained compounds were determined by MP90 digital melting point apparatus (Mettler Toledo, OH, USA) and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds were registered by a Bruker 300 MHz digital FT-NMR spectrometer and Bruker 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d_6, respectively. Splitting patterns were designated as follows: s: singlet; d: doublet; t: triplet; m: multiplet in the NMR spectra. Coupling constants (*J*) were reported as Hertz. M + 1 peaks were determined by Shimadzu LC/MSMS system (Shimadzu, Tokyo, Japan). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany) (Figure 3).

Chemistry

Synthesis of 2-chloro-1-(4-substitutedpiperazin-1-yl)ethan-1-one (1a–1f) derivatives:

Chloroacetyl chloride (0.014 mol, 1.056 mL) in THF (15 mL) was taken into ice bath. 4-Substituted piperazine derivatives (0.012 mol) and TEA (0.0132 mol, 1.90 mL) in THF (50 mL) were added dropwise to this solution. After completion of dropping the reaction mixture was stirred at room temperature for 1 h. The precipitated product was filtered washed with water and dried.[35,36]

The general synthesis method of target compounds (2a–2f)

5-mercapto-1-methyl-1*H*-tetrazole (0.001 mol), potassium carbonate (0.001 mol, 0.138 g) and 2-chloro-1-(4-substituted-piperazin-1-yl)-ethane-1-one (0.0015 mol) derivative were dissolved in acetone (15 mL) and refluxed for 6 h. After TLC control, the solvent was evaporated, the residue was washed with water, dried, and then recrystallized from ethanol to afford final compounds (**2a–2f**).

1-(4-Ethylpiperazin-1-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethan-1-one (2a)

Yield: 78%, M.p. = Semi-solid. ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.07$ (3H, t, $J = 7.17$ Hz, CH_3), 2.56–2.59 (4H, m, piperazine CH_2), 2.66–2.68 (2H, m, CH_2), 3.52–3.60 (4H, m, piperazine CH_2), 3.98 (3H, s, $-CH_3$), 4.47 (2H, s, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 11.36$ (CH_3), 37.83 (N-CH_3), 41.22 (S-CH_2), 43.27 (CH_2), 44.83 (piperazine C), 45.91 (piperazine C), 51.64 (piperazine C), 51.99 (piperazine C), 165.29 (tetrazole C), 169.63 (C = O). HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{10}H_{18}N_6OS$: 271.1320; found: 271.1328. Anal. calcd. For $C_{10}H_{18}N_6OS$, C, 44.43; H, 6.71; N, 31.09. Found: C, 44.56; H, 6.70; N, 31.11.

1-(4-Cyclohexylpiperazin-1-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethan-1-one (2b)

Yield: 77%, M.p. = Semi-solid. ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.18$–1.21 (4H, m, cyclohexyl CH), 1.55–1.59 (2H, m, cyclohexyl CH), 1.74–1.79 (5H, m, cyclohexyl CH), 3.03–3.07 (4H, m, piperazine CH_2), 3.49–3.52 (4H, m, piperazine CH_2), 3.97 (3H, s, $-CH_3$), 4.45 (2H, s, CH_2), ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 25.15$ (cyclohexyl C), 25.60 (cyclohexyl C), 26.16 (cyclohexyl C), 28.29 (cyclohexyl C), 28.47(cyclohexyl C), 37.89 (N-CH_3), 42.03 (S-CH_2), 43.91 (piperazine C), 45.86 (piperazine C), 48.40 (piperazine C), 48.77 (piperazine C), 63.42 (cyclohexyl C), 165.12 (tetrazole C), 167.23 (C = O). HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{24}N_6OS$: 325.1805; found: 325.1797. Anal. calcd. For $C_{14}H_{24}N_6OS$, C, 51.83; H, 7.46; N, 25.90. Found: C, 51.90; H, 7.45; N, 25.84.

1-(4-(Pyridin-2-yl)piperazin-1-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethan-1-one (2c)

Yield: 74%, M.p. = 82.4–84.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.24$–2.27 (2H, m, piperazine CH_2), 2.31–2.35 (2H, m, piperazine CH_2), 3.42–3.49 (4H, m, piperazine CH_2), 3.97 (3H, s, $-CH_3$), 4.45 (2H, s, CH_2), 7.31–7.40 (4H, m, pyridine CH). ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 37.94$ (N-CH_3), 42.06 (S-CH_2), 45.67 (piperazine C), 46.05 (piperazine C), 54.54 (piperazine C),

54.90 (piperazine C), 127.05 (pyridine C), 130.44 (pyridine C), 137.94 (pyridine C), 142.36 (pyridine C), 153.80 (tetrazole C), 165.17 (C = O). HRMS (m/z): $[M+H]^+$ calcd for $C_{13}H_{17}N_7OS$: 320.0776; found: 320.0779. Anal. calcd. For $C_{13}H_{17}N_7OS$, C, 48.89; H, 5.36; N, 30.70. Found: C, 48.96; H, 5.35; N, 30.76.

1-(4-(4-Fluorophenyl)piperazin-1-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethan-1-one (2d)

Yield: 69%, M.p. = 114.4–115.9 °C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 3.18–3.23 (2H, m, piperazine CH_2), 3.58–3.66 (4H, m, piperazine CH_2), 3.98 (3H, s, –CH_3), 4.51 (2H, s, CH_2), 6.96 (2H, d, J = 8.67 Hz, 1,4-disubstituted benzene), 7.22 (2H, d, J = 8.70 Hz, 1,4-disubstituted benzene). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 37.87 (N-CH_3), 41.99 (S-CH_2), 45.61 (piperazine C), 46.11 (piperazine C), 48.56 (piperazine C), 48.92 (piperazine C), 116.35 (4-fluorophenyl 2 C), 119.84 (4-fluorophenyl 2C), 165.30 (tetrazole C), 168.15 (C = O). HRMS (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{17}FN_6OS$: 337.0076; found: 337.0091. Anal. calcd. For $C_{14}H_{17}FN_6OS$, C, 49.99; H, 5.09; N, 24.98. Found: C, 50.11; H, 5.10; N, 25.04.

