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FACILE SYNTHESIS OF AMIDE INCORPORATED 1,2,3-TRIAZOLE DERIVATIVES, THEIR ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

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ABSTRACT

A library of nineteen new amide incorporated 1,2,3-triazole derivatives have been synthesized and evaluated for antioxidant and antimicrobial activities. Firstly, 1,2,3-triazole derivative, (2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzoic acid) **4** was prepared by click chemistry using methyl salicylate **1** as starting material. This key intermediate **4** was coupled with different aromatic amines, aliphatic amines and acid hydrazide using HoBt and EDC as coupling agent to get the amide incorporated 1,2,3-triazole derivatives as target compounds **5-23.** All the new compounds were evaluated for antioxidant and antimicrobial activities. The antioxidant activity was performed by DPPH assay. The antimicrobial activity was done by disc diffusion method against bacterial and fungal strains. Further MIC was calculated for six compounds (**6, 9, 10, 18, 19** and **22**) against two gram positive (*S.aureus, E.faecalis*) and two gram negative (*E.coli, P.aerugenosa*) bacterial strains. Among the tested compounds, two compounds **6** and **19** showed superior DPPH scavenging activity with 94.12 and 95.64% inhibition, respectively compared to the standard molecule ascorbic acid which showed 91.80% inhibition. Compounds **6** and **19** revealed strong antimicrobial effect. Compound **6** showed MIC 25 µg/disk against *E. coli and E.faecalis,* compared to the standard drug Amoxicillin which showed MIC 12.5 and >25 µg/disk respectively. Compound **19** showed MIC 25 µg/disk against *E. coli* and 50 µg/disk against *P.aerugenosa* compared to standard drug Amoxicillin which showed MIC 12.5 and 25

µg/disk, respectively. From the antioxidant and antimicrobial results, it can be concluded that compounds **6** and **19** may be used as a lead molecule in the development of more potent antibacterial agent in future.

Keywords: Amide incorporated 1,2,3-Triazole, Click Chemistry, Antioxidant, Antibacterial, Antifungal; Minimum inhibitory concentration

1. INTRODUCTION

Antimicrobial resistance (AMR) is the ability of a microorganism like bacteria, fungi and viruses to stop the functions of antimicrobial agents such as antibiotics [1-2]. The increase in antibiotic resistance leads to ineffective treatments and persistence of infections which sometimes lead to death [3-4]. As listed by World Health Organization, antimicrobial resistance is the biggest concerns about human health today [5]. Microbial infections and their rapid increase in the resistance towards available antibiotics pose a serious problem to human health [6]. Therefore, design and development of effective antimicrobial agents is a challenge for the medicinal chemists [7-8]. Triazoles are the basic heterocycles which form the skeleton of many important drugs such as Tazobactum [9], carboxyamidotriazole [10], Cefatrizine [11]. 1,2,3-Triazoles, due to presence of three adjacent nitrogen atoms forms hydrogen bonds which leads to increase in solubility and interaction with the bimolecular targets [12]. 1,2,3-Triazoles are stable towards extreme conditions indicating its aromatic character [13]. 1,2,3-Triazoles has been conjugated with therapeutically important agents like ciplofloxacin, sulphanilamide, fluconazole, etc which exhibited significant antibacterial [14,15], antifungal [16], anticancer [17], anti-inflammatory [18] and antitubercular activity [19]. There are very few 1,2,3-triazole-containing molecules in 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. Various amide derivatives of naphthoquinone, benzothiazole, salinomycin, naphthoquinones have been reported which exhibited diverse biological activity viz. antimicrobial [20], anticonvulsant [21], antitumour [22], antiviral [23] and antitubercular [24]. The biological potential of 1,2,3-triazole and amide derivatives prompted us to synthesize new compounds wherein the two active moiety is conjugated under one construct to enhance the antimicrobial activity. Therefore, in this paper, we have design, synthesized and evaluated antioxidant and antimicrobial activities of

new amide incorporated 1,2,3-triazole conjugates **5-23** as shown in **SCHEME.**

2. MATERIAL AND METHODS

2.1 Chemistry

All the chemicals (reagent grade) used were purchased from Sigma-Aldrich (Germany) and Merck Co. (Germany), Loba (India). All the chemicals used directly without further purification. IR spectra were recorded on thermos scientific iS-50 by ATR method. NMR was recorded on a Bruker 300 MHz and 850 MHz instruments in $CDCl_3$/DMSO-d_6 using TMS as an internal standard, chemical shift and coupling constant are given in Hertz (Hz) and parts per million (ppm). 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*. Melting points were recorded by using automatic melting point (Stuart SMP40). Elemental analysis was carried out using LEECO/Elementar Elemental Analyzer. Elemental analysis data is reported in % standard and were within ±0.4% of the calculated values.

2.2 Experiment

2.2.1 Synthesis of methyl 2-(prop-2-ynyloxy)benzoate (2)

In a 250 ml round bottom flask, added methyl salicylate **1** (5.0g, 0.032 mol), dry acetone (40 ml) and anhydrous potassium carbonate (6.8g, 0.049 mol). The reaction mixture was refluxed with stirring for 1h. It was then cool to 20-30^{0}C and added propargyl bromide (4.6g, 0.038 mol) gradually. The reaction mass was refluxed for 12h, after completion of the reaction monitored by TLC, the reaction mixture was filtered. The filtrate was concentrated to 20 ml and pour into water (50 ml) and extracted with chloroform (50 mLX2). The organic layer was separated, dried over anhydrous sodium sulphate and evaporated under reduced pressure to get light yellow liquid. Yield: 90%;

2.2.2 Synthesis of methyl 2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzoate (3)

In a 250 ml round bottom flask, charged 2-(prop-2-ynyloxy) benzoate **2** (4.0g, 0.021 mol), tert-butanol: water (1:1, 40 mL), copper sulphate pentahydrate (5.2g, 0.021 mol) and sodium ascorbate (4.1g, 0.021 mol) followed by the addition of 2-chlorophenyl azide (3 .2g, 0.021 mol). The reaction mass was stirred for 12 h at room temperature. The reaction was monitored by TLC, after completion of the reaction, the reaction mass was poured into water (100 ml) and extracted with ethyl acetate (100 mlX2). The organic layer was separated, washed with water (100ml) and dried over anhydrous sodium sulphate. The organic layer was evaporated under reduced pressure to give crude product,

which was recrystallized in methanol. Yield: 6.0 g 83% m.p.: 91-93℃. Spectroscopic analysis: IR (ATR): v (cm^{-1}): 3074, 2941, 2064, 2036, 1707, 1603, 1582, 1491, 1454, 1426, 1417, 1303, 1247, 1237, 1190, 1167, 1133, 1095, 1071, 1036; ^{1}H NMR, δ, ppm (300 MHz, $CDCl_3$): 3.88 (s, 3H, -O-CH_3), 5.43 (s, 2H, -O-CH_2-), 7.04 (t, J=7.8 Hz, 1H, Ar-H) 7.16 (d, J= 8.4 Hz, 1H, Ar-H), 7.53-7.43 (m, J=9.9, Hz, 2H, Ar-H) 7.64 (m, J= Hz, 3H, Ar-H) 7.85-7.82 (m, J= Hz, 1H, Ar-H) 8.19 (s, 1H, triazole-proton); ^{13}C NMR, δ, ppm (75 MHz, $SDCl_3$): 52.02 (-O-CH_3), 63.55 (-O-CH_2-), 114.19, 120.68, 121.14, 124.99, 127.85, 127.92, 130.79, 130.84, 131.83, 133.71, 144.28, 157.78, 166.41 (C=O); ESI +ve MS (m/z): 343.76 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{17}H_{14}O_3N_3Cl$ Calculated: C: 59.40, H: 4.10, O: 13.96, N: 12.22% found: C: 59.50, H: 4.18, O: 13.99, N: 12.23%.

