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Antimicrobial activity of novel substituted 1,2,4-triazole and 1,3-thiazole derivatives

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ABSTRACT

In this work, thirteen new compounds have been synthesized, characterized and evaluated for their antifungal and antimicrobial activities. Seven of them which contain the thiazole ring were obtained by reacting 2-(2-(2-methoxyphenoxy)propanoyl)-*N*-phenylhydrazine-1-carbothioamide (**3**) with phenylacyl bromide derivatives, while the other six compounds that have the 1,2,4-triazole ring (**6a-6f**) were prepared through couple of steps starting from the same intermediate 2-(2-(2-methoxyphenoxy)acetyl)-*N*-phenylhydrazine-1-carbothioamide (**3**). All final compounds were characterized using LC-MS, ^{1}H—NMR and ^{13}C—NMR spectroscopic techniques. All compounds were tested against twelve bacterial strains and ten fungus species. Derivative (**5a**) that is *N*'-(3,4-diphenylthiazol-2(3*H*)-ylidene)-2(methoxyphenoxy)propanehydrazide had shown the best activity and selectivity toward Yersinia enterocolitica bacterial type. All compounds demonstrated good activity against filamentous fungi species compared to yeast species. Molecular docking studies also showed that derivatives 2-(2-Methoxyphenoxy)-*N*'-(4-(4-methoxyphenyl)-3-phenylthiazol-2(3*H*)-ylidene)propanehydrazide (**5c**), *N*'-(4-(4-chlorophenyl)-3-phenylthiazol-2(3*H*)-ylidene)-2-(2-methoxyphenoxy)propanehydrazide (**5d**) and *N*-(4-(4-fluorophenyl)-3-phenylthiazol-2(3*H*)-ylidene)-2-(2-methoxyphenoxy)propanehydrazide (**5e**) interacted with the active cavity of lanosterol 14α-demethylase enzyme.

1. Introduction

Antimicrobial resistance (AMR) is considered a critical trouble to public health systems around the globe concerning its potential to cause serious infections, extended hospitalization, higher healthcare expenditures and treatment failures. The rate of AMR is being accelerated by several distinct factors, including the misuse of antibiotics, the use of antibiotics in food-production industries, increasing income levels particularly in developing countries, facile transportation routes for people, animals, and goods, biological factors through mutation and bacterial evolution, and paucity of awareness about antibiotic resistance [1]. The resistance to antibiotics might be developed by microorganisms in several ways according to their mechanisms of antimicrobial action. For instance, various microorganisms can acquire resistance to β-lactams antibiotics that interfere with the synthesis of the bacterial cell walls by enzymatic inactivation and/or by alteration in the target site of the antibiotic resulting in lower attraction forces between the antibiotics and the target penicillin-binding proteins (PBPs) [2]. Similarly, the resistance to azole drugs in yeasts and molds might be induced by various factors such as drug transporter upregulation, overexpression or alteration of the drug target, cellular alterations and modification of Cyp51A [3]. Therefore, there is a great demand to develop novel treatment techniques to combat antimicrobial resistance and address its subsequent hazards [4]. Several strategies have been suggested to manage and command bacterial resistance such as targeting the enzymes that induce the resistance against treatments namely the well-known β-lactamase inhibitor, clavulanic acid; or increasing the spectrum by prescribing a combination of antibiotics that exhibit different modes of action exerting synergistic activity such as the clinically effective combination of β-lactam with fluoroquinolone or tetracycline [5].

In recent years, medicinal chemists have been trying intensively to synthesize novel molecules by incorporating different heterocyclic scaffolds that are known to exhibit antimicrobial activity within the same structure [6–9]. According to the literature, thiazole-containing

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structures have displayed antidiabetic [10], antitumor (Largazole) [11, 12] antimicrobial and antifungal activities [13]. Additionally, triazole rings possess intriguing medicinal activities such as antimigraine, antimicrobial, antioxidant, anti-inflammatory, diuretic, anticancer, and antidiabetic activities [14,15]. Currently, the most prescribed antifungals in clinical therapy are triazole-containing medications including fluconazole, voriconazole and itraconazole [16]. Accordingly, medicinal chemists have shown a growing interest in triazoles, mainly because of their distinct attributes, including rigidity, robust hydrogen-bond properties, *in-vivo* stability, and intriguing pharmacokinetic profiles [17]. Furthermore, several biologically active natural compounds include the 2-imino-1,3-thiazoline nucleus, which exhibits pronounced antibacterial activity against bacteria, yeast, and mold [18]. It is also proposed that the presence of Schiff base plays a role in antibacterial activity due to its ability to interact with metal ions within the active site of many enzymes forming a complex [19].

Furthermore, it has been proved that the thiazole ring serves as a fundamental structural component in various natural compounds, such as vitamin B1 thiamine, thiamine pyrophosphate TPP co-enzyme within the Krebs cycle, carboxylase, as well as the extensive family of macrocyclic thiopeptide antibiotics including micrococcin and thiostrepton [20]. Likewise, several studies have demonstrated the role of benzothiazole nucleus as an antimicrobial against various species such as *Staphylococcus* (*S.*) *aureus, Escherichia (E.) coli,* and *Plasmodium* [21] (Fig. 1).

In this work, we intended to synthesize six 1,2,4 triazole-containing compounds connected by an amide-thioether bridge to different substituted benz/thiazole derivatives, in addition to other seven thiazole-containing structures with hydrazide-Schiff base at the 2nd position and different substituted phenyl ring at the 4th position of the thiazole core and tested them against different bacterial and fungus species. After that, based on the biological results, the active compounds were further studied *in-silico* for their pharmacokinetics properties and molecular interactions with one of the most important targets for antifungal agents lanosterol 14α-demethylase enzyme. In conclusion, the findings of this study are expected to have an impact on the course of discovering new antimicrobial agents highlighting the major heterocyclic cores that are predicted to exhibit this action. Specifically, the results of our study emphasized that the presence of 1,2,4-triazole and thiazole rings play a significant role in the antimicrobial activity of many compounds supporting previous studies that examined their action against several pathogens. Therefore, the integration of these rings might provide a promising strategy to develop more effective and targeted treatments for infectious diseases caused by drug-resistant strains of bacteria and fungi that have deleterious impacts not only on humans but also on animals and plants.

2. Materials and methods

2.1. Chemistry

All chemicals were obtained from Merck Chemicals (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA). All uncorrected melting points (m.p.) were measured using the MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA). All reactions were controlled by thin-layer chromatography (TLC) using Silica Gel 60 F_{254} TLC plates (Merck KGaA, Darmstadt, Germany). The mobile phase of TLC was petroleum ether-ethyl acetate (3:1). ^{1}H-NMR, ^{13}C-NMR data were obtained using NMR, Bruker DPX- 300 FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA); in DMSO-d_6, using TMS as internal standard. While Shimadzu LC/MS-IT-TOF system (Shimadzu, Kyoto, Japan) was used to determine the mass spectra HRMS of all products.

2.1.1. Synthesis of ethyl 2-(2-methoxyphenoxy)propanoate (1)

0.10 mol of Guaiacol (2-methoxyphenol) was stirred with the base potassium carbonate in acetone for 15 mins then ethyl 2-bromopropionate (0.11 mol) was added and refluxed for 3 hrs. After cooling, the solvent was evaporated and the residue was treated with water, filtered, dried and recrystallized from the ethanol.

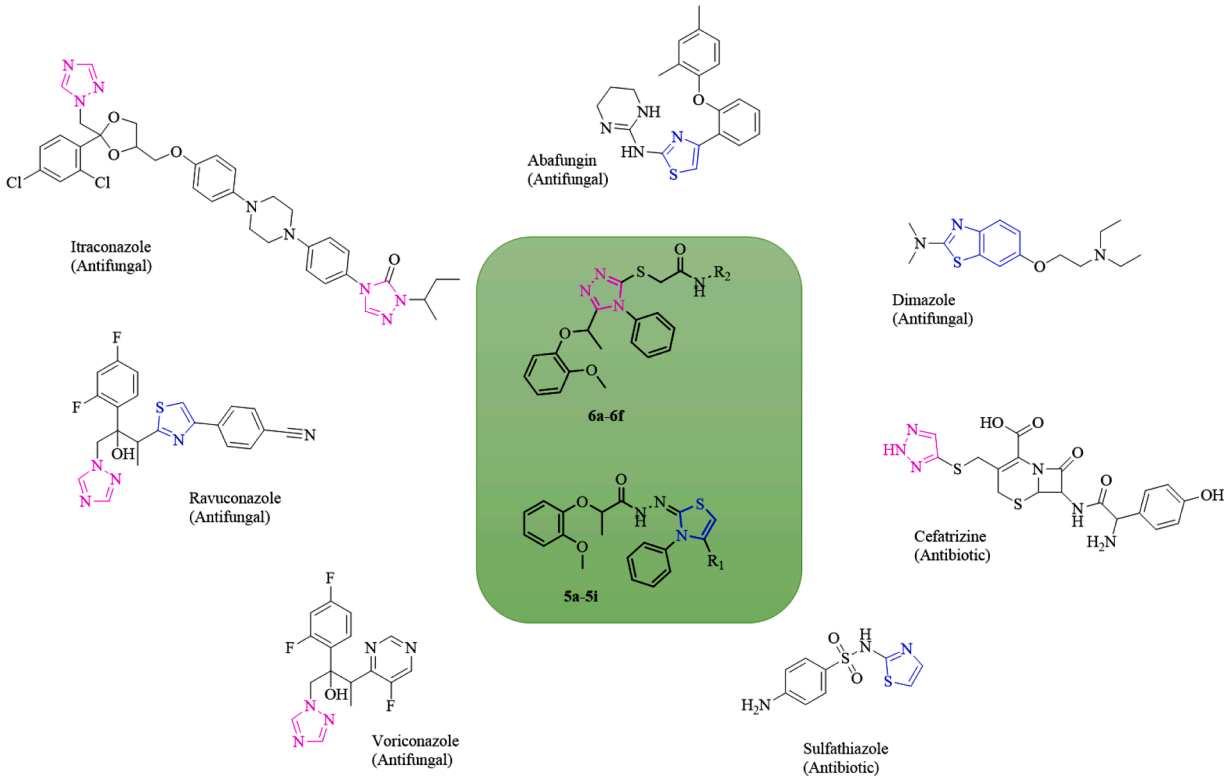


Fig. 1. Structures of synthesized targeted derivatives (**5a-5i** and **6a-6f**) and some approved triazole and benzo/thiazole-containing antimicrobial agents.

