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A FACILE SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW BIS-TRIAZOLE COMPOUNDS

AL-SHSMRANI JAWAHER MOHAMED[*], MOHAMMAD MAHBOOB ALAM AND FAWY MOHAMED ABD EL LATIF

Chemistry Department, Faculty of Science, Albaha University, Kingdom of Saudi Arabia

***Corresponding author: E Mail: j-s-az@hotmail.com; Phone: +966 534833691; Fax: 0096677247272**

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ABSTRACT

New 1,2,4-triazole and 1,2,3-triazole as bis heterocyclic were synthesized using click chemistry approach. The chemical structures of the new triazolo compounds were confirmed by spectroscopic (IR), ^{1}H-NMR, ^{13}C-NMR, mass spectrometry (MS) and elemental analysis techniques. The antibacterial and antifungal activities of the new compounds have been evaluated. Some gram positive and gram negative bacterial strains were sensitive against some tested compounds. The compounds **7**, **10** and **20** were active against most of the tested bacterial strains. Compound **10** showed highest activity among the series of tested compounds than the standard drug amoxicillin against *S.a: Staphylococcus aureus, S.e: Staphylococcus Epidermidis* and *P.m: Proteus Merabilis*. Also, compound **11**, **12** and **18** were active against all the tested gram positive bacterial strains whereas inactive towards the tested gram negative bacterial strains. The five compounds **7**, **10**, **12**, **18** and **20** were active against the *candida albicans.*

Keywords: 1,2,3-Triazole,1,2,4-Triazole, Bis-triazole, Aromatic azide and Biological evalution

1. INTRODUCTION

Triazole and its derivatives consider as an important group of heterocyclic compounds which have been searched and still its scope is inescapable. In addition, triazole nucleus has broad potential as medicinal agents because their pharmacological activity, low toxicity, less adverse effects, high bioavailability, and diversity of drug administration **[1]**. The chemistry of 1,2,4-triazoles and their fused heterocyclic compounds has been attracting widespread attention due to their synthetic utility as agricultural **[2]** and industrial applications **[3]**. Also, many pharmaceutical drugs consist from a number of 1,2,4-triazole rings, including anticancer **[4]**, anti-inflammatory **[5]**, antimicrobial **[6]**, analgesic **[7]**, antiviral **[8]**, antifungal **[9]**, antibacterial **[10]** and anticonvulsant agent **[11]** such as Alprazolam, Anastrazole, Voriconazole, Estazolam (tranquilizer hypnotic, sedative) , Fluconazole, Itraconazole, Trazodone, Propiconazole, Myclobutanil, Triazolam, Tebuconazole, Trazodone (antidepressant, anxiolytic), Terconazole, Ribavirin, and Rizatriptan **[12]**. Furthermore, biheterocyclic compounds such as 1,2,4-triazole ring fused to other heterocyclic rings are as antimicrobial agents **[13, 14]**. 1,2,4-Triazole derivatives also can incorporating Schiff base employ for discovery of new antimicrobial agent **[15]**.1,2,3-Triazoles also are therapeutically important agents such as antitubercular **[16]**, anticancer **[17]**, anti-inflammatory **[18]**, antifungal **[19]**, antimicrobial activity **[20, 22]** and antiviral **[23]**. 1,2,3-Triazole is synthesized using click chemistry approach **[24]**. There are very few 1,2,4-triazole and 1,2,3-triazole-containing molecules in the market. Therefore, the biological potential of 1,2,4-triazole and 1,2,3-triazole derivatives prompted us to synthesize new compounds wherein the two active moieties is conjugated under one construct to enhance their antimicrobial activity. This work aimed to designing and synthesis of new heterocyclic compounds containing bis-triazole moiety and screen their biological evaluation as antimicrobial activities.

2. MATERIAL AND METHODS

All chemicals were purchased from Loba Chemie (India), Scharlau (Germany), Laboratory Reagent, and Sigma-Aldrich (Germany). All the chemicals used directly without recrystallization or distillation. All the compounds were determined for progress of the reactions and purities via thin layer chromatography (TLC) using Silica gel plates stationary phase and iodine vapors. Melting points were measured by open capillary tube method using electrothermal type 9100 melting point apparatus. Mass spectra were measured on thermo scientific-LCQ Fleet (LCF10605) using electron spray ionization method at 75 eV at Faculty of Science, Albaha University. Mass-spectrometric (MS) data is reported in *m/z*. IR spectra were taken on Thermo Scientific Instrument via iS50 ATR method and ν expressed at cm^{-1}. ^1H-NMR and ^{13}C-NMR spectra were measured on a Bruker 300 MHz and 850 MHz instruments in DMSO-d6 and $CDCl_3$ using TMS as an internal standard, chemical shift $\delta$and coupling constant are given in Hertz (Hz) and parts per million (ppm) at King Abdulaziz University, Jeddah, KSA. Elemental analysis was performed on LEECO/Elementary and is reported in % standard and were within ±0.4% of the calculated values.

2.1. EXPERIMENT

The compounds **2**, **3**, **4**, **5** and **6a** have been prepared similar to the methods reported in references **[25, 26]**.

2.2.1. *General procedure for the synthesis of Schiff bases (6b-e).*

In a (50 ml) clean and dry round bottom flask a mixture of compound **5** (0.005 mol) and the corresponding different aromatic aldehydes (0.0056 mol) was refluxed for 8 - 12 h in absolute ethanol (25 ml) in the presence of few drops of glacial acetic acid. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was concentrated, cooled and the formed precipitate was filtered and recrystallized from the proper solvents.

N-(4-Methoxybenzylidene)-3-phenyl-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-amine (6b)

White crystals (chloroform); m.p.: 152-153 °C; yield: 66%; IR: ν (cm^{-1}): 3295, 3221, 3120, 2960, 2117, 1683, 1596, 1474, 1242, 1043, 1026, 691. MS (*m/z*): 349 $(M+H)^+$. Analytical calc. for $C_{19}H_{16}N_4OS$: C: 65.51, H: 4.59, N: 16.09, O: 4.59, S: 9.19 %. Found: C: 65.50, H: 4.57, N: 16.08, O: 4.57, S: 9.17 %.

2-Methoxy-4-[((3-phenyl-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-yl) imino) methyl]phenol (6c)

Off white crystals (chloroform); m.p.: 153-154 °C; yield: 76%; IR: ν (cm^{-1}): 3300, 3223, 3113, 2959, 2117, 1646, 1596, 1558, 1521, 1475, 1451, 1432, 1338, 1285, 1243, 1188, 1106, 1076, 1043, 1026, 692. MS (*m*/z): 365 $(M+H)^+$. Analytical calc. for $C_{19}H_{16}N_4OS$: C: 62.63, H: 4.39, N: 15.38, O: 8.79, S: 8.79 %. Found: C: 62.62, H: 4.40, N: 15.37, O: 8.77, S: 8.38 %.

