IJBPAS, January, 2019, 8(1): 37-52

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

SYNTHESIS AND CHARACTERIZATION OF 1,2,3-TRIAZOLE AND HYDRAZONE DERIVATIVES AS POTENT ANTIMICROBIAL AGENT

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Received 13th July 2018; Revised 14th Aug. 2018; Accepted 30th Oct. 2018; Available online 1st Jan. 2019

https://doi.org/10.31032/IJBPAS/2019/8.1.4606

ABSTRACT

A library of eleven new conjugates of 1,2,3-triazole-hydrazone hybrid molecules have been synthesized using click chemistry. All the novel compounds were confirmed by different spectroscopic techniques and were evaluated for their antimicrobial activity. Most of the compounds were active against tested gram positive bacterial strains, whereas resistant towards two gram negative bacterial strains *P.m* and *P.a*. Compound **7** showed superior activity than the standard drug amoxicillin against *E.c and K.p* with zone of inhibition **30 mm** and **32 mm** respectively compared to standard drug amoxicillin which showed zone of inhibition **29 mm** each at concentration of 200 µg/disc. Compound **6** also showed promising activity with zone of inhibition **28 mm** and **30 mm** against *E.c and K.p* respectively comparable to standard drug. Five compounds showing significant activity were further evaluated for their minimum inhibition concentration (MIC). Compound **7** showed **MIC 25 µg/disk** against *E.c and K.p,* compound **12** showed **MIC 25 µg/disk** against *E.c.* Fungal strain *Candida albicans* was found to be resistant against most of the tested compounds. From the results it can be concluded halogen group present in the aromatic ring enhance the activity and need further work to synthesize more compounds to get some lead compound for drug candidate

Keywords: 1,2,3-Triazole hydrazone hybrid, Phenyl azide, Click Chemistry, Antibacterial, Antifungal; Minimum inhibitory concentration

1. INTRODUCTION

1,2,3-triazole and 1,2,4-triazole are the basic heterocyclic rings present in many medicinal agents like carboxyamidotriazole (CAI), β-lactum antibiotic Tazobactum, the cephalosporine Cefatrizine, etc. [1,2]. 1,2,3-Triazole moieties are attractive connecting units because they form hydrogen bonding and stable to metabolic degradation which favours the binding of biomolecular targets thus improve solubility.[3,4] A large volume of research has been carried out on 1,2,3-triazole and their derivatives, thereby proving the pharmacological significance of this important nucles [5-16]. There are very few 1,2,3-triazole-containing molecules on the market or are in the last stage of clinical trials. Therefore, it is a challenge for medicinal chemists to synthesize better drugs possessing drug likeness, better pharmacokinetic properties, selectivity and activity

The synthesis of 1,2,3-triazole derivatives using click chemistry is an emerging field [17]. Click chemistry is a powerful tool in number of applications like combinatorial chemistry,and target-templated in vitro chemistry for lead discovery. The formation of triazole by click chemistry accelerates lead finding as well as lead optimization therefore click chemistry has diverse impact in the area of drug discovery and development [18].

In the last two decades, there is increase in the incidence of life-threatening fungal infections because of greater use of immunosuppressive drugs and prolonged use of broad spectrum antibiotics [19]. Due to the multi drug resistance and toxicity of the presently marketed antimicrobial drugs there is necessity to develop new class of antimicrobial agents with high efficacy, low toxicity and varied mode of action from present clinical drugs. We therefore herein report the synthesis of 1,2,3-triazole –hydrazone hybrid using click chemistry and also evaluation of their antimicrobial activity (Scheme 1).

2. MATERIALS AND METHODS

2.1 Chemistry

All the chemicals (reagent grade) used were purchased from Sigma-Aldrich (Germany) and Merck Co. (Germany). They were used without further purification. Melting points were measured on an electro thermal melting point apparatus (Fisher Scientific) and are uncorrected. ^{1}H NMR was recorded on a Jeol 600 instruments in $CDCl_3$/Methanol-d_4 using TMS as an internal standard. Chemical shifts (δ) and coupling constants (*J*) are given in ppm and Hertz respectively. Mass spectra were recorded on thermo scientific-LCQ Fleet (LCF10605) using electron spray ionization method at 75 eV. Mass-

spectrometric (MS) data is reported in *m/z*. Elemental analysis was carried out using LEECO Elemental Analyser. Elemental analysis data is reported in % standard and were within ±0.4% of the calculated values.

2.2 Experiment

2.2.1 General procedures for the synthesis of methyl 2-(prop-2-ynyloxy)benzoate (2)

In a 250 ml clean dry round bottom flask equipped with a thermometer, magnetic stirrer and magnetic bar, charged methylsalisylate (0.005 mol), dry acetone (50 ml) and anhydrous potassium carbonate (0.0075 mol). The solution was refluxed with stirring for 1h and then allowed to cool to room temperature followed by addition of propargyl bromide (0.006 mol) slowly to the solution. The reaction mixture was further refluxed for 12h, after completion of the reaction monitored by TLC, the reaction mass was filtered; filtrate was concentrated to get light yellow liquid.

Yield: 90% ; IR (ATR): v (cm^{-1}) : 3140, 2930, 2119, 1611, 1588, 1385, 1467, 1256, 1009; ESI +ve MS (m/z) : 190 $(M+1)^+$

2.2.2 General procedures for the synthesis of methyl 2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)benzoate (3)

Compound 2 (0.005 mol) was suspended in 20 ml of tert-butanol : water (2:1) at ambient temperature , to this suspension Copper sulphate pentahydrate , Sodium ascorbate and phenyl azide (0.006 mol) were added. The reaction mixture was allowed to stir for 12 h at ambient temperature. The progress of the reaction was monitored by TLC, after completion of the reaction, the reaction mass was poured into the water and extracted with ethylacetate, seperated ethylacetate layer was washed with water and dried with anhydrous sodium sulphate. The ethylacetate was evaporated and the crude product was recrystalized in methanol.