2.1.2. Synthesis of 2-(2-methoxyphenoxy)acetohydrazide (2)

Ethyl 2-(2-methoxyphenoxy)propanoate (**1**) (0.07 mol, 14.7 g) was dissolved in ethanol (65 mL). After that, a solution of hydrazine hydrate (0.14 mol, 6.7 mL) in ethanol (35 mL) was slowly added, stirred, and refluxed for 4 hrs. The reaction was controlled using TLC. The solvent was evaporated, washed, and filtered.

2.1.3. Synthesis of 2-(2-(2-methoxyphenoxy)acetyl)-N-phenylhydrazine-1-carbothioamide (3)

2-(2-Methoxyphenoxy)acetohydrazide (**2**) (0.05 mol, 10.5 g) was refluxed with phenylisothiocyanate (0.06 mol, 7.37 ml) for 6 hrs. After that, the mixture was cooled to room temperature, filtered and dried.

2.1.4. General synthesis of (N'-(substituted phenyl)−3-phenylthiazol-2 (3H)-ylidene)−2-(2-methoxyphenoxy)propanehydrazide derivatives (5a-5 g)

Compound **3** was refluxed with the corresponding substituted phenylacyl bromide in a 1:1 equivalent ratio to obtain their respective products. After the reaction was finished, the mixture was filtered out of ethanol while it was hot to acquire the final product in the pure form.

2.1.4.1. N'-(3,4-Diphenylthiazol-2(3H)-ylidene)−2(methoxyphenoxy) propanehydrazide (5a). Yield=75%, m.p.=225 °C.^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.05–1.54 (3H, m, CH—CH_3), 3.71–3.80 (3H, q, J_1=14, J_2=3.5 Hz, O—CH_3), 4.48–4.88 (1H, m, CH—CH_3), 6.53–6.75 (1H, m, Ar-H), 6.95–6.99 (3H, m, Ar-H), 7.10–7.37 (4H, m, Ar-H), 7.49–7.54 (7H, m, Ar-H), 11.39 (H, s, N—H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.61 (CH—CH_3), 55.94 (O—CH_3), 73.93 (CH—CH_3), 100.56, 106.45, 112.84, 113.13, 116.19, 116.38, 116.75, 117.36, 121.06, 122.83, 123.22, 123.60, 128.77, 129.08, 129.90, 130.68, 140.95, 146.75, 149.97, 170.91(C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{25}H_{23}N_3O_3S$) calculated: 446.1533; found: 446.1542. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5a** were given in Supplementary; Figures 1, 2, and 3, respectively.

2.1.4.2. 2-(2-Methoxyphenoxy)-N'-(3-phenyl-4-(p-tolyl)thiazol-2(3H)-ylidene)propanehydrazide (5b). Yield 82%, M.P.= 71 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 1.19–1.30 (3H, m, CH—CH_3), 2.34–2.38 (3H, m, phenyl-CH_3), 3.71 (3H, s, O—CH_3), 4.83 (1H, dq, J_1=6.6, J_2=24.11 Hz, CH—CH_3), 6.50.6.73 (2H, m, Ar-H), 6.89–7.10 (4H, m, Ar- H), 7.24–7.30 (3H, m, Ar- H), 7.32–7.41 (3H, m, Ar-H), 7.43–7.53 (2H, m, Ar- H), 11.54 (H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 19.28 (CH—CH_3), 21.39 (CH_3-Ar), 55.92 (O—CH_3), 74.26 (CH—CH_3), 113.09, 116.16, 116.82, 120.76, 121.25, 122.56, 122.87, 123.70, 124.4, 125.18, 129.00, 129.61, 129.79, 130.15, 130.69, 138.75, 139.89, 147.00, 149.15, 149.78, 150.44, 154.43, 171.51 (C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{26}H_{25}N_3O_3S$) calculated: 460.1689; found: 460.1697. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5b** were given in Supplementary Figures 4, 5 and 6, respectively.

2.1.4.3. 2-(2-Methoxyphenoxy)-N'-(4-(4-methoxyphenyl)−3-phenylthiazol-2(3H)-ylidene)propanehydrazide (5c). Yield 79%, M.P.=222 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 1.21–1.32 (3H, m, CH—CH_3), 3.72 (3H, s, 4–OCH_3 phenyl), 3.80 (3H, s, OCH_3), 4.48–4.84 (1H, dq, J_1=6.6, J_2=23 Hz, CH—CH_3), 6.50–6.58 (1H, m, Ar-H), 6.71–6.75 (1H, m, Ar- H), 6.89–7.10 (6H, m, Ar-H), 7.29–7.53 (6H, m, Ar-H), 11.5 (H, NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.69 (CH—CH_3), 55.88 (O—CH_3), 74.46 (CH—CH_3), 112.76, 113.12, 114.50, 116.15, 116.84, 120.17, 120.91, 121.22, 122.90, 123.76, 124.40, 130.71, 131.55, 138.62, 140.95, 146.13, 147.01, 150.01, 150.49, 160.97, 170.95, 171.50 (C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{26}H_{25}N_3O_4S$) calculated: 476.1639; found: 476.1643. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5c** were given in Supplementary Figures 7, 8, and 9 respectively.

2.1.4.4. N'-(4-(4-Chlorophenyl)−3-phenylthiazol-2(3H)-ylidene)−2-(2-methoxyphenoxy)propanehydrazide (5d). Yield 87%, M.P. = 123 °C.^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 1.25–1.53 (3H, m, CH—CH_3), 3.72–3.79 (3H, m, O—CH_3), 4.71–4.80 (1H, m, CH—CH_3), 6.68 (1H, s, Ar-H), 6.85 (1H, s, Ar-H), 6.91–6.98 (3H, m, Ar-H), 7.32–7.37 (4H, m, Ar-H), 7.51 (5H, m, Ar-H), 11.54 (1H,NH). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ: 18.93 (CH—CH_3), 55.97 (O—CH_3), 74.24 (CH—CH_3), 112.83, 113.01, 116.56, 120.81, 122.86, 128.76, 128.91, 129.11, 129.66, 130.17, 130.48, 130.57, 131.04, 131.70, 132.54, 134.97, 139.39, 146.77, 171.27 (C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{25}H_{22}N_3O_3SCl$) calculated: 480.1143; found: 480.1159. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5d** were given in Supplementary; Figures 10, 11 and 12 respectively.

2.1.4.5. N'-(4-(4-Fluorophenyl)−3-phenylthiazol-2(3H)-ylidene)−2-(2-methoxyphenoxy)propanehydrazide (5e). Yield 77%, M.P.= 193 °C.^{1}H-NMR (300 MHz, DMSO-*d6,* ppm): δ: 1.03- 1.51 (3H, m, CH—CH_3), 3.42–3.79 (3H, m, O—CH_3), 4.71–4.74 (1H, m, CH—CH_3), 6.69–7.01 (4H, m, Ar-H), 7.09–7.15 (1H, m, Ar-H), 7.21–7.73 (5H, m, Ar-H), 7.48–7.57 (4H, m, Ar-H), 11.25 (1H, N—H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ:19.28 (CH—CH_3), 56.03 (O—CH_3), 74.41 (CH—CH_3), 112.80, 113.02, 115.93, 116.22, 116.46, 116.89, 120.83, 121.10, 123.08, 128.79, 129.61, 130.16, 130.48, 131.36, 132.46, 146.79, 161.61, 164.89, 171.28 (C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{25}H_{22}N_3O_3F$) calculated: 464.1439; found: 464.1436. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5e** were given in Supplementary; Figures 13, 14 and 15 respectively.

2.1.4.6. 2-(2-Methoxyphenoxy)-N'-(4-(4-nitrophenyl)−3-phenylthiazol-2 (3H)-ylidene)propanehydrazide (5f). Yield 73%, M.P. = 133 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 1.41–1.50 (3H, m, CH—CH_3), 3.73 (3H, s, O—CH_3), 4.60–4.71 (1H, m, CH—CH_3), 6.52–6.75 (2H, m, Ar-H), 6.83–7.11 (5H, m, Ar-H), 7.31–7.48 (4H, m, Ar-H), 7.64–7.74 (1H, m, Ar-H), 8.02–8.27 (2H, m, Ar-H), 11.33 (1H, N—H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ:19.51 (CH—CH_3), 55.97 (O—CH_3), 74.49 (CH—CH_3), 99.00, 105.01, 112.67, 112.99, 115.45, 116.03, 120.64, 121.04, 121.42, 122.36, 122.69, 122.99, 124.12, 128.85, 130.06, 136.62, 137.87, 147.09, 147.62, 179.99, 171.93 (C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{25}H_{22}N_4O_5S$) calculated: 491.1384; found: 491.1376. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5f** were given in Supplementary; Figure 16, 17 and 18 respectively.