2-[((3-Phenyl-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-yl) imino) methyl] phenol (6d)

White crystals (chloroform); m.p.: 150-152 °C; yield: 74.8%; IR: ν (cm^{-1}): 3301, 3226, 2960, 2117, 1608, 1575, 1506, 1521, 1475, 1455 1249, 1046, 693. MS (*m/z*): 335 $(M+H)^+$. Analytical calc. for $C_{18}H_{14}N_4OS$: C:

64.67, H: 4.19, N: 16.76, O: 4.79, S: 9.58 %. Found: C: 64.65, H: 4.18, N: 16.75, O: 4.78, S: 9.57 %.

N-(2-Bromobenzylidene)-3-phenyl-5-(prop-2-yn-1-ylthio)-4H-1,2,4-triazol-4-amine (6e)

Pale yellow crystals (chloroform); m.p.: 127-130 °C; yield: 87%; IR: *ν* (cm^{-1}): 3221, 3063, 2960, 2116, 1683, 1596, 1521, 1473, 1451, 1423, 1401, 1376, 1339, 1270, 1242, 1201, 1105, 1076, 1042, 1026, 690, 583. ^1H NMR ($CDCl_3$), δ (ppm): 2.28 (s, 1H, CH), 4.09 (s, 2H, CH_2), 7.46-7.44 (m, 5H, Ar-H), 7.63-7.65 (m, 1H, Ar-H), 7.86-7.89 (m, 2H, Ar-H), 8.13-8.15 (m, 1H, Ar-H), 8.93 (s, 1H, N=CH). ^{13}C NMR ($CDCl_3$), δ (ppm): 22.10 (CH_2), 72.97 (CH), 76.73 (-C-), 126.02, 126.55, 128.13, 128.63, 128.75, 128.93, 130.46, 130.74, 133.69, 134.25, 146.63 (C=N of triazole), 152.13 (Ar-C=N-N-), 163.53(C-S of triazole); MS (*m/z*): 399 $(M+2H)^+$. Analytical calc. for $C_{18}H_{13}BrN_4S$: C: 54.40, H: 3.27, N: 14.10, Br: 19.89, S: 8.06 %. Found: C: 54.38, H: 3.25, N: 14.11, Br: 19.88, S: 8.04 %.

2.2.7 *General procedure for synthesis of 1,2,4-triazole-1,2,3-triazole*(7-20)

The new bis-triazole compounds **7-20** were prepared according to the procedure reported [24].

The compounds **(6a-e)**, (0.004 mol,1g) were dissolved in 30 ml of a warm mixture of *t*-BuOH: water (2:1) and the reaction mixture was cooled to room temperature, sodium ascorbate (0.004 mol, 0.8 g), $CuSO_4$ (0.004mol, 0.6 g) were added with stirring for 30 min. and then the benzoazide derivative**7**(0.005 mol, 0.6 g) was added. The reaction mixture was stirred for 20 h. at room temperature. After completion of the reaction monitored by TLC, the reaction mixture was poured into the cold water, stirred for 30 min. and extracted twice with ethyl acetate. Ethyl acetate layer was separated and dried over anhydrous sodium sulphate. The ethyl acetate was filtered, evaporated to get the crude product and recrystallized as **7**. The same above procedure was applied to prepare the new bis-triazoles **8-20,** respectively.

N-Benzylidene-3-[(((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl) methyl] thio)-5-phenyl-4H-1,2,4-triazol-4-amine (7)

Light green crystals (dichloromethane); m. p.: 123-125 °C; yield: 67%; IR: *ν* (cm^{-1}): 1593, 1558, 1540, 1507, 1488, 1473, 1465, 1448, 1429, 1395, 1339, 1278, 1233, 1187, 1080, 1047, 1045, 1023, 784, 698. ^1H NMR (DMSO-d_6), δ (ppm): 4.56 (s, 2H, CH_2), 7.66-7.45 (m, 8H, Ar-H), 7.82-7.86 (m, 5H, Ar-H), 7.96-7.97 (t, 1H, Ar-H), 8.78 (s, 1H, -C=CH-N-), 8.85 (s, 1H, N=CH-Ar). ^{13}C NMR (DMSO-d_6), δ (ppm): 27.92 (CH2), 125.88, 126.30, 127.77, 128.19, 128.28, 128.42, 128.81, 129.08, 129.33, 130.06, 130.53, 131.37, 131.62 (C of triazole), 133.42 (C of triazole), 134.30 (C of triazole), 142.63 (Ar-C=N-N-), 168.80 (C-S of triazole); MS (*m/z*): 472 $(M+H)^+$. Analytical calc. for $C_{24}H_{18}ClN_7S$: C: 61.14, H: 3.82, Cl: 7.43, N: 20.80, S: 6.79%. Found: C: 61.12, H: 3.80, Cl: 7.42, N: 20.78, S: 6.78 %.

N-Benzylidene-3-phenyl-5-[((1-(o-tolyl)-1H-1,2,3-triazol-4-yl) methyl) thio]-4H-1,2,4-triazol-4-amine (8)

Green crystals (dichloromethane); m. p.: 121-122 °C; yield: 58%; ^1H NMR (DMSO-d_6), δ (ppm): 2.32 (s, 3H, CH_3) 4.56 (s, 2H, CH_2), 7.33-7.70 (m, 10H, Ar-H), 7.85-7.91 (m, 4H, Ar-H), 8.28 (s, 1H, -C=CH-N-), 8.87 (s, 1H, N=CH-Ar). ^{13}C NMR (DMSO-d_6), δ (ppm): 17.28 (CH_3) 28.17 (CH_2), 125.28, 125.86, 126.33, 126.92, 127.77, 128.80, 129.08, 129.33, 129.80, 130.05, 131.33, 131.40, 132.96 (C of triazole), 133.40 (C of triazole), 136.07 (C of triazole), 142.42 (Ar-C=N-N-), 168.87 (C-S of triazole); MS (*m/z*): 452 $(M+H)^+$. Analytical calc. for $C_{25}H_{21}N_7S$: C: 66.51, H: 4.65, N: 21.72, S: 7.09 %. Found: C: 66.50, H: 4.64, Cl: 7.42, N: 21.70, S: 7.08 %.

N-Benzylidene-3-[((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio]-5-phenyl-4H-1,2,4-triazol-4-amine (9)

Green crystals (dichloromethane); m. p.: 126-127 °C; yield: 63%; IR: *ν* (cm^{-1}): 1593, 1558, 1540, 1507, 1488, 1473, 1465, 1448, 1429, 1395, 1339, 1278, 1233, 1187, 1045, 1023, 784, 698. ^1H NMR (DMSO-d_6), δ (ppm): 4.61 (s, 2H, CH_2), 7.76-7.52 (m, 10H, Ar-H), 7.91-7.85 (m, 4H, Ar-H), 8.40 (s, 1H, -C=CH-N-), 8.84 (s, 1H, N=CH-Ar). ^{13}C NMR (DMSO-d_6), δ: 27.92 (CH2), 125.88, 126.30, 127.77, 128.19, 128.28, 128.42, 128.81, 129.08, 129.33, 130.06, 130.53, 131.37, 131.62 (C of triazole), 133.42 (C of triazole), 134.30 (C of triazole), 142.63 (Ar-C=N-N-), 168.80 (C-S of triazole); MS (*m/z*): 472 $(M+H)^+$. Analytical calc. for $C_{24}H_{18}ClN_7S$: C: 61.14, H: 3.82, Cl: 7.43, N: 20.80, S: 6.79%. Found: C: 61.13, H: 3.80, Cl: 7.42, N: 20.79, S: 6.77 %.