Yield: 74.0% ; IR (ATR): v (cm^{-1}): 3050, 2915, 1728, 1220, 1048; ^1H NMR ($CDCl_3$ 300 MHz) δ (ppm): 3.9 (s, 3H), 5.41 (s, 2H), 7.05 (t, 1H, J = 7.5 Hz), 7.15 (d, 1H, J = 8.4 Hz), 7.51 (t, J = 7.5Hz, 1H), 7.84-8.02 (m, 3H), 8.30-8.41 (m, 4H); ESI +ve MS (m/z): 309 $(M)^+$ Elemental analysis: Calculated for molecular formullae $C_{17}H_{15}N_3O_3$ Calculated : C, 66.01; H, 4.89; N, 13.58; Found: C, 66.05; H, 4.90; N, 13.56%.

2.2.3. Procedure for synthesis of 2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)benzohydrazide (4)

To a 250 mL clean and dry round bottom flask compound 3 (0.005 mol) was charged followed by addition of absolute alcohol 150 mL and hydrazine monohydrate (0.0075 mol), the reaction mass was refluxed for 6 hrs, after

completion of the reaction, the reaction mass was concentrated to around 30 mL, cool to room temperature and pour on to the crushed ice, stirred for 30 minutes, the precipitated solid mass was filtered, washed with cold water and dried.

Yield: 90% ; IR (ATR): ν (cm^{-1}): 3312, 3222, 3025, 2915, 1652, 1590, 1473, 1333, 1270, 1187, 1000; ESI +ve MS (m/z) : 309 $(M)^+$

2.2.4. General Methods For Preparation of triazole-hydrazone hybrid (5-15)

In a clean and dry 100 mL round botom flask compound 4 (0.001 mol) was charged, then added 50 mL absolute alcohol, followed by addition of dfferent aromatic aldehydes (0.0011 mol) and few drops of glacial acetic acid. The reaction mass was then refluxed for 3-8 hrs, the progress of the reaction was monitored by TLC, after completion of the reaction, the reaction mass was concentrated to 15-20 mL and cool to 10-20 ^{0}C to get white to off white crystals, the crystals was filtered washed with cold 10 ml ethanol:water (1:1) and dried to get 47-87.5% yield.(Table 1)

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(4-nitrobenzylidene)benzohydrazide (5)

Light yellow crystals; m.p. 209-211 ^{0}C; Yield: 87.5%. IR (ATR): ν (cm^{-1}) :3258, 3096, 2915, 1727, 1651, 1523, 1508, 1450, 1338, 1292, 1110, 1048; ^{1}H NMR (DMSO-d_6, 850 MHz) δ (ppm)**:** δ 5.43 (s, 2H), 7.14 (t, 7.6Hz, 1H), 7.40-7.45 (m, 2H), 7.47-7.52 (m, 1H), 7.57 (t, J = 7.6 Hz, 2H), 7.66 (dd, J = 5.1 Hz, J = 1.7 Hz, 1H), 7.86 (d, J = 7.6 Hz, 2H), 7.90 (d, J = 9.3Hz, 2H), 8.29 (d, J = 8.5 Hz, 2H), 8.38 (s, 1H), 8.89 (s, 1H), 11.89 (s, 1H); ^{13}C NMR (DMSO-d_6 213 MHz): 62.09 (-O-CH_2-), 112.96, 114.27, 114.94, 120.64, 121.22, 122.88, 123.20, 123.28, 124.11, 124.28, 126.33, 127.50, 129.03, 130.17, 133.01, 136.91, 144.22, 148.30, 156.07, 163.01; ESI -ve MS (m/z) : 441 $(M-1)^+$;Elemental analysis :Calculated for molecular formullae $C_{23}H_{18}N_6O_4$ Calculated: C, 62.44; H, 4.10 N, 19.00; Found : C, 62.42; H, 4.11; N, 18.98%.

2-((1-phenyl-1H-1,2,3-triazol-4-yl) methoxy)-N'-(4-chlorobenzylidene) benzohydrazide (6):

Off white crystals; m.p. 168-170 ^{0}C; Yield: 86.90%; IR (ATR): ν (cm^{-1}) :3258, 3096, 2915, 1727, 1651, 1596, 1524, 1508, 1491, 1339, 1292, 1237, 1110, 1085,1035; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): 5.40 (s, 2H), 7.06-7.14 (m, 2H), 7.28 (d, J = 7.2Hz, 2H), 7.51-7.53 (m, 4H), 7.73-7.81 (m, 5H), 8.16 (s, 1H, triazole ring proton), 8.28 (s, 1H), 11.40 (s, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz): 62.7 (O-CH_2), 112.62, 120.60, 128.79, 128.89, 129.28, 129.95, 132.96, 133.37, 136.52, 143.11, 147.61, 155.65,

162.03; ESI -ve MS (m/z) : 429 $(M-1)^+$ Elemental analysis :Calculated for molecular formullae $C_{23}H_{18}ClN_5O_2$ Calculated : C, 63.96; H, 4.20; N, 16.22. Found : C, 63.94; H, 4.21; N, 16.20%.