2.2.3 Synthesis of 2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzoic acid (4)

In a 250 mL round bottom flask, charged Methyl 2-[(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy]benzoate **3** (5.0 g, 0.045 mol) , methanol (30 mL) and water (30 mL) followed by addition of sodium hydroxide (2.5 g, 0.0625 mol). The reaction mass was refluxed for 10 h. After complete hydrolysis, monitored by TLC, methanol was evaporated and then acidified by HCl (1N) to pH 2-3 to get solid precipitate. The solid mass was filtered, washed with water (20 mL), dried and recrystalized in methanol. Yield: 4.2 g; 87.5%; m.p.: 133-135 ℃. IR (ATR): v (cm^{-1}): 3156, 2878, 1702, 1604, 1581, 1494, 1470, 1457, 1419, 1394, 1320, 1373, 1256, 1213, 1172, 1091, 1074, 1045. ^{1}H NMR, δ, ppm (300 MHz, $CDCl_3$): 5.57 (s, 2H, O-CH_2-), 7.17 (t, J=7.8 Hz, 1H, Ar-H), 7.29 (t, J=8.1 Hz, 1H, Ar-H), 7.44-7.50 (m, 2H, Ar-H), 7.57-7.66 (m, 4H, Ar-H), 8.15 (s, 1H, triazole-proton), 8.17 (s, 1H, -O-H); ^{13}C NMR, δ, ppm (75 MHz, $CDCl_3$): 63.43 (-O-CH2-), 113.44, 122.74, 125.41, 127.77, 128.08, 128.57, 130.87, 131.16, 133.85, 134.47, 135.05, 141.83, 156.92, 165.56 (C=O); ESI +ve MS (m/z): 329.34 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{16}H_{12}O_3N_3Cl$ Calculated: C: 58.28, H: 3.67, O: 14.56, N: 12.74 found: C: 58.26, H: 3.69, O: 14.58, N: 12.78%.

2.2.4 General procedures for the synthesis of 2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-(substituted)benzamide (5-23)

In a 100 mL round bottom flask, charged compound **4** (0.2 g, 0.06 mmol), dichloromethane (50 mL) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.12g, 0.06 mmol) portion wise at 0-5 ^{0}C, stir for 10 min. Then, HOBt (0.090g, 0.06 mmol) was added portion wise at 0-5 ^{0}C, followed

by the addition of amine portion wise at 0-5 ^{0}C. The reaction mass was stirred for 10 hrs at 0-5 ^{0}C. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mass was poured into water and extracted with MDC (50 mLX2) and combined organic layer was washed with HCl (1N, 20 mL), then sodium hydroxide solution (1M 20 mL) and finally with water (20 mL×2) and dried over anhydrous sodium sulphate. The organic layer was evaporated to get crude products. The desired products were purified by crystallization or column chromatography using petroleum ether and ethyl acetate as eluents.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-(4-fluorophenyl)benzamide (5)

Off white crystals; m.p.: 146-148℃; Yield: 56%; IR (ATR): v (cm^{-1}): 3342 (N-H), 3067, 1653 (C=O), 1600, 1577, 1538, 1508, 1484, 1449, 1403, 1312, 1289, 1217, 1160, 1126, 1089, 1067, 1047, 1024. ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 5.49 (s, 2H, -O-CH_2-), (t, J=6.3 Hz, 2H, Ar-H), 7.17-7.21 (m, 1H, Ar-H), 7.44-7.54 (m, 3H, Ar-H), 7.56-7.563 (m, 5H, Ar-H), 8.10 (s, 1H, Ar-H), 8.28-8.31 (m, 1H, triazole-proton), 9.93 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 213 MHz): 62.55 (-O-CH_2-), 112.74, 121.79, 121.83, 122.41, 122.45, 125.08, 127.76, 128.12, 128.75, 130.92, 131.28, 132.72, 133.25, 134.50, 134.63, 142.15, 155.92, 159.73, 163.00 (C=O). ESI +ve MS (m/z): 422 $(M+H)^+$ Element analysis : Calculated for molecular formulae for $C_{22}H_{16}O_2N_4FCl$ Calculated: C, 62.49; H, 3.81; O, 7.57; N, 13.25 found: C, 62.50; H, 3.87; O, 7.55; N, 13.28%.

N-(4-chlorophenyl)-2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzamide (6)

Off white crystals; m.p.: 178-180 ℃; Yield: 80%; IR (ATR): v (cm-1): 3348 (N-H), 3078, 1647 (C=O), 1593, 1533, 1492, 1448, 1398, 1315, 1293, 1246, 1209, 1180, 1165, 1133, 1118, 1090, 1078, 1045; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 5.48 (s, 2H, O-CH_2-), 7.17-7.23 (m, 3H, Ar-H), 7.49-7.54 (m, 3H, Ar-H), 7.59-7.82 (m, 5H, Ar-H), 8.10 (s, 1H, Ar-H), 8.30 (t, J=1.5 Hz, 1H, triazole-proton), 9.98 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 213 MHz): 62.54 (-O-CH_2-), 112.72, 121.41, 122.34, 122.43, 125.07, 127.76, 128.14, 128.73, 128.77, 128.85, 130.93, 131.29, 132.75, 133.37, 134.49, 137.20 (C-NH), 142.08, 155.92, 163.08 (C=O). ESI +ve MS (m/z): 439.25 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{22}H_{16}O_2N_4Cl_2$ Calculated: C, 60.15; H, 3.67; O, 7.28; N, 12.75 Found: C, 60.17; H, 3.69; O, 7.30; N, 12.74%.

N-(3-chlorophenyl)-2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzamide (7)

Off white crystals; m.p.: 138-140℃; Yield: 76%; IR (ATR): v (cm^{-1}): 3337 (N-H), 3052, 1658 (C=O), 1594, 1550, 1498,

1481, 1450, 1406, 1324, 1298, 1285, 1255, 1217, 1156, 1128, 1100, 1076, 1045; ^{1}H NMR ($CDCl_3$ 850 MHz) δ (ppm): δ 5.50 (s, 2H, O-CH_2-), 7.04 (t, J= 7.6 Hz, 1H, Ar-H), 7.18-7.20 (m, 2H, Ar-H), 7.26 (s, 1H, Ar-H), 7.43 (d, J= 7.6 Hz, 1H, Ar-H), 7.47-7.51 (m, 2H, Ar-H), 7.54-7.55 (m, 1H, Ar-H), 7.60 (t, J= 7.6 Hz, 1H, Ar-H), 7.65 (d, J= 7.6 Hz, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 8.12 (s, 1H,triazole ring H), 8.29 (d, J= 6.8 Hz, 1H, Ar-H), 9.96 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 213 MHz): 62.61 (-O-CH_2-), 112.75, 118.16, 120.19, 122.27, 122.47, 123.96, 125.10, 127.86, 128.11, 128.67, 129.85, 130.88, 131.22, 132.81, 133.45, 134.49, 134.51, 139.71, 142.07, 155.94, 163.15 (C=O); ESI +ve MS (m/z): 438$(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{22}H_{16}O_2N_4Cl_2$ Calculated: C: 60.15, H: 3.67, O: 7.28, N: 12.75 found: C, 60.19; H, 3.70; O, 7.29; N, 12.74%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-(m-tolyl)benzamide (8)

White crystals; m.p.: 123-125℃; Yield: 81%; IR (ATR): ν (cm^{-1}): 3385 (N-H), 3073, 1671 (C=O), 1592, 1548, 1450, 1373, 1303, 1241, 1162, 1124, 1089, 1072, 1048; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 2.30 (s, 3H, -CH_3), 5.50 (s, 2H, O-CH_2-), 6.88 (d, J=7.2 Hz, 1H, Ar-H), 7.13-7.21 (m, 3H, Ar-H) 7.30-7.33 (m, 1H, Ar-H), 7.43-7.56 (m, 2H, Ar-H), 7.55-7.62 (m, 4H, Ar-H), 8.10 (s, 1H, Ar-H), 8.27-8.31 (m, 1H, triazole-proton), 9.79 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 213 MHz): 21.53 (-CH_3), 62.65 (-O-CH_2-), 112.78, 117.19, 120.80, 122.36, 122.70, 124.81, 125.22, 127.76, 128.04, 128.70, 128.74, 130.87, 131.17, 132.68, 133.15, 134.55, 138.77, 142.24, 155.91, 163.00 (C=O); ESI +ve MS (m/z): 418 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{19}O_2N_4Cl$ Calculated: C, 65.95; H, 4.57; O, 7.64; N, 13.38 found: C, 65.93; H, 4.59; O, 7.67; N, 13.40%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-(p-tolyl)benzamide (9)