N-(4-Methoxybenzylidene)-3-phenyl-5-[((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)meth-yl)thio]-4H-1,2,4-triazol-4-amine (10)

Off white crystals (dichloromethane); m.p.: 194-195 °C, yield: 50%;IR: *ν* (cm^{-1}): 3323, 3064, 1597, 1520, 1476, 1448, 1428, 1393, 1340, 1259, 1232, 1191, 1177, 1109, 1054, 1074, 1045, 1022, 693. ^1H NMR (DMSO-d_6), δ (ppm): 2.38 (s, 3H, CH_3), 3.88(s, 3H, OCH_3), 4.60 (s, 2H, CH_2), 7.39-7.41 (d, *J* =8.6 Hz, 2H, Ar-H), 7.52 -7.53 (m, 3H, Ar-H), 7.74-7.76 (d, *J* =8.4 Hz, 3H, Ar-H), 7.99-8.01 (m, 5H, Ar-H), 8.69 (s, 1H, -C=CH-N-), 9.88 (s, 1H, N=CH-Ar). ^{13}C NMR (DMSO-d_6), δ (ppm): 20.54 (CH_3), 27.86 (CH_2), 55.58 (-O-CH_3), 110.74, 115.71, 118.88, 119.94, 121.91, 122.59, 125.12, 126.33, 127.69, 128.76, 129.95, 130.20, 130.45, 134.20, 138.41 (C of triazole), 148.21 (C=C of triazole), 152.22 (C of triazole), 169.15 (Ar-C=N-N-), 191.01 (C-S of triazole); MS (*m/z*): 482 $(M+H)^+$. Analytical calc. for $C_{26}H_{23}N_7OS$: C: 64.86, H: 4.78, N: 20.37, O: 3.32, S: 6.65 %. Found: C: 64.84, H: 4.76, N: 20.35, O: 3.30, S: 6.63 %.

N-(4-Methoxybenzylidene)-3-[((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio]-5-phenyl-4H-1,2,4-triazol-4-amine (11)

Deep yellow crystals (dichloromethane); m.p.: 204-206 °C; yield: 66%; IR: ν (cm^{-1}): 3344, 3090, 1595, 1520, 1506, 1473, 1452, 1426, 1337, 1257, 1170, 1074, 1043, 1021, 689. ^{1}H NMR (DMSO-d_6), δ (ppm): 3.88 (s, 3H, -OCH_3), 4.62 (s, 2H, CH_2), 7.49-7.53 (m, 5H, Ar-H), 7.80-7.90 (m, 1H, Ar-H), 7.99-8.01 (m, 3H, Ar-H), 8.21-8.23 (d, *J*= 9.2, 2H, Ar-H), 8.44-8.46 (d, *J*=8.8, 2H, Ar-H), 8.72 (s, 1H, -C=CH-N-), 8.89 (s, 1H, N=CH-Ar); MS (*m/z*): 512 $(M+H)^+$. Analytical calc. for $C_{25}H_{20}N_8O_3S$: C: 58.59, H: 3.90, N: 21.87, O: 9.37, S: 6.25 %. Found: C: 58.57, H: 3.87, N: 21.87, O: 9.35, S: 6.23 %.

2-Methoxy-4-[((3-phenyl-5-(((1-(p-tolyl)-1H-1,2,3-triazol-4-yl) methyl) thio)-4H-1,2,4-triazol-4-yl) imino) methyl] phenol (12)

Light brown crystals (dichloromethane); m.p.: 188-189 °C; yield: 73%; ^{1}H NMR (DMSO-d_6), δ (ppm): 2.38 (s, 3H, CH_3), 3.80(s, 3H, OCH_3), 4.58 (s, 2H, CH_2), 7.00-7.02 (d, *J* =8.4 Hz, 1H, Ar-H), 7.22 -7.50 (m, 7H, Ar-H), 7.69-7.71 (d, *J* =8.4 Hz, 2H, Ar-H), 7.84-7.86 (m, 2H, Ar-H), 8.62 (s, 1H, -C=CH-N-), 8.63 (s, 1H, N=CH-Ar), 10.22 (s, 1H, OH); MS (*m/z*): 498 $(M+H)^+$. Analytical calc. for $C_{26}H_{23}N_7O_2S$: C: 62.77, H: 4.62, N: 19.71, O: 6.43, S: 6.43 %. Found: C: 62.75, H: 4.61, N: 19.70, O: 6.41, S: 6.42 %.

2-Methoxy-4-[((3-(((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio)-5-phenyl-4H-1,2,4-triazol-4-yl) imino) methyl] phenol (13)

Light yellow crystals (dichloromethane); m.p.: 199-200 °C; yield: 68%; IR: ν (cm^{-1}): 3341, 3125, 1595, 1519, 1473, 1453, 1426, 1337, 1259, 1173, 1108, 1074, 1043,690; ^{1}H NMR (DMSO-d_6): δ (ppm) = 3.85 (s, 3H, OCH_3), 4.58 (s, 2H, CH_2), 7.44 -7.53 (m, 5H, Ar-H), 7.94-8.04- (m, 3H, Ar-H), 8.21-8.23 (d, *J* =9.2 Hz, 2H, Ar-H), 8.44-8.47 (d, *J* =9.2, 2H, Ar-H), 8.90 (s, 1H, -C=CH-N-), 8.97 (s, 1H, N=CH-Ar), 10.19 (s, 1H, OH);MS (*m/z*): 529 $(M+H)^+$Element analysis: Calculated for molecular formulae $C_{25}H_{20}N_8O_4S$, C: 56.81, H: 3.78, N: 21.21, O: 12.12 S: 6.06. Found: C: 56.80, H: 3.76, N: 21.20, O: 12.11 S: 6.05 %.