2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-N'-(4-N,N-dimethylbenzylidene) benzohydr azide (7)

Off white crystals; m.p. 171-173 ^{0}C; IR (ATR): ν (cm^{-1}) :3302, 3076, 2915, 1727, 1644, 1596, 1523, 1508, 1480, 1339, 1292, 1251, 1110, 1046,1034; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm) : 2.99 (s, 6H), 5.41 (s, 2H), 6.62 (d, J = 9.0Hz, 2H), 7.07-7.18 (m, 2H), 7.43-7.60 (m, 5H), 7.72 (d, J = 7.2 Hz, 2H), 8.04 (s, 1H), 8.15 (s, 1H), 8.28 (d, J = 6.6 Hz, 2H), 10.94 (s, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) : 40.16, 62.78, 111.60, 112.67, 120.62, 120.91, 121.58, 122.31, 129.08, 129.30, 129.87, 136.61, 142.53, 143.40, 145.07, 149.25, 151.76, 155.79, 162.08; ESI +ve MS (m/z) : 441 $(M+1)^+$ Elemental analysis: Calculated for molecular formullae $C_{25}H_{24}N_6O_2$ Calculated: C, 68.17; H, 5.49; N, 19.08. Found: C, 68.15; H, 5.50; N, 19.07%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(4-hydroxybenzylidene) benzohydrazide (8)

White crystals; m.p. 192-194 ^{0}C; IR (ATR): ν (cm^{-1}) :3302, 3090, 2915, 1728, 1642, 1596, 1508, 1499, 1449, 1369, 1287, 1240, 1162, 1104, 1086, 1018; ^{1}H NMR(DMSO-$_{d6}$300 MHz) δ (ppm): δ 5.44 (s, 2H), 6.83 (d, J = 8.1Hz, 2H), 7.05-7.24 (m, 2H), 7.45-7.59 (m, 6H), 7.78 (d, J = 7.2 Hz, 2H), 8.12-8.33 (m, 1H), 8.45 (s, 1H), 9.39 (s, 1H), 11.33 (s, 1H); ^{13}C NMR (DMSO-$_{d6}$75 MHz,) : 62.05, 115.02, 119.73, 119.97, 121.26, 124.67, 128.58, 129.10, 131.30, 133.54, 134.05, 145.07, 149.25, 151.76, 155.79, 162.08; ESI +ve MS (m/z) : 414 $(M+1)^+$ Elemental analysis: Calculated for molecular formullae $C_{23}H_{19}N_5O_3$ Calculated: C, 66.82; H, 4.63; N, 19.94. Found: C, 68.79; H, 5.51; N, 19.09%.

2-[[2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}benzoylimino]methyl]benzoic acid (9)

White crystals; m.p. 173-175 ^{0}C; IR (ATR): ν (cm^{-1}):3248, 3091, 2915, 1709, 1621, 1537, 1501, 1479, 1368, 1286, 128, 1162, 1127, 1071, 1019; ^{1}H NMR (DMSO-$_{d6}$850 MHz) δ (ppm) : 5.33 (s, 2H), 7.11 (t, J = 7.6 Hz, 1H), 7.40- 7.42 (m, 2H), 7.45-7.59 (m, 6H), 7.62-7.64 (m, 1H), 7.80 (d, J = 7.6 Hz, 2H), 7.90 (dd, J = 7.6 Hz, J = 0.85Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 8.81 (s, 1H), 9.05 (s, 1H), 11.83 (s, 1H); ^{13}C NMR (DMSO-$_{d6}$213 MHz) : 62.17 (s, 2H), 113.65, 120.06, 120.18, 120.85, 124.56, 126.73, 128.28, 129.81, 129.99, 132.0, 132.05, 134.62, 136.54, 143.82, 146.05, 155.47, 162.67, 167.99. ESI +ve MS (m/z) : 442 $(M+1)^+$ Elemental analysis :Calculated for molecular formullae $C_{24}H_{19}N_5O_4$ Calculated : C,

65.30; H, 4.34; N, 15.86. Found: C, 65.28; H, 4.33; N, 15.84%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(4-hydroxy-3-methoxybenzylidene) benzo hydrazide (10)

Light brown crystals; m.p. 194-196 ^{0}C; IR (ATR): ν (cm^{-1}) :3264, 3058, 2960, 1638, 1591, 1552, 1524, 1500, 1479, 1287, 1227, 1122, 1073, 1034; ^{1}H NMR ($DMSO_{d6}$-850 MHz) δ (ppm): 3.79 (s, 3H), 5.42 (s, 2H), 6.80 (d, J = 8.0 Hz, 1H), 6.96 (dd, J = 8.5 Hz, J = 2.4Hz, 1H), 7.12 (t, J = 7.6 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.49-7.58 (m, 5H), 7.67 (dd, J = 6.8 Hz, J = 1.7 Hz, 1H), 7.86 (d, J = 6.8 Hz, 2H), 8.11 (s, 1H), 8.91 (s, 1H), 9.57 (s, 1H), 11.41 (s, 1H); ^{13}C NMR (DMSO-$_{d6}$213 MHz) : 55.95 (s, 3H), 62.64, 109.34, 114.12, 114.94, 115.88, 120.63, 121.75, 122.47, 123.16, 123.19, 124.32, 125.99, 129.35, 130.40, 130.43, 132.67, 136.93, 144.23, 148.06, 148.49, 149.44, 155.97, 162.16; ESI -ve MS (m/z) : 442 $(M-1)^+$ Elemental analysis :Calculated for molecular formullae $C_{24}H_{21}N_5O_4$ Calculated: C, 65.00; H, 4.77; N, 15.79. Found: C, 64.98; H, 4.79; N, 15.82%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(2-bromobenzylidene)benzohydrazide (11)

