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New sulfamethoxazole derivatives contain 1,2,3-triazoline ring in Vitro antibacterial agents

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ABSTRACT

Triazoles stand out among the heterocyclic nitrogen-containing chemicals for their exceptional pharmacological uses. In this study, 6 novel sulfamethoxazole-triazole derivatives were synthesized and their antibacterial activities were investigated. Using melting point and spectroscopic methods (1H and 13C NMR), the produced compounds were thoroughly analyzed. Compounds (9 and 12) showed the greatest efficacy against E. coli with an inhibitory zone of 18 µg/mL when the in vitro antibacterial activities of synthesized compounds were assessed against two bacterial strains, S. aureus and E. coli. In addition, compound (12) with a MIC value of 22 µg/mL.had the strongest antibacterial activity against Staphyloccocus aureus.

Keywords: *1,2,3-triazoline, Sulfametoxazole, Antibacterial activity, Click chemistry, Alkyl chain*

INTRODUCTION

Multidrug-resistant (MDR) pathogens are becoming more prevalent, and resistance to triazole medications in particular makes microbiological treatment problematic, less effective, and gives infections a worse prognosis [1]. For instance, the most prevalent azole medication, fluconazole, is ineffective against the Candida albicans and Candida krusei strains, which account for 75-88% of fungal infections [2]. Several medications that are azole-derived have also developed resistance to A. fumigatus and C. glabrata strains [3]. In addition, a number of serious side effects, such as heart failure, renal failure, liver difficulties, Stevens-Johnson syndrome, and others, have been linked to numerous triazole medications [4]. These side effects include rash, diarrhea, headaches, hepatotoxicity, and gastrointestinal disorders.

Thus, to combat MDR pathogens and lessen the negative effects of currently available medications, it is imperative to design novel triazole medications carefully using molecular hybridization, bioisosteric replacement, and replacement of an isostere. Sulfonamides, also referred to as sulfa medicines, are the oldest medications that are regularly and consistently utilized as bioactive agents. Sulfamethoxazole, sulfathiazole, sulfadiazine, sulfamoxole, and sulfafurazole are a few of these medications that include the sulfonamide molecule [5]. Diverse medicinal uses for sulfa drug derivatives with heterocyclic scaffolds exist, including as antibacterial [6], antifungal [7], anti-inflammatory [5], and cytotoxic agents [8]. Derivatives of the triazole are essential components of medicines and agrochemicals [9]. Due to its wide range of uses in the biomedical, biological, and material sciences,

the triazole moiety is crucial in organic chemistry [10]. Over the past few decades, the chemistry of molecules containing this moiety has significantly expanded [11]. These substances are frequently utilized in industrial settings for things like dyes, photographic materials, photostabilizers, agrochemicals, and corrosion inhibitors (copper alloys) [12]. In the current experiment, our goal was to create some brand-new 1,2,3-triazoline-sulfamethoxazole hybrids that would be powerful antibacterial agents against specific strains. To demonstrate the enzymatic inhibition, antibacterial activity, biodistribution, and safety of the newly developed hybrids, in vitro tests were conducted.

MATERIALS AND METHODS

Multiple supply chemical firms such as Sigma Aldrich chemicals, Thomas baker, Merck, Fluke, and industrial suppliers have purchased all reagents, solvents and starting materials. For the aforementioned progress of all reactions, supplied on silica gel SG-40 by Merck Company, TLC plates were used. At Bruker ALPHA, University of Kufa, Faculty of science, Fourier transformation infrared was used to record FTIR spectra. NMR spectrum, 400MHz for 1HNMR and 100 MHz for ^{13}CNMR, Mashhed University, were confirmed on the Bruker apparatus.

Synthesis 4-azido-N-(5-methylisoxazol-3-yl)benzenesulfonamide (1)

In a mixture of distilled water and Hydrochloric acid in a ratio of (1:3) mL, (15mmol) of sulfamethoxazole is dissolved, and then the mixture cooled with a salt-ice bath to a temperature of 0 °C. During this, an aqueous solution of sodium nitrite was prepared (15mmol) and it is also cooled to 0 °C , after which a nitrite solution is added to sulfadiazine solution a drop by drop where after several additions the color of the solution turned to a slightly-yellow color, after completing the addition the solution is left for stirring for 45 min during this an aqueous solution of sodium azide was prepared (2eq), after which the sodium azide solution was added in batches where bubbles were observed during the addition. After the completion of the addition of the solution is left for stirring for 2hrs. The sediment that is formed is filtered and washed several times with distilled water.