2-[((3-Phenyl-5-[((1-(p-tolyl)-1H-1,2,3-triazol-4-yl) methyl) thio)-4H-1,2,4-triazol-4-yl) imino] methyl] phenol (14)

Light green crystals (dichloromethane); m.p.: 174-175 °C; yield: 71%; IR: *ν* (cm^{-1}): 3342, 3090, 2971, 1596, 1519, 1452, 1427, 1337, 1259, 1191, 1151, 1108, 1074, 1043, 691. ^1H NMR (DMSO-d_6), *δ* (ppm): 2.38 (s, 3H, CH_3), 4.60 (s, 2H, CH_2), 7.22 -7.50 (m, 8H, Ar-H), 7.70-7.82 (m, 5H, Ar-H), 8.65 (s, 1H, -C=CH-N-), 8.93 (s, 1H, N=CH-Ar), 10.57 (s, 1H, OH). ^{13}C NMR (DMSO-d_6), *δ* (ppm): 20.55 (CH3), 31.26 (CH2), 116.79, 117.58, 119.69, 120.09, 120.54, 122.36, 125.54 126.41, 127,27, 127.82, 128.49, 128.76, 129.97, 134.99, 140.67 144.05, 144.52, 145.79 (C of triazole), 146.67 (C of triazole), 151.28 (C of triazole) 158.81 (Ar-C=N-N-), 164.28 (C-S of triazole); MS (*m/z*): 468 $(M+H)^+$. Analytical calc. for $C_{25}H_{21}N_7OS$: C: 64.23, H: 4.49, N: 20.98, O: 3.42, S: 6.85 %. Found: C: 64.22, H: 4.48, N: 20.97, O: 3.41, S: 6.84 %.

2-[(((3-(((1-(4-Nitrophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio)-5-phenyl-4H-1,2,4-triazol-4-yl) imino) methyl] phenol hydrate (15)

Deep yellow crystals (dichloromethane); m.p.: 186-187 °C; yield: 66%; ^1H NMR (DMSO-d_6), *δ* (ppm): 4.60 (s, 2H, CH_2), 7.00-7.02 (d, *J* =8.4, 1H, Ar-H), 7.35-7.38 (d, *J* =9.2, 1H, Ar-H), 7.50-7.51 (m, 3H, Ar-H), 7.78-7.84 (m, 3H, Ar-H), 8.17-8.19 (d, *J* =9.2, 1H, Ar-H), 8.25-8.28 (m, 2H, Ar-H), 8.42-8.44 (m, 2H, Ar-H) 8.92 (s, 1H, -C=CH-N-), 8.93 (s, 1H, N=CH-Ar), 10.67 (s, 1H, OH). ^{13}C NMR (DMSO-d_6), *δ* (ppm): 27.57 (CH2), 116.79, 117.58, 119.69, 120.09, 120.54, 122.36, 125.54, 126.41, 127,27, 127.82, 128.49, 128.76, 129.97, 134.99, 140.67, 144.05 144.52, 145.79 (C of triazole), 146.67 (C of triazole), 151.28 (C of triazole) 158.81 (Ar-C=N-N-), 164.28 (C-S of triazole); MS (*m/z*): 497 $(M-H)^-$. Analytical calc. for $C_{24}H_{18}N_8O_3S$: C: 57.83, H: 3.61, N: 22.48, O: 9.63, S: 6.42%. Found: C: 57.80, H: 3.60, N: 22.47, O: 9.62, S: 6.41 %.

N-(2-Bromobenzylidene)-3-phenyl-5-[((1-(p-tolyl)-1H-1,2,3-triazol-4-yl) methyl) thio]-4H-1,2,4-triazol-4-amine (16)

Deep brown crystals (dichloromethane); m. p.: 169-170 °C; yield: 60%; IR: *ν* (cm^{-1}): 3133, 2925, 1597, 1520, 1475, 1447, 1432, 1393, 1332, 1274, 1253, 1231, 1178, 1109, 1074, 1045, 1022, 697, 518. ^1H NMR (DMSO-d_6), *δ* (ppm): 2.38 (s, 3H, CH_3), 4.61 (s, 2H, CH_2), 7.37-7.39 (d, *J*=8, 2H, Ar-H), 7.53-7.58 (m, 5H, Ar-H), 7.69-7.71 (d, *J*=8.2, 2H, Ar-H), 7.78-7.83 (m, 3H, Ar-H), 8.00-8.02 (m, 1H, Ar-H), 8.64 (s, 1H, -C=CH-N-), 8.99 (s, 1H, N=CH-Ar). ^{13}C NMR (DMSO-d_6), *δ* (ppm): 20.54 (CH_3), 28.04 (CH_2), 119.86, 121.86, 125.45, 126.33, 128.18, 128.50, 128.56, 128.85, 130.14, 130.19, 130.45, 133.68, 134.20, 134.70 (for C of triazole), 138.34 (C=C of triazole), 151.10 (C of triazole), 159.00 (Ar-C=N-N-), 164.84(C-S of triazole); MS (*m/z*): 532 $(M+2H)^+$. Analytical calc. for $C_{25}H_{20}BrN_7S$: C: 56.60, H: 3.77, N: 18.49, Br: 14.90, S: 6.03 %. Found: C: 56.58, H: 3.75, N: 18.47, Br: 14.89, S: 6.02 %.

N-(2-Bromobenzylidene)-3-[((1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio]-5-phenyl-4H-1,2,4-triazol-4-amine (17)

Light yellow crystals (dichloromethane); m. p.: 194-195 °C, yield: 70%; IR: ν (cm^{-1}): 3140, 2090, 1661, 1597, 1522, 1506, 1475, 1447, 1392, 1336, 1276, 1261, 1236, 1177, 1109, 1092, 1074, 1045, 1020, 696, 598. ^1H NMR (DMSO-d_6), δ (ppm): 4.63 (s, 2H, CH_2), 7.53 -7.54 (m, 5H, Ar-H), 7.77 -7.82 (m, 3H, Ar-H), 7.97-8.00 (m, 1H, Ar-H), 8.14-8.17 (d, J = 8.8 Hz, 2H, Ar-H), 8.42-8.44 (d, J = 8.8 Hz, 2H, Ar-H), 8.91 (s, 1H, -C=CH-N-), 8.98 (s, 1H, N=CH-Ar). ^{13}C NMR (DMSO-d_6), δ (ppm): 27.95 (CH_2), 112.36, 120.10, 120.47, 122.35, 125.14, 125.45, 125.53, 126.32, 126.37, 128.17, 128.43, 128.55, 128.86, 130.15, 130.40, 134.70, 140.61 (for C of triazole), 133.68 (C=C of triazole), 144.54 (C of triazole), 146.68 (Ar-C=N-N-), 164.81(C-S of triazole); MS (*m/z*): 563 $(M+2H)^+$. Analytical calc. for $C_{24}H_{17}BrN_8O_2S$: C: 51.42, H: 3.03, N: 20.00, Br: 14.10, O: 5.71, S: 5.71 %. Found: C: 51.41, H: 3.02, N: 19.99, Br: 14.09, O: 5.70, S: 5.69 %.

