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Original Article

Synthesis, Molecular Docking Study and Antimicrobial Evaluation of Some 1,2,4-Triazole Compounds

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ABSTRACT

Aim: The objective of this study is synthesis of 1,2,4-triazole derivatives, evaluation there *in vitro* antimicrobial activity and showing the molecular docking. **Materials and Methods:** Two series of 5-alkylthio-3-aryl-4-phenyl-1,2,4-triazoles were successfully synthesized. The 1,2,4-triazole thiol was produced via cyclization reaction using the hydrazide compounds (2-hydroxybenzohydrazide and 5-bromofuran-2-carbohydrazide), which were then employed as nucleophilic species to attract various kinds of alkyl halides to synthesis the final compounds. All produced compounds underwent spectroscopic characterization and tested their antibacterial and antifungal activities. With synthetic ligands, molecular docking studies were conducted against the proteins 1AJ0, 1JIJ, and 4ZA5 to identify the crucial interactions underlying antimicrobial activity. **Results:** UV-visible, FTIR, 1H-NMR and CHNS analysis were confirmed the chemical structures and purity. Initial antibacterial screening results showed that several produced compounds **1e**, **1f**, **2e**, and **2f** have good inhibitory effects, whilst some compounds **2e** demonstrated high antifungal activity when compared to the control drug fluconazole. Two active substances, **1e** and **2e**, showed a moderate level of toxicity, with LD_{50} values of 3.5 and 2.3 g/kg, respectively. The strongest compounds, **1e**, **1f**, **2e**, and **2f**, demonstrated high binding energy antibacterial and antifungal activity, which were supported by docking analysis. **Conclusion:** Triazole derivatives were synthesized by multi-reaction steps with high yields. Some synthesized triazole derivatives showed promising antibacterial and antifungal activities as compared with parent compounds and standard drugs. The results of antimicrobial activities of compounds were enhanced by good affinity of molecular docking of these compounds with the active site of proteins as compared with standard drugs amoxicillin and fluconazole.

Keywords: Triazole, Antibacterial, Antifungal, Molecular docking, MIC, Modification of drug.

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INTRODUCTION

Because of their excellent therapeutic applications, triazoles stand out among heterocyclic nitrogen-containing compounds. There are two structural subtypes of five-membered triazoles: 1,2,3-triazole and 1,2,4-triazole.[1] Triazoles and their derivatives exhibit potent biological effects, including antimicrobial,[2-4] anti-TB,[5] anticancer,[6] anti-inflammatory,[7] and many other biological activities.[8-11] Contrarily, the special nature of the triazole ring also makes it frequently utilized in construct supramolecular drugs by producing supramolecular aggregates, which have shown substantial promise and garnered especial attention.[12,13] Triazole rings can also be used to link parts of pharmacophore parts, as well as produce hybrid molecules by linking two molecules of the drug, thus enhancing the activity of the molecule biologically and structurally. The triazole ring is one of essential azole like oxazole and thiazole used to synthesis various novel medicinal drugs.[14]

Many triazole derivatives were synthesized and evaluated their biological activities, five derivatives of coumarin triazole were synthesized and evaluated their activities as 5-LOX/ COX inhibitory effect, free-radical neutralizing effects, apoptotic-inducing effect, evaluated against four tumorous cell lines,[15] and antibacterial effect.[16]

The major cause of death for immunocompromised people with conditions like AIDS, cancer, and tuberculosis has been infections brought on by bacteria and fungus.[17] Amphotericin B is one example of an antibacterial triazole molecule that is used as a conventional antibiotic but also has negative effects on people. Despite being often used to treat fungal infections, antimicrobial drugs with distinct structural variants are losing their efficacy.[18] Other antifungals that are successful in treating persons

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with impaired resistance, such as those with AIDS, include itraconazole, allylamines, thiocarbamates, fluoropyrimidines, and the oral triazole antibiotic fluconazole.[19]

These observations prompted to synthesis a new class of antimicrobial agents with different chemical characteristics from those now in use in order to combat the emergence of antibiotic resistance. 2-Subtitiuted thio-1,2,4-triazole containing a 5-substituted aromatic (2-hydroxyphenyl and 5-bromofuran-2-yl) side chain is an ideal heterocyclic for antifungal activity. All newly synthesized triazole derivatives were screened for their antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and fungi *Aspergillus niger.* Also, these newly synthesized triazole derivatives were studied the molecular docking using 1AJ0, 1JIJ and 4ZA5 proteins.

MATERIALS AND METHODS

The chemicals were supplied by Fluka and Aldrich chemicals. Melting points were determined by open tube capillary method and were uncorrected. The purity of the compounds was checked on Thin Layer Chromatography (TLC) plates (silica gel G) in ethyl acetate:ethanol (8:2) and ethyl acetate:ethanol:hexane (7:2:1) solvent systems, the spots were located under iodine vapors and UV light. IR spectra were obtained on an 8400S SHIMADZU (Japan) FT-IR spectrometer (KBr pellets).[1] H-NMR spectra were recorded by a Bruker model ultra shield 300MHz (Switzerland) spectrometer using TMS as internal standard in DMSO-d_6 and UV spectra were recorded on a UV-visible Spectrophotometer Phoenix Range.

Synthesis of compounds

Synthesis of 3-(2-hydroxyphenyl)-4-phenyl-1,2,4-triazole-5-thiol, 1a and 3-(5-bromofuran-2-yl)-4-phenyl-1,2,4- triazole-5-thiol, 2a

a- Synthesis of thiosemicarbazide

At 60°C, phenyl iso-thiocyanate (0.01 mol) was added to a solution of acid hydrazide (2-hydroxybenzohydrazide or 5-bromofuran-2-carbohydrazide, 0.01 mol), in dioxane:ethanol (4:1) mixture. At room temperature, the reaction was stirred for 1 hr then at 60°C for 30 min. The separated crystals were filtered, washed with cold ethanol and dried at room temperature. The products were used in the next step without further purification.[20] The melting points of 2-(2-hydroxybenzoyl)-N-phenylhydrazinecarbothioamide and 2-(5-bromofuran-2-carbonyl)-N-phenylhydrazinecarbothioamide are 189-192°C and 169-173°C, respectively, as shown in Scheme 1.

b- Synthesis of compounds 1a and 2a

Equimolar of thiosemicarbazide and sodium hydroxide in 100 mL of distilled water was refluxed for 2 hr. The resulting solution was filtered to remove unreacted materials then treated with 2M HCl cold solution to reach pH 4. The crystals were separated, filtered and washed with water. The resultant solid was then dried at room temperature after recrystallization from methanol,[20] as shown in Scheme 1.

1a: Yellowish white crystals, 85.93% yield, m. p. 192-194°C, R_f 0.82, UV λ_{max} (Ethanol) 228, 267, 303 nm, IR (KBr): ν (cm^{-1}) = 3278 (O-H), 3100 (C-H, aromatic), 2761 (S-H), 1616 (C=N, triazole ring), 1583, 1537 (C=C), 1242 (C-N). ^{1}H NMR (DMSO-d_6): δ (ppm) = 10.25 (s, 1H, OH), 7.20-6.72 (m, 4H, *J* = 7.2 Hz, 2-hydroxyphenyl), 7.45-7.31 (m, 5H, phenyl-N). Anal. Calc. (Found) for $C_{14}H_{11}N_3O_S$ (269.32): C, 62.44 (62.31), H, 4.12 (4.17), N, 15.60 (15.57), S, 11.90 (11.84).

2a: Yellowish white crystals, 85.93% yield, m. p. 192-194°C, R_f 0.72, UV λ_{max} (Ethanol) 214, 258, 300 nm, IR (KBr): ν (cm^{-1}) = 3090 (C-H, aromatic), 2768 (S-H), 1650 (C=N, triazole ring), 1595, 1523 (C=C), 1278 (C-N), 1130, 989 (C-O-C, sym. & asym. Furan ring).[1] HNMR (DMSO-d_6): δ (ppm) = 7.62-7.35 (m, 5H, phenyl-N), 6.63 (d, 1H, *J* = 3.6 Hz, furan ring), 6.06 (d, 1H, *J* = 3.6 Hz, furan ring). Anal. Calc. (Found) for $C_{12}H_8BrN_3OS$ (322.18): C, 44.74 (44.62), H, 2.50 (2.46), N, 13.04 (12.99), S, 9.95 (9.98).