Light brown crystals; m.p. 160-162 ^{0}C; IR (ATR): ν (cm^{-1}) :3282, 3076, 2916, 1663, 1597, 1530,1502, 1482, 1351, 1292, 1227, 1167, 1038; ^{1}H NMR ($CDCl_3$ 300 MHz)δ (ppm) : 5.41 (s, 2H), 6.85 (d, J = 8.7 Hz, 2H), 7.00-7.18 (m, 2H), 7.44-7.54 (m, 4H), 7.67-7.84 (m, 4H), 8.15-8.22 (m, 3H),11.13 (s, 1H); ESI -ve MS (m/z) : 476 $(M)^+$ Elemental analysis: Calculated for molecular formullae $C_{23}H_{18}$ BrN_5O_2 Calculated: C, 58.00; H, 3.81; N, 14.70. Found: C, 58.03; H, 3.83; N, 14.72%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(4-methoxybenzylidene)benzohydrazide (12):

Off white crystals; m.p. 184-186 ^{0}C; IR (ATR): ν (cm^{-1}) :3285, 3075, 2930, 1644, 1597, 1550,1502, 1480, 1450, 1295, 1248, 1229, 1171, 1106, 1024; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): 3.86 (s, 3H), 5.41 (s, 2H), 6.85 (d, J = 8.4 Hz, 2H), 6.99-7.18 (m, 2H), 7.44-7.54 (m, 4H), 7.66 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 7.5 Hz, 2H), 8.15-8.22 (m, 2H), 8.28 (d, J = 7.5 Hz, 1H), 11.13 (s, 1H); ^{13}C NMR ($CDCl_3$75 MHz) : 55.19, 62.73, 110.79, 112.78, 120.39, 120.49, 121.96, 126.70, 128.86, 129.63, 131.37, 132.20, 133.00, 136.70, 143.83, 155.86, 157.94, 161.70; ESI +ve MS (m/z): 428 $(M+1)^+$ Elemental analysis : Calculated for molecular formullae $C_{24}H_{21}N_5O_3$ Calculated: C, 67.44; H, 4.95; N, 16.38. Found: C, 67.42; H, 4.92; N, 16.37%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(2-

methoxybenzylidene)benzohydrazide (13):

Off white crystals; m.p. 168-170 ^{0}C; IR (ATR): v (cm^{-1}) : 3296, 3127, 3066, 2930, 1659, 1598, 1530, 1500, 1484, 1448, 1349, 1293, 1250, 1163, 1143, 1046; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 3.89 (s, 3H), 5.45 (s, 2H), 6.79 (d, J = 7.2 Hz, 1H), 6.94-7.03 (m, 1H), 7.15-7.18 (m, 3H), 7.46-7.81 (m, 5H), 8.04-8.06 (brd m, 2H), 8.20-8.56 (m, 3H), 11.13 (s, 1H); ESI +ve MS (m/z) : 428 $(M+1)^+$ Elemental analysis :Calculated for molecular formullae $C_{24}H_{21}N_5O_3$ Calculated: C, 67.44; H, 4.95; N, 16.38. Found: C, 67.41; H, 4.93; N, 16.36%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-((furan-2-yl)methylene}benzohydrazide (14):

Light yellow crystals; m.p. 163-165 ^{0}C; IR (ATR): v (cm^{-1}) :3282, 3079, 2930, 1655, 1598, 1542,1502, 1480, 1449, 1295, 1234, 1155, 1144, 1055, 1037; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): 5.54 (s, 2H), 6.47 (s, 1H), 6.58 (d, J = 9.0 Hz, 1H), 6.89-7.05 (m, 2H), 7.29-7.50 (m, 5H), 7.74 (d, J= 8.4 Hz, 2H), 8.11 (s, 1H), 8.20-8.38 (m, 2H), 11.28 (s, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) : 62.75, 111.83, 112.74,113.15, 114.19, 120.67, 120.85, 122.32, 129.15, 129.86, 132.83, 133.26, 136.75, 138.32, 143.27, 144.41, 149.71,155.84, 161.91; ESI +ve MS (m/z : 388 $(M+1)^+$ Elemental analysis :Calculated for molecular formullae $C_{21}H_{17}N_5O_3$ Calculated: C, 65.11; H, 4.42; N, 18.08 Found: C, 65.08; H, 4.40; N, 18.10%.

2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(2-methoxybenzylidene)benzohydrazide (15):

White crystals; m.p. 188-190 ^{0}C; IR (ATR): v (cm^{-1}) :3285, 3083, 2930, 1654, 1598, 1548,1501, 1480, 1449, 1295, 1235, 1146, 1052, 1032; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): 5.41 (s, 2H), 7.00-7.18 (m, 2H), 7.34 (brd, s, 3H), 7.43-7.54 (m, 4H), 7.73-7.75 (m, 4H), 8.15 (s, 1H), 8.27-8.29 (m, 2H), 11.25 (s, 1H); ^{13}C NMR ($CDCl_3$ 75 MHz) : 62.74, 112.66, 120.61,120.76, 122.37, 127.78, 128.49, 129.19, 129.75, 129.90, 130.12,132.93, 133.24, 134.08, 136.73, 148.60, 155.91, 161.82; ESI +ve MS (m/z) : 398 $(M+1)^+$ Elemental analysis : Calculated for molecular formullae $C_{23}H_{19}N_5O_2$ Calculated: C, 69.51; H, 4.82; N, 17.62 Found: C, 69.47; H, 4.80; N, 17.60%.