N-(2-Bromobenzylidene)-3-(((1-(3-chlorophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio)-5-phenyl-4H-1,2,4-triazol-4-amine (18)

Off white crystals (dichloromethane); m.p.: 157-158 °C; yield: 72%; IR: ν (cm^{-1}): 1593, 1489, 1475, 1465, 1447, 1430, 1394, 1338, 1278, 1234, 1046, 1022, 785, 677, 598. ^1H NMR ($CDCl_3$), δ (ppm): 4.83 (s, 2H, CH_2), 7.25-7.77 (m, 13H, Ar-H), 8.06 (s, 1H, -C=CH-N-), 9.06 (s, 1H, N=CH-Ar). ^{13}C NMR ($CDCl_3$), δ (ppm): 27.92 (CH2), 116.79, 117.58, 119.69, 120.09, 120.54, 122.36, 125.54, 126.41, 127,27, 127.82, 128.49, 128.76, 129.97, 134.99, 140.67, 144.05, 144.52, 145.79, 146.67 (C of triazole), 151.28 (C of triazole) 158.81 (C of triazole), 164.28 (Ar-C=N-N-), 168.80 (C-S of triazole); MS (*m/z*): 552 $(M+H)^+$. Analytical calc. for $C_{24}H_{17}BrClN_7S$: C: 52.26, H: 3.08, Cl: 6.35, Br: 14.33, N: 17.78, S: 5.80 %. Found: C: 52.23, H: 3.04, Cl: 6.32, Br: 14.31, N: 17.74, S: 5.77 %.

N-(2-Bromobenzylidene)-3-[((1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl) methyl) thio]-5-phenyl-4H-1,2,4-triazol-4-amine (19)

Light green crystals (dichloromethane); m.p.: 148-149 °C; yield: 67%; IR: ν (cm^{-1}): 1593, 1489, 1475, 1465, 1447, 1430, 1338, 1278, 1234, 785, 677, 598. ^1H NMR ($CDCl_3$), δ

(ppm): 5.65 (s, 2H, CH_2), 7.64-7.16 (m, 13H, Ar-H), 8.03 (s, 1H, -C=CH-N-), 9.40 (s, 1H, N=CH-Ar). ^{13}C NMR ($CDCl_3$), δ (ppm): 27.92 (CH2), 116.79, 117.58, 119.69, 120.09, 120.54, 122.36, 125.54, 126.41, 127,27, 127.82, 128.49, 128.76, 129.97, 134.99, 140.67, 144.05, 144.52, 145.79, 146.67 (C of triazole), 151.28 (C of triazole) 158.81 (C of triazole), 164.28 (Ar-C=N-N-), 168.80 (C-S of triazole); MS (*m/z*): 552 $(M+H)^+$. Analytical calc. for $C_{24}H_{17}BrClN_7S$: C: 52.26, H: 3.08, Cl: 6.35, Br: 14.33, N: 17.78, S: 5.80 %. Found: C: 52.24, H: 3.06, Cl: 6.34, Br: 14.30, N: 17.76, S: 5.77 %.

N-(2-Bromobenzylidene)-3-phenyl-5-(((1-phenyl-1H-1,2,3-triazol-4-yl) methyl) thio)-4H-1,2,4-triazol-4-amine (20)

Light green crystals (dichloromethane); m.p.: 140-141 °C; yield: 67%; IR: ν (cm^{-1}): 677, 583. ^{1}H NMR ($CDCl_3$), δ (ppm): 4.91 (s, 2H, CH_2), 7.25-7.75 (m, 14H, Ar-H), 8.04 (s, 1H, -C=CH-N-), 9.15 (s, 1H, N=CH-Ar); MS (*m/z*): 516 $(M+H)^+$. Analytical calc. for $C_{24}H_{18}BrN_7S$: C: 55.92, H: 3.49, Br: 15.33, N: 19.02, S: 6.21 %. Found: C: 55.89, H: 3.46, Cl: 6.34, Br: 15.30, N: 18.99, S: 6.19 %.

2.3. MICROBIOLOGY

2.3.1 *In vitro antimicrobial assay*

The antimicrobial activities of the prepared compounds were carried out against the following different bacterial and fungal strains, the Gram Positive Bacterial *Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228),* Gram Negative Bacterial Strains: *Escherichia coli (ATCC 25922), Proteus mirabilis (ATCC 13376), Pseudomonas aeruginosa (ATCC 27853),* and Fungal Strain: *Candida albicans* (*ATCC 10231*) were used for antimicrobial studies.

2.3.1.1 Disc Diffusion Method

All the antimicrobial studies were performed at Albaha reagonal research laboratory, Albaha, Kingdom of Saudi Arabia. All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare chemicals of stock solution of 2 mg/mL and simple susceptibility screening test was carried out using reported disc diffusion method **[27]**. Each microbial strain was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to 10^6 colony forming unit (cfu)/mL. They were ''flood-inoculated'' onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) and then dried.For *Candida albicans*, SDA were used. For *Pseudomonas aeruginosa*, and for *Escherichia coli* and *Staphylococcus aureus* Muller Hinton agar were used. Six-millimeter diameter disc were prepared and 200 μg of the compounds were loaded. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organism. Amoxicillin (200 µg) and Fluconazole (100 µg) were used as standard drugs. Dimethyl sulfoxide was used as solvent (negative controls).

3. RESULTS AND DISCUSSION

3.1 Chemistry

A [1,2,3] triazole ring connected to [1,2,4] triazole moiety through a thiomethylene (–CH_2-S-) linkage and the main structure incorporate a Schiff base which as far we know represents a new bis-triazole derivatives which showed a promising biological activity. However, most of the chemicals used in this preparation are laboratory available and inexpensive, as well as the methods used are facile general and can repeat easily. The key intermediate 4-amino-5-phenyl-4*H*-1, 2, 4-triazole -3-thiol **(4)** prepared via condense methylbenzoate with hydrazine hydrate to afford the benzohydrazide **(2)**. The hydrazide **2** further reacted with carbon disulfide under basic conditions to afford the corresponding potassium salt of thiocarbazinate **(3)**. Finally, reaction of potassium salt of thiocarbazinate **3** with hydrazine mono hydrate under reflux conditions result in formation of the thiol **4**, as shown in Scheme **1**. The IR spectra of compound **4** showed absorption bands at ν= 3299, 3096 due to NH_2, and at ν= 1037 cm^{-1} assigned for C=S. Whereas its ^{1}HNMR spectrum reveals peaks at δ 13.93 ppm due to S-H and at δ 5.80 ppm, respectively for two protons of NH_2, whereas the ^{13}C-NMR spectrum of compound **4** shows 6 signals at δ 125.74-139.44 due to carbons of phenyl group, at δ 149.44 assigned for (C=N) of triazole ring, atδ 166.86 assigned to (C-S) of triazole ring and its mass spectrum showed its *m/z* [M^+]at 193.