Off white crystals; m.p.: 156-158℃; Yield: 85%; IR (ATR): ν (cm^{-1}): 3362 (N-H), 3077, 1644 (C=O), 1595, 1533, 1512, 1498, 1486, 1448, 1405, 1321, 1294, 1209, 1164, 1133, 1096, 1077, 1044, 1023; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): 2.29 (s, 3H, -CH_3), 5.49 (s, 2H, O-CH_2-), 7.08 (d, J=8.1 Hz, 2H, Ar-H), 7.18 (t, J=7.8 Hz, 2H, Ar-H) 7.44-7.55 (m, 3H, Ar-H), 7.57-7.61 (m, 4H, Ar-H), 8.09 (s, 1H, Ar-H), 8.27-8.31 (m, 1H, triazole-proton), 9.79 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$ 75 MHz): 23.64 (-CH_3), 62.67 (O-CH_2), 112.80, 120.11, 122.36, 125.15, 127.80, 128.03, 128.81, 129.38, 130.86, 131.18, 132.69, 133.05, 133.51, 135.95, 142.26, 155.88, 162.86 (C=O). ESI +ve MS (m/z): 418 $(M+H)^+$ Element analysis : Calculated for molecular

formulae $C_{23}H_{19}O_2N_4Cl$ Calculated: C, 65.95; H, 4.57; O, 7.64; N, 13.38 found: C, 65.99; H, 4.58; O, 7.66; N, 13.42%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-(4-methoxyphenyl)benzamide (10)

Off white crystals; m.p.: 133-135°C; Yield: 60%; IR (ATR): v (cm^{-1}): 3344 (N-H), 3072, 1651 (C=O), 1600,1532, 1511, 1495, 1450, 1408, 1325, 1292, 1237, 1188, 1174, 1160, 1128 1094, 1076, 1048; ^1H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 3.78 (s, 3H, O-CH_3), 5.49 (s, 2H, O-CH_2), 6.81-6.84 (m, 2H, Ar-H), 7.18 (t, J=7.2 Hz, 2H, Ar-H), 7.46-7.54 (m, 4H, Ar-H), 7.56-7.61 (m, 3H, Ar-H), 8.09 (s, 1H, Ar-H), 8.28-8.31 (m, 1H, triazole-proton), 9.76 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 75 MHz): 55.48 (-O-CH_3), 62.69 (-O-CH_2), 112.80, 114.06, 121.66, 122.37, 125.09, 127.78, 128.04, 128.80, 130.87, 131.18, 132.66, 132.98, 142.29, 155.92, 162.76 (C=O); ESI +ve MS (m/z): 434 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{19}O_3N_4Cl$ Calculated: C, 63.52; H, 4.40; O, 11.04; N, 12.88 found: C, 63.55; H, 4.42; O, 11.06; N, 12.86%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-phenylbenzamide (11)

Off white crystals; m.p.: 131-133°C; Yield: 84%; IR (ATR): v (cm^{-1}): 3370 (N-H), 3055, 1667 (C=O), 1596, 1533, 1486, 1450, 1437, 1375, 1322, 1290, 1246, 1226, 1201, 1179, 1163, 1129, 1099, 1075, 1049, 1036; ^1H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 5.49 (s, 2H, O-CH_2-), 7.08 (t, J=7.5 Hz, 1H, Ar-H), 7.18 (t, J=7.8 Hz, 2H, Ar-H), 7.21-7.30 (m, 2H, Ar-H), 7.43-7.53 (m, 3H, Ar-H), 7.57-7.61 (m, 4H, Ar-H), 8.10 (s, 1H, Ar-H), 8.28-8.31 (m, 1H, triazole-proton), 9.90 (s, 1H, N-H);^{13}C NMR ($CDCl_3$ 213 MHz): 62.68 (-O-CH_2), 112.81, 120.15 122.39, 122.65, 122.65, 125.27, 127.82, 128.06, 128.82, 128.91, 130.88, 131.22, 132.73, 133.21, 134.55, 138.51, 142.23, 155.92, 163.05 (C=O). ESI +ve MS (m/z): 404 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{22}H_{17}O_2N_4Cl$ Calculated: C, 65.27; H, 4.23; O, 7.90; N, 13.84 Found: C, 65.30; H, 4.24; O, 7.92; N, 13.88%.

N-(2-chlorophenyl)-2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzamide (12)

Off white crystals; m.p.: 112-114°C; Yield: 76%; IR (ATR): v (cm^{-1}): 3355 (N-H), 3074, 1674 (C=O), 1588, 1522, 1493, 1442, 1313, 1227, 1160, 1122, 1094, 1075, 1034; ^1H NMR ($CDCl_3$ 850 MHz) δ (ppm): δ 5.63 (s, 2H, O-CH_2-), 7.04 (t, J= 7.6 Hz, 1H, Ar-H), 7.19 (t, J= 7.6 Hz, 1H, Ar-H), 7.31 (t, J= 7.6, 1H, Ar-H), 7.35 (d, J= 7.6 Hz, 2H, Ar-H), 7.46-7.49 (m, 2H, Ar-H), 7.55-7.58 (m, 2H, Ar-H), 7.64 (d, J= 6.8 Hz, 1H, Ar-H), 8.11 (s, 1H, triazole proton), 8.32 (d, J= 7.6 Hz, 1H, Ar-H) 8.64 (d, J= 8.5 Hz 1H, Ar-H), 10.32 (s, 1H, N-

H); ^{13}C NMR ($CDCl_3$ 213 MHz): 62.84 (-O-CH_2-), 113.21, 122.22, 122.27, 123, 124.38, 125.75, 127.68, 127.79, 128.01, 128.59, 129, 130.82, 131.02, 132.84, 133.61, 134.61, 135.60, 142.46, 155.97, 163.27 (C=O); ESI +ve MS (m/z): 438$(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{22}H_{16}O_2N_4Cl_2$ Calculated: C, 60.15; H, 3.67; O, 7.28; N, 12.75 Found: C, 60.12; H, 3.70; O, 7.30; N, 12.72%.

N-(3-bromophenyl)-2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzaamide (13)

Off white crystals; m.p.: 152-154℃; Yield: 70%; IR (ATR): v (cm^{-1}): 3340 (N-H), 3109, 1661 (C=O), 1586, 1523, 1497, 1483, 1449, 1417, 1308, 1296, 1251, 1223, 1165, 1128, 1103, 1067, 1041; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 5.49 (s, 2H, O-CH_2-), 7.13-7.22 (m, 4H, Ar-H), 7.45-7.55 (m, 3H, Ar-H), 7.57-7.68 (m, 3H, Ar-H), 7.92 (t, J=1.5 Hz, 1H, Ar-H), 8.12 (s, 1H,triazole proton), 8.27-8.30 (m, 1H, Ar-H), 9.96 (s, 1H, N-H). ^{13}C NMR ($CDCl_3$ 75 MHz): 62.73 (-O-CH_2-), 112.74, 122.46, 123.27, 125.07, 126.89, 127.86, 130.15, 130.87, 131.20, 132.78, 133.59, 139.78, 142.07, 155.95, 172.82 (C=O); ESI +ve MS (m/z): 483 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{22}H_{16}O_2N_4ClBr$ Calculated: C, 54.62; H, 3.33; O, 6.61; N, 11.58 Found: C, 54.60; H, 3.36; O, 6.63; N, 11.60%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-(o-tolyl)benzamide (14)

Off white crystals; m.p.: 101-103℃; Yield: 77%; IR (ATR): v (cm^{-1}): 3363 (N-H), 3071, 2922, 1654 (C=O), 1598, 1587, 1528, 1496, 1456, 1380, 1310, 1290, 1255, 1216, 1162, 1132, 1114, 1089, 1074, 1044; ^{1}H NMR ($CDCl_3$ 850 MHz) δ (ppm): δ 2.08 (s, 3H, Ar-Me), 5.54 (s, 2H, O-CH_2-), 7.03 (t, J= 7.6 Hz, 1H, Ar-H), 7.12 (d, J= 6.8 Hz, 1H, Ar-H), 7.18 (t, J= 7.6 Hz, 1H, Ar-H), 7.21 (t, J= 7.6 Hz, 1H, Ar-H), 7.27 (d, J= 7.65 Hz, 1H, Ar-H), 7.48-7.44 (m, 2H, Ar-H), 7.53 (t, J= 6.8 Hz, 1H, Ar-H), 7.56 (t, J= 8.5 Hz, 2H, Ar-H), 8.07 (s, 1H, triazole proton), 8.11 (d, J= 7.6 Hz, 1H, Ar-H), 8.32 (d, J= 7.6 Hz, 1H, Ar-H), 9.50 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 213 MHz): 17.91 (Ar-CH_3), 62.71 (-O-CH_2), 112.94, 122.35, 122.76, 122.80, 124.58, 125.61, 126.65, 127.70, 128.07, 128.42, 128.54, 130.27, 130.88, 131.12, 132.95, 133.18, 134.49, 136.47, 142.22, 155.92, 163.14 (C=O); ESI +ve MS (m/z): 418 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{19}O_2N_4Cl$ Calculated: C, 65.95; H, 4.57; O, 7.64; N, 13.38 Found: C, 65.91; H, 4.60; O, 7.66; N, 13.36%.