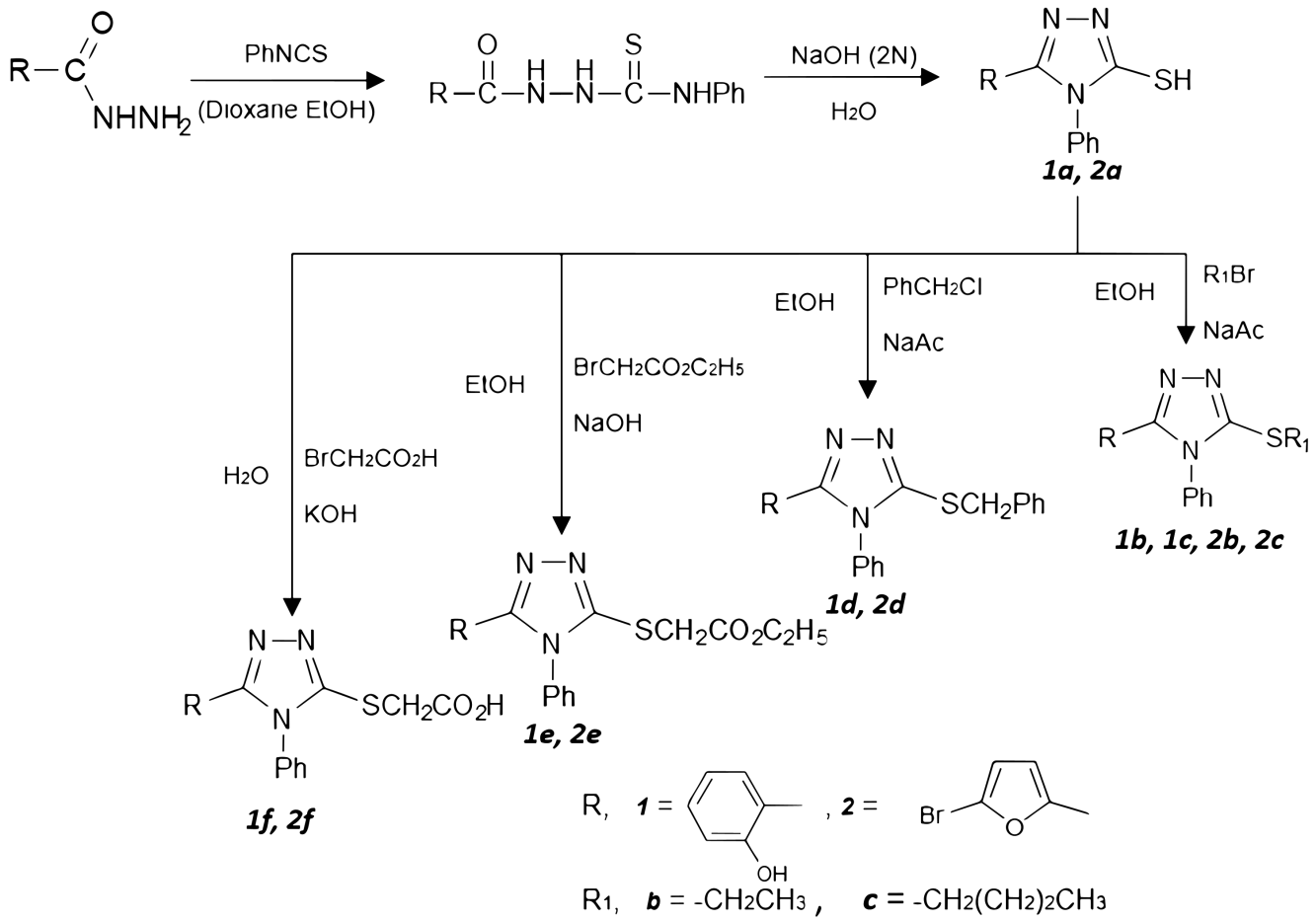
Synthesis of alkylthio-triazole derivatives (General procedure)

The compounds, 5-ethylthio-3-(2-hydroxyphenyl)-4-phenyl-1,2,4-triazole (**1b**), 5-butylthio-3-(2- hydroxyphenyl)-4-phenyl-1, 2,4-triazole (**1c**), 5-ethylthio-3-(5-bromofuran-2-yl)-4-phenyl-1,2,4-triazole (**2b**) and 5-butylthio-3-(5-bromofuran-2-yl)-4 -phenyl-1,2,4-triazole (**2c**) were synthesized by the same procedure.[21]

A mixture containing **1a** or **2a** 0.018 mol of ethyl or butyl bromide mixed with 0.02 mol of sodium acetate in 100 mL round bottom flask containing 50 mL of absolute ethanol was refluxed for 3 hr. The reaction mixture was poured into 100 mL of ice-filled cold water after being allowed to cool. The resulting product was recrystallized from ethanol, as shown in Scheme 1.

1b: White crystals, 72.46% yield, m. p. 134-136.5°C, R_f 0.65, UV λ_{max} (Ethanol) 225, 268, 300 nm, IR (KBr): ν (cm^{-1}) = 3246 (O-H), 3037 (C-H, aromatic), 2956, 2933 (C-H, aliphatic), 1600 (C=N, triazole ring), 1596, 1490 (C=C), 1265 (C-N).[1] HNMR (DMSO-d_6): δ (ppm) = 10.24 (s, 1H, OH), 7.23-6.71 (m, 4H, *J* = 7.2 Hz, 2-hydroxyphenyl), 7.42-7.33 (m, 5H, phenyl-N), 3.14 (q, 2H, -CH_2-S-), 0.88 (t, 3H, -CH_3). Anal. Calc. (Found) for $C_{16}H_{15}N_3OS$ (297.38): C, 64.62 (64.62), H, 5.08 (5.15), N, 14.13 (14.21), S, 10.78 (10.69).

1c: Bright white crystals, 62.57% yield, m. p. 149-151°C, R_f 0.64, UV λ_{max} (Ethanol) 224, 269, 302 nm, IR (KBr): ν (cm^{-1}) = 3262 (O-H), 3037 (C-H, aromatic), 2956, 2933 (C-H, aliphatic), 1598 (C=N, triazole ring), 1598, 1490 (C=C), 1271 (C-N).[1] HNMR (DMSO-d_6): δ (ppm) = 10.23 (s, 1H, OH), 7.13-6.73 (m, 4H, *J* = 7.2 Hz, 2-hydroxyphenyl), 7.47-7.29 (m, 5H, phenyl-N),



Scheme 1: Synthesis of the compounds.

3.15 (t, 2H, CH_2-S-, *J*=7.2 Hz), 1.65 (q, 2H, S-CH_2CH_2), 1.42 (sx, 2H, -CH_2CH_3), 0.91 (t, 3H, -CH_3). Anal. Calc. (Found) for $C_{18}H_{19}N_3OS$ (325.43): C, 66.47 (66.38), H, 5.88 (5.76), N, 12.92 (12.95), S, 9.85 (9.85).

2b: White crystals, 82.82% yield, m. p. 107-109°C, R_f 0.67, UV λ_{max} (Ethanol) 217, 271, 295 nm, IR (KBr): ν (cm^{-1}) = 3060 (C-H, aromatic), 2965, 2923 (C-H, aliphatic), 1617 (C=N, triazole ring), 1593, 1504 (C=C), 1267 (C-N), 1122, 1027 (C-O-C, sym. & asym. Furan ring).[1] H NMR (DMSO-d_6): δ (ppm) = 7.63-7.33 (m, 5H, phenyl-N), 6.62 (d, 1H, *J* = 3.6 Hz, furan ring), 6.06 (d, 1H, *J* = 3.6 Hz, furan ring), 3.16 (q, 2H, -CH_2-S-), 0.92 (t, 3H, -CH_3). Anal. Calc. (Found) for $C_{14}H_{12}BrN_3OS$ (350.23): C, 48.01 (48.10), H, 3.45 (3.47), N, 12.00 (11.97), S, 9.15 (9.16).

2c: White plated crystals, 70.98% yield, m. p. 90-93 °C, R_f 0.65, UV λ_{max} (Ethanol) 218, 271, 290 nm, IR (KBr): ν (cm^{-1}) = 3103 (C-H, aromatic), 2958, 2922 (C-H, aliphatic), 1613 (C=N, triazole ring), 1592, 1502 (C=C), 1269 (C-N), 1121, 1117 (C-O-C, sym. & asym. Furan ring).[1] H NMR (DMSO-d_6): δ (ppm) =7.62-7.47 (m, 5H, phenyl-N), 6.62 (d, 1H, *J* = 3.6 Hz, furan ring), 6.06 (d, 1H, *J* = 3.6 Hz, furan ring), 3.12 (t, 2H, CH_2-S-, *J*=7.2 Hz), 1.63 (q, 2H, S-CH_2CH_2), 1.34 (sx, 2H, -CH_2CH_3), 0.85 (t, 3H, -CH_3). Anal. Calc. (Found) for $C_{16}H_{16}BrN_3OS$ (378.29): C, 50.80 (50.73), H, 4.26 (4.26), N, 11.11 (11.20), S, 8.48 (8.57).