However, treatment of **4** with propargyl bromide in presence of dimethylformamide (DMF) and potassium carbonate afforded the 3-phenyl- 5- (prop-2-yn-1-ylthio)- 4H-1,2,4-triazol-4-amine **5**via elimination of hydrogen bromide (HBr) as shown in Scheme **2**. Formation of compound **5**was supported by appearance two bands at ν 3294, 3240 cm^{-1}due to NH_2, intense absorption band characteristics for triple carbon-carbon bond atν 2117cm^{-1} in its IR and absorption signal at δ 6.19 ppm assigned for two protons of NH_2 in ^{1}HNMR spectrum. The structure of compound **5**further confirmed from mass spectrometry that showed molecular ion peak at *m/z* at 231 [M^+]. The intermediacy of **5** allowed to condense with different aromatic aldehydes such as (benzaldehyde, anisaldehyde, vanillin, salicyladehyde, and 2-bromobenzaldehyde) to afford new different substituents of Schiff bases as *N*-(substituted benzylidene)-3- phenyl-5- (prop- 2- yn- 1-

ylthio)-4H-1, 2, 4 triazol-4-amine **(6a-e)** as shown in Scheme **2**.

The IR spectra of all five compounds **6a-e** showed the disappearance of absorption peak due to amino function as indication to form the Schiff bases compounds, whereas showed absorption peak at *v* 2117 cm^{-1} due to presence of triple bonded carbon of propargyl terminal group. The ^1H NMR spectra of the new Schiff bases, e.g. compound

(6a) showed the disappearance of absorption signal due to the two proton of NH_2 and the appearance of singlet signals due to azomethine group (CH=N-) at δ 8.55 ppm. As well as, the chemical structure of the new Schiff bases were confirmed via their ^{13}C NMR ($CDCl_3$) spectrum and mass spectra measurements.

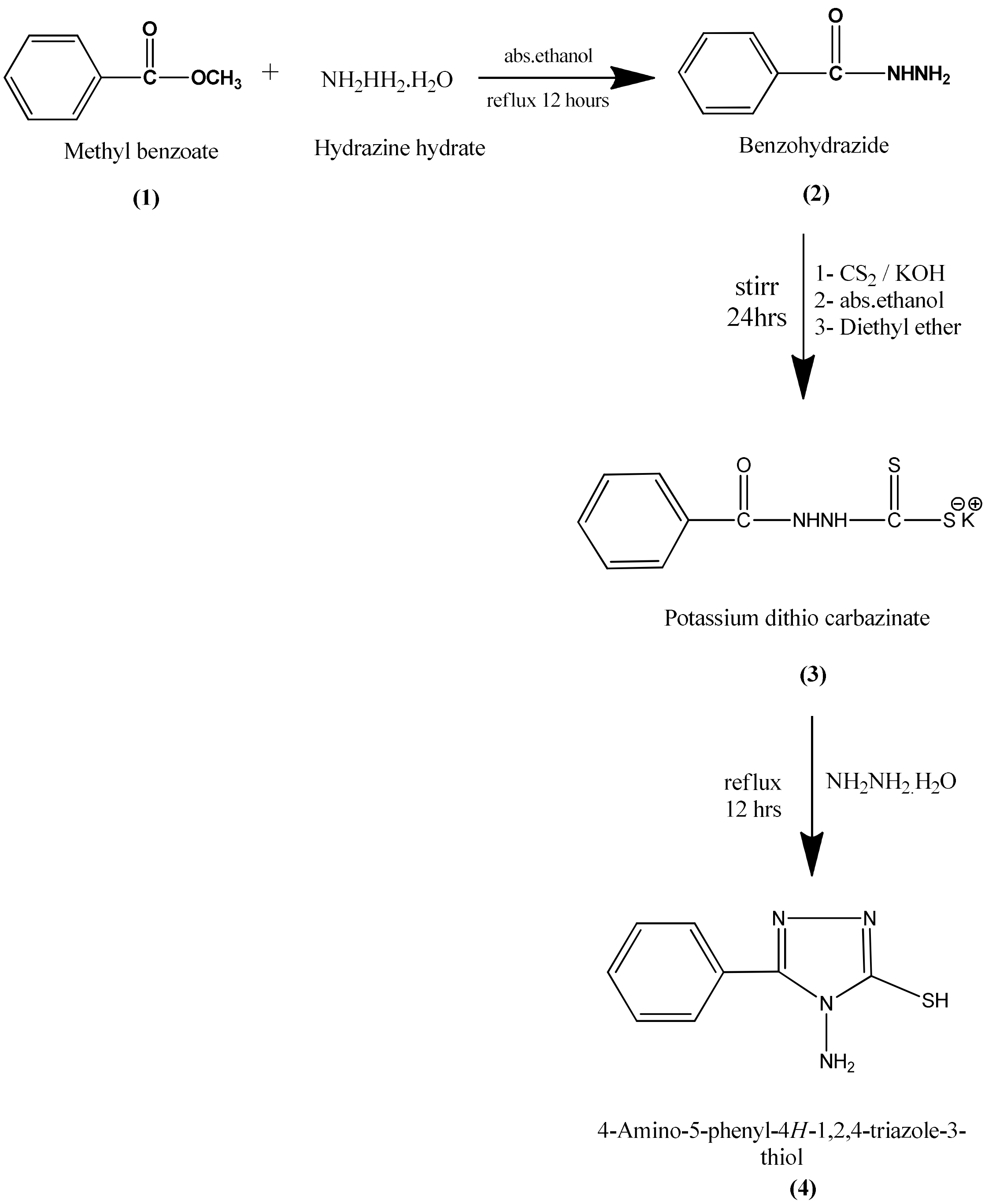
Finally, the new intermediate 1,2,4-triazole derivatives **6a-e** further reacted with different azides as click chemistry in presence of tertiary butanol: water (2:1) as solvent, copper sulphate and sodium ascorbate as cyclizing agent yielded 1,2,4-triazolo-1,2,3-triazole as new bis heterocycles **(7-20)**, as shown in **Scheme 3**.

The chemical structures of the new compounds were established through analytical and spectral data such as infrared (IR), ^1H NMR, ^{13}C NMR, mass spectrometry (MS) and elemental analysis. The IR spectra the characteristic absorption bands due to alkyenyl triple bond around 2115-2200 cm^{-1} were disappeared, however, an absorption stretching bands in the rang 1428-1661 cm^{-1} have been observed which assigned for C=N and C=C of triazole ring. The ^1H NMR of compounds **7-20** show the presence of a distinguish singlet signal attributed for methylene group ($-CH_2-S$), and disappearance of signal characteristic for proton of terminal alkyenyl proton (≡CH), whereas showed a singlet signal for each of triazole proton and for Schiff base protons. However, revealed the presence of the corresponding absorption peaks of substituents groups attached to benzene rings in the molecule. Also, the ^{13}C NMR spectrum shows all the expected signals with the disappearance of terminal alkyenyl carbon. Finally, the mass spectra supported the proposed chemical structure for the new compounds 7-20 as listed in the experimental part. However, The mass spectra of the final new compound **7** showed *m/z*at 472 $(M+H)^+$**.** The fragmentation pattern of the compound **7**was proposed to demonstrate the chemical structure as in the following **Chart (1)**.