4-azido-N-(5-methylisoxazol-3-yl)benzenesulfonamide (1)

It was prepared as a white crystalline, Chemical formula: $C_{10}H_9N_5O_3S$; 88% yield; mp 93-95 ºC; FTIR, ν (cm^{-1}) 3242(N-H), 3077(C-H, aromatic), 2986, 2842(C-H, aliphatic), 2103(N_3),1686(C=C), 1344(asy SO_2 group) , 1169(sy SO_2), ^{1}H NMR (300 MHz, DMSO-d6) δ 11.50 (s, 1H, N-H, sulfonamide), 7.90–7.30 (m, 4H, Ar-H), 6.11 (s, 1H, C-H-sulfamethoxazole ring), 2.29 (s, 3H, -CH_3).

Synthesis of (E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (2)

1.2 Equivalents of an aqueous solution of 30% sodium hydroxide is added to a solution of (8mmol, 0.93mL) acetophenone and (8mmol) hydroxybenzyldehyde dissolved in ethanol, after completing the addition the solution is left for stirring at laboratory temperature where the completion of the reaction is followed by using a TLC, after cooling the reaction, the precipitate is filtered and washed several times with distilled water, the products from different derivatives were recrystallized using hot ethanol.

(E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (2)

It was prepared as a white crystalline, Chemical formula: $C_{15}H_{12}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 3386 (OH phenol), 2067 (C-H aromatic), 1638(C=O ketone), 1558,1505(C=C aromatic),

Synthesis ether derivatives (3-8)

To the prepared (E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (2) (10mmol) in DMSO, 1.5 equivalents of sodium hydroxide NaOH were added, and stirred the reaction mixture, then 1.2 equivalents of alkyl halide was added in small quantities and in batches, after which the reaction was left under reflux, the completion of the reaction was monitored using TLC. The solvent is removed under reduced pressure. The residue is dissolved in distilled water, and extraction is accomplished by adding chloroform twice. Anhydrous magnesium sulfate was used to dry the organic layer. To obtain the desired product, the solvent is removed under reduced pressure, and purified by colum chromatography (4:1.5) using a mixture of hexane and ethyl acetate as the eluent**.**

(E)-3-(4-(decyloxy)phenyl)-1-phenylprop-2-en-1-one (3)

It was prepared as a white crystalline, Chemical formula: $C_{25}H_{32}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 2100(C-H aromatic), 3021, 2928 (C-H aliphatic), 1610(C=O ketone), 1558,1505(C=C aromatic), 1322(C-O aromatic), 1131(C-O aliphatic),

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.81 – 7.18 (m, 9H, Ar-H), 7.93 (d, 1H, J = 15.6 Hz, Ph-C<u>H</u>=CH-CO-Ph), 7.59(d, 1H, *J* = 15.6 Hz, Ph-CH=<u>CH</u>-CO-Ph), 4.06 (t, J = 6.1 Hz, 2H, -O-C<u>H</u>$_2$-alkyl chain),1.74 (m, 2H, β-CH_2-), 1.33 –1.22 (m, 14H, -(CH_2-)$_7$), 0.87 (t, J = 6.1 Hz, 3H, -CH_3). ^{13}C-NMR (75 MHz, DMSO-d_6) δ 189.68(-C=O), 160.11, 144.51, 137.93, 133.30, 129.18, 128.56, 128.36, 127.53, 122.76, 114.75 (10 C, Ar-C), 144.51 (-<u>C</u>=CH-CO), 122.76 (Ph-CH=<u>C</u>-CO-Ph), 68.15 (-O-CH_2-, alkyl chain),31.78, 29.52, 29.47, 29.35, 29.22, 26.09, 22.65(7 -CH_2-), 14.08 (CH_3).