Synthesis of benzythiol-triazole derivatives (General procedure)

The compounds 5-benzylthio-3-(2-hydroxyphenyl)-4-phenyl-1,2,4-triazole (**1d**) and 5-benzylthio-3-(5-bromofuran-2-yl)-4-phenyl-1,2,4-triazole (**2d**) were synthesized by the same procedure.

An ethanolic solution of benzyl chloride 0.01 mol was added gradually to a solution containing mixture of 0.01 mol of **1a** or **2a** and 0.05 mol of sodium acetate dissolved in 30 mL of absolute ethanol. The total solution was heated under reflux for 4 hr. After the content was put into crushed ice, it separated into a solid mass that was filtered and recrystallized from ethanol,[22] as shown in Scheme 1.

1d: White needle crystals, 78.94% yield, m. p. 180-183°C, R_f 0.51, UV λ_{max} (Ethanol) 228, 257, 294 nm, IR (KBr): ν (cm^{-1}) = 3260 (O-H), 3058 (C-H, aromatic), 2933, 2837 (C-H, aliphatic), 1600 (C=N, triazole ring), 1585, 1487 (C=C), 1247 (C-N).[1] H NMR (DMSO-d_6): δ (ppm) = 10.21 (s, 1H, OH), 7.43-6.74 (m, 14H, *J* = 7.2 Hz, aromatic systems), 4.41 (s, 2H, -CH_2-S-). Anal. Calc.

(Found) for $C_{21}H_{17}N_3OS$ (325.43): C, 70.20 (70.43), H, 4.76 (4.69), N, 11.69 (11.60), S, 8.92 (8.89).

2d: Yellowish white crystals, 86.36% yield, m. p. 115-118°C, R_f 0.49, UV λ_{max} (Ethanol) 219, 272, 290 nm, IR (KBr): ν (cm^{-1}) = 3055 (C-H, aromatic), 2930 (C-H, aliphatic), 1595 (C=N, triazole ring), 1590, 1503 (C=C), 1276 (C-N), 1128, 1016 (C-O-C, sym. & asym. Furan ring). ^{1}H NMR (DMSO-d_6): δ (ppm) =7.61-7.27 (m, 10H, phenyl-N and benzyl ring), 6.62 (d, 1H, *J* = 3.6 Hz, furan ring), 6.06 (d, 1H, *J* = 3.6 Hz, furan ring), 4.39 (s, 2H, CH_2-S-). Anal. Calc. (Found) for $C_{19}H_{14}BrN_3OS$ (412.31): C, 55.35 (55.42), H, 3.42 (3.38), N, 10.19 (10.26), S, 7.78 (7.84).

Synthesis of ethoxycarbonylmethylthio-triazole derivatives (General procedure)

The compounds 5-ethoxycarbonylmethylthio-3-(2-hydroxyphenyl)-4-phenyl-1,2,4-triazole (**1e**) and 5-ethoxycarbonylmethylthio-3-(5-bromofuran-2-yl)-4-phenyl-1,2,4-triazole (**2e**) were synthesized by the same procedure.

In 50 mL round bottom flask, equimolar **1a** or **2a** and sodium hydroxide was dissolved in 30 mL of absolute ethanol was stirred vigorously with heating for 30 min. An amount of 0.01 mol ethyl bromoacetate was added dropwise and the solution was heated under reflux for 4 hr. A white solid was appeared when the solution was added to crushed ice water. The pure product was formed after recrystallization from ethanol,[23] as shown in Scheme 1.

1e: White cotton crystals, 79.23% yield, m. p. 154-156°C, R_f 0.61, UV λ_{max} (Ethanol) 222, 254, 288 nm, IR (KBr): ν (cm^{-1}) = 3275 (O-H), 3039 (C-H, aromatic), 2974, 2935 (C-H, aliphatic), 1735 (C=O, ester), 1605 (C=N, triazole ring), 1598, 1494 (C=C), 1288 (C-N).[1] H NMR (DMSO-d_6): δ (ppm) = 10.13 (s, 1H, OH), 7.26-6.76 (m, 4H, *J* = 7.2 Hz, 2-hydroxyphenyl), 7.50-7.29 (m, 5H, phenyl-N), 4.11 (s, 2H, -CH_2-S-), 4.13 (q, 2H, -O-CH_2-, *J*=7.5 Hz), 1.20 (t, 3H, -CH_3, *J*=7.5 Hz). Anal. Calc. (Found) for $C_{18}H_{17}N_3O_3S$ (355.41): C, 60.83 (60.75), H, 4.82 (4.83), N, 11.82 (11.91), S, 9.02 (8.97).

2e: White cotton crystals, 81.68% yield, m. p. 62-64°C, R_f 0.51, UV λ_{max} (Ethanol) 215, 270, 295 nm, IR (KBr): ν (cm^{-1}) = 3125 (C-H, aromatic), 2940 (C-H, aliphatic), 1730 (C=O, ester), 1615 (C=N, triazole ring), 1600, 1510 (C=C), 1280 (C-N), 1143, 1010 (C-O-C, sym. & asym. Furan ring). ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.63-7.34 (m, 5H, phenyl-N), 6.62 (d, 1H, *J* = 3.6 Hz, furan ring), 6.05 (d, 1H, *J* = 3.6 Hz, furan ring), 4.23 (s, 2H, -CH_2-S-), 4.10 (q, 2H, -O-CH_2-, *J*=7.5 Hz), 1.27 (t, 3H, -CH_3, *J*=7.5 Hz). Anal. Calc. (Found) for $C_{16}H_{14}BrN_3O_3S$ (408.27): C, 47.07 (47.11), H, 3.46 (3.45), N, 10.29 (10.31), S, 7.85 (7.82).

Synthesis of carboxymethylthio-triazole derivatives (General procedure)

The compounds 5-carboxymethylthio-3-(2-hydroxyphenyl)-4-phenyl-1,2,4-triazole (**1f**) and 5-carboxymethylthio-3-(5-bromofuran-2-yl)-4-phenyl-1,2,4-triazole (**2f**) were synthesized by the same procedure.

A mixture containing equimolar of **1a** or **2a**, monobromoacetic acid and 30 mL of ethanolic KOH solution was subjected to heating under reflux for 3 hr. The resulting hot solution was left to cool then treated with 2 M of HCl to achieve pH (3-4). The precipitated solid product was filtered out, washed twice with distilled water and finally recrystallized from mixture of ethanol:water (5:1).[24]

1f: White powder, 61.30% yield, m. p. 219-221°C, R_f 0.66, UV λ_{max} (Ethanol) 222, 259, 297 nm, IR (KBr): ν (cm^{-1}) = 3270 (O-H), 3068 (C-H, aromatic), 2925 (C-H, aliphatic), 1726 (C=O, carboxyl), 1600 (C=N, triazole ring), 1587, 1492 (C=C), 1250 (C-N).[1] H NMR (DMSO-d_6): δ (ppm) = 12.52 (s, 1H, COOH), 10.18 (s, 1H, OH), 7.13-6.65 (m, 4H, *J* = 7.2 Hz, 2-hydroxyphenyl), 7.34-7.28 (m, 5H, phenyl-N), 4.32 (s, 2H, -CH_2-S-). Anal. Calc. (Found) for $C_{16}H_{13}N_3O_3S$ (359.45): C, 58.70 (58.77), H, 4.00 (3.92), N, 12.84 (12.91), S, 9.80 (9.87).

2f: Yellowish white powder, 64.97% yield, m. p. 210-213°C, R_f 0.58, UV λ_{max} (Ethanol) 220, 270, 297 nm, IR (KBr): ν (cm^{-1}) = 3115 (C-H, aromatic), 2960 (C-H, aliphatic), 1724 (C=O, carboxyl), 1612 (C=N, triazole ring), 1594, 1504 (C=C), 1276 (C-N), 1140, 1016 (C-O-C, sym. & asym. Furan ring). ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.60-7.42 (m, 5H, phenyl-N), 6.63 (d, 1H, *J* = 3.6 Hz, furan ring), 6.07 (d, 1H, *J* = 3.6 Hz, furan ring). 4.51 (s, 2H, -CH_2-S-). Anal. Calc. (Found) for $C_{14}H_{10}BrN_3O_3S$ (380.22): C, 44.23 (44.16), H, 2.65 (2.62), N, 11.05 (11.01), S, 8.43 (8.47).

Antimicrobial activity

Microorganisms

The antibacterial activity for all synthesized compounds was investigated by using the standard bacterial species, Gram-negative *Escherichia coli* (ATCC 25922) and Gram-positive *Staphylococcus aureus* (ATCC 25923), whereas antifungal activity investigated by using the isolated pathogenic fungus *Aspergillus niger*.