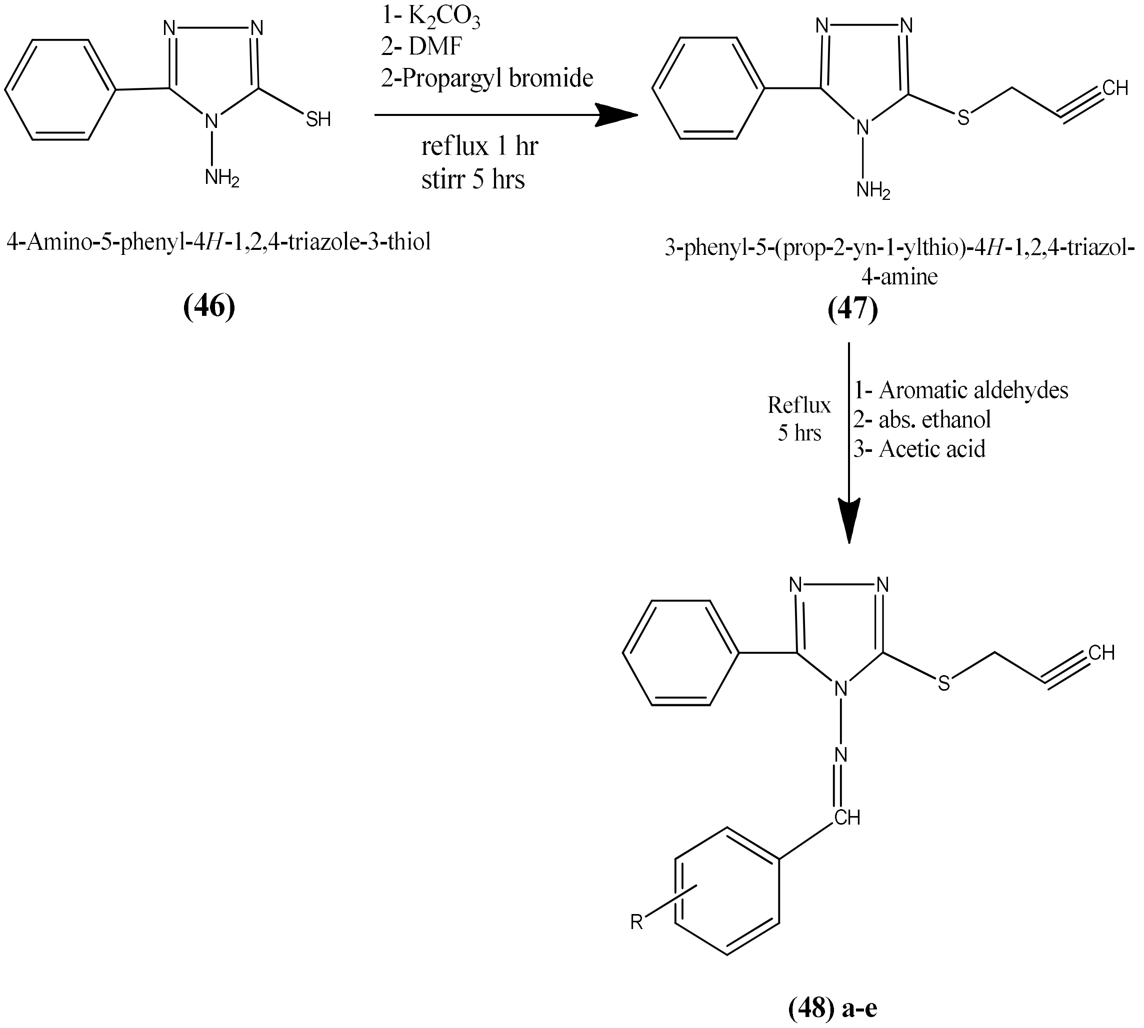
BIOLOGICAL ACTIVITY

3.2 In vitro antimicrobial activity

In order to check the biological activity, all the target compounds **7-20** from this series were screened for their *in vitro* antimicrobial activity against standard bacterial and fungal strains. For the antibacterial studies two gram positive and three gram negative bacterial strains *S.a: Staphylococcus aureus (ATCC 25923) S.e: Staphylococcus Epidermidis (ATCC 12228); and E.c: Escherichia Coli (ATCC25922); P.m: Proteus merabilis (ATCC 13376); P.a: Pseudomonas aerugenosa (ATCC 27853)* respectively were used, and for antifungal activity *Candida albicans* (ATCC10231) was used. Some gram positive and gram negative bacterial strains were sensitive against some tested compounds. The compounds **7**, **10** and **20** were active against most of the tested bacterial strains (gram positive and gram negative). However, compound **10** was the most active compounds among the series of tested compounds showed superior activities than the standard drug amoxicillin against *S.a, S.e and P.m* with inhibition zone of 28 mm, 25 and 25 mm, respectively compared to standard drug amoxicillin which showed zone of inhibition 29 mm, each at concentration of 200 µg/disc. Meanwhile, compounds **11**, **12** and **18** were active against all the tested gram positive bacterial strains whereas inactive towards the tested gram negative bacterial strains. From the results it can be concluded that substituent such as methoxy, bromine at *ortho* position, chlorine at *meta* position in the aromatic ring enhance the antibacterial activity. Most of the tested compounds were resistant against the *candida albicans*, among the tested compounds, five compounds were active and showed zone of inhibition 8-10 mm at concentration of 200 µg/di.

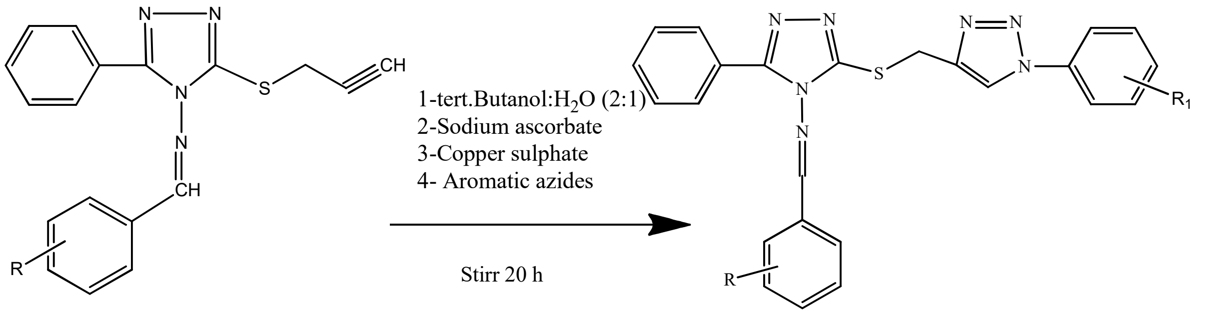


SCHEME 1: Synthesis of 1,2,4-triazole-3-thiol



a) R= H , b) R= *p*-OCH_3 , c) R= *p*-OH , *m*-OCH_3 , d) R= *o*-OH, e) R= *o*-Br