2.1.4.7. N'-(4-(4-Cyanophenyl)−3-phenylthiazol-2(3H)-ylidene)−2-(2-methoxyphenoxy)propanehydrazide(5g). Yield 88%, M.P.= 98 °C.^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 1.38 (3H, dd, J_1=2.67, J_2= 6.6 Hz, CH—CH_3), 3.74 (3H, s, OCH_3), 4.60 (1H, m, CH—CH_3), 6.64 (1H, s, Ar-H), 6.58–6.65 (2H, m, Ar-H), 6.76 (1H, d, J = 7.8 Hz, Ar-H), 6.87–6.97 (4H, m, Ar-H), 7.06 (1H, t, J = 6.9 Hz, Ar-H), 7.33–7.38 (2H, m, Ar-H), 7.59–7.59 (1H, dd, J_1=2.2, J_2= 6.9 Hz,Ar-H), 7.63–7.66 (1H, dd, J_1=2.2, J_2= 8.1 Hz, Ar-H), 7.76–7.80 (1H, dd, J_1=2.2, J_2= 8.1 Hz,Ar-H), 7.90–7.93 (1H, dd, J_1=2.3, J_2= 8.1 Hz, Ar-H), 11.27 (1H, brd, N—H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 19.45 (CH—CH_3), 55.94 (O—CH_3), 73.54 (CH—CH_3), 97.80 (thiazole-C), 111.76, 112.85, 115.81, 119.00, 120.74, 121.22, 122.53, 123.78, 128.55, 129.98, 132.75, 171.94 (C = O). HRMS (*m/z*): $[M + H]^+$ for ($C_{26}H_{22}N_4O_3S$) calculated: 471.1485; found: 471.1481. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **5g** were given in Supplementary; Figures 19, 20 and 21 respectively.

2.1.5. Synthesis of 5-((2-methoxyphenoxy)methyl)−4-phenyl-4H-1,2,4-triazole-3-thiol (4)

2-(2-(2-Methoxyphenoxy)acetyl)-*N*-phenylhydrazine-1-carbothioamide **(3)** (15 mmol, 5.17 g) was refluxed for 6 hrs in a freshly prepared ethanolic potassium hydroxide solution (2 M). After the reaction was completed, the mixture was cooled to room temperature before being

added to ice water. The pH was adjusted to 5 by the addition of the aqueous HCl solution. The precipitated product was collected, dried and recrystallized.

2.1.6. General synthesis of N-(substituted-benzo [d]thiazol-2-yl)−2-((5-(1-(2-methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio) acetamide (6a-6f)

2.1.6.1. 2-Chloro-N-(thiazol-2-yl/benzothiazol-2-yl)acetamide derivatives. Thiazole/benzothiazole derivatives (10 mmol) were dissolved in THF (100 mL). After that, triethylamine (12 mmol, 1.67 mL) was added to the reaction and stirred for 15 mins. Then, chloroacetyl chloride (12 mmol, 0.96 mL) was drop-wisely added at 0–5 °C. After 3 hrs, the reaction was completed and the mixture was poured into ice-water and the crude raw product was filtered, dried and recrystallized in ethanol.

In the next step, equimolar of the corresponding 2-chloro-*N*-(thiazol-2-yl/benzothiazol-2-yl)acetamide derivative and 5-(1-(2-methoxyphenoxy)ethyl)−4-phenyl-4*H*-1,2,4-triazole-3-thiol (1 mmol) were reacted under basic conditions of potassium carbonate (1.5 mmol) in acetone at room temperature for 24–36 hrs. The reaction was controlled by TLC, the solvent was evaporated and the resulting precipitate was washed with distilled water, filtered, dried and recrystallized in ethanol.

2.1.6.2. N-(Benzo [d]thiazol-2-yl)−2-((5-(1-(2-methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (6a). Yield 73%, M.P.=158 °C ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.53 (3H, d, J = 6.51 Hz, CH—CH_3), 3.60 (3H, s, O—CH_3), 4.16 (2H, s, S-CH_2), 5.36 (1H, q, J = 6.48 Hz, CH—CH_3), 6.65–6.74 (2H, m, Ar-H), 7.16 (2H, t, J = 7.98 Hz, Ar-H), 7.31 (3H, t, J = 6.95 Hz, Ar-H), 7.46–7.50 (3H, m, Ar-H), 7.60 (1H,d, J = 8.02 Hz, Ar-H), 7.82 (1H,d, J = 7.5 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.47 (CH—CH_3), 38.09 (S-CH_2), 55.68 (O—CH_3), 68.17 (CH—CH_3), 112.78, 117.39, 120.16, 120.88, 121.72, 122.66, 123.22, 125.77, 127.87, 129.97, 130.39, 132.52, 133.06, 145.73, 149.80, 150.71, 152.40, 155.30, 162.75 (C = O), 169.35 (benzothiazole C_2). HRMS (m/z): $[M + H]^+$ for ($C_{26}H_{23}N_5O_3S_2$) calculated: 518.1315; found: 518.1312. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **6a** were given in Supplementary; Figures 22, 23 and 24 respectively.

2.1.6.3. 2-((5-(1-(2-Methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(6-methylbenzo [d]thiazol-2-yl)acetamide (6b). Yield 77%, M.P.=201 °C ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.53 (3H, d, J = 6.45 Hz, CH—CH_3), 3.40 (1H, s, Ar-CH_3), 3.50 (2H, s, Ar-CH_3), 3.59 (3H, s, O—CH_3), 4.26 (2H, s, S-CH_2), 5.35 (1H, q, J = 6.48 Hz, CH—CH_3), 6.64–6.73 (2H, m, Ar-H), 6.85–6.90 (2H, t, J = 7.98 Hz, Ar-H), 7.23–7.33 (3H, m, Ar-H), 7.41–7.49 (4H,m, Ar-H), 7.63–7.75 (2H, m, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.41 (CH—CH_3), 21.47, 36.46 (S-CH_2), 55.65 (O—CH_3), 68.20 (CH—CH_3), 112.81, 117.44, 120.88, 121.79, 123.27, 126.79, 127.83, 130.00, 130.48, 132.07, 132.92, 133.57, 145.69, 146.94, 148.07, 150.74, 151.65, 155.57, 157.49, 160.11, 167.37, 168.17 (benzothiazole C_2). HRMS (m/z): $[M + H]^+$ for ($C_{27}H_{25}N_5O_3S_2$) calculated: 532.1472; found: 532.1486. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **6b** were given in Supplementary; Figures 25, 26 and 27 respectively.

2.1.6.4. N-(6-Methoxybenzo [d]thiazol-2-yl)−2-((5-(1-(2-methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (6c). Yield 78%, M.P.=205 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm): δ 1.53 (3H, d, J = 6.52 Hz, CH—CH_3), 3.60 (3H, s, O—CH_3), 3.80 (3H, s, OCH_3), 4.26 (2H, s, S-CH_2), 5.35 (1H, q, J = 6.48 Hz, CH—CH_3), 6.64–6.75 (2H, m, Ar-H), 6.88–6.90 (1H, m, Ar-H), 7.01–7.04 (1H, m, Ar-H), 7.21–7.33 (3H,m, Ar-H), 7.44–7.49 (3H, m, Ar-H), 7.54 (1H, d, J = 2.52 Hz, Ar-H), 7.64 (1H, d, J = 8.85 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.40 (CH—CH_3), 36.13 (S-CH2), 55.65(O—CH_3), 56.09(O—CH_3), 68.21 (CH—CH_3), 105.17, 112.81, 115.39, 117.45, 120.89, 121.66, 123.27, 127.83, 130.00, 130.48, 132.91, 133.28, 143.10, 145.69, 150.75, 151.57, 155.61, 156.60, 165.20, 167.28 (benzothiazole C_2). HRMS (m/z): $[M + H]^+$ for ($C_{27}H_{25}N_5O_4S_2$) calculated: 548.1421; found: 548.1426. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **6c** were given in Supplementary; Figures 28, 29 and 30 respectively.

2.1.6.5. N-(6-Fluorobenzo [d]thiazol-2-yl)−2-((5-(1-(2-methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (6d). Yield 74%, M.P.=181 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ:1.53 (3H, d, J = 6.54 Hz, CH—CH_3), 3.59 (3H, s, OCH_3), 4.24 (2H, s, S-CH_2), 5.35 (1H, q, J = 6.51 Hz, CH—CH_3), 6.64–6.75 (2H, m, Ar-H), 6.85–6.90 (2H, m, Ar-H), 7.21–7.33 (3H,m, Ar-H), 7.45–7.50 (3H, m, Ar-H), 7.68–7.73 (1H, q, J = 4.8 Hz, Ar-H), 7.64 (1H, dd, J_1=2.67 Hz, J_2= 8.6 Hz Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.43 (CH—CH_3), 36.71 (S-CH2), 55.69 (O—CH_3), 68.20 (CH—CH_3), 108.66, 112.80, 114.53, 117.44, 120.88, 121.86, 123.26, 127.84, 130.00, 130.46, 132.95, 133.28, 145.70, 150.74, 151.78, 155.53, 157.34, 160.51, 168.27 (benzothiazole C_2). HRMS (m/z): $[M + H]^+$ for ($C_{26}H_{22}N_5O_3FS_2$) calculated: 536.1221; found: 536.1221. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **6d** were given in Supplementary; Figures 31, 32 and 33 respectively.