Preliminary antibacterial assay

By using the diffusion process using filter paper discs, the antibacterial properties of the produced compounds were studied. Each component was dissolved in 1000 µg/mL of dimethyl sulfoxide solvent to create a stock solution, which was then kept at 4 to 8°C until it was needed. A sterile cotton swab was dipped into the inoculum and evenly streaked over the entire surface of the agar plates in the Petri dishes to inoculate them. Filter paper discs (6 mm in diameter) were impregnated with a 1000 µg/mL solution of the investigated chemicals, dried, and then

put on an agar plate with lawn cultures of certain bacteria. The plates were incubated for 24 hr at the recommended temperature for growth (37°C), after which the zone of microbial growth inhibition surrounding the discs was measured (in mm), and its results were compared with those obtained using amoxicillin and streptomycin.[25,26]

Determination of the produced compounds' inhibitory zone

The MIC for first active substances was determined using the filter paper disc diffusion method. By dilution from stock solutions (1000 µg/mL), various concentrations of each component (500, 400, 300, 200, and 100 µg/mL) in dimethyl sulfoxide were created. Filter paper discs (6 mm in diameter) were impregnated with various concentrations of the active compound's solution, dried, and then put on Mueller Hinton agar plates with lawn cultures of certain bacteria. The visible zone of microbial growth inhibition surrounding the discs was measured in millimeters following a 24 hr incubation period at 37°C.[27]

Preliminary antifungal assay

The fungus *Aspergillus niger* was used as a test subject for the synthetic compounds' antifungal activity, which was determined using the agar diffusion method at a concentration of 1000 µg/mL in dimethyl sulfoxide solvent. Dextrose agar made of Sabouraud was the medium in this case. Using a stainless steel, sterile cutting tool (cork borer), wells measuring 6 mm in diameter were created. 100 µL of each compound was then put to each well. Inhibition zone diameters in mm were measured after 5-7 days of incubation at 25°C, and the results were compared to those obtained with fluconazole.[28]

Medium lethal dose (LD_{50})

Swiss albino mice BALB/c, weighing about 25 g, were housed under controlled conditions and were used to test the medium lethal dosage (LD_{50} research of various active compounds). Six groups of five mice each were created by dividing the mice. The other five groups served as the tested groups, while one of the six groups served as the control. The control group received 0.5 mL of the olive oil orally through a stomach tube, whereas the other groups received dosages of each component dissolving in 0.5 mL of olive oil in the range of 1-5 g/kg body weight. The mortality data were gathered after a 72 hr observation period in each group.[29]

Docking studies

Using ChemDraw Software 2016, all compounds were predicted. Using MOE 2015 v10, all ligands and water molecules were eliminated during the molecular docking of all triazole compounds. Crystal structure of a ternary complex of *E. coli* dihydropteroate synthase DHPS (protein ID: 1AJ0), crystal structure of *S. aureus* tyrosyl-tRNA synthetase TyrRS in complex with SB-239629 (proteins ID: 1JIJ) and crystal structure of *A. niger* ferulic acid decarboxylase (Fdc1) (proteins ID: 4ZA5) were obtained from RCSB protein data bank.

RESULTS AND DISCUSSION

Chemistry

A series of triazoles was prepared using a series of successive reactions, starting with hydrazide compounds. The first reaction involves the reaction of hydrazide with phenyl isothiocyanate using a mixture of ethanol and dioxane as solvents to give thiosemicarbazide compounds. The reaction involves a nucleophilic attack of the amine group in the hydrazide on the thionyl carbon atom of the phenyl isothiocyanate compound.

The second reaction includes the cyclization of open compound thiosemicarbazide in alkaline medium (2N NaOH) to give 1,2,4-triazole thiolate intermediate which converted to 1,2,4-triazole thiol by treating with diluted acid, Scheme 2 depicted the mechanism pathway.[30]

The final compounds **1a** and **2a** were used as starting materials to synthesis their S-substituted derivatives (**1b**-**1f** and **2b**-**2f**) using different substituted halide compounds in the presence of sodium acetate as a weak base. The reactions involved nucleophilic substitution reaction where compounds **1a** or **2a** were attack the electron deficient carbon of halide compounds. The resulting final derivatives were white to yellowish white solid and the yields of reactions were 61.30-86.36%.

The UV-visible spectrophotometer was used to evaluate all of the produced compounds. It revealed three primary absorption bands in the wavelength ranges of 214-228 nm, 254-272 nm, and 288-303 nm, which were attributed to the electronic transition of the different types of aromatic systems in the compounds.[31,32]

The synthesized compounds were identified using FT-IR spectroscopy, the non-substituted compounds were characterized by a medium absorption band at 2761 cm^{-1} and 2768 cm^{-1} which attributed to S-H stretching vibration of **1a** and **2a**, respectively. All IR spectra of compounds showed strong-medium bands at 1650-1595 cm^{-1}, 1600-1487 cm^{-1} and 1288-1242 cm^{-1} which referred to stretching vibrations of C=N, C=C and C-N of the (triazole) N-C(phenyl), respectively.[33] The spectra of salicylic-triazole compounds showed a broad band at 3278-3246 cm^{-1} attributed to O-H stretching of 2-hydroxyphenyl group which at the lower frequency due to intramolecular H-bonding[34] with N atom of triazole ring. Strong absorption bands at 1735-1724 cm^{-1}, which are linked to the ester and carboxyl groups in the compounds **1e**, **1f**, **2e**, and **2f**, are a distinguishing feature of these compounds.

^{1}H-NMR spectra of the butylthio-triazole compounds **1c** and **2c**, these compounds had the typical aliphatic system that produced triplet signals at 0.859-0.874 ppm in relation to the protons of the -CH_3 group, multiplet signals at 1.340-1.359 ppm due to the

protons of -CH_2- group which coupled with adjacent protons of -CH_2- and -CH_3 groups, multiplet signals at 1.632-1.651 ppm related to the protons -CH_2- which coupled with adjacent protons of -CH_2- and -CH_2-S groups and triplet signals at 3.129-3.150 ppm related to the protons of -CH_2-S group coupled with adjacent protons of -CH_2- group. The fourth signal resonated at the lowest field due to the deshielding of adjacent sulfur atom[35] as compared with the first three signals. The coupling constant for these four signals was *J*=7.2 Hz.

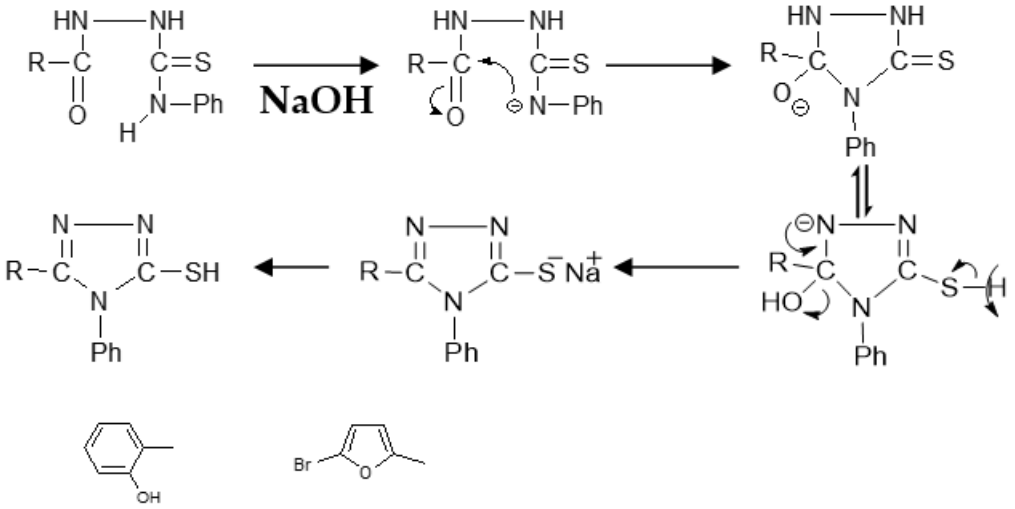
The aromatic system of compound **1c** (Figure 1-a) exhibited multiplet signal at 6.733-6.810 ppm related to the H-5 and H-3, doublet signal at 7.134 ppm and multiplet signal at 7.224 ppm due to the H-6 ($J_{6,5}$=7.2 Hz) and H-4 protons of 2-hydroxyphenyl group, respectively. Two multiplet signals appeared, the first at 7.297-7.328 ppm which attributed to the two protons H-3' and H-5', and the second at 7.458-7.479 ppm related to protons H-2', H-4' and H-6' of phenyl ring. The last two multiplet signals of protons of the phenyl ring reflected the electron withdrawing effect of triazole ring on the chemical shift of these five protons. The singlet signal at 10.239 ppm was due to intramolecular hydrogen bonded proton of OH of 2-hydroxyphenyl group.[36]

The ^{1}H-NMR spectra of compound **2c** (Figure 1-b) showed characteristic doublet signals at 6.067 ppm and 6.627 ppm related to the protons H-a and H-b of 5-bromofuran ring with $J_{a,b}$=$J_{b,a}$= 3.6 Hz. The protons H-a and H-b exhibited AX coupling system. Another multiplet signals at 7.478-7.509 ppm and 7.575-7.623 ppm related to the protons (H-3 and H-5) and (H-2, H-4 and H-6) of phenyl ring, respectively.