(E)-3-(4-(dodecyloxy)phenyl)-1-phenylprop-2-en-1-one (4)

It was prepared as a white crystalline, Chemical formula: $C_{27}H_{36}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 2104 (C-H aromatic), 3089, 2831 (C-H aliphatic), 1599(C=O ketone), 1540,1456(C=C aromatic), 1254(C-O aromatic), 1127(C-O aliphatic),

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.80 – 7.21 (m, 9H, Ar-H), 7.95 (d, 1H, J = 15.6 Hz, Ph-C<u>H</u>=CH-CO-Ph), 7.58(d, 1H, *J* = 15.6 Hz, Ph-CH=<u>CH</u>-CO-Ph), 4.05 (t, J = 6.1 Hz, 2H, -O-C<u>H</u>$_2$-alkyl chain),1.76 (m, 2H, β-CH_2-), 1.34 –1.21 (m, 18H, -(CH_2-)$_9$), 0.86 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 189.78(-C=O), 160.36, 144.69, 137.47, 133.78, 129.98, 128.47, 128.12, 127.11, 122.74, 114.87 (10 C, Ar-C), 144.69 (-<u>C</u>=CH-CO), 122.74 (Ph-CH=<u>C</u>-CO-Ph), 66.14 (-O-CH_2-, alkyl chain), 31.71, 29.89, 29.68, 29.55, 29.44, 29.32, 29.20, 26.03, 22.35(9 -CH_2-), 14.13 (CH_3).

(E)-3-(4-(tetradecyloxy)phenyl)-1-phenylprop-2-en-1-one (5)

It was prepared as a white crystalline, Chemical formula: $C_{29}H_{40}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 2010(C-H aromatic), 3048, 2852 (C-H aliphatic), 1620(C=O ketone), 1572,1491(C=C aromatic), 1282(C-O aromatic), 1174(C-O aliphatic),

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.79 – 7.21 (m, 9H, Ar-H), 7.92 (d, 1H, J = 15.6 Hz, Ph-C<u>H</u>=CH-CO-Ph), 7.60(d, 1H, *J* = 15.6 Hz, Ph-CH=<u>CH</u>-CO-Ph), 4.04 (t, J = 6.1 Hz, 2H, -O-C<u>H</u>$_2$-alkyl chain),1.76 (m, 2H, β-CH_2-), 1.34 –1.21 (m, 22H, -(CH_2-)$_{11}$), 0.85 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 189.17(-C=O), 160.35, 144.74, 137.04, 133.74, 129.28, 128.01, 127.96, 127.28, 122.65, 114.47 (10 C, Ar-C), 144.74 (-<u>C</u>=CH-CO), 122.65 (Ph-CH=<u>C</u>-CO-Ph), 65.98 (-O-CH_2-, alkyl chain), 31.89, 29.97, 29.86, 29.71, 29.59, 29.51, 29.48, 29.37, 29.20,26.14, 22.57(11 -CH_2-), 14.21 (CH_3).

(E)-3-(4-(hexadecyloxy)phenyl)-1-phenylprop-2-en-1-one (6)

It was prepared as a white crystalline, Chemical formula: $C_{31}H_{44}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 2059(C-H aromatic), 3053, 2858 (C-H aliphatic), 1613(C=O ketone), 1585,1561(C=C aromatic), 1276(C-O aromatic), 1150(C-O aliphatic),

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.80 – 7.19 (m, 9H, Ar-H), 7.93 (d, 1H, J = 15.6 Hz, Ph-C<u>H</u>=CH-CO-Ph), 7.58(d, 1H, *J* = 15.6 Hz, Ph-CH=<u>CH</u>-CO-Ph), 4.06 (t, J = 6.1 Hz, 2H, -O-C<u>H</u>$_2$-alkyl chain),1.76 (m, 2H, β-CH_2-), 1.36 –1.23 (m, 26H, -(CH_2-)$_{13}$), 0.85 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 189.35(-C=O), 161.05, 144.67, 136.94, 134.14, 130.21, 129.31, 128.54, 127.18, 122.71, 114.47 (10 C, Ar-C), 144.67 (-<u>C</u>=CH-CO), 122.71 (Ph-CH=<u>C</u>-CO-Ph), 65.17 (-O-CH_2-, alkyl chain), 31.97, 29.98, 29.87, 29.74, 29.64, 29.58, 29.52, 29.46, 29.35, 29.26, 29.13,26.21, 22.63(13 -CH_2-), 14.14 (CH_3).