2.1.6.6. N-(6-Chlorobenzo [d]thiazol-2-yl)−2-((5-(1-(2-methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio)acetamide (6e). Yield 73%, M.P.=205 °C. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ:1.53 (3H, d, J = 6.42 Hz, CH—CH_3), 3.60 (3H, s, O—CH_3), 4.22 (2H, s, S-CH_2), 5.32–5.39 (1H, q, J = 6.51 Hz, CH—CH_3), 6.64–6.75 (2H, m, Ar-H), 6.85–6.90 (2H, m, Ar-H), 7.32 (2H, s, Ar-H), 7.37–7.41 (1H, dd, J_1=1.95 Hz, J_2= 8.4 Hz, Ar-H), 7.48 (1H, d, J = 5.4 Hz, Ar-H), 7.66 (1H, d, J = 8.6 Hz, Ar-H), 8.02 (1H, d, J = 1.98 Hz, Ar-H).^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.44 (CH—CH_3), 37.07 (S-CH_2), 55.69 (O—CH_3), 68.20 (CH—CH_3), 112.81, 117.45, 120.88, 121.77, 123.26, 126.50, 127.35, 127.84, 129.99, 130.45, 132.97, 133.95, 145.70, 148.32, 150.74, 151.91, 155.49, 161.44, 168.78 (benzothiazole C_2). HRMS (m/z): $[M + H]^+$ for ($C_{26}H_{22}N_5O_3S_2Cl$) calculated: 552.0925; found: 552.0923. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **6e** were given in Supplementary; Figures 34, 35 and 36 respectively.

2.1.6.7. 2-((5-(1-(2-Methoxyphenoxy)ethyl)−4-phenyl-4H-1,2,4-triazol-3-yl)thio)-N-(4-phenylthiazol-2-yl)acetamide (6f). Yield 70%. ^{1}H-NMR (300 MHz, DMSO-d_6, ppm) δ: 1.54 (3H, d, J = 6.50 Hz, CH—CH_3), 3.60 (3H, s, OCH_3), 4.11 (2H, s, S-CH_2), 5.32–5.39 (1H, q, J = 6.52 Hz, CH—CH_3), 6.66–6.77 (2H, m, Ar-H), 6.86–6.91 (2H, m, Ar-H), 7.26–7.29 (2H, m, Ar-H), 7.35–7.40 (4H, m, Ar-H), 7.48–7.50 (3H, m, Ar-H), 7.87 (1H, d, J = 7.17 Hz, Ar-H). ^{13}C-NMR (75 MHz, DMSO-d_6, ppm): δ 18.51 (CH—CH_3), 38.02 (S-CH_2), 55.70 (O—CH_3), 68.21 (CH—CH_3), 101.93, 107.22, 112.82, 117.44, 120.89, 123.22, 126.02, 127.63, 127.89, 128.98, 129.52, 129.96, 130.35, 133.12, 135.62, 145.76, 148.72, 150.74, 152.51, 155.26, 168.21 (benzothiazole C_2). HRMS (m/z): $[M + H]^+$ for ($C_{28}H_{25}N_5O_3S_2$) calculated: 544.1472; found: 544.1468. The HRMS, ^{1}H-NMR and ^{13}C-NMR spectra of compound **6f** were given in Supplementary; Figures 37, 38 and 39 respectively.

2.2. ADME parameters

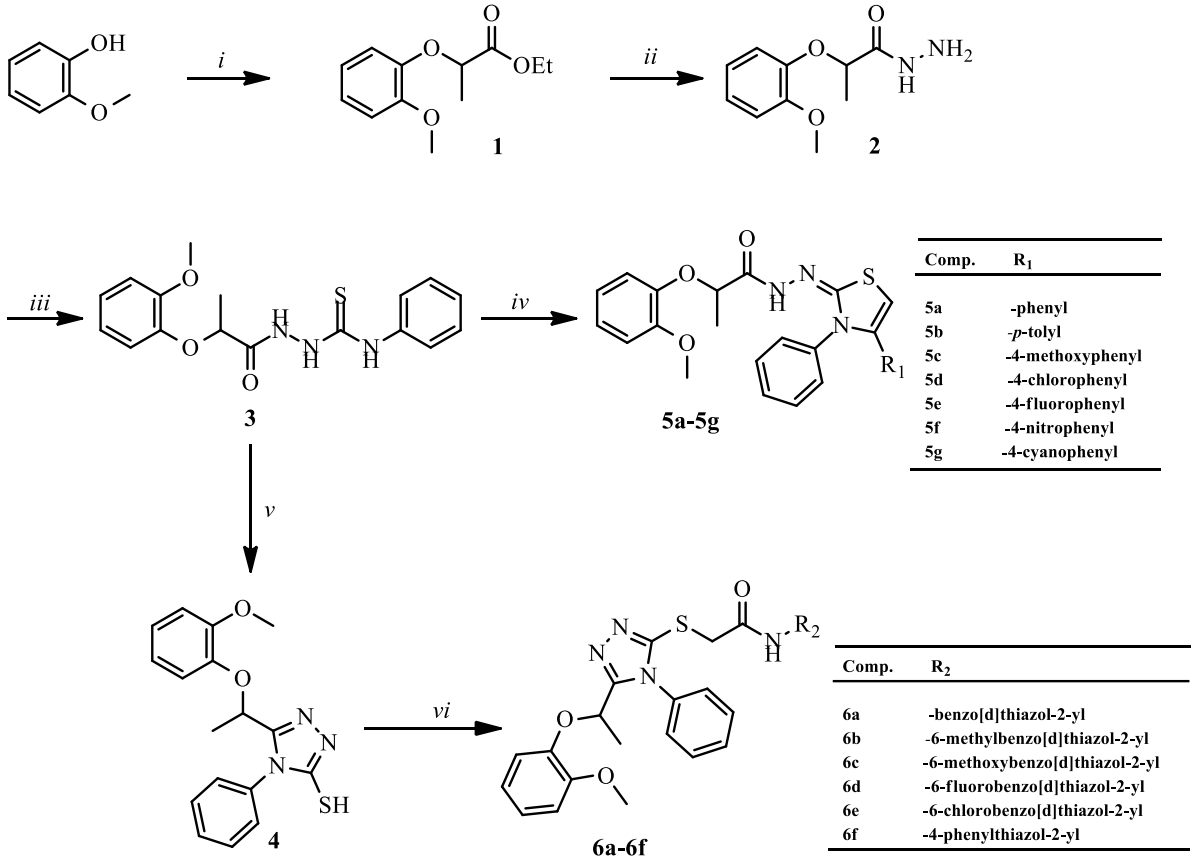
Absorption, distribution, metabolism and elimination (ADME) are all among the important pharmacokinetic parameters that should be estimated besides the biological activity evaluation, the SWISSADME program was used to obtain the theoretical calculations of the active compounds and reference drugs chloramphenicol and ketoconazole.

2.3. Antimicrobial activity

The antibacterial activity of the synthesized compounds was tested on twelve bacterial species that are *Bacillus cereus* (ATCC 10,876), *Bacillus subtilis* (NRRL NRS-744), *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (NRRL B-4375), *Listeria monocytogenes* (ATCC 19,111) and *Streptococcus faecalis* (ATCC 9790) as gram-positive bacteria; *Escherichia coli* (ATCC 25,922), *Salmonella typhimurium* (ATCC 14,028), *Proteus vulgaris* (NRRL B-123), *Yersinia enterocolitica* (Y53), *Enterobacter aerogenes* (NRRL B-3567) and *Aeromonas hydrophila* (laboratory culture) as gram-negative bacteria while the antifungal activity was tested on ten fungal species that are *Candida parapsilosis* (ATCC 22,019), *Candida albicans* (ATCC 90,028), *Candida glabrata* (ATCC 90,030) and *Candida krusei* (ATCC 6258) as yeasts; *Aspergillus niger* (ATCC 16,404), *Aspergillus flavus* (ATCC 9807), *Aspergillus parasiticus* (NRRL 465), *Aspergillus fumigatus* (NRRL 113), *Penicillium citrinum* (ATCC 9849) and *Penicillium expansum* (ATCC 24,692) as filamentous fungi species.

Bacterial cultures were maintained using Mueller-Hinton agar plates while fungal cultures were maintained using Potato dextrose (PD) agar and Sabourad dextrose (SD) agar. The tested bacteria were taken from bacterial cultures that were prepared by first incubating on Mueller-Hinton agar substrate for 24 hrs at 37 °C, followed by dilution using the 0.5 McFarland standard to a concentration of approximately 10^8 CFU/mL while fungal spore suspensions were prepared from 3 to 7 days aged fresh mature cultures that were growing at 30 °C on a PD agar substrate followed by rinsing with sterile 0.1% Tween 80 and further dilution using the procedural recommendations of Clinical and Laboratory Standards Institute (CLSI) to a concentration of approximately 10^6 CFU/mL [22].

A serial dilution technique utilizing 96-well microtitre plates was used to obtain the minimum inhibitory concentration (MIC) of the synthesized compounds (**5a-5g** and **6a- 6f**) and the reference drugs chloramphenicol as an antibacterial and ketoconazole as an antifungal. A stock solution of each of the compounds was first prepared by dissolving 80 mg of the compound in 6 mL DMSO (20%), then a two-fold serial dilution method was applied ten times using either Mueller-Hinton broth for bacterial cultures or SD broth for fungal cultures to finally get several diluted solutions with concentrations in the range of 8 mg/mL and 15.625 μg/mL for each compound. Using microtitration plates, 100 μL from each bacterial and fungal previously prepared diluted solution was incubated for 24–48 hrs. at 35–37 °C for bacteria/yeasts and 25 °C for filamentous fungi. Three positive control samples were prepared, the first containing 100 μL of microorganism solution and 20% DMSO solution, the second containing 100 μL of microorganism solution and 100 μL Mueller-Hinton broth and the third containing chloramphenicol or ketoconazole for bacteria and fungi species, respectively. On the other hand, negative control samples were prepared using dilute solutions of the synthesized compounds without the presence of microorganisms. After incubation for 24–48 hrs, the observed turbidity is used to evaluate positive and negative results by comparing them to the control samples. The MIC at which there is no visible growth was determined for each microorganism using resazurin which is a non-fluorescent blue dye that turns into fluorescent pink upon reduction with oxidoreductases inside viable cells, thus the MIC for the microorganism studied at the determined concentration was characterized as the boundary dilution of resazurin with no color change [23].