^{1}H-NMR spectra of the benzylthio-triazole compounds (**1d** and **2d**) exhibited characteristic singlet signal at 4.392-4.410 ppm attributed to the protons of methylene in benzyl group. The ^{1}H-NMR spectrum of **1d** (Figure 1-c) exhibited multiplet signal at 6.741-6.795 ppm related to the protons H-3 and H-5 of salicylic ring. Another multiplet signal appeared at 7.128-7.223 ppm related to the protons H-4, H-6 (2-hydroxyphenyl ring), H-3' and H-5' (phenyl ring). Multiplet signals appeared at 7.248-7.367 ppm and at 7.418-7.435 ppm related to the aromatic protons in fragment of benzyl and protons (H-2', H-4' and H-6') of phenyl ring, respectively. Singlet signal appeared at 10.21 ppm related to proton of hydroxyl group of 2-hydroxyphenyl ring.

The NMR spectra of compound **2d** (Figure 1-d) showed characteristic doublet signals, the first signal at 6.067 ppm and the second signal at 6.622 ppm. The first signal related to the proton H-a and the second signal related to the proton H-b of 5-bromofuran ring and these two proton exhibited AX coupling system[37,38] with coupling constant $J_{a,b}$=$J_{b,a}$=3.6 Hz, as shown in Figures 3-59 to 3-60. Multiplet signal as 7.273-7.356 ppm which attributed to the protons H-3 and H-5 of phenyl ring and the protons of the benzyl ring. Another multiplet signal appeared at 7.541-7.612 ppm which attributed to the protons H-2, H-4 and H-6 of phenyl ring.

The compound **1e** (Figure 1-e) exhibited characteristic aliphatic signals, triplet and quartet signals at 1.203 and 4.161 ppm related to the protons of -CH_3 group -O-CH_2- groups, respectively, and singlet signal at 4.275 ppm attributed to the protons of -S-CH_2- group. The aromatic system gave multiplet signals at 6.760-6.808 ppm and 7.162-7.264 ppm which attributed to the protons (H-3 and H-5) and (H-4 and H-6) of 2-hydroxyphenyl group, respectively. Another Multiplet signals appeared at 7.299-7.348 ppm and 7.460-7.506 ppm which referred to the protons (H-3' and H-5') and (H-2', H-4' and H-6') of phenyl ring, respectively. A singlet signal appeared at 10.133 ppm which referred to the proton of OH of 2-hydroxyphenyl group.



Scheme 2: Mechanism pathway of 1,2,4-triazole synthesis.

Antibacterial activity of triazole compounds

From Table 1, we observed that the compound **1e** exhibited good activity against *S. aureus* and *E. coli* with inhibition zones 22 mm and 28 mm, respectively, and this compound was more potent that the parent triazole **1a**, whereas it had less activity comparable to that of streptomycin and amoxicillin. The compounds **2e** and **1f** exhibited inhibition zones 18 and 21 mm, respectively, against Gram-positive bacteria and these compounds have more potent than their parents triazole.

Antifungal activity of triazole compounds

The compound **2e** gave good fungicidal activity with inhibition zone 27 mm against the fungus as compared with the standard drug fluconazole 23 mm and the parent **2a** 10 mm, whereas the compound **1e** and **2f** exhibited inhibition zone 12 and 15 mm, respectively, which was less than fluconazole and greater than the parent triazole, as shown in Table 1.

Minimum Inhibitory Concentration (MIC)

The MIC activities of the triazole series were performed against Gram-negative and Gram-positive bacterial strains. We found that compound **1e** had the best activity against *E. coli* at low concentrations (200 µg/mL), but compounds **1f** and **2e** had an activity against *S. aureus* at 300 µg/ml. In general, S-substituted compounds of triazole gave an activity against the two bacterial strains, specially the derivatives of mercaptoesters and mercapto acids and these two types of compounds which have ester or carboxyl ends can open an area for further modification at these

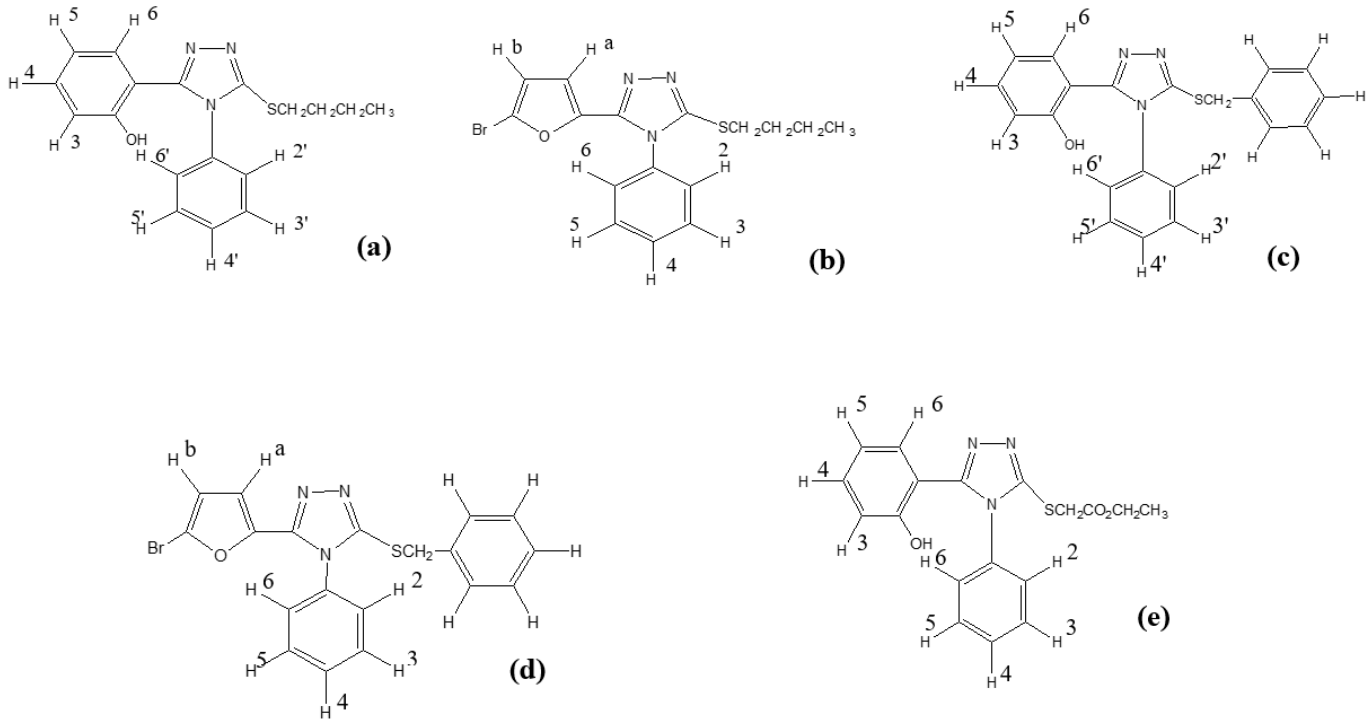


Figure 1: Chemical structures of compounds, a: 1c, b: 2c, c: 1d, d: 2d, and e: 1e.