2.4 Microbiology

2.4.1 In vitro antimicrobial assay

The antimicrobial studies of the synthesized compounds were carried out against following different bacterial and fungal strains, the Gram Positive bacterial strains: *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603) *Escherichia coli* (ATCC 25922),

Staphylococcus aureus (ATCC 25923), Gram Negative Bacterial Strains: *Proteus mirabilis (ATCC 13376), Sreptococcus pneumonie (ATCC49619), Enterococcus faecalis (ATCC 29212), Staphylococcus epidermidis (ATCC12228)* and Fungal Strain: *Candida albicans* (ATCC 10231) were used for antimicrobial studies.

2.4.1.1. 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 dimethylformamide (DMF) to prepare chemicals of stock solution of 2 mg/mL and simple susceptibility screening test was carried out using reported disc diffusion method [20]. 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*, *Klebsiella pneumoniae,* Macconcy agar was used and for *Escherichia coli* and *Staphylococcus aureus* Muller Hinton agar were used. Six-millimeter diameter disc were prepared and 200 µg and 100 µg of the compounds were loaded. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organism. Amoxicillin (200, 100 µg) and Fluconazole (100, 50 µg) were used as standard drugs. Dimethylformamide was used as solvent (negative controls).

2.4.1.2. Minimum Inhibition Concentration (MIC):

The minimum inhibitory concentration (MIC) was determined by the conventional paper disc diffusion method [21]. The compounds showing promising zone of inhibition were dissolved in dimethylformamide (DMF) and loaded on the disks by micropipette with different concentrations (200, 100, 50, 25, 12.50, 6.25, 3.125 and 1.56 µg/disk). The loaded disks were kept on microbes inoculated agar plate surface. The plates were kept at 37 ^{0}C for 24 h, each experiment was repeated three times and MIC was expressed as the lowest concentration at which inhibition of test organism takes place.

3. RESULTS AND DISCUSSION

3.1 Chemistry

Treatment of methylsalisylate 1 with propargyl bromide in presence of dry acetone and potassium carbonate yielded methyl 2-(prop-2-ynyloxy)benzoate **2** which upon click chemistry with phenyl azide using tertiary butanol and water as solvent and sodium ascorbate and copper sulphate penta hydrate as cyclising agent yielded an intermediate 1,2,3-triazole

methyl ester **3,** this intermediate was then refluxed with hydrazine monohydrate in absolute alcohol yielded a key intermediate 1,2,3-triazole hydrazide **4.** The key intermediate **4** was finally reacted with different aromatic aldehydes to yield 1,2,3-triazole-hydrazone hybrid novel molecules 2-[{1-(phenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N'-(substituted benzylidene) benzohydrazide **5-15 (Table 1)**.

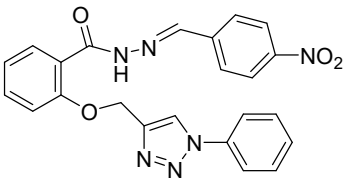
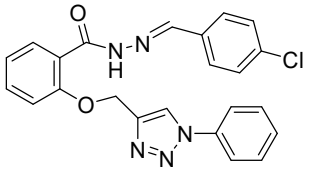
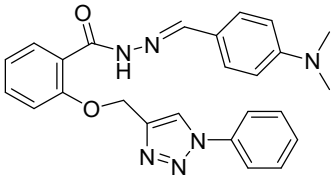
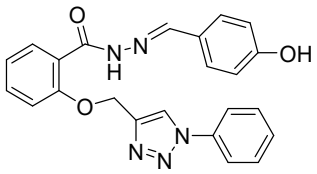
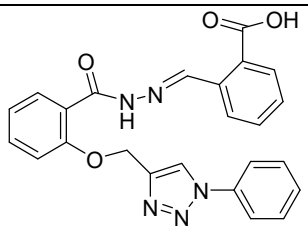
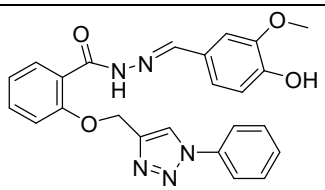
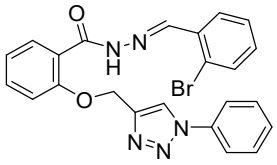
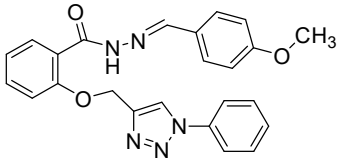
The proposed structures of the compounds were confirmed by different analytical techniques such as IR, NMR, CHNS analyzer and mass spectrometry. All the spectral data were found to be in agreement with the proposed structures of all the target compounds. Formation of methyl 2-(prop-2-ynyloxy)benzoate **2** was confirmed by the presence of a strong peak at 2119 cm^{-1} in IR spectra for triple bonded carbon of propargyl group supporting the propargylation of hydroxyl group. The formation of compound **2** was further confirmed from mass spectrometry which showed molecular ion peak at 109. Formation of the cyclised product methyl 2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)benzoate **3** was confirmed by the absence of strong peak at 2119 cm^{-1} in FT-IR due to triple carbon-carbon bond of compound 2 and appearance of peak at 1450-1523 cm^{-1} due to 1,2,3-triazole ring stretching, also presence of additional peaks in aromatic region in ^1H NMR suggested the presence of phenyl ring in the molecule. Further confirmation of the proposed structure of the compound **3** was obtained from mass spectra which exhibited molecular ion peak at 309. Formation of compound **4** was supported by appearance of peak at 3192 cm^{-1} and 3300 cm^{-1} in FT-IR due to NH_2 and N-H stretching and absence of singlet at δ 3.9 in ^1H NMR confirming the substitution of methoxy group by hydrazine group. The target compounds **5-15** was confirmed by the presence of peak at 1621-1663 cm^{-1} for carbonyl carbon of amide (CO-N-H) and peak at 1591-1598 cm^{-1} due to C=N stretching of benzilidine group in FT-IR. The ^1H NMR spectrum revealed a singlet in range δ 5.33-5.53 confirming the presence of $–O-CH_2-$ in the vicinity of aromatic ring which deshielded the methylene proton. The extra proton due to extra aromatic ring and substituents of the different aldehydes confirm the structure of the compounds. Finally all the compounds were confirmed by mass spectrometry and elemental analysis, where all the results were in agreement with the proposed structure of the synthesized compounds.