Comp.	R_1
5a	-phenyl
5b	-*p*-tolyl
5c	-4-methoxyphenyl
5d	-4-chlorophenyl
5e	-4-fluorophenyl
5f	-4-nitrophenyl
5g	-4-cyanophenyl

Comp.	R_2
6a	-benzo[d]thiazol-2-yl
6b	-6-methylbenzo[d]thiazol-2-yl
6c	-6-methoxybenzo[d]thiazol-2-yl
6d	-6-fluorobenzo[d]thiazol-2-yl
6e	-6-chlorobenzo[d]thiazol-2-yl
6f	-4-phenylthiazol-2-yl

Scheme 1. i: Ethyl 2-bromopropionate, acetone, K_2CO_3, reflux; *ii:* $NH_2NH_2.H_2O$, EtOH, r.t; *iii:* phenylisothiocyanate, EtOH, reflux; *iv:* substituted phenylacyl bromide derivatives, EtOH, reflux; *v:* ethanol, KOH, reflux; *vi:* 2-chloro(benzo)/thiazolyl acetamide derivatives, acetone, potassium carbonate, rt.

Table 1
The calculated ADME parameters.

Molecule	MW	HBA	HBD	TPSA	Log P	Log S	Log Kp	BBB	NoV
5a	445.53	4	1	93.09	4.57	−6.07	−5.11	No	0
5b	459.56	4	1	93.09	4.87	−6.37	−4.94	No	0
5c	475.56	5	1	102.32	4.56	−6.14	−5.32	No	0
5d	479.98	4	1	93.09	5.08	−6.66	−4.88	No	0
5e	463.52	5	1	93.09	4.84	−6.23	−5.15	No	0
6b	531.65	6	1	144.7	4.74	−6.69	−5.38	No	1
6d	535.61	7	1	144.7	4.71	−6.55	−5.59	No	1
Chloramphenicol	323.13	5	3	115.38	0.53	−2.32	−7.46	No	0
Ketoconazole	531.43	5	0	69.06	3.57	−5.69	−6.46	Yes	1

MW: Molecular weight, HBA: Number of hydrogen acceptors, HBD: Number of hydrogen donors, TPSA: Topological polar surface area (Å^2) Log P: Octanol/water partition coefficient, Log S: Aqueous solubility (the decimal logarithm of the molar solubility in water), Log Kp: Skin permeation constant (cm/s), NoV: Number of Violation (Lipinski's rule). All properties were calculated by http://www.swissadme.ch/index.php.

2.4. Docking methods

In silico approaches are a useful tool to predict their physicochemical properties and possible binding mode of the target molecules [7,24–27]. The docking studies were performed using the Schrodinger Maestro Suite program. The interfaces of this program are used for the protein preparation process, ligand preparation process, grid generation, docking, and visualization studies [28–31]. The crystal structure of the enzymes was retrieved from the Protein Data Bank server (PDB codes: *5TZ1* for lanosterol 14α-demethylase). All ligands were set to the physiological pH (pH = 7.4) at the protonation step. The proteins were prepared according to the mentioned previous study.

3. Results and discussion

3.1. Chemistry

A total of fifteen compounds were synthesized as illustrated in Scheme 1 from 2-methoxyphenol as the first starting material which was refluxed for 3 hrs with ethyl 2-bromopropionate in acetone and K_2CO_3 to produce ethyl 2-(2-methoxyphenoxy)propanoate (**1**) that was further reacted with hydrazine monohydrate in ethanol to produce 2-(2-methoxyphenoxy) propane hydrazide (**2**). Afterward, compound (**2**) was refluxed for 2 hrs with phenyl isothiocyanate in ethanol to afford 2-(2-(2-methoxyphenoxy)propanoyl)-*N*-phenylhydrazine-1-carbothioamide (**3**) [32]. After that compound (**3**) was reacted in two different pathways to afford either 1,2,4-triazole or 1,3-thiazole-containing compounds. The reaction of compound (**3**) with substituted phenyl acyl bromides in ethanol afforded thiazole-containing compounds **5a-5g**, while 1,2,4-triazole products **6a-6f** were obtained through a couple of steps from compound (**3**). The 1,2,4-triazole ring was first cyclized by refluxing intermediate **3** under basic conditions of alcoholic potassium hydroxide. After the reaction was terminated, the pH was adjusted to pH=5–6 where the desired product (**4**) was precipitated. The obtained compound (**4**) was then reacted with 2-chloro-*N*-substituted acetamide derivatives and potassium carbonate in acetone at room temperature producing **6a-6f** compounds [33].

3.2. ADME parameters evaluation

Nowadays, an early expectation about the pharmacokinetic profile of chemical compounds is a necessity for drug candidates besides effective pharmacological activity. The SwissADME program was used to get the theoretical calculation of ADME-related properties for the active compounds and reference drugs chloramphenicol and ketoconazole as shown in Table 1.

The molecular weight of compounds containing the thiazole ring ranged from 445.53 to 479.98 while the molecular weight of the active compounds containing the triazole ring **6b** and **6d** were above 500 g/mol. The maximum number of hydrogen bond acceptors for synthesized compounds was 7, however, all synthesized compounds have only one hydrogen bond donor. Lipid solubility which was expressed as partition coefficient values between octanol and water (log P) has a range between 4.71 and 5.08 which is considered an important factor that plays a significant role in antifungal activity. Based on all these calculations, thiazole-containing compounds are consistent with Lipinski's rule with no violation making them good orally available drugs. All compounds have good skin permeability as their skin permeation constant (Log Kp) ranged from −4.88 to −5.59. Additionally, all compounds cannot cross the blood-brain barrier providing them an advantage in terms of central side effects.

Table 2
The antibacterial activity of the compounds (μg/mL).

Comp.	A	B	C	D	E	F	G	H	I	J	K	L
5a	–	400	400	–	–	800	200	400	–	–	–	400
5b	–	–	–	–	800		–	400	400	800	–	800
5c	–	–	–	–	–	400	–	400	–	–	–	400
5d	–	400	–	–	–	400	–	800	800	–	–	400
5e	–	–	–	–	–	–	–	800	–	–	–	–
5f	–	–	1600	–	–	–	–	–	–	–	–	–
5 g	–	–	1600	800	1600	–	–	–	–	–	–	–
6a	–	–	–	–	800	–	–	–	–	800	–	–
6b	–	–	400	–	–	–	–	–	–	–	–	–
6c	–	–	–	–	–	–	–	800	–	–	–	–
6d	–	–	–	–	200	–	–	800	–	–	–	–
6e	–	–	–	–	800	–	–	–	–	–	–	–
6f	–	–	400	800	800	–	–	–	–	–	–	–
Ref.	25	100	25	50	50	100	100	50	50	25	50	25

A: E. coli, B: Staphylococcus aureus, C: Micrococcus luteus, D: Proteus vulgaris, E: Listeria monocytogenes, F: S. typhimurium, G: Y. enterocolitica, H: Bacillus cereus, I: Enterobacter aerogenes, J: Bacillus subtilis, K: Streptococcus faecalis, L: Aeromonas hydrophila,Reference: Chloramphenicol.

Table 3
The antifungal activity of the compounds (μg/mL).

Comp.	A	B	C	D	E	F	G	H	I	J
5a	400	400	400	200	200	200	100	100	200	100
5b	400	400	400	200	100	200	200	200	200	200
5c	100	100	400	–	200	200	200	200	200	200
5d	100	100	400	400	200	100	200	200	100	100
5e	–	400	400	–	100	100	200	200	100	100
5f	–	–	–	–	200	200	200	200	200	200
5 g	–	–	–	–	200	200	200	100	200	200
6a	–	–	–	–	400	400	200	200	200	200
6b	–	–	–	–	200	200	200	200	200	100
6c	–	–	–	–	200	200	200	200	200	200
6d	–	400	400	–	100	200	100	200	100	200
6e	–	–	–	–	200	400	200	100	200	200
6f	–	–	–	–	200	200	200	200	200	200
Ref.	50	50	25	50	25	12.5	25	12.5	25	25

A: Candida albicans, B: Candida glabrata, C: Candida krusei, D: Candida parapsilosis, E: Penicillium citrinum, F: Penicillium expansum, G: Aspergillus niger, H: Aspergillus flavus, I: Aspergillus paraciticus, J: Aspergillus fumigatus, Reference: Ketoconazole.