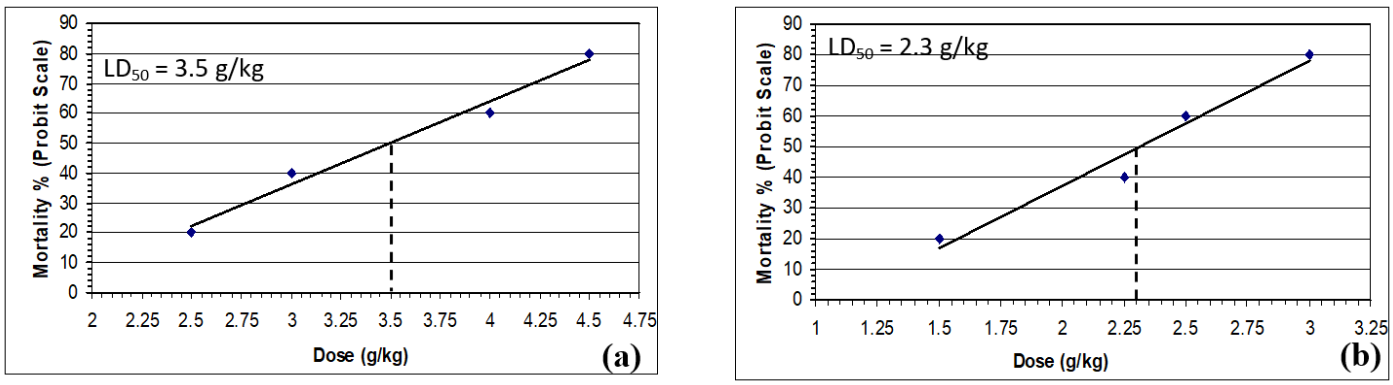
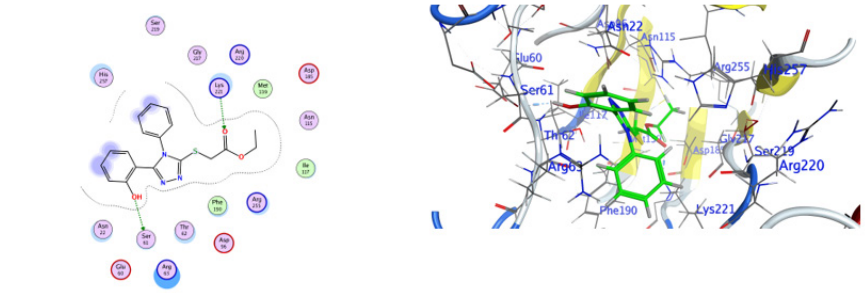
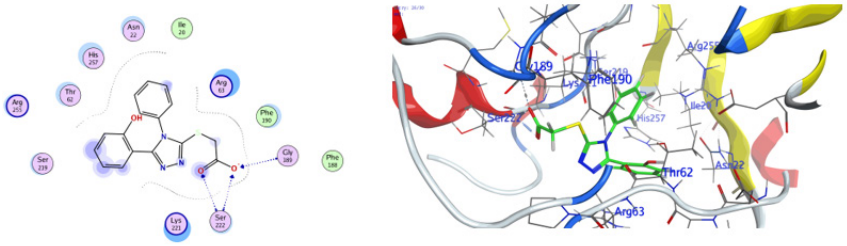


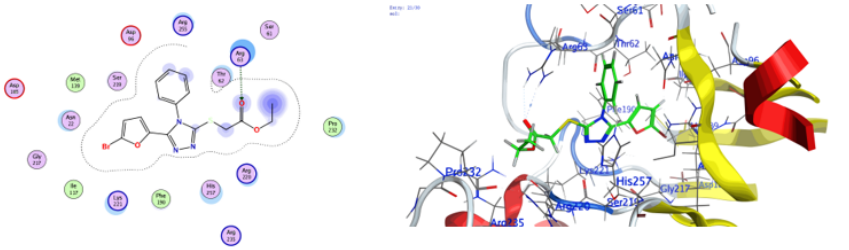
Figure 2: The LD_{50} of the compound a: 1e and b: 2e.



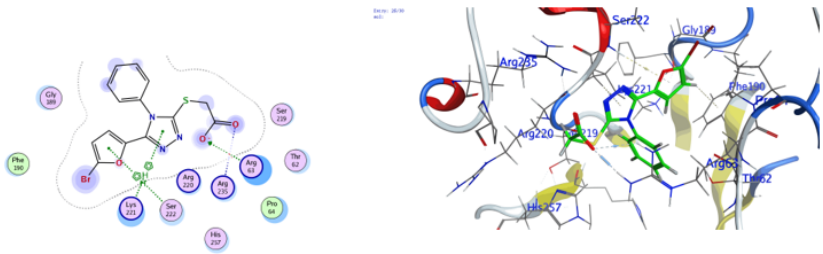
1e



1f



2e



2f

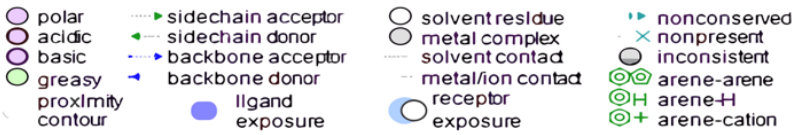


Figure 3: 2D and 3D binding affinity of 1e, 1f, 2e, and 2f compound with 1AJ0.

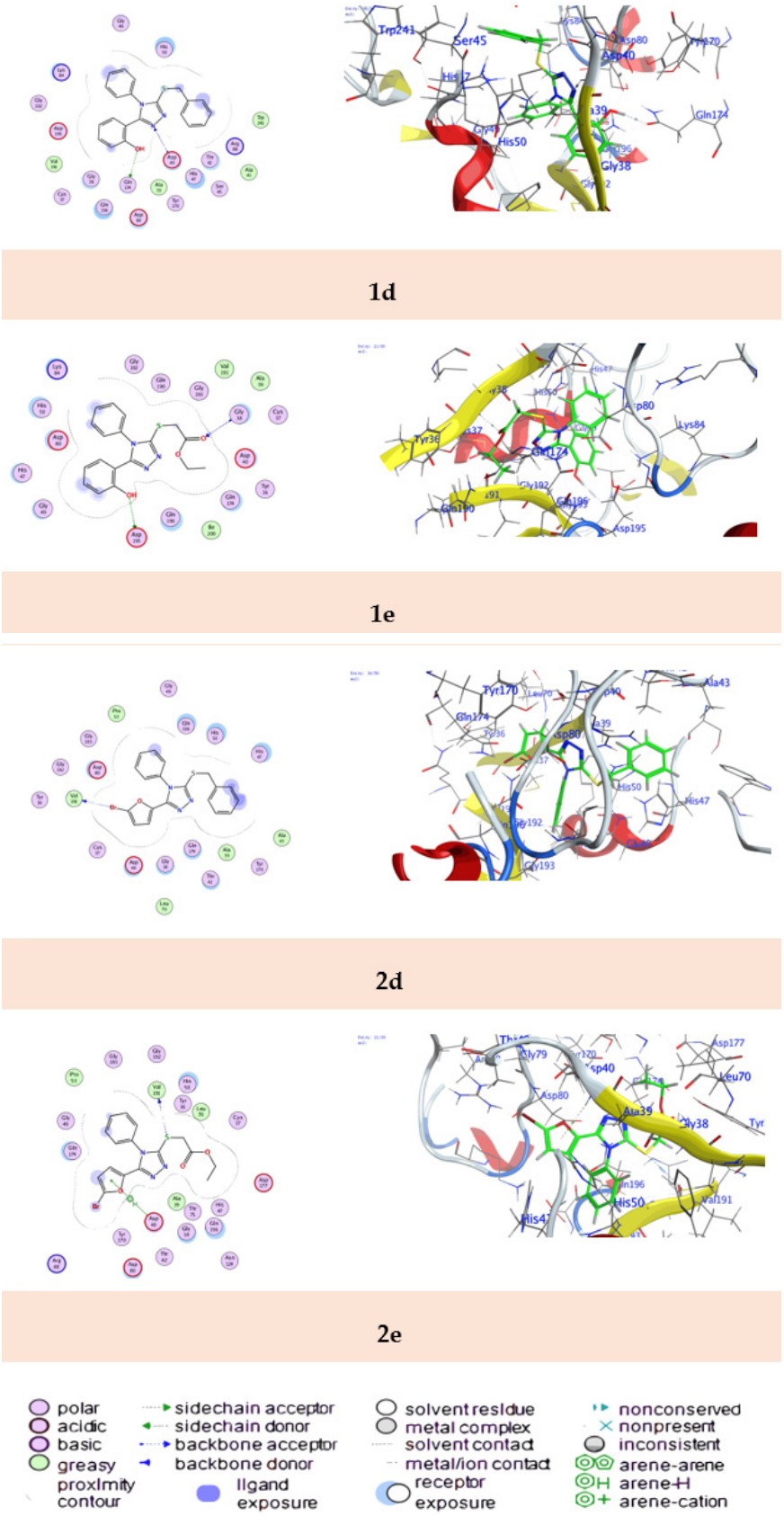


Figure 4: 2D and 3D binding affinity of 1d, 1e, 2d, and 2e compound with 1JIJ.

Table 1: Antimicrobial evaluation of triazoles at 1000 µg/mL against *A. niger, E. coli* and *S. aureus.*

Compd.	Zone of inhibition (mm)			Compd.	Zone of inhibition (mm)		
	A. niger	*S. aureus*	*E. coli*		*A. niger*	*S. aureus*	*E. coli*
1a	Nil	10	14	2a	10	Nil	Nil
1b	Nil	Nil	15	2b	10	Nil	Nil
1c	11	10	12	2c	Nil	Nil	Nil
1d	Nil	10	12	2d	Nil	8	Nil
1e	12	22	28	2e	27	18	14
1f	Nil	21	17	2f	15	15	12
Amoxicillin	-	39	38	Fluconazole	23	-	-
Streptomycin	-	34	28				

Nil= No inhibition.

points, so we decided to modify the structure at the SH group by other agents.