(E)-3-(4-(octadecyloxy)phenyl)-1-phenylprop-2-en-1-one (7)

It was prepared as a white crystalline, Chemical formula: $C_{33}H_{48}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 2077 (C-H aromatic), 3089, 2975 (C-H aliphatic), 1613(C=O ketone), 1590,1500(C=C aromatic), 1269(C-O aromatic), 1186(C-O aliphatic),

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.78 – 7.14 (m, 9H, Ar-H), 7.89 (d, 1H, J = 15.6 Hz, Ph-C<u>H</u>=CH-CO-Ph), 7.58(d, 1H, *J* = 15.6 Hz, Ph-CH=<u>CH</u>-CO-Ph), 4.05 (t, J = 6.1 Hz, 2H, -O-C<u>H</u>$_2$-alkyl chain),1.76 (m, 2H, β-CH_2-), 1.37 –1.20 (m, 30H, -(CH_2-)$_{15}$), 0.87 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 188.78(-C=O),

161.15, 144.58, 136.98, 134.18, 130.74, 129.48, 128.51, 127.74, 122.87, 114.65 (10 C, Ar-C), 144.58 (-C=CH-CO), 122.87 (Ph-CH=C-CO-Ph), 65.14 (-O-CH_2-, alkyl chain), 31.89, 29.98, 29.89, 29.79, 29.76, 29.67,29.59, 29.51, 29.49, 29.47, 29.33, 29.24, 29.08,26.47, 22.61(15 -CH_2-), 14.24 (CH_3).

(E)-3-(4-(benzyloxy)phenyl)-1-phenylprop-2-en-1-one (8)

It was prepared as a white crystalline, Chemical formula: $C_{22}H_{18}O_2$; 85% yield; mp 68-70 ºC; FTIR data (cm^{-1}): 3097(C-H aromatic), 3052, 2867(C-H aliphatic), 1635(C=O ketone), 1612,1578(C=C aromatic), 1283(C-O aromatic), 1150(C-O aliphatic),

^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.84 – 7.17 (m, 14H, Ar-H), 7.95 (d, 1H, J = 15.6 Hz, Ph-CH=CH-CO-Ph), 7.59(d, 1H, *J* = 15.6 Hz, Ph-CH=CH-CO-Ph), 5.04 (s, J = 6.1 Hz, 2H, -O-CH_2-alkyl chain),^{13}C-NMR (75 MHz, DMSO-d_6) δ 188.60(-C=O), 156.74, 144.47, 137.93, 135.84, 133.78, 130.27, 128.97, 128.44, 128.06,127.64, 126.24,122.38, 114.82,69.87 (14 C, Ar-C), 144.47 (-C=CH-CO), 122.38 (Ph-CH=C-CO-Ph), 69.87 (-O-CH_2-, alkyl chain),

Synthesis of 1,2,3-triazoline derivatives (9-14) [13]

In 17 mL of DMF containing (0.54mmol) from compound **(1)** (1.2equiv) of the prepared chalcones (**3-8**) are added after several minutes, the catalyst is added (monovalent copper and sodium ascorbate in a ratio of 5%mol and 10%mol respectively). Then the temperature is raised to 50 ºC and the reaction is left to when finished (as indicated by TLC, ethylacetate:n-hexane:methanol 2:1:0.35), the solvent is then evaporated using a rotary evaporator and the product is washed with distilled water. The products were recrystallized using a mixture of glacial acetic acid and acetone (2:3).

4-(4-benzoyl-5-(4-(decyloxy)phenyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (9)

It was prepared as a white crystalline, Chemical formula: $C_{35}H_{41}N_5O_5S$; 85% yield; mp 68-70 ºC; FT-IR data (cm-1): 3154 (NH) 3088 (C-H aromatic), 2939(C-H aliphatic),1613(C=O group), 1469(C=C aromatic), 1414(N=N group), 1303(asy SO_2 group), 1236(N-N group), 1196(sy SO_2), 893(S-N), 812(C-S)

^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.45 (s, 1H, -NH-), 7.53-7.23 (m, 13H, Ar-H), 6.14 (s, 1H, C-H-methoxazole ring), 8.56 (d, 1H, triazoline ring), 8.34 (d, 1H, triazoline ring), 4.05 (t, J = 6.1 Hz, 2H, -O-CH_2-alkyl chain), 2.31 (s, 3H, methoxazole ring -CH_3), 1.79 (m, 2H, β-CH_2-), 1.34 –1.23 (m, 14H, -$(CH_2)_7$), 0.87 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 169.97 (CO), 158.47(CH_3-C-methoxazole ring),153.45 (sulfonamide-C-methoxazole ring),147.87, 145.72, 145.47, 143.28, 140.61, 138.78, 135.49, 129.57, 128.49, 124.87, 122.87, 122.78,120.68,115.78,114.17,111.48(16C, Ar-C), 96.68(CH-methoxazole ring), 61.27 (C4, triazoline ring), 56.69 (C5, triazoline ring), 32.45 (-O-CH_2-, alkyl chain),29.98, 29.82, 29.55, 29.41, 29.30, 26.74, 22.41, 14.31(7 -CH_2-), 12.28 (methoxazole ring-CH_3).