3.3. Antimicrobial activity evaluation

The minimum inhibitory concentration (MIC) of the synthesized compounds (**5a-5g** and **6a- 6f**) were evaluated against bacterial and fungal species as illustrated in Table 2 and Table 3, respectively. The tested compounds showed generally more activity against filamentous fungi species compared to other bacterial or yeast species. Among these filamentous fungi species, *Aspergillus fumigatus* was the most sensitive one as four compounds that are **5a, 5d, 5e** and **6b** showed the same highest inhibition activity against it with MIC of 100 μg/mL. However, compound **5e** is the one that had the most significant activity (100 μg/mL) against four out of six filamentous fungi species that are *Penicillium*

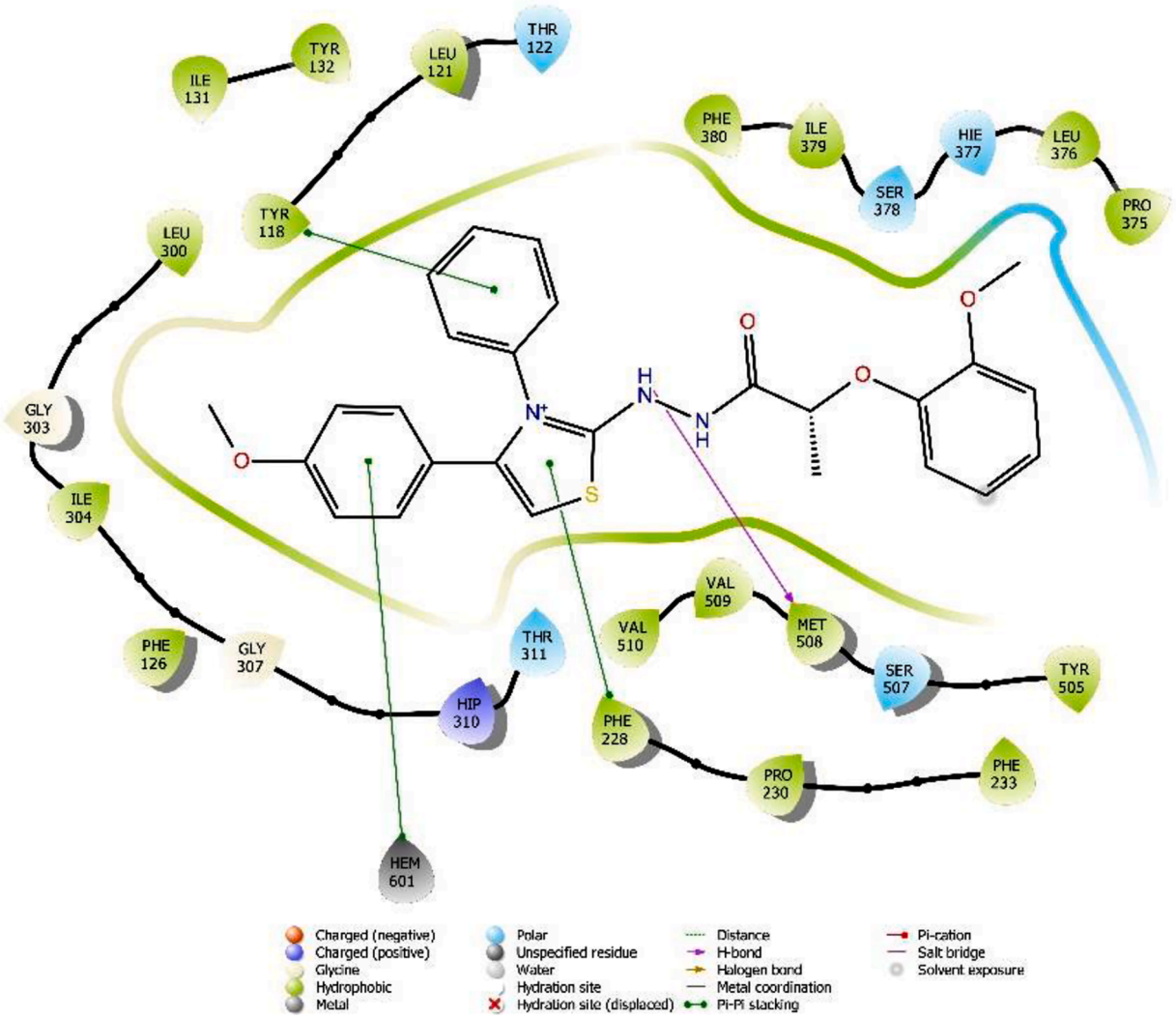


Fig. 2. 2D pose of **5c** in the active cavity of lanosterol 14α-demethylase (LDM, PDBID: *5TZ1*).

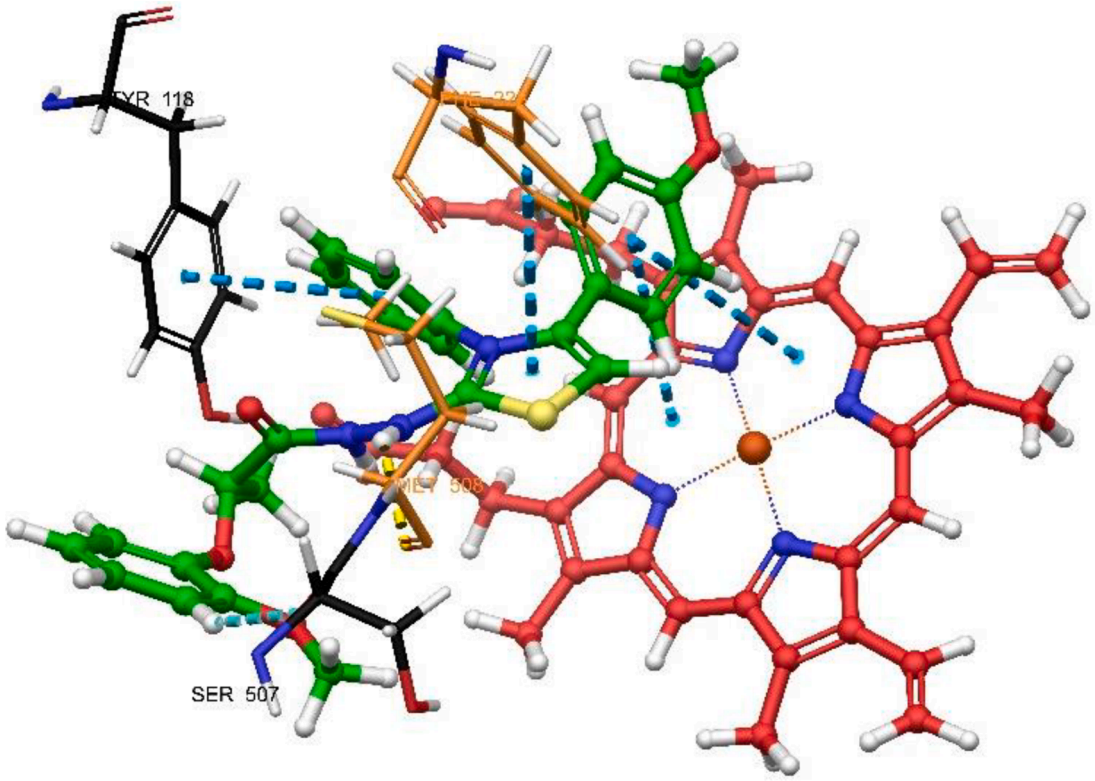


Fig. 3. 3D pose of **5c** in the active cavity of lanosterol 14α-demethylase (LDM, PDBID: *5TZ1*).

citrinum, Penicillium expansum, Aspergillus paraciticus and *Aspergillus fumigatus*. Compound **6d** showed the highest inhibition against *Penicillium citrinum, Aspergillus niger* and *Aspergillus flavus* with MIC of 100 μg /mL. In addition, compound **5b** showed high inhibition against *Penicillium citrinum,* compound **5a** against *Aspergillus niger* and compound **5d** against *Aspergillus flavus* with the same MIC value. On the other hand, both compounds **5c** and **5d** had the most significant activity (100 μg/mL) against *Candida albicans* and *Candida glabrata* as yeast species.

Among bacterial species, *Bacillus cereus* was the most sensitive one toward synthesized compounds. Remarkably, compound 5a is the most active one (200 μg/mL) compared to all other compound's activity, and more importantly, it is the only active one against *Y. enterocolitica* species which give it an advantage in term of selectivity profile.

The compounds chemically have two main structures; those containing thiazole **5a-5g** and those containing triazole **6a-6f**. In the compounds of the first group, the para substituents of the phenyl ring attached to the 3rd position of the thiazole constitute the difference. When the electronic effects of the substituents were evaluated among these compounds, **5c, 5d** and **5e** compounds containing inductively electron-withdrawing and resonance electron-donating substituents such as methoxy, chloro and fluoro exhibit higher antifungal activity than others. Compounds **5f** and **5g** containing resonance and inductively electron-withdrawing substituents (NO_2, CN) show the least antifungal potential. In the derivatives containing triazoles in the second group, the substituents in the sixth position of the benzothiazole in the acetamidobenzothiazole moiety connected from the 3rd position of the 1,2,4-triazole ring are different. When comparing the biological activity and the substituents among these compounds, it was seen that the fluoro-containing derivative (**6d**) has remarkable activity among others. When all compounds were evaluated in two main groups, it was determined that the compounds containing thiazole showed higher potential.

3.4. Molecular docking evaluation

In this study, possible interactions between ligands and enzymes were investigated to understand why derivatives have only moderate or poor, not better or excellent antifungal activity. In our previous studies [34–36], inhibitor agents against cytochrome enzymes like aromatase, MAO, 14-α lanosterol, etc., have similar shapes: there is one center atom, one azole and two other aromatic rings attached to that center atom.

Per the investigations conducted in relevant literature concerning lanosterol 14α-demethylase enzyme crystals, the enzymatic catalytic domain encompasses an excess of 450 amino acids. This domain encompasses a concealed iron-containing porphyrin moiety, contributing to a distinct region within the extensively embedded catalytic site [37]. Moreover, Tyr118 and HEM601 are essential for the activity of antifungal agents [27], they have been displayed in 5c and 5e within the catalytic site. Furthermore, the binding of **5c** and **5e** with the enzyme was strengthened by H-bonding of the hydrazide with Met508, while the thiazole ring reserved the interaction with Phe226 but they are insufficient to exhibit high activity and additional interactions are required.