N-benzyl-2-[{1-(-2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzamide (15)

Off white crystals; m.p.: 113-115℃; Yield: 70%; IR (ATR): v (cm^{-1}): 3392 (N-H), 3067, 1637 (C=O), 1597, 1583, 1521, 1497, 1479, 1464, 1450, 1386, 1292, 1266, 1220, 1164, 1133, 1103, 1077, 1045; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm) : δ 4.62 (d, J=5.1 Hz, 2H, -CH_2-NH), 5.39 (s, 2H, O-CH_2-), 7.02 (t, J=7.6 Hz, 1H, Ar-H), 7.15-7.18 (m, 4H, Ar-H), 7.25-7.27 (m, 3H, Ar-H), 7.46-7.62 (m, 4H, Ar-H), 7.64 (s, 1H, Ar-H), 8.24-8.27 (m, 2H, triazole-proton, N-H); ^{13}C NMR ($CDCl_3$ 75 MHz): 43.84 (-NH-CH_2-), 62.94 (-O-CH_2-), 112.91, 122.20, 124.58, 127.15, 127.73, 127.85, 127.98, 128.47, 128.71, 130.80, 131.07, 132.57, 132.85, 138.61, 142.46, 156.14, 165.01 (C=O); ESI +ve MS (m/z) : 419 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{19}O_2N_4Cl$ Calculated: C, 65.95; H, 4.57; O, 7.64; N, 13.38 Found: C, 65.98; H, 4.59; O, 7.65; N, 13.35%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N-methylbenzamide (16)

Off white crystals; m.p.: 83-85℃; Yield: 77%; IR (ATR): v (cm^{-1}): 3343 (N-H), 1652 (C=O), 1600, 1538, 1496, 1456, 1402, 1373, 1290, 1222, 1160, 1127, 1101, 1075, 1047; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 2.96 (d, J=4.8 Hz, 3H, NH-CH_3), 5.44 (s, 2H, O-CH_2-), 7.11 (d, J=7.2 Hz, 1H, Ar-H), 7.27 (d, J=8.1 Hz, 1H, Ar-H), 7.44-7.64 (m, 6H, Ar-H), 8.05 (s, 1H, triazole-proton), 8.15-8.33 (m, 1H, N-H); ^{13}C NMR ($CDCl_3$ 75 MHz): 24.00 (NH-CH_3), 55.43 (-O-CH_2-), 122.18, 124.99, 127.86, 128.09, 131.05, 132.70, 136.64, 139.86, 140.20, 146.28, 151.42, 159.86 (C=O); ESI +ve MS (m/z): 342 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{17}H_{15}O_2N_4Cl$ Calculated: C, 59.57; H, 4.41; O, 9.34; N, 16.34 Found: C, 59.58; H, 4.43; O, 9.35; N, 16.36%.

N-butyl-2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzamide (17)

Off white crystals; m.p.: 77-79℃; Yield: 55%; IR (ATR): v (cm^{-1}): 3391 (N-H), 3071, 2935, 2877, 1630 (C=O), 1597, 1539, 1497, 1473, 1447, 1398, 1299, 1233, 1217, 1156, 1130, 1102, 1072, 1039; ^{1}H NMR ($CDCl_3$ 300 MHz) δ (ppm): δ 0.85 (t, J=7.5 Hz, 3H, -CH_3), 1.25-1.34 (m, 2H, -CH_2-Me), 1.48 (pentet, 2H, -CH_2-), 3.37-3.44 (m, 2H, N-CH_2-), 5.42 (s, 2H, O-CH_2-), 7.12 (t, J=7.5 Hz, 2H, Ar-H), 7.44-7.52 (m, 3H, Ar-H), 7.59-7.65 (m, 2H, Ar-H), 7.88 (s, 1H, Ar-H), 8.07 (s, 1H, triazole-proton), 8.19-8.23 (m, 1H, N-H); ^{13}C NMR ($CDCl_3$ 75 MHz): 13.73 (-CH_3) 20.14 (-CH_2-Me), 31.42 (-CH_2-), 39.55 (-NH-CH_2-), 62.56 (-O-CH_2-), 112.79, 122.10, 124.93, 127.72, 128.10, 130.90, 131.11, 132.49, 142.53, 156.08, 165.27 (C=O); ESI +ve MS (m/z): 384 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{20}H_{21}O_2N_4Cl$ Calculated: C, 62.42; H,

5.50; O, 8.31; N, 14.56 Found: C, 62.46; H, 5.51; O, 8.32; N, 14.57%.

N`-benzoyl-2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzohydrazide (18)

Off white crystals; m.p.: 231-233 °C; Yield: 58%; IR (ATR): v (cm^{-1}): 3313 (N-H), 1673 (C=O), 1623 (C=O), 1603, 1578, 1540, 1497, 1457, 1380, 1288, 1220, 1196, 1160, 1125, 1100, 1073, 1047; ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): δ 5.49 (s, 2H, O-CH_2-), 7.14 (t, J=7.2 Hz, 1H, Ar-H), 7.34-7.66 (m, 7H, Ar-H), 7.75 (t, J=7.5 Hz, 3H, Ar-H) 7.87 (d, J=7.8 Hz, 2H, Ar-H), 8.80 (s, 1H, triazole-proton), 10.06 (s, 1H, -N-H), 10.63 (s, 1H, -N-H); ^{13}C NMR (DMSO-d_6 213 MHz): 62.44, (-O-CH_2-), 113.84, 121.26, 122.70, 126.88, 126.92, 127.48, 128.39, 128.48, 128.53, 130.44, 130.62, 131.64, 131.89, 132.37, 132.63, 134.47, 142.99, 155.72, 164.79 (C=O), 165.29 (C=O); ESI +ve MS (m/z): 447 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{18}O_3N_5Cl$ Calculated: C: 61.68, H: 4.05, O: 10.72, N: 15.64%; Found: C: 61.65, H: 4.06, O: 10.75, N: 15.66%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N'-(3-hydroxybenzoyl) benzohydrazide (19)

White crystals; m.p.: 199-201°C; Yield: 50%; IR (ATR): v (cm^{-1}): 3373 broad peak (N-H and OH), 3073, 1699 (C=O), 1623 (C=O), 1601, 1574, 1558, 1500, 1471, 1435, 1289, 1225, 1160, 1132, 1095, 1070, 1034; ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): δ 5.50 (s, 2H, O-CH_2-), 6.95-7.26 (m, 3H, Ar-H), 7.47 (s, 3H, Ar-H), 7.60 (t, J=6 Hz, 4H, Ar-H), 7.76-7.88 (m, 4H, Ar-H), 8.84 (s, 1H, N-H), 10.43 (s, 1H, -OH); ^{13}C NMR (DMDO-d_6 213 MHz): 62.53 (-O-CH_2-), 113.84, 114.93, 117.26, 119.09, 121.36, 121.70, 127.00, 128.44, 128.51, 130.61, 131.74, 132.93, 133.96, 134.48, 142.73, 155.80, 158.70, 162.98 (C=O), 165.38 (C=O); ESI +ve MS (m/z): 464 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{18}O_4N_5Cl$ Calculated: C, 56.55; H, 3.91; O, 13.80; N, 15.10 Found: C, 56.58; H, 3.93; O, 13.82; N, 15.12%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N'-(4-bromobenzoyl)benzohydrazide (20)