1-(4-(Pyrimidin-2-yl)piperazin-1-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethan-1-one (2e)

Yield: 79%, M.p. = Semi-solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.54–3.61 (5H, m, piperazine CH_2), 3.80–3.83 (3H, m, piperazine CH_2), 3.99 (3H, s, –CH_3), 4.52 (2H, s, CH_2), 6.67 (1H, t, J = 4.74 Hz, pyrimidine CH), 8.38 (2H, d, J = 4.74 Hz, pyrimidine CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 33.28 (N-CH_3), 38.07 (S-CH_2), 41.90 (piperazine C), 43.36 (piperazine C), 43.61 (piperazine C), 46.07 (piperazine C), 110.98 (pyrimidine 2C), 158.48 (pyrimidine 2C), 161.49 (pyrimidine 2C), 165.50 (tetrazole C), 166.38 (C = O). HRMS (m/z): $[M+H]^+$ calcd for $C_{12}H_{16}N_8OS$: 321.1241; found: 321.1246. Anal. calcd. For $C_{12}H_{16}N_8OS$, C, 44.99; H, 5.03; N, 34.98. Found: C, 45.08; H, 5.04; N, 35.07.

1-(4-(4-Chlorobenzyl)piperazin-1-yl)-2-((1-methyl-1H-tetrazol-5-yl)thio)ethan-1-one (2f)

Yield: 71%, M.p. = Semi-solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.42–3.50 (8H, m, piperazine CH_2), 3.64 (2H, s, CH_2), 3.97 (3H, s, CH_2), 4.44 (2H, s, CH_2), 7.35–7.38 (4H, m, Aromatic CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 34.51 (N-CH_3), 37.96 (S-CH_2), 42.14 (piperazine C), 45.85 (piperazine C), 52.36 (piperazine C), 52.82 (piperazine C), 61.21 (CH_2), 128.67 (4-chlorobenzyl 2 C), 131.14 (4-chlorobenzyl 2 C), 165.15 (tetrazole C), 169.45 (C = O). HRMS (m/z): $[M+H]^+$ calcd for $C_{15}H_{19}N_6OSCl$: 367.1102; found: 367.1112. Anal. calcd. For $C_{15}H_{19}N_6OSCl$, C, 49.11; H, 5.22; N, 22.91. Found: C, 49.23; H, 5.20; N, 22.94.

Antimicrobial activity

The antimicrobial activity of final compounds (**2a**–**2f**) compounds was screened on eight bacterial and three fungal strains according to the standard procedure of CLSI as described in the previous study.[37] The antibacterial activities of the synthesized compounds were tested against *E. coli* (ATCC 25922), *K. pneumonia* (ATCC 13883), *P. aeruginosa* (ATCC 27853), *E. faecalis* (ATCC 2942), *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 29213). *C. albicans* (ATCC 24433), *C. krusei* (ATCC 6258), *C. parapsilopsis* (ATCC 22019), and *C. glabrata* (ATCC 9) were used to test the antifungal activity of the same compounds. Azithromycin (against bacterial strains) and voriconazole, fluconazole (against candida strains) was used as standard reference drugs.

Cytotoxicity assay

Cell culture

L929, the fibroblast cell line is purchased from American Type Culture Collection and grown in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (FBS; Sigma Aldrich), 1% L-glutamine (Sigma-Aldrich), and 1% penicillin/streptomycin (Sigma-Aldrich). The cultured cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. All newly synthesized compounds were dissolved in DMSO, and stock solutions were diluted with DMEM as the final concentration of DMSO did not exceed 0.5%.

Cell viability assay

The effect of the compounds between **2a–2f** on the viability of L929 cell line was analyzed by MTT assay. The cells were seeded at a density of 1×10^4 cells/well and treated with 100 µM concentrations for each and incubated for 48 h. Untreated cells were used as control. Following incubation, the cells were treated with 20 μL of MTT solution (5 mg/mL in PBS, Sigma) and incubated at 37 °C for 3 h to let the metabolically active cells reduce MTT dye into formazan crystals. The formazan crystals were dissolved in DMSO (Sigma). The reduction of MTT was quantified by measuring the absorbance at 540 nm with a microplate reader (Thermo, Germany). Datas were represented as mean ± standard deviation (± SD).

Molecular docking

Molecular docking studies were carried out as in the method previously published by Cevik et al..[37] Molecular modeling was carried out with Schrödinger Maestro 12.8. Sterol 14-alpha demethylase enzyme structure was imported with PDB ID: 5TZ1 and prepared using the OPLS4 force field with 'Protein Preparation Wizard' default settings.[38] 3D structures of the compounds **2a**–**2f** were prepared using the OPLS4 force field with 'LigPrep' at pH:7 ± 2. The active site coordinates file was created with the 'Receptor Grid Generation' module based on the cocrystal ligand VT1 on the 5TZ1 structure. Molecular docking was performed with the Glide SP module.[39] 2D and 3D visualization were

carried out with the Maestro 'Ligand Interaction' module and the PyMOL Molecular Graphics System v2.5.2, respectively.

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Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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