Furthermore, clinically employed antifungal drugs (-conazoles) possess precisely this pharmacophore conformation. Despite the diversity in shapes reported across different studies [38–40], all these investigations uniformly underscore the indispensability of the π–cation interaction with the HEM moiety for exerting inhibitory activity.

Our docking results showed that the thiazole ring interacted with Phe226 via π-π stacking in all three compounds **5c, 5e** and **5d** while the phenyl ring at the 4th position of the thiazole interacted with HEM via π-π stacking in **5c** and **5e,** not **5d** The possible explanation is simply chlorine volume at the para position of the phenyl ring hindered the phenyl ring interaction with HEM as shown in Fig. 4.

However, all three compounds interacted with Tyr118 (π-π stacking), Phe226 (π-π stacking), Ser507 (Ar H-bond), and Met508 (H-bond). In

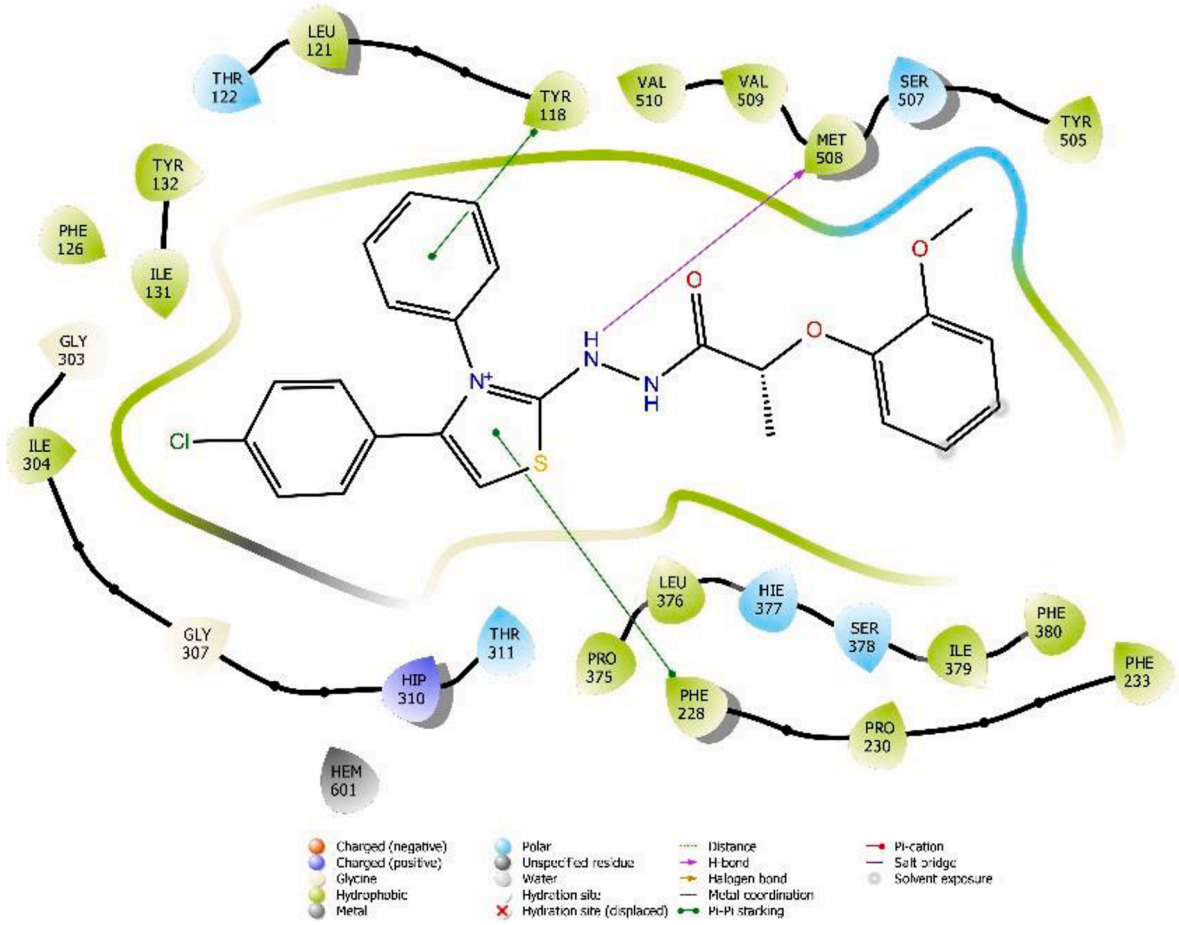


Fig. 4. 2D pose of **5d** in the active cavity of lanosterol 14α-demethylase (LDM, PDBID: *5TZ1*).

addition to that, compound **5d** interacted with Gly307 (Ar H-bond) while **5e** interacted with Tyr132 (Ar H-bond) and Phe380 (Ar H-bond).

In order to enhance efficacy in subsequent academic pursuits, it is suggested that the attachment of *N*-phenyl-4-(4-fluorophenyl) or *N*-phenyl-4-(4-methoxyphenyl)thiazole moieties, along with the hydrazide functional group, should be maintained at the fifth position of the thiazole ring. This strategic arrangement aims to facilitate the interaction of the ligand with the iron atom of HEM through π-cation bonding (Figs. 2–7).

3.5. Structure-activity relationship evaluation

The outcomes stemming from the analysis of biological efficacy and docking investigations have unveiled that the structural framework comprising a thiazole ring, wherein a hydrazide-Schiff base resides at the 2nd position and a distinct substituent-bearing phenyl ring at the 4th position of the thiazole nucleus, has demonstrated superior antifungal potency when contrasted with 1,2,4-triazole and benz/thiazole derivatives featuring an amide-thioether linkage.

Four notable attributes, demonstrating noteworthy efficacy, have been identified (Fig. 8). These attributes comprise the ionizable thiazole ring, an unsubstituted phenyl ring linked to the nitrogen of the thiazole ring, a hydrogen bond donating group like the hydrazide positioned at the 2nd position of the thiazole ring, and notably, the pivotal characteristic, the substituted phenyl ring at the 4th position of the thiazole ring.

The better activity and the broader spectrum have been demonstrated when the phenyl ring substituted with chloride at the *para* position (**5d**) exhibiting antifungal activity against *Candida glabrata, Candida krusei, Aspergillus paraciticus* and *Aspergillus fumigatus*. However, the interactions of compound **5d** have not been stabilized properly within the active cavity of lanosterol 14α-demethylase due to the absence of the interaction between the ligand and the porphyrin ring of the enzyme. Therefore, it is suggested that the activity of compound **5d** might be developed by another mechanism rather than interacting with the lanosterol 14α-demethylase enzyme.

Furthermore, it has been observed that compound **5c**, characterized by *p*-methoxyphenyl substitution, exhibits noteworthy efficacy against two Candida species, namely *Candida glabrata* and *Candida krusei*, at a concentration of 100 μg/mL. Notably, the activity and spectrum have been sharply decreased with unsubstituted and 4-fluoro substituted phenyl rings.

Additionally, **5f** and **5g** compounds which incorporate resonance and inductively electron-withdrawing substituents (NO_2, CN) have demonstrated the antifungal potential.

In conclusion, insertions of inductively electron-withdrawing and resonance electron-donating substituents such as methoxy, chloro and fluoro at the 4th position of the phenyl ring resulted in outstanding activity against different fungal species, as evidenced by the notable activity exhibited by compounds **5c, 5d**, and **5e**.

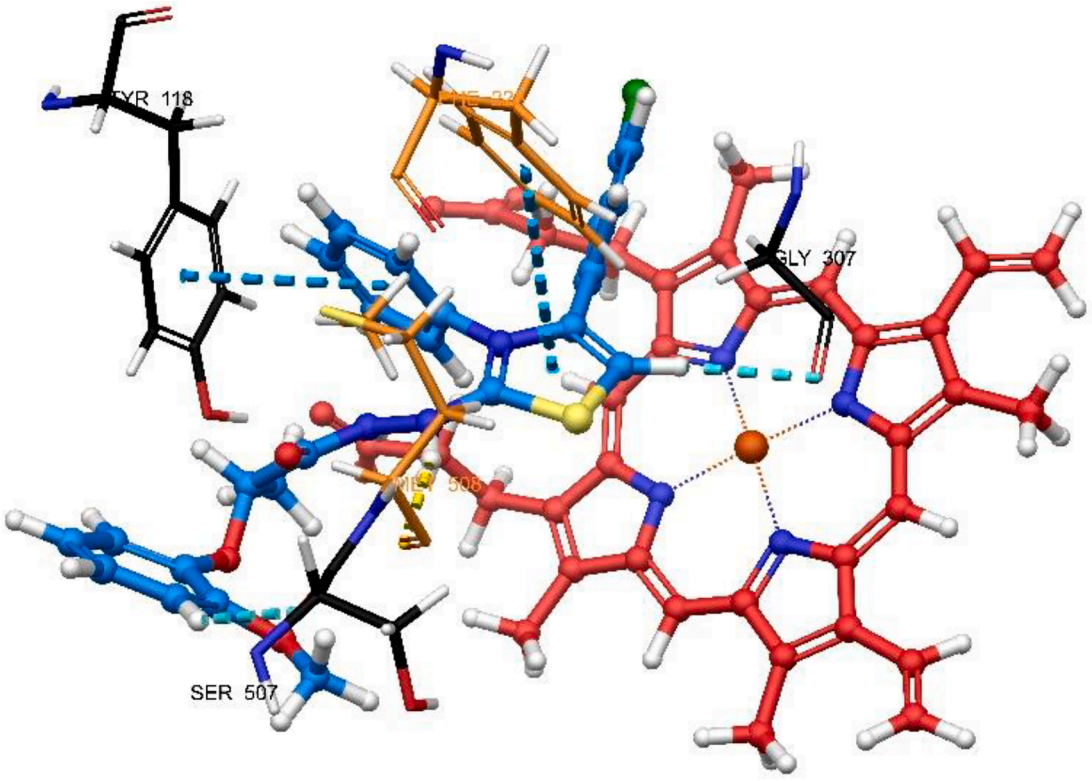


Fig. 5. 3D pose of **5d** in the active cavity of lanosterol 14α-demethylase (LDM, PDBID: *5TZ1*).