SCHEME 2: Synthesis of Schiff bases compounds (6a-e)



(6a-e) **(7-20)**

Compound No	Substituents R	Substituents R$_1$
7	**H**	***m*-Cl**
8	**H**	***o*-CH3**
9	**H**	***o*-Cl**
10	***p*-OCH$_3$**	***p*-CH3**
11	***p*-OCH$_3$**	***p*-NO$_2$**
12	***m*-OCH$_3$, *p*-OH**	***p*-CH3**
13	***m*-OCH$_3$, *p*-OH**	***p*-NO$_2$**
14	***o*-OH**	***p*-CH3**
15	***o*-OH**	***p*-NO$_2$**
16	***o*-Br**	***p*-CH$_3$**
17	***o*-Br**	***p*-NO$_2$**
18	***o*-Br**	***m*-Cl**
19	***o*-Br**	***o*-Cl**
20	***o*-Br**	**H**

SCHEME 3: Synthesis of bis-triazole derivatives (7-20)

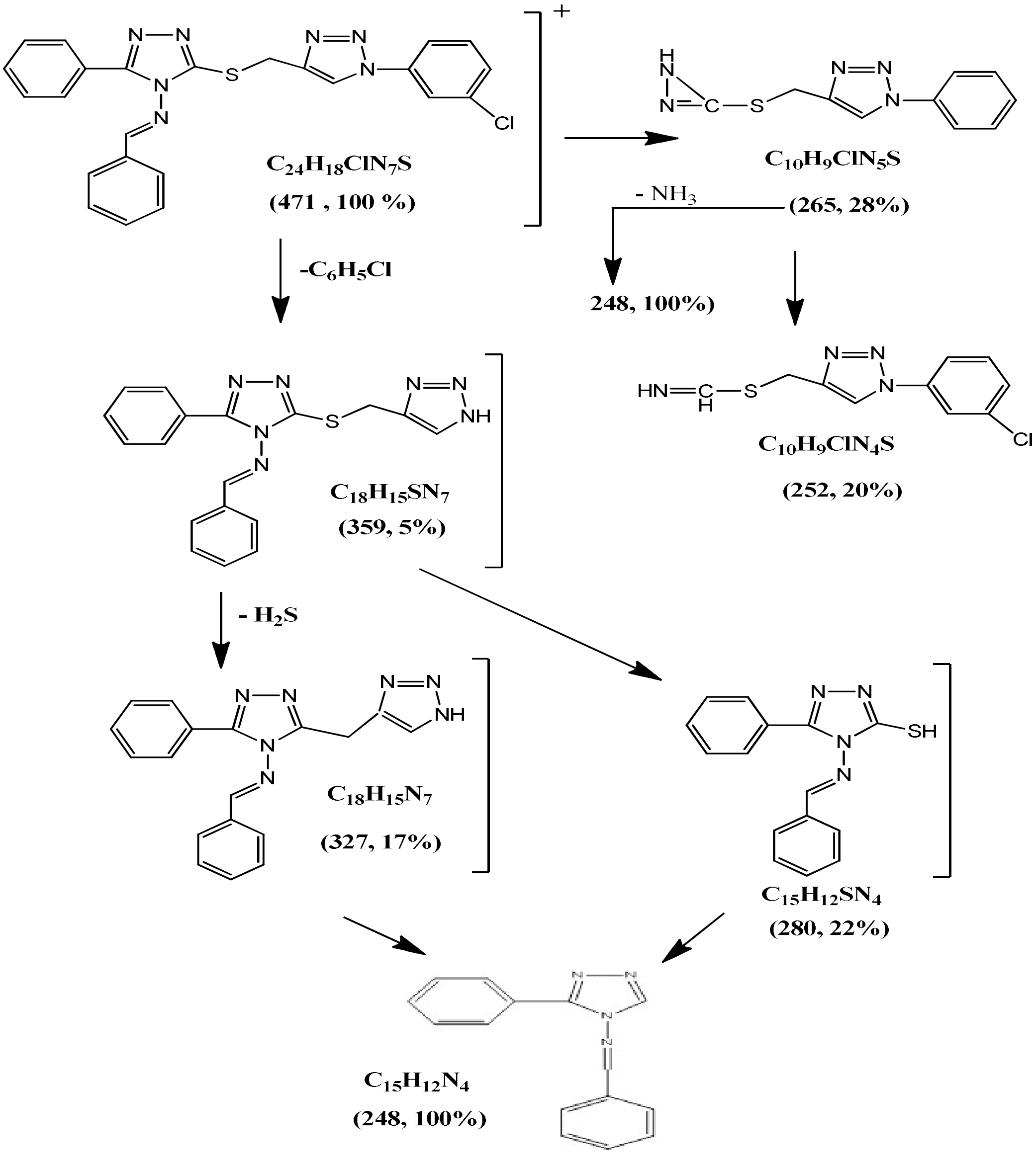


Chart 1

Mass Fragmentation of Compound 55

Table 2: Antimicrobial activity of the synthesized compounds

Antimicrobial Activity at concentration of 200 µg/disc (bacterial strains), 100 µg/disc (Fungal strain)						
Comp. No.	**Gram Positive Bacteria**		**Gram Negative Bacteria**			**Fungal Strain**
	S.a	***S.e***	***E.c***	***P.m***	***p.a***	***C.a***
7	29	----	24	20	08	**10**
8	----	----	----	----	----	----
9	----	----	----	----	----	----
10	28	25	19	25	07	**10**
11	08	07	----	----	----	----
12	08	19	----	----	----	**8**
13	----	----	----	----	----	----
14	----	----	----	----	----	
15	----	----	----	----	----	----
16	----	----	----	----	----	----
17	----	----	----	----	----	----
18	08	08	----	----	----	**8**
19	----	----	----	----	----	----
20	**24**	19	----	07	07	**8**
Amoxicillin	**26**	**23**	**18**	**22**	**15**	----
Fluconazole	**NT**	**NT**	**NT**	**NT**	**NT**	**18**

S.a: Staphylococcus aureus (ATCC 25923) S.e: Staphylococcus Epidermidis (ATCC 12228); E.c: Escherichia Coli (ATCC25922); P.m: Proteus Merabilis (ATCC 13376); P.a: Pseudomonas Aerugenosa (ATCC 27853); C.a: Candia albican(ATCC10231); **NT: Not Tested*****; ---:*** **No zone of inhibition**

CONCLUSION

New heterocyclic compounds based on 1,2,4-triazole moiety have been designed and synthesized. The chemical structures of the new compounds have been confirmed by analytical and spectral analysis, IR, ^{1}H-NMR, ^{13}C-NMR and MS. The synthesized compounds **7-20** were evaluated for antimicrobial activities showed promising activities comparing to standard drug amoxicillin.

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