3.2 In vitro antimicrobial activity

In order to check the biological activity, all the target compounds from this series were screened for their *in vitro* antimicrobial activity against standard bacterial and fungal strains. For the antibacterial studies

four gram positive and four gram negative bacterial strains *S.e: Staphylococcus Epidermidis (ATCC 12228); S.a: Staphylococcus aureus (ATCC 25923); E.f:Enterococcus Faecalis (ATCC 29212); S.p: Streptococcus Pneomoniae (ATCC 49619) and E.c: Escherichia Coli (ATCC25922); K.p: Klebsiella Pneumoniae (ATCC 700603); P.m: Proteus Merabilis (ATCC 13376); P.a: Pseudomonas Aerugenosa (ATCC 27853)* respectively were used, and for antifungal activity *Candida albicans* (ATCC10231) was used. From the results it was observed that most of the gram positive strains were sensitive towards the all the tested compounds, but some gram negative bacterial strains *p.m* and *p.a* were resistant against all the tested compounds. Compound **7** showed superior activities than the standard drug amoxicillin against *E.c and K.p* with zone of inhibition **30 mm** and **32 mm** respectively compared to standard drug amoxicillin showed zone of inhibition **29 mm** each at concentration of 200 µg/disc. Compound **6** also showed promising activity with zone of inhibition **28 mm** and **30 mm** against *E.c and K.p respectively* comparable to standard drug. Gram positive bacterial strain *S.e* was more sensitive toward compound **6** and showed zone of inhibition **22 mm** where standard drug showed zone of inhibition **16 mm.** From the results it can be concluded that substituents such as chloro, hydroxy, N,N,-dimethyl in the aromatic ring enhance the antibacterial activity and aromatic ring without the substituents have least activity. Most of the tested compounds were resistant against the *candida albicans*. Among the tested compounds, compound **9** was only active and showed zone of inhibition **10 mm** at concentration of 200 µg/disc. The compounds **5**, **6**, **7**, **11** and **12** showing more zone of inhibition were further evaluated for their minimum inhibition concentration (MIC). Compound **7** showed MIC 25 µg/disk against *E.c and K.p,* compound **12** showed MIC 25 µg/disk against *E.c.* The antimicrobial activity results are summarized in Table 2, Table 3 and Figure 1.

Table 1: Physical data of the phenyl based 1,2,3-triazole-hydrazone hybrid

Compd. No.	Structure	Chemical Name	%Yield
5		**)2-{(1-phenyl-1H-1,2,3-triazol-4-yl}methoxy]-N'-(4-nitrobenzylidene)benzohydrazide**	**87.50**
6		**)2-{(1-phenyl-1H-1,2,3-triazol-4-yl}methoxy)-N'-(4-chlorobenzylidene)benzohydrazide**	**86.90**
7		**)2-{(1-phenyl-1H-1,2,3-triazol-4-yl}methoxy)-N'-(4-(dimethylamino)benzylidene)benzohydrazide**	**75.00**
8		**)2-{(1-phenyl-1H-1,2,3-triazol-4-yl}methoxy)-N'-(4-hydroxybenzylidene)benzohydrazide**	**81.00**
9		**-2})2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)benzoylimino)methyl)benzoic acid**	**83.00**
10		**)2-{(1-phenyl-1H-1,2,3-triazol-4-yl}methoxy]-N'-(4-hydroxy-3-methoxybenzylidene)benzohydrazide**	**75.00**
11		**)2-{(1-phenyl-1H-1,2,3-triazol-4-yl}methoxy)-N'-(2-bromobenzylidene) benzohydrazide**	**60.00.**
12		**2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(4methoxybenzylidene) benzohydrazide**	**65.83**

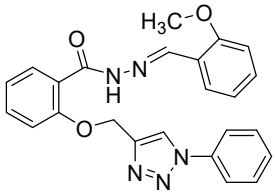
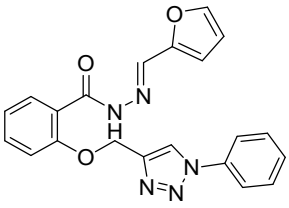
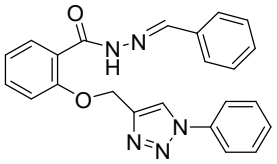
13		**2-{(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy}-N'-(2-methoxybenzylidene) benzohydrazide**	**65.00**
14		**)2-[{(1-phenyl-1H-1,2,3-triazol-4-yl}met hoxy]-N'-{(furan-2-yl)methylene} benzohydrazide**	**47.00**
15		**2-{(1-phenyl-1H-1,2,3-triazol-4-yl)meth oxy}-N'-benzylidenebenzohydrazide**	**76.00**