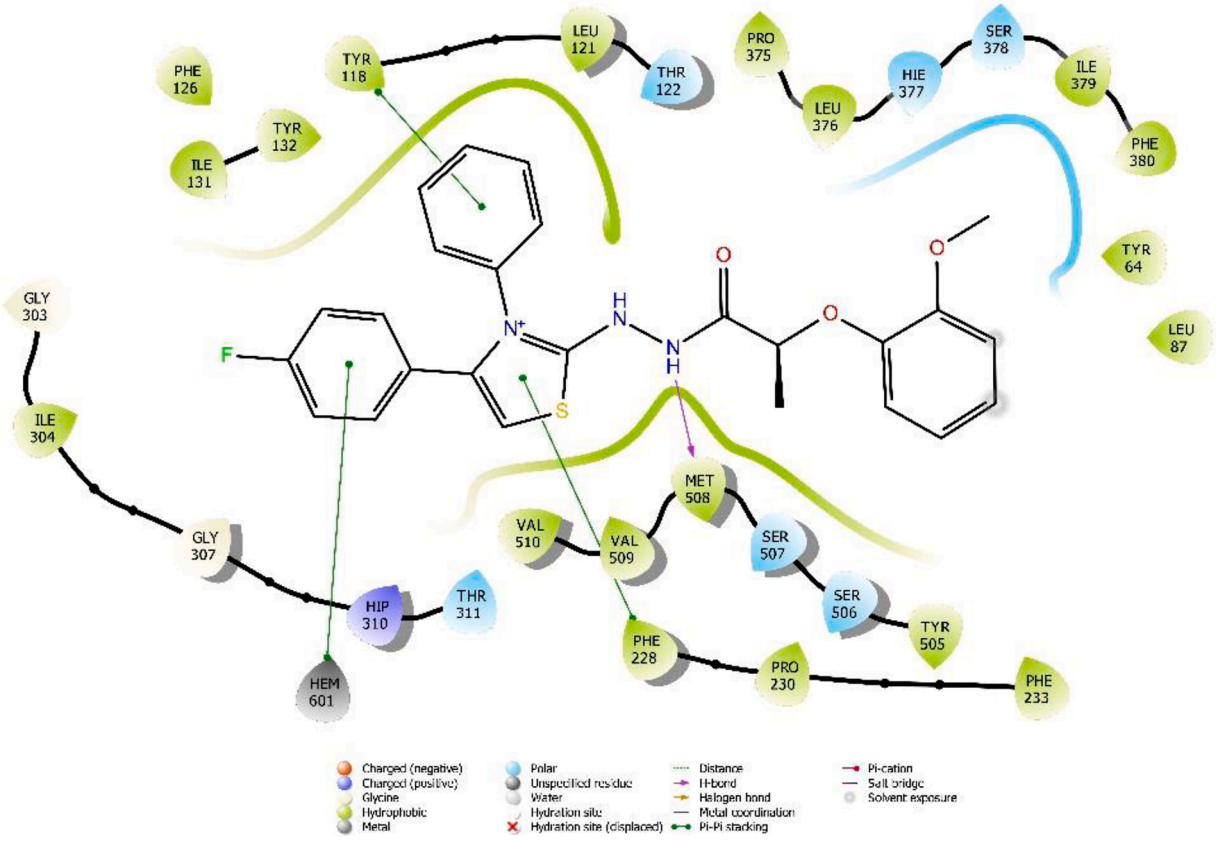


Fig. 6. 2D pose of **5e** in the active cavity of lanosterol 14α-demethylase (LDM, PDBID: *5TZ1*).

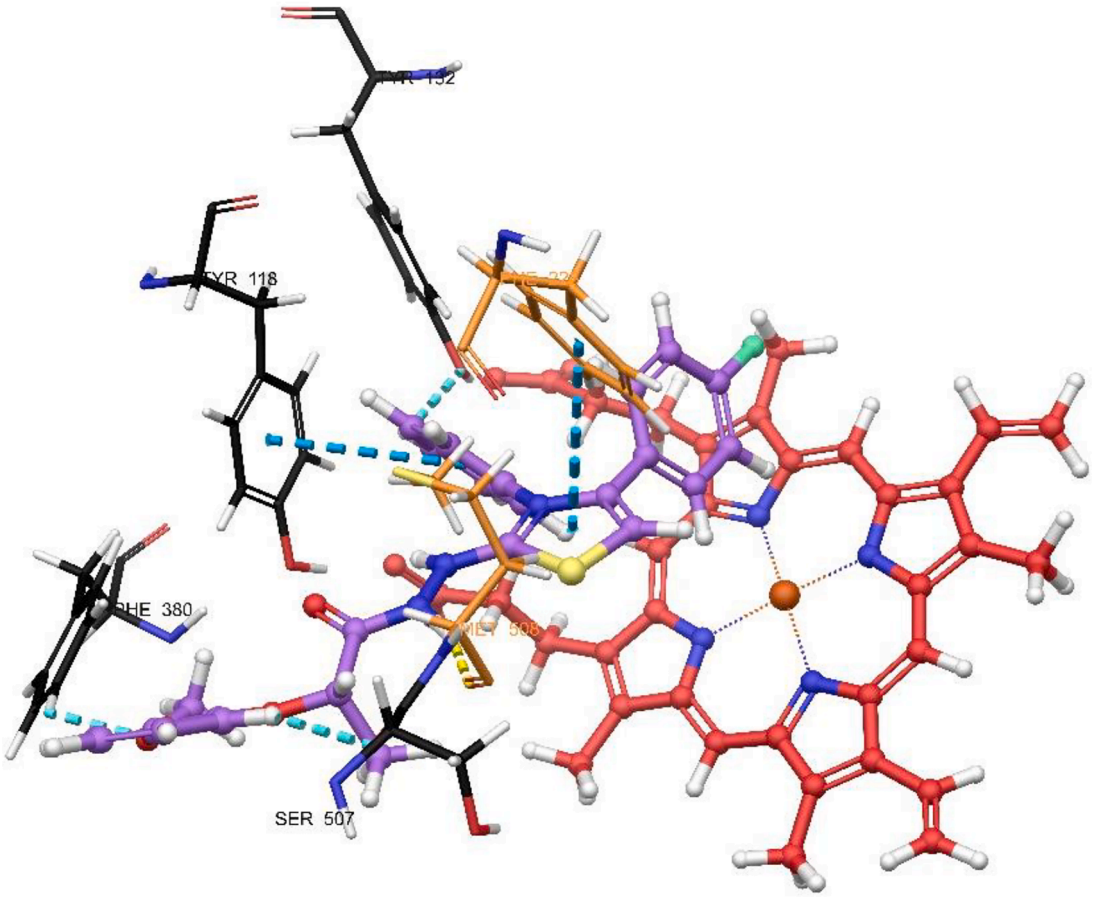


Fig. 7. 3D pose of **5e** in the active cavity of lanosterol 14α-demethylase (LDM, PDBID: *5TZ1*).

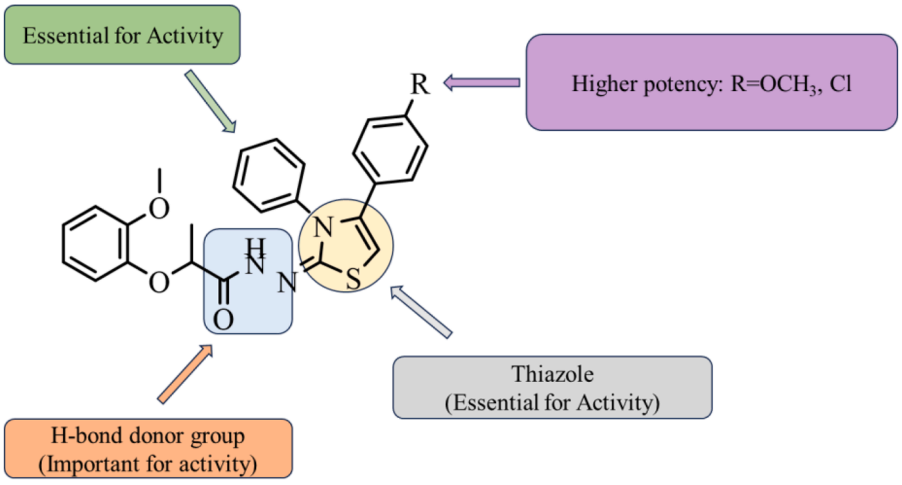


Fig. 8. Structure-activity relationship of thiazole derivatives.

4. Conclusion

A total of 13 compounds have been synthesized, characterized and biologically screened against twelve bacterial strains and ten fungal species. Seven compounds contain a thiazole ring and six have 1,2,4-triazole-benz/thiazole in their skeleton. Biological studies showed that all final compounds have demonstrated acceptable antimicrobial activities. Compound (**5a**) exhibited better activity and selectivity compared to other compounds against *Y. enterocolitica* bacterial strain. All compounds demonstrated good activity against filamentous fungi species especially compounds **5c, 5d**, and **5e** which have electronically similar substituents (–OCH_3, -Cl and -F) compared to yeast species. Molecular docking studies also supported the interactions of these compounds with lanosterol 14α-demethylase enzyme.

Declaration of Competing Interest

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 paper.

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2023.136675.

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