White crystals; m.p.: 228-230°C; Yield: 69%; IR (ATR): v (cm^{-1}): 3318 (N-H), 3104, 1697 (C=O), 1621 (C=O), 1601, 1591, 1560, 1499, 1466, 1442, 1394, 1294, 1219, 1159, 1133, 1111, 1070, 1050; ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): δ 5.46 (s, 2H, O-CH_2-), 7.13 (t, J=7.5 Hz, 1H, Ar-H), 7.42 (d, J=8.4 Hz, 1H, Ar-H), 7.54-7.64 (m, 3H, Ar-H), 7.66-7.80 (m, 7H, Ar-H), 8.77 (s, 1H, triazole-proton), 10.12 (s, 1H, N-H), 10.80 (s, 1H, N-H); ^{13}C NMR ($CDCl_3$ 213 MHz): 62.41 (-O-CH_2-), 113.83, 121.25, 122.60, 125.71, 126.87,

128.08, 128.37, 128.50, 129.55, 130.41, 130.62, 131.47. 131.58, 131.76, 132.66, 134.45, 142.96, 155.70, 164.72 (C=O); ESI +ve MS (m/z): 526 $(M+H)^+$, 528 $(M+2)^+$ Element analysis : Calculated for molecular formulae $C_{23}H_{17}O_3N_5ClBr$ Calculated: C, 52.44; H, 3.25; O, 9.11; N, 13.29 Found: C, 52.43; H, 3.26; O, 9.13; N, 13.31%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N'-[2-(2,4-dichlorophenoxy) acetyl]benzohydrazide (21)

White crystals; m.p.: 167-169℃; Yield: 68%; IR (ATR): v (cm^{-1}): 3392 (N-H), 3326 (N-H), 1697 (C=O), 1624 (C=O), 1598, 1558, 1501, 1470, 1433, 1393, 1360, 1290, 1266, 1248, 1266, 1248, 1232, 1162, 1103, 1073, 1045; ^{1}H NMR (DMSO-d_6 850 MHz): 4.77 (s,2H, -O-CH_2-), 5.44 (s, 2H, -O-CH_2-), 7.10-7.13 (m, 2H, Ar-H), 7.25-7.26 (m, 1H, Ar-H), 7.43 (t, J= 8.5 Hz, 1H, Ar-H), 7.53-7.56 (m, 2H, Ar-H), 7.59 (d, J=2.55 Hz, 1H, Ar-H), 7.62-7.64 (m, 1H, Ar-H), 7.68-7.69 (m, 1H, Ar-H), 7.71-7.72 (m, 1H, Ar-H), 7.75-7.76 (m, 1H, ArH), 8.72 (s, 1H, triazole-proton), 10.14 (s, 1H, N-H), 10.55 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6 213 MHz): 62.39 (-O-CH_2-), 66.55 (-O-CH_2-), 113.89, 115.41, 121.30, 122,11, 122.53, 125.15, 126.78, 127.97, 128.36, 128.42, 128.54, 129.39, 130.45, 130.57, 131.73, 132.77, 134.42, 142.72, 152.57, 155.69, 163.80 (C=O), 165.44 (C=O); ESI +ve MS (m/z): 546.7 $(M+H)^+$, 548.7 $(M+2)^+$ Element analysis : Calculated for molecular formulae $C_{24}H_{18}O_4N_5Cl_3$ Calculated: C, 52.72; H, 3.32; O, 11.70; N, 12.81 found: C, 52.75; H, 3.34; O, 11.72; N, 12.79%.

2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]-N'-(2-phenoxyacetyl)benzohydrazide (22)

White crystals; m.p.: 125-127℃; Yield: 66%; IR (ATR): v (cm^{-1}): 3306 (N-H), 3199 (N-H),3084, 1699 (C=O), 1627 (C=O), 1601, 1559, 1496, 1447, 1388, 1294, 1220, 1173, 1161, 1114, 1073, 1045; ^{1}H NMR ($CDCl_3$-d_6 300 MHz) δ (ppm): δ 4.77 (s, 2H, O=C-CH_2-O-), 5.46 (s, 2H, O-CH_2-), 7.12 (t, J=7.2 Hz, 2H, Ar-H), 7.33 (d, J=8.1 Hz, 1H, Ar-H), 7.41-7.70 (m, 10H, Ar-H), 8.71 (s, 1H, triazole-proton), 10.11 (s, 1H, N-H), 10.51 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6 75 MHz,): 62.20 (-O-CH_2-), 66.90 (-O-CH_2-), 113.97, 116.11, 121.73, 127.19, 128.85, 131.01, 132.15, 133.16, 134.70, 143.0, 153.03, 164.26, 165 (C=O), 171 (C=O); ESI +ve MS (m/z): 477 (M+H Element analysis : Calculated for molecular formulae $C_{24}H_{20}O_4N_5Cl$ Calculated: C, 60.32; H, 4.22; O, 13.39; N, 14.65 Found: C, 60.30; H, 4.25; O, 13.41; N, 14.63%.

N`-[2-(3-chlorophenoxy)acetyl]-2-[{1-(2-chlorophenyl)-1H-triazol-4-yl}methoxy] benzohydrazide (23)

White crystals; m.p.: 189-191℃; Yield: 68%; IR (ATR): v (cm^{-1}): 3287 (N-H), 3158 (N-H), 1653 (C=O), 1625 (C=O), 1593, 1540, 1505, 1473, 1366, 1313, 1288, 1217, 1166, 1115, 1102, 1074, 1046; ^{1}H NMR (DMSO-d_6 300 MHz) δ (ppm): δ 4.66 (s, 2H, O-CH_2-C=O), 5.45 (s, 2H, O-CH_2-), 6.99-7.14 (m, 3H, Ar-H), 7.32 (t, J=8.1 Hz, 1H, Ar-H), 7.42 (d, J=8.4 Hz, 1H, Ar-H), 7.55-7.64 (m, 3H, Ar-H), 7.67-7.77 (m, 4H, Ar-H), 8.73 (s, 1H, triazole-proton), 10.04 (s, 1H, N-H), 10.48 (s, 1H, N-H); ^{13}C NMR (DMSO-d_6 213 MHz,): 62.35 (-O-CH_2-), 66.12 (-O-CH_2-), 133.78, 115.00, 121.25, 122.38, 126.78, 128.40, 128.47, 130.43, 130.60, 130.89, 131.74, 132.69, 133.67, 134.44, 142.81, 155.68, 158.69, 164.18 (C=O), 166.02 (C=O). ESI +ve MS (m/z): 511 $(M+H)^+$ Element analysis : Calculated for molecular formulae $C_{24}H_{19}O_4N_5Cl_2$ Calculated: C, 56.26; H, 3.74; O, 12.49; N, 13.67 found: C, 56.23; H, 3.76; O, 12.51; N, 13.71%.

2.3. Microbiology

2.3.1 DPPH radical scavenging activity

The antioxidant activity was evaluated by measuring the reduction of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The test samples were prepared with concentrations 25µM, 50µM, 75µM, 100µM, 125µM and 250µM in methanol. The DPPH solution (60 mM) was prepared in methanol. The DPPH solution was added to the sample solution [25]. After 30 min, the UV absorbance of the resulting solutions was recorded at λ 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. Vitamin C was used as the positive control. The free radical scavenging activity was calculated as a percentage inhibition of the DPPH radical by the sample or positive control.

$$\% \text{ scavenging} = [(A_0 - A_1)/(A_0)] \times 100$$

A_0 = control absorbance; A_1 = sample absorbance

2.3.2 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), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), Gram Negative Bacterial Strains: *Proteus mirabilis (ATCC 13376), Enterococcus faecalis (ATCC 29212), Staphylococcus epidermidis (ATCC12228)* and Fungal Strain: *Candida albicans* (ATCC 10231) were used for antimicrobial studies.

2.4.2.1 Disc Diffusion Method

All the antimicrobial studies were performed at Albaha regional research laboratory, Albaha, Kingdom of Saudi Arabia. All the newly synthesized compounds were dissolved in dimethyl formamide (DMF) to prepare chemicals of

stock solution of 2 mg/mL and simple susceptibility screening test was carried out using reported disc diffusion method [26]. 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 100 µg of the compounds were loaded. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organism. Amoxicillin (100 µg) and Fluconazole (50 µg) were used as standard drugs. Dimethyl formamide was used as solvent (negative controls).