Table 2: Antimicrobial activity of phenyl based 1,2,3-triazole-hydrazone hybrid

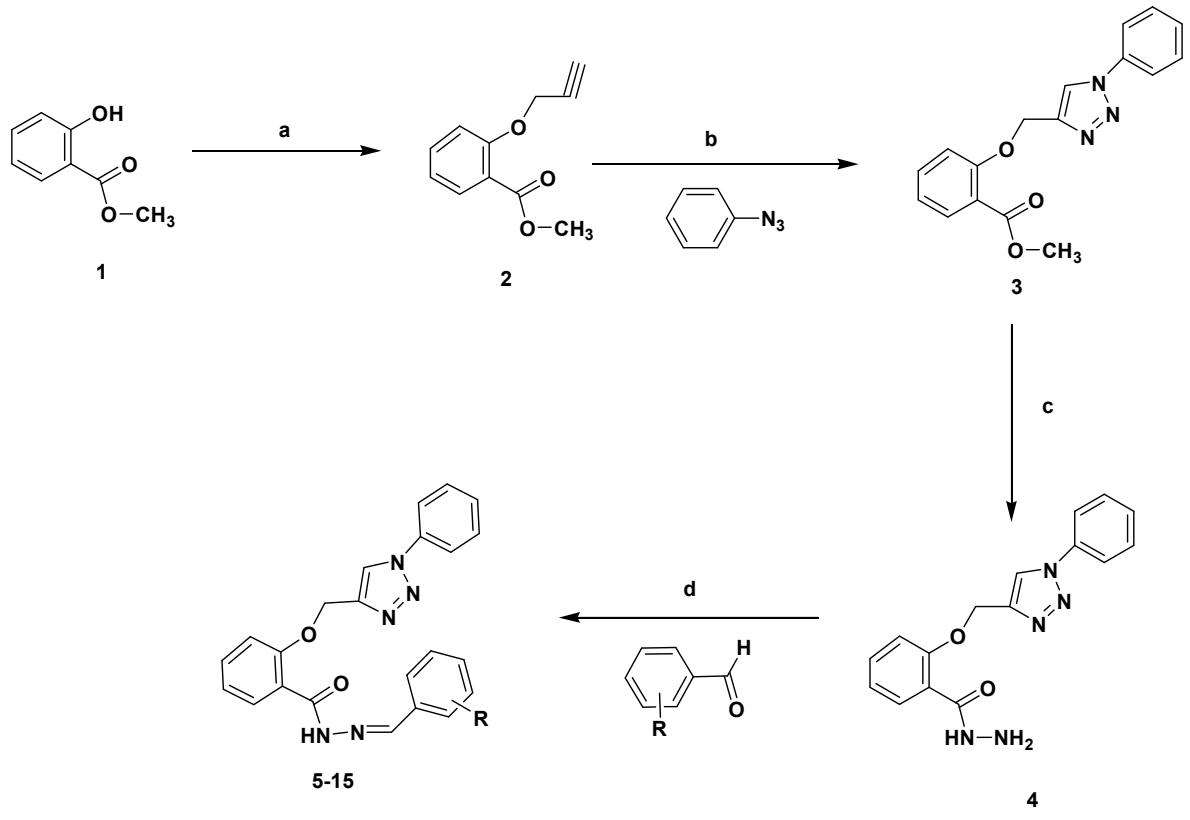
Antibacterial Activity 200 (100) µg/disc								**Antifungal Activity**
Comp.No.	**Gram Positive Bacteria**				**Gram Negative Bacteria**				***Yeast***
	S.e	***S.a***	***E.f***	***S.p***	***E.c***	***K.p***	***P.m***	***P.a***	***C.a***
5	**---**	**---**	**---**	**10 (--)**	**29 (15)**	**29 (14)**	**----**	**---**	**---**
6	**---**	**---**	**22 (12)**	**12 (--)**	**28 (14)**	**30 (12)**	**-----**	**---**	**---**
7	**18 (10)**	**12 (---)**	**18 (13)**	**10 (--)**	**30 (14)**	**32 (16)**	**-----**	**---**	**---**
8	**12 (8)**	**10 (---)**	**16 (13)**	**14 (8)**	**15 (10)**	**15 (10)**	**-----**	**---**	**---**
9	**15 (8)**	**15 (8)**	**16 (12)**	**13 (--)**	**-----**	**----**	**-----**	**---**	**10**
10	**---**	**---**	**14 (--)**	**12 (--)**	**-----**	**-----**	**-----**	**---**	**---**
11	**12 (---)**	**14 (---)**	**12 (---)**	**10 (--)**	**27 (14)**	**22 (12)**	**-----**	**---**	**---**
12	**10 (---)**	**12 (---)**	**14 (8)**	**---**	**34 (16)**	**30 (16)**	**-----**	**---**	**---**
13	**10 (---)**	**10 (---)**	**---**	**---**	**-----**	**-----**	**-----**	**---**	**---**
14	**10 (---)**	**10 (---)**	**---**	**11 (--)**	**-----**	**-----**	**-----**	**---**	**---**
15	**10 (---)**	**10 (--)**	**---**	**12 (--)**	**-----**	**-----**	**-----**	**---**	**---**
Amoxicilin	**18 (12)**	**>30**	**16 (12)**	**18 (13)**	**29 (18)**	**29 (14)**	**24 (16)**	**22 (11)**	**NT**
Fluconazole	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**16**

S.e: Staphylococcus Epidermidis (ATCC 12228); S.a: Staphylococcus aureus (ATCC 25923); E.f:Enterococcus Faecalis (ATCC 29212); S.p: Streptococcus Pneomoniae (ATCC 49619)E.c: Escherichia Coli (ATCC25922); K.p: Klebsiella Pneumoniae (ATCC 700603); P.m: Proteus Merabilis (ATCC 13376); P.a: Pseudomonas Aerugenosa (ATCC 27853); C.a: Candia albican(ATCC10231); **NT: Not Tested*; ---:* No zone of inhibition**