Medium Lethal Dose (LD_{50})

Medium lethal dose is the dose that is likely to cause the death of 50% of the animals. For the two active substances **1e** and **2e** that were chosen, the LD_{50} test in mice yielded 3.5 and 2.3 g/kg, respectively. Figure 2 depicted the test findings reveals that the LD_{50} values for these substances were less than 5 g/kg. We can classify these chemicals as moderately hazardous materials in accordance with Klassen and Doull's classification.[39]

Molecular docking studies

We used three types of proteins according to this descriptions, *E. coli* dihydropteroate synthase is the first one. Dihydropteroate synthase, or DHPS (protein ID: 1AJ0), catalyzes the condensation of para-aminobenzoic acid with 6,8-hydroxymethyl-7,8-dihydropteridine pyrophosphate to generate 7,8-dihydropteroate. The three-step process from 6-hydroxymethyl-7,8-dihydropterin to 7,8-dihydrofolate includes this as the second stage. Sulfonamides are substrate analogues that compete with para-aminobenzoic acid, and they target DHPS. Bacterial DHPS is a 275-315 amino acid protein that can either be found on different antibiotic resistance plasmids or is encoded on the chromosome.[40]

The second one is the TyrRS complex of the *S. aureus* tyrosyl-tRNA synthetase and SB-239629 (protein ID: 1JIJ). By creating charged tRNAs, aminoacyl-tRNA synthetases contribute significantly to the production of proteins. An amino acid is transported onto a corresponding tRNA to create the desired product after being condensed with an ATP molecule to create a stable aminoacyl-adenylate intermediate. Because the synthetases are so crucial, substances that selectively inhibit the bacterial aminoacyl-tRNA synthetases could develop into powerful antibacterial drugs.[41]

The third one is the ferulic acid decarboxylase Fdc1 from *A. niger* (protein ID: 4ZA5), which belongs to the broad family of (de) carboxylases known as the microbial UbiD superfamily and is capable of reversible decarboxylation on, -unsaturated acids. *In vivo* production of hydrocarbons like isobutene and 1,3-butadiene as well as C-H activation via CO_2 fixation are recent applications of Fdc.[42]

The binding free energies are used to assess the accuracy of the prediction of binding affinity between ligands and target proteins, lower values of binding energy represent stronger ligand binding.[43] Tables 2-4 showed the docking results of those compounds where series (**1a**-**1f**) possessed binding energy values (-6.90 to -5.30 kcal/mol), (-7.54 to -5.89 kcal/mol), and (-8.54 to -7.42 kcal/mol) with proteins dihydropteroate synthase DHPS of *E. coli*, tyrosyl-tRNA synthetase of *S. aureus*, and ferulic acid decarboxylase of *A. niger*, respectively. Whereas, compounds (**2a**-**2f**) gave (-7.07 to -5.50 kcal/mol), (-7.55 to -5.90 kcal/mol), and (-9.28 to -6.53 kcal/mol) with the same proteins compared with standard drugs amoxicillin (7.56 kcal/mol) and fluconazole (-6.11 kcal/mol).

As a result of the structural similarity of compounds **1a**-**1f** and **2a**-**2f**, it was found that the binding energy values followed a pattern that was likewise similar. Total energy values ranged from -24.74 to -53.87 kcal/mol in all docking affinity values, demonstrating that the synthesized ligands were extremely stable in the protein's active pocket.

Based on the molecular docking results, the best binding energy was the synthesized ligand **1e** (-6.90 and -7.54 kcal/mol) with 1AJ0 and 1JIJ, respectively and the synthesized ligand **1f** (-9.03 kcal/mol) with 4ZA5. In the same direction, the synthesized ligand **2e** showed the best binding affinity (-7.07 and -7.55 kcal/mol) with 1AJ0 and 1JIJ, respectively, whereas, compound **2f** gave -9.28 kcal/mol with 4ZA5, as mentioned in Tables 3-5.

Table 2: Molecular docking results for the synthesized ligands with 1AJ0.

Compd.	S Score kcal/mol	RMSD Å	Interaction between atoms in synthesized ligands and active site of protein						
			Compd. Atoms	Receptor Atoms	Receptor Residues	Interaction	d (Å)	E (kcal/mol)	Total E (kcal/mol)
1a	-5.30	1.65	S18	OG1	THR24	H-donor	3.73	-0.7	-26.29
			6-ring	5-ring	HIS257	pi-pi	3.99	0	
1b	-5.88	1.50	5-ring	CE1	HIS257	pi-H	3.63	-0.7	-30.30
1c	-6.42	1.26	O12	OG	SER61	H-donor	3.11	-1.3	-32.11
1d	-6.56	1.74	O12	OG	SER61	H-donor	2.96	-1.7	-33.48
1e	-6.90	1.30	O12	OG	SER61	H-donor	2.93	-1.8	-38.63
			O27	NZ	LYS221	H-acceptor	3.05	-2.4	
1f	-6.58	1.79	O23	CA	GLY189	H-acceptor	3.59	-0.7	-39.15
			O23	N	SER222	H-acceptor	3.10	-3.7	
			O24	N	SER222	H-acceptor	2.96	-4.9	
2a	-5.50	1.23	S6	OD1	ASN22	H-donor	3.25	-1.2	-28.26
			S6	NH1	ARG255	H-acceptor	3.87	-0.7	
			5-ring	CG	LYS221	pi-H	3.85	-1.3	
			5-ring	CE	LYS221	pi-H	4.41	-0.8	
2b	-5.96	1.06	S 6	OD1	ASN22	H-donor	3.49	-0.5	-32.33
			5-ring	CG	LYS221	pi-H	3.78	-1.4	
			5-ring	CE	LYS221	pi-H	4.40	-0.9	
2c	-6.49	1.14	Br 27	O	PRO232	H-donor	3.56	-1.7	-37.02
			5-ring	CA	LYS221	pi-H	4.13	-0.6	
			5-ring	CG	LYS221	pi-H	4.06	-0.6	
2d	-6.53	1.66	Br 28	O	PRO232	H-donor	3.55	-2.2	-36.11
			5-ring	CG	LYS221	pi-H	4.15	-0.7	
2e	-7.07	1.32	O15	NH2	ARG63	H-acceptor	3.37	-1.0	-41.70
2f	-6.27	1.58	O11	NH1	ARG63	H-acceptor	3.39	-0.9	-40.40
			O11	NH2	ARG63	H-acceptor	2.94	-8.5	
			O11	NH1	ARG63	Ionic	3.39	-2.3	
			O11	NH2	ARG63	Ionic	2.94	-4.9	
			O12	NH1	ARG235	Ionic	3.21	-3.2	
			5-ring	CA	LYS221	pi-H	3.75	-1.6	
			5-ring	N	SER222	pi-H	4.53	-1.1	
Amox.	-7.56	1.47	*	*	*	*	*	*	*

Amox. = Amoxicillin, * = Did not calculate.

The binding affinities of the ligands 1e, 1f, 2e, and 2f into dihydropteroate synthase DHPS (protein ID: 1AJ0) active sites of *E. coli*

Table 2 shows the binding pose of synthesized ligands in the pocket of DHPS. Ligand **1e** exhibited H-donor bond of oxygen of hydroxyl group with binding pocket residues SER61 and H-accepter bond of oxygen of carbonyl ester with LYS221. Whereas, ligand **1f** gave three H-accepter interactions between oxygen atoms of carboxyl group and other residues of the binding pocket which GLY189, SER222, and SER222.

Ligand **2e** possessed H-accepter interaction for carbonyl oxygen of ester fragment and amino group of ARG63 residue, as compared with different types and sites of interaction between ligand **2f** and residues of the binding pocket which gave H-acceptor and ionic interactions oxygens of carboxyl group of **2f** ligand with amino group of ARG63 and ARG235, meanwhile pi-H bonds between five-member rings for furane and triazole of **2f** ligand with LYS221 and SER222, respectively, of residues, as shown in Figure 3.

Table 3: Molecular docking results for the synthesized ligands with 1JIJ.