2.4.2.2 Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by the conventional paper disc diffusion method [27]. The compounds showing promising zone of inhibition were dissolved in dimethylformamide (DMF) and loaded on the disks by micropipette with different concentrations (100, 50, 25, 12.50, 6.25 and 3.125 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

A library of nineteen new amide incorporated 1,2,3-triazole derivatives **5-23** have been synthesized according to the synthetic route described in **Scheme**. Treatment of methyl salicylate **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 2-chlorophenyl azide using tertiary butanol and water as solvent and sodium ascorbate and copper sulphate penta hydrate as cyclizing agent yielded intermediate 2-[(1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzoate **3**. This intermediate **3** was then hydrolyzed using sodium hydroxide in methanol-water to yield a key intermediate 2-[{1-(2-chlorophenyl)-1H-1,2,3-triazol-4-yl}methoxy]benzoic acid **4**. The key intermediate **4** was finally reacted with different aromatic amines, aliphatic amines and acid hydrazides to yield amide incorporated 1,2,3-triazole derivatives **5-**

23. The proposed structures of the compounds were confirmed by different analytical techniques such as IR, ^{1}H NMR, ^{13}C NMR, elemental analysis 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 absorption band at 2121 cm^{-1} for triple bonded carbon of propargyl group and 3288 cm^{-1} for C-H stretching in IR spectra which supports the propargylation of hydroxyl group. The formation of compound **2** was further confirmed from mass spectrometry which showed molecular ion peak at 190. Formation of the cyclized product methyl 2-[(1-phenyl-1H-1,2,3-triazol-4-yl)methoxy]benzoate **3** was supported by the disappearance of absorption band at 2121 cm^{-1} and 3288 cm^{-1} of triple carbon-carbon bond and terminal alkyne, respectively and appearance of absorption band at 1454-1603 cm^{-1} due to 1,2,3-triazole ring stretching in FT-IR. The presence of additional aromatic protons signals in ^{1}H NMR suggested the presence of 2-chlorophenyl ring in the molecule. Further confirmation of compound **3** was obtained from mass spectra which exhibited molecular ion peak at 343 $[M+H]^{+}$, 345 $[M+H+2]^{+}$. Formation of compound **4** was supported by appearance of broad band at 3156 cm^{-1} in FT-IR due to COOH stretching and absence of singlet at δ 3.88 in ^{1}H NMR for O-CH_3 confirming the hydrolysis of methoxy group. The target compounds **5-14, 15-17 and 18-23** were confirmed by the presence of absorption band in the range 1644-1674 cm^{-1}, 1630-1652 cm^{-1} and 1621-1627 cm^{-1} for carbonyl carbon of amide (CO-N-H) stretching and absorption band in the range 3287-3387 cm^{-1} due to N-H stretching of amide group in FT-IR. In case of target compounds **18-23**, two absorption band in the range 1621-1627 cm^{-1} and 1653-1699 cm^{-1} in FT-IR and two peaks in the range 163-171 ppm in ^{13}C NMR supported the presence of two carbonyl groups in the molecule. The ^{1}H NMR of target compounds **5-23** showed presence of additional protons peaks of different amine used and absence of signals of carboxylic protons. Finally, the target compounds **5-23** were confirmed by the appearance of molecular ion peak in mass spectrometry.

Biological Activity

3.2.1 Antioxidant activity

All the target compounds **5-23** were evaluated for their DPPH scavenging effect at different concentration 25, 50, 75, 100, 125 and 250 μM. Most of the compound showed moderate scavenging effect compared to the positive control ascorbic

acid. The results show that scavenging effect was good at 250 µM concentration, where compound **6** and **19** showed significant scavenging effect with 94.12 and 95.64% inhibition, respectively compared to the ascorbic acid showing 91.80% inhibition. The results are shown in **Figure 1**.

3.2.2*In vitro* antimicrobial assay

In order to check the antimicrobial activity, all the target compounds **5-23** were screened for their *in vitro* antimicrobial activity against standard bacterial and fungal strains. From the results it was observed that most of the gram-positive strains were sensitive towards all the tested compounds, whereas gram negative bacterial strains were less sensitive towards the tested compounds. Among the tested compounds, compounds **6, 9, 10, 18, 19** and **22** showed significant activity against the tested bacterial strains. Compounds **6, 10** and **19** exhibited zone of inhibition 24, 22, 20 mm, respectively compared to standard drug Amoxycillin which showed zone of inhibition 16 mm against gram negative bacterial strain *Escherichia Coli.* The tested Compounds **6, 10** and **19** revealed comparable zone of inhibition compared to the standard drug amoxicillin against *Enterococcus Faecalis*. The result is shown in **Table 1**.

3.2.2.1 Minimum Inhibitory Concentration (MIC)

The MIC of the active compounds (**6, 9, 10, 18, 19 and 22**) was evaluated using disc diffusion methods. Compound **6** showed MIC 25 µg/disk against *E. coli and E.faecalis,* compound **19** showed MIC 25 against *E.coli* and 50 µg/disk against *P.aerogenosa*. The MIC result is summarized in **Table 3**.

From the series of amide incorporated 1,2,3-triazole conjugates, it was observed that compounds **5-14** and compounds **18-23** were more active in scavenging DPPH compared to compounds **15-17**. Compounds **5-14** substituted with chlorine, methoxy group at *para* position showed significant activity. Compound **19** having hydroxy group in aromatic ring showed highest DPPH scavenging effect with 95.64 % inhibition compared to positive control ascorbic acid which showed 91.80% inhibition at 250µ concentration.

The antimicrobial result of the screened compounds **5-23** showed that most of the compounds were active against the tested gram-positive bacterial strains. Among the tested compounds, six compounds (**6, 9, 10, 18, 19 and 22**) were found to be more active against the bacterial strains. Compounds 5-14 and 18-23 having aromatic ring in the structure were found to be potent in inhibiting the growth of the

tested microorganisms. Compounds having chlorine (**6**), methoxy (**10**) and Methyl group (**9**) at *para* position in the aromatic ring were more active and showed comparable zone of inhibition with that of standard drug Amoxicillin. Compounds **6, 10 and 19** exhibited showed zone of inhibition **24, 22, 20** mm, respectively which was more significant than the standard drug amoxicillin showing zone of inhibition **16 mm** against gram negative bacterial strain *Escherichia Coli.* The tested Compounds **6, 10 and 19** showed comparable zone of inhibition with the standard drug Amoxicillin against *Enterococcus Faecalis*. Compounds **6** and **19** revealed strong antimicrobial effect. Compound **6** showed MIC 25 µg/disk against *E. coli and E.faecalis,* compared to the standard drug Amoxicillin which showed MIC 12.5 and >25 µg/disk respectively. Compound **19** showed MIC 25 µg/disk against *E. coli* and 50 µg/disk against *P.aerugenosa* compared to standard drug Amoxicillin which showed MIC 12.5 and 25 µg/disk, respectively

Table 1: *In vitro* antimicrobial activity of the compounds 5-23

	Antibacterial Activity (100 µg/disc)						
Comp.No.	**Gram Positive Bacteria Strains**			**Gram Negative Bacteria Strains**			***Fungal Strain***
	S.e	***S.a***	***E.f***	***E.c***	***P.m***	***P.a***	***C.a***
5	---	---	---	---	---	---	---
6	**30**	**36**	**40**	**24**	**40**	**34**	**10**
7	---	---	---	---	---	---	---
8	**10**	**18**	**26**	**----**	**18**	**16**	---
9	**27**	**29**	**38**	**18**	**35**	**31**	---
10	**30**	**33**	**40**	**22**	**38**	**35**	**10**
11	**17**	**20**	**40**	**--**	**14**	**17**	---
12	**10**	**12**	**14**	**10**	**---**	**12**	**8**
13	**12**	**14**	**12**	**13**	**12**	**11**	---
14	**13**	**14**	**16**	**14**	**--**	**---**	---
15	**14**	**15**	**14**	---	---	---	---
16	---	**12**	---	---	---	---	---
17	**10**	---	**12**	---	---	---	---
18	**29**	**28**	**40**	**16**	**33**	**31**	---
19	**30**	**32**	**40**	**20**	**40**	**32**	**8**
20	**12**	---	**12**	---	---	---	---
21	**15**	**15**	**28**	---	---	**12**	---
22	**26**	**26**	**38**	**19**	**29**	**32**	**NA**
23	**18**	**18**	**29**	---	**22**	**16**	**--**
Amoxicillin	**36**	**36**	**40**	**16**	**40**	**35**	**NT**
Fluconazole	**NT**	**NT**	**NT**	**NT**	**NT**	**NT**	**26**