Table 3: Minimum Inhibition Concentration (MIC)

Compounds	Bacterial Strains			
	E.c	*K.p*	*S.a*	*E.f*
5	>50	>100	100	>100
6	100	50	>100	>100
7	25	25	50	>100
11	>50	>100	>50	50
12	25	50	100	>50
Amoxicillin	25	50	12.5	25

MIC measured in µg/disk



a. Dry Acetone, K_2CO_3, reflux; b. t-BuOH, $CuSO_4.5H_2O$. sodium ascorbate; c. NH_2NH_2, EtOH, Reflux ; d. CH_3COOH, EtOH, reflux

Scheme: Synthesis of 1,2,3-triazole-hydrazone hybrid

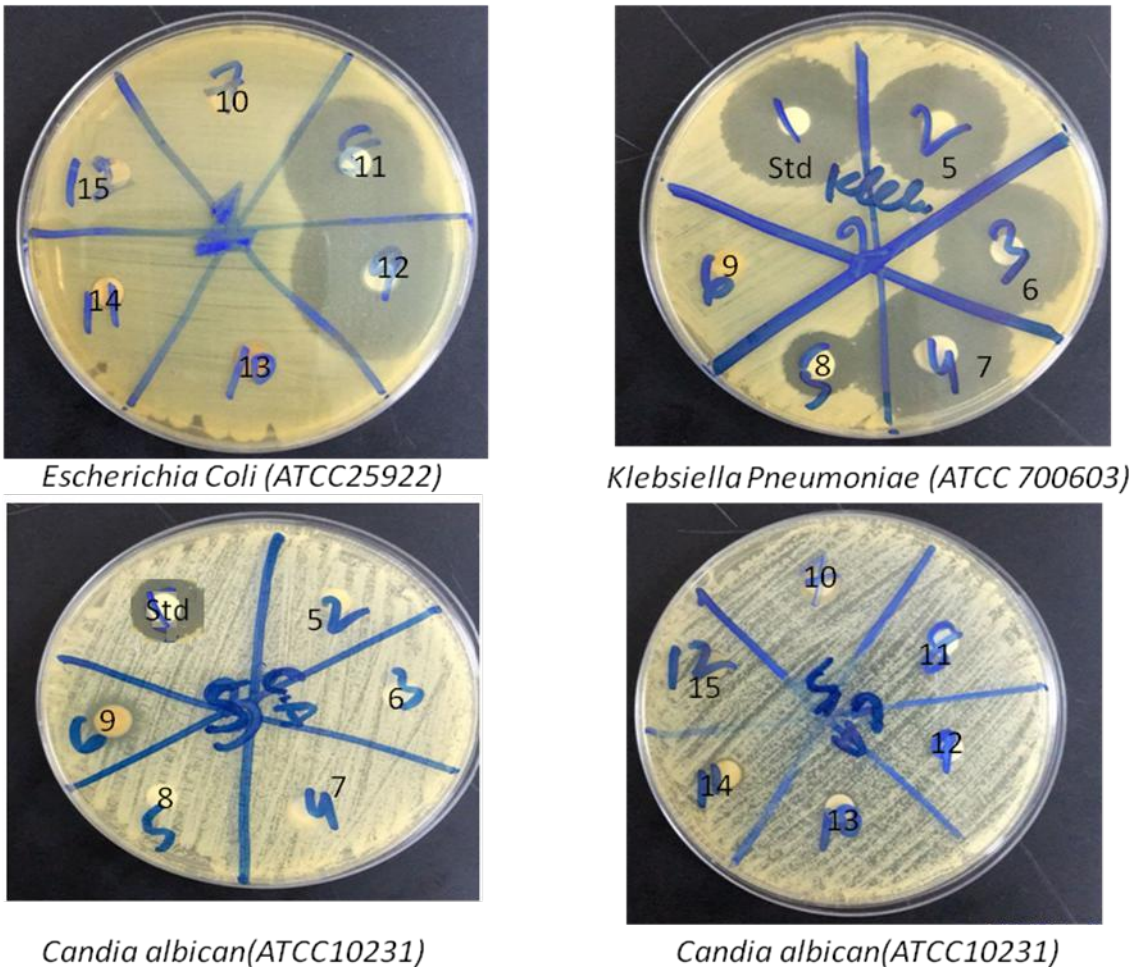


Figure 1: Zone of inhibition of tested compounds at concentration 200 µg/disc

4. CONCLUSION

A library of eleven new conjugates of 1,2,3-triazole-hydrazone hybrid molecules have been synthesized and confirmed by different analytical techniques. Most of the compounds were active against tested gram positive bacterial strains, whereas resistant towards two gram negative bacterial strains *P.m* and *P.a*. Five compounds showing significant activity were further evaluated for their minimum inhibition concentration (MIC). Compound **7** showed MIC 25 µg/disk against *E.c and K.p,* compound **12** showed MIC 25 µg/disk against *E.c.* Fungal strain *Candida albicans* was resistant against most of the tested compounds.

From the results it can be concluded halogen group present in the aromatic ring enhance the activity and need further work to synthesize more compounds to generate structure activity relationship to get some lead compound for drug candidate and also these molecules can be studies for anticancer and antioxidant studies.

5. ACKNOWLEDGEMENTS

The authors are thankful to King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia for providing the financial support to this

project (Grant No. 1-17-01-013-0005) and Albaha University for providing the necessary facilities to Department of Chemistry. The authors are also thankful to Dr T S Thakur, microbiologist, regional laboratory and central blood bank , Albaha Kingdom of Saudi Arabia for evaluating the antimicrobial activity.

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