Compd.	S Score kcal/mol	RMSD Å	Interaction between atoms in synthesized ligands and active site of protein						
			Compd. Atoms	Receptor Atoms	Receptor Residues	Interaction	d (Å)	E (kcal/mol)	Total E (kcal/mol)
1a	-5.89	1.05	S18 5-ring	OD2 CA	ASP40 ALA39	H-donor pi-H	3.07 3.86	-7.8 -0.6	-24.74
1b	-6.78	1.61	O12	OD2	ASP195	H-donor	2.84	-4.3	-36.60
1c	-7.14	1.58	S18	O	VAL191	H-donor	4.05	-1.1	-35.23
1d	-7.41	1.58	O12 N14	OE1 N	GLN174 ASP40	H-donor H-acceptor	3.23 3.14	-1.1 -3.1	-43.41
1e	-7.54	1.79	O12 O27	OD2 N	ASP195 GLY38	H-donor H-acceptor	2.99 2.84	-3.6 -0.8	-40.57
1f	-6.77	1.27	O23 O24	NE2 NE2	HIS47 HIS50	H-acceptor H-acceptor	3.07 3.03	-0.7 -1.0	-37.29
2a	-5.90	0.81	S6 5-ring	OD2 CA	ASP40 ALA39	H-donor pi-H	3.10 3.97	-7.5 -0.6	-26.93
2b	-6.58	1.91	Br21	O	VAL191	H-donor	3.31	-0.5	-36.01
2c	-7.22	1.50	BR27 S6 6-ring	OD2 NZ N	ASP40 LYS84 ASP40	H-donor H-acceptor pi-H	3.43 3.92 4.72	-0.6 -0.7 -0.9	-40.66
2d	-7.43	1.35	BR28	O	VAL191	H-donor	3.26	-0.3	-44.91
2e	-7.55	1.00	S6 5-ring	O N	VAL191 ASP40	H-donor pi-H	3.50 4.26	-2.6 -1.4	-37.26
2f	-6.84	1.37	BR20 O11 O11	O NZ NZ	VAL191 LYS84 LYS84	H-donor H-acceptor ionic	3.32 3.07 3.07	-0.4 -4.5 -4.0	-39.77
Amox.	-7.56	1.47	*	*	*	*	*	*	*

Amox. = Amoxicillin, * = Did not calculate.

The binding affinities of the ligands 1d, 1e, 2d, and 2e into aminoacyl-tRNA synthetases TyrRS (proteins ID: 1JIJ) active sites of *S. aureus*

Table 3 shows the binding pose of synthesized ligands in the pocket of TyrRS. Ligand **1d** had two interactions, H-donor interaction with amino acid GLN174 and H-acceptor with amino acid ASP40. Ligand **1e** had H-donor between oxygen of hydroxyl group and amino acid ASP195, furthermore H-acceptor bond between oxygen atom of carbonyl and amino acid GLY38, as shown in Figure 4.

Synthesized ligand **2d** exhibited H-donor bond interaction between bromide and binding pocket residue, VAL191. Two types of interactions were found for ligand **2e**, H-donor between sulfur atom and VAL191, meanwhile pi-H between furane ring and ASP40 (Figure 4).

The binding affinities of the ligands 1d, 1f, 2d, and 2f into ferulic acid decarboxylase Fdc1 (proteins ID: 4ZA5) active sites of *A. niger*

Table 4 shows the binding pose of synthesized ligands in the pocket of Fdc1. Ligand **1d** also exhibited appreciable interaction to the binding pocket of Fdc1. H-donor and pi-H bonds between SER224 and GLN190 with the oxygen of hydroxyl group and phenyl ring, respectively, was observed. Excellent binding score of **1f** with binding pocket of Fdc1was observed to be -9.03 kcal/mol. Binding pocket residues ILE171, LYS391, LYS391, ASN168, HIS191, LYS391, and ASN168 were also found interacting with **1f**.

Synthesized ligand **2d** gave H-donor and pi-H bond interactions with binding pocket residues SER314 and GLN190, respectively. Whereas, ligand **2f** showed perfect binding affinity of -9.28 kcal/mol as compared with other synthesized ligands and standard drug fluconazole (-6.11 kcal/mol). **2f** exhibited H-acceptor interactions with ILE171, LYS391, LYS391, and ASN168, ionic

Table 4: Molecular docking results for the synthesized ligands with 4ZA5.

Compd.	S Score kcal/mol	RMSD Å	Interaction between atoms in synthesized ligands and active site of protein						
			Compd. Atoms	Receptor Atoms	Receptor Residues	Interaction	d (Å)	E (kcal/mol)	Total E (kcal/mol)
1a	-7.42	1.52	N14	NZ	LYS391	H-acceptor	2.83	-10.1	-36.24
1b	-8.03	1.55	O12 N14 S18	O NZ N	LYS391 LYS391 ILE171	H-donor H-acceptor H-acceptor	3.15 2.90 3.90	-1.1 -4.9 -0.9	-36.27
1c	-8.09	1.38	N14	N	ILE171	H-acceptor	3.17	-1.2	-30.53
1d	-8.54	1.43	O12 6-ring	OG CG	SER224 GLN190	H-donor pi-H	2.85 3.60	-2.1 -0.7	-38.99
1e	-8.34	1.52	N14	N	ILE171	H-acceptor	3.24	-0.9	-33.12
1f	-9.03	1.51	N14 S18 O23 O24 O23 O23 O24	N NZ NZ ND2 NE2 NZ ND2	ILE171 LYS391 LYS391 ASN168 HIS191 LYS391 ASN168	H-acceptor H-acceptor H-acceptor H-acceptor Ionic Ionic ionic	3.10 3.50 2.92 2.97 3.43 2.92 2.97	-4.4 -1.3 -6.1 -17.3 -2.2 -5.0 -4.7	-53.86
2a	-6.53	1.72	S6 BR15	OE1 SG	GLN190 CYS316	H-donor H-donor	3.66 3.62	-1.6 -1.1	-26.78
2b	-7.24	1.42	BR21	O	LYS391	H-donor	3.72	-0.5	-30.23
2c	-7.83	0.78	S6 5-ring	OE1 CB	GLN190 ILE171	H-donor pi-H	3.64 3.80	-1.2 -1.5	-40.39
2d	-8.48	1.48	BR28 6-ring	OG CG	SER314 GLN190	H-donor pi-H	3.75 3.58	-0.7 -0.7	-37.84
2e	-8.26	1.70	S6 O15 5-ring 6-ring 5-ring	OE1 NE2 CB CA CG1	GLN190 GLN190 ILE171 ALA172 ILE327	H-donor H-acceptor pi-H pi-H pi-H	3.17 3.05 4.44 4.62 3.66	0.6 -1.3 -0.6 -0.7 -0.7	-43.54
2f	-9.28	1.85	N2 S6 O11 O12 O11 O11 O12 5-ring	N NZ NZ ND2 NE2 NZ ND2 CB	ILE171 LYS391 LYS391 ASN168 HIS191 LYS391 ASN168 ILE171	H-acceptor H-acceptor H-acceptor H-acceptor Ionic Ionic Ionic pi-H	3.06 3.40 2.97 2.97 3.42 2.97 2.97 3.82	-5.5 -1.0 -5.2 -17.4 -2.2 -4.7 -4.7 -0.8	-53.87
Fluc.	-6.11	1.90	*	*	*	*	*	*	*

interactions with HIS191, LYS391, and ASN168, and pi-H interaction with ILE171 (Figure 5).

CONCLUSION

Two series of 1,2,4-triazole derivatives, **1a-f** and **2a-f**, were produced; they were then characterized using FT-IR, 1H NMR, and CHNS methods. The well diffusion method was used to investigate their antibacterial properties against *E. coli, S. aureus*, and *A. niger*. Against the examined bacteria, some of the reported compounds demonstrated notable antimicrobial capabilities. When compared to fluconazole, the compounds **1e** and **2e** were found to be much more effective against *S. aureus* and *E. coli*, as well as showing potential efficacy against *A. niger*. According to the binding mode studies, some of the designed compounds displayed interactions that were comparable to those produced by the standard medication used in bacterial receptors. All compounds displayed interactions that were superior to those produced by fluconazole drug with active site of fungal protein. Compounds **1e, 1f, 2e** and **2f** showed good interactions with the active site of dihydropteroate synthase (-6.90, -6.58, -7.07 and -6.27 kcal/mol, respectively), and compounds **1d, 1e, 2d** and **2e** with the active site of aminoacyl-tRNA synthetases (-7.41, -7.54, -7.43 and -7.55 kcal/mol, respectively) compared with amoxicillin (-7.56 kcal/mol), whereas, compounds **1d, 1f, 2d** and **2f** showed excellent interactions with the active site of ferulic acid decarboxylase (-8.54, -9.03, -8.48 and -9.28 kcal/mol, respectively) than the fluconazole drug (-6.11 kcal/mol).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UV: Ultra violate; **FT-IR:** Fourier transform infra-red; **^{1}H-NMR:** Proton nuclear magnetic resonance; **TMS:** Tetramethylsilane; **DMSO-d_6:** Deuterated dimethyl sulfoxide; **δ:** Chemical shift; **ppm:** Part per million; **RMSD:** Root mean square deviation.

SUMMARY

The research goaled to synthesis antibacterial and antifungal analogues using 1,2,4-triazole derivatives. Spectroscopic techniques were used to characterize the synthesized compounds to correspond the chemical structure and test the purity. The molecular docking was used to investigate the matching between the practical and theoretical antimicrobial activity.

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