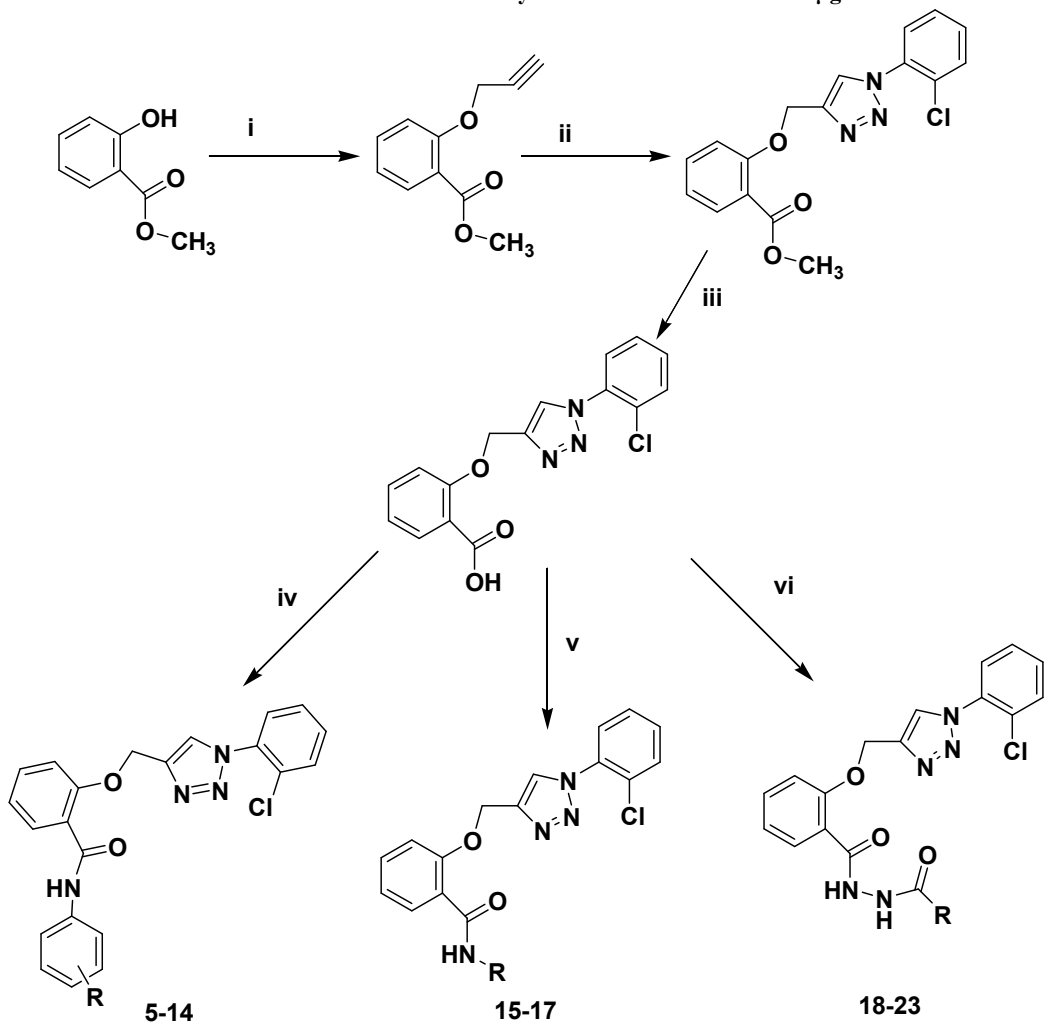
a Activity is expressed as zone of inhibition in mm at concentration of 100 µg/disc;

For antibacterial activity, Amoxicillin (100 µg/disc) is used as a standard drug; for antifungal activity, Fluconazole (50 µg/disc) is used as a standard drug. S.e: *Staphylococcus Epidermidis* (ATCC 12228); S.a: *Staphylococcus aureus* (ATCC 25923); E.f:*Enterococcus faecalis* (ATCC 29212); E.c: *Escherichia coli* (ATCC25922); P.m: *Proteusmerabilis* (ATCC 13376); P.a: *Pseudomonas aerugenosa* (ATCC 27853); C.a: *Candia albicans* (ATCC10231); NT: Not Tested; ---: No zone of inhibition.

Table 3: Minimum inhibitory concentration (MIC) of the compounds

Compounds	Bacterial Strains			
	S.a	*E.f*	*E.c*	*p.a*
6	>50	25	25	>100
9	100	50	>100	>100
10	50	>25	50	>100
18	>50	>100	>50	50
19	25	50	25	50
22	>100	>100	>25	>100
Amoxicillin	>25	>25	12.5	25

Minimum inhibitory concentration measured in µg/disc



SCHEME: Synthesis of amide incorporated 1,2,3-triazole derivatives 5-23

i. dry acetone, K_2CO_3, Propargyl bromide; ii. ter.Butanol:Water (1:1), $CuSO_4.5H_2O$, Sodium Ascorbate 2-Chlorophenyl azide; iii.Methanol:Water (1:1), NaOH; iv. EDC, HOBt, MDC, Aromatic amines; v. EDC, HOBt, MDC, Aliphatic amines; vi. EDC, HOBt, MDC, Acid hydrazides

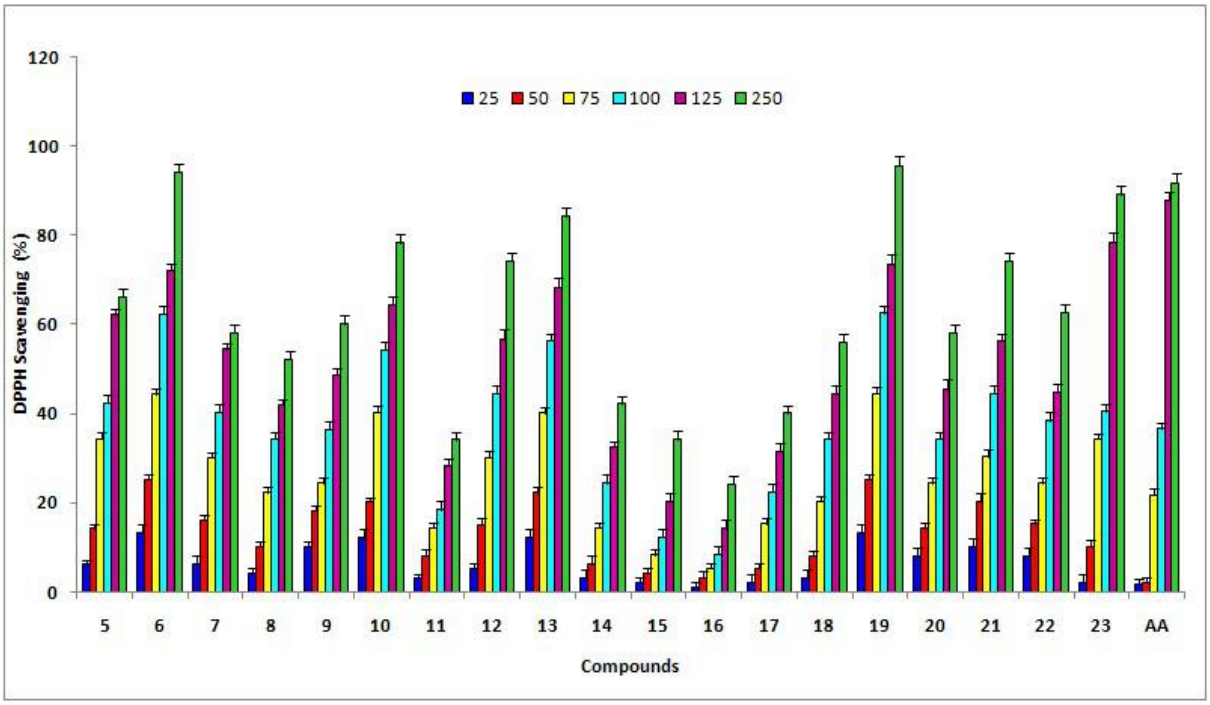


Figure 1: DPPH Scavenging effect of compounds 5-23. Each value represents a mean ±SD (n=3). AA: Ascorbic acid (Vitamin C as standard)

4. CONCLUSION

A library of nineteen new amide incorporated 1,2,3-triazole derivatives have been synthesized and confirmed by different analytical techniques. The target compounds **5-23** were evaluated for antioxidant and antimicrobial activities. Two compounds **6** and **19**, among the tested compounds showed significant DPPH scavenging activity with 94.12% and 95.64% inhibition, respectively whereas the positive control ascorbic acid showed 91.80% inhibition. Most of the compounds were active against tested gram-positive bacterial strains, whereas some tested aliphatic amide of 1,2,3-triazole derivatives were resistant towards some gram-negative bacterial strains. Compound **6** showed MIC 25 µg/disk against *E.c and E.f,* compound **19** showed MIC 25 µg/disk against *E.c.* and 50 µg/disk against *p.a*. Fungal strain *Candida albicans* was resistant against most of the tested compounds. From the results it can be concluded that halogen, methoxy, methyl group present in the aromatic ring at *para* position 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 their toxicological studies.

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REFERENCES

[1] Viswanathan V.K. Off-label abuse of antibiotics by bacteria. Gut Microbes, 2014; 5: 3-4.

[2] Read A. F, Woods, R.J. Antibiotic resistance management. Evol. Med. Public Health, 2014, 2014:147.

[3] Hof H. Is there a serious risk of resistance development to azoles among fungi due to the widespread use and long-term application of azole antifungals in medicine? Drug Res. Updat, 2008; 11: 25-31. (Check it)

[4] Upmanyu N, Kumar S, Shah K, Mishra P. Synthesis and Antimicrobial Studies of Some 4-(Substituted)-Ethanoylamino-3-Mercapto-5- (4-Substituted) Phenyl-1,2,4-Triazoles. Dhaka Univ. J. Pharm. Sci, 2012; 11(1): 7-18.

[5] Holpuch A. UN meeting tackles the "fundamental threat" of antibiotic-resistant superbugs. Guardian 2016.

[6] Jones K.E, Patel N.G, Levy M.A, Storeygard A, Balk D, Gittleman J.L, Daszak P. Global trends in emerging infectious diseases. Nature, 2008; 451: 990-993.

[7] Rezaei Z, Khabnadideh S, Pakshir K, Hossaini Z Amiri F, Assadpour E. Design, synthesis and antifungal activity of triazole and benzotriazole derivatives. Eur. J. Med. Chem, 2009; 44: 3064-3067.

[8] Chua T, Moore C.L, Perri M.B, Donabedian S.M, Masch W, Vager D, Davis S.L, Lulek K, zimnicki B, Zervos M.J. Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus Bloodstream Isolates in Urban Detroit. J. Clin. Microbiol, 2008; 46: 2345-2352.

[9] Yang Y, Rasmussen B.A, Shlaes D.M. Class A β-lactamases-enzyme-inhibitor interactions and resistance, Pharmacol. Ther, 1999; 83: 141-151.

[10] Soltis M, Yeh H, Cole K, Whittaker N, Wersto R, Kohn E. Identification and characterization of human metabolites of CAI [5-amino-1-1(4'-chlorobenzoyl-3,5-dichlorobenzyl)-1,2,3-triazole- 4-carboxamide). Drug Metab. Dispos, 1996; 24: 799-806.

[11] Weinstein A. The Cephalosporins: Activity and Clinical Use. Drugs, 1980; 19: 137-154.

[12] Zhang H.-Z, Wei J.-J, Kumar K. V, Rasheed S, Zhou C.-H. Synthesis and biological evaluation of novel d-glucose-derived 1,2,3-triazoles as potential antibacterial and antifungal agents. Med. Chem. Res, 2015; 24: 182-196.

[13] Ramesh S, Ashok D, Goud G.L, Reddy V.P. Microwave assisted synthesis of novel methylenebis{2-[(1-benzyl/cyclohexyl-1H-1,2,3-triazol-4-yl)methoxy]chalcones} and their antibacterial activity. Russ. J. Gen. Chem, 2014; 84: 1608-1614.

[14] Wang X.-L, Wan K, Zhou C.-Z. Synthesis of novel sulfanilamide-derived 1,2,3-triazoles and their evaluation for antibacterial and antifungal activities. Eur. J. Med Chem, 2010; 45: 4631-4639.

[15] Kant R, Singh V, Nath G, Awasthi S.K, Agarwal A. Design, synthesis and biological evaluation of ciprofloxacin tethered bis-1,2,3-triazole conjugates as potent antibacterial agents. Eur. J. Med. Chem, 2016; 124: 218-228.

[16] Aher N.G, Pore V.S, Mishra N.N, Kumar A, Shukla P.K, Sharma A, Manoj K. Bhat M.K. Synthesis and antifungal activity of 1,2,3-triazole containing fluconazole analogues. Bioog. & Med. Chem. Lett, 2009; 19: 759-763.

[17] Yan S.-J, Liu Y.-J, Chen Y.-L, Liu L, Lin J. An efficient one-pot synthesis of heterocycle-fused 1,2,3-triazole derivatives as anti-cancer agents. Bioog. & Med. Chem. Lett, 2010; 20: 5225-5228.

[18] Rao P.S, Kurumurthy C, Veeraswamy B, Kumar G.S, Poornachandra Y, Kumar C.G, Vasamsetti S.B, Kotamraju S, Narsaiah B. Synthesis of novel 1,2,3-triazole substituted-N-alkyl/aryl nitrone derivatives, their anti-inflammatory and anticancer activity. Eur. J. Med. Chem, 2014; 80: 184-191.

[19] Shaikh M.H, Subhedar D.D, Shingate B.B, Khan F.A.K, Sangshetti J.N, Khedkar V.M, Nawale L, Sarkar D, Navale G.R, Shinde S.S. Synthesis, biological evaluation and molecular docking of novel coumarin incorporated triazoles as antitubercular, antioxidant and antimicrobial agents. Med. Chem. Res, 2016; 25: 790-804.

[20] Sreelatha T, Kandhasamy S, Dinesh R, Shruthy S, Shweta S, Mukesh D, Karunagaran D, Balaji R, Mathivanan N, Perumal P.T. Synthesis and SAR study of novel anticancer and antimicrobial naphthoquinone amide derivatives. Bioog & Med. Chem. Lett, 2014; 24 :3647-3651.

[21] Zhao Z, Bai Y, Chen X, Wu S, He X, Bai Y, Sun Y, Zheng X. Design, synthesis and biological evaluation of (E)-3-(3,4,5- trimethoxyphenyl) acrylic acid (TMCA) amide derivatives as anticonvulsant and sedative agents. Med. Chem. Res, 2018; 27: 2387-2396.

[22] El-Damasy A.K, Lee J-H, Seo S.H, Cho N-C, Pae A.N, Keum G. Design and synthesis of new potent anticancer benzothiazole amides and ureas featuring pyridylamide moiety and possessing dual B-Raf^{V600E} and C-Raf kinase inhibitory activities. Eur. J. Med. Chem, 2016; 115: 201-216.

[23] Lan X, Xie D, Yin L, Wang Z, Chen J, Zhang A, Song B, Hu D. Novel α,β-unsaturated amide derivatives bearing α-amino phosphonate moiety as potential antiviral agents. Bioog. & Med. Chem. Lett, 2017; 27: 4270-4273**.**

[24] Rawal R.K, Tripathi R, Katti S.B, Pannecouque C, Clercq E.D. Design, synthesis, and evaluation of 2-aryl-3-heteroaryl-1,3- thiazolidin-4-ones as anti-HIV agents. Bioog & Med. Chem, 2007; 15: 1725-1731.

[25] Alves C.Q, David J.M, David J.P, Mahia M.V, Aguiar R.M. Methods for determination of *in vitro* antioxidant activity for extracts and organic compounds. Quim. Nova, 2010; 33: 2202-2210.

[26] Bauer A.W, Kirby W.M.M, Sherris J.C, Turck M. Antibiotic susceptibility testing by a standardised single disc method. Am. J. Clin. Pathol, 1966; 45: 493-496.

[27] Jorgensen J.H, Turnidge J.D. Susceptibility test method: dilution and disc diffusion methods. In Murray PR (ed) Manual of Clinical Microbiology, 8th ed, ASM International, pp: 1108-1127.