4-(4-benzoyl-5-(4-(dodecyloxy)phenyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (10)

It was prepared as a white crystalline, Chemical formula: $C_{37}H_{45}N_5O_5S$; 85% yield; mp 68-70 ºC; FT-IR data (cm-1):3120 (NH) 3082(C-H aromatic), 2925(C-H aliphatic),1615(C=O group), 1455(C=C aromatic), 1413(N=N group), 1370(asy SO_2 group), 1277(N-N group), 1143(sy SO_2), 966(S-N), 809(C-S)

^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.44 (s, 1H, -NH-), 7.40-7.22 (m, 13H, Ar-H), 6.18 (s, 1H, C-H-methoxazole ring), 8.36 (d, 1H, triazoline ring), 8.34 (d, 1H, triazoline ring), 4.05 (t, J = 6.1 Hz, 2H, -O-CH_2-alkyl chain), 2.34 (s, 3H, methoxazole ring -CH_3), 1.78 (m, 2H, β-CH_2-), 1.33 –1.27 (m, 18H, -$(CH_2)_9$), 0.83 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 186.97 (CO), 167.88(CH_3-C-methoxazole ring),153.77 (sulfonamide-C-methoxazole ring),152.22, 146.76, 144.54, 143.22, 142.25, 137.98, 134.49, 128.55, 128.52, 123.33, 122.53, 121.50,121.12,117.10,116.77,114.14(16C, Ar-C), 97.67(CH-methoxazole ring), 60.13 (C4, triazoline ring), 55.12 (C5, triazoline ring), 33.86 (-O-CH_2-, alkyl chain), 32.12,29.77, 29.67, 29.60, 29.49, 29.47, 29.35,25.16, 20.75, 13.33(9 -CH_2-), 13.22 (methoxazole ring-CH_3).

4-(4-benzoyl-5-(4-(tetradecyloxy)phenyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (11)

It was prepared as a white crystalline, Chemical formula: $C_{39}H_{49}N_5O_5S$; 85% yield; mp 68-70 ºC; FT-IR data (cm-1): 3154 (NH)3086(C-H aromatic), 2999(C-H aliphatic),1632(C=O group), 1468(C=C aromatic), 1409(N=N group), 11360(asy SO_2 group), 1252(N-N group), 1125(sy SO_2), 925(S-N), 878(C-S)

^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.42 (s, 1H, -NH-), 7.79-7.15 (m, 13H, Ar-H), 6.16 (s, 1H, C-H-methoxazole ring), 7.82 (d, 1H, triazoline ring), 7.80 (d, 1H, triazoline ring), 4.05 (t, J = 6.1 Hz, 2H, -O-CH_2-alkyl chain), 2.33 (s, 3H, methoxazole ring -CH_3), 1.81 (m, 2H, β-CH_2-), 1.36 –1.22 (m, 22H, -$(CH_2)_{11}$), 0.84 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 170.88 (CO), 157.75(CH_3-C-methoxazole ring),155.21 (sulfonamide-C-methoxazole ring),149.78, 147.55, 146.12, 145.22, 144.23, 139.97, 138.43, 129.55, 128.55, 126.30, 123.54, 122.57,121.12,114.12,112.74,111.14(16C, Ar-C), 95.55(CH-methoxazole ring), 62.12 (C4, triazoline ring), 54.12 (C5, triazoline ring), 33.88 (-O-CH_2-, alkyl chain), 31.13,29.77, 29.59, 29.48, 29.43, 29.36, 29.33,29.29,28.26,26.16, 21.75, 15.12, (11 -CH_2-), 12.23 (methoxazole ring-CH_3).

4-(4-benzoyl-5-(4-(hexadecyloxy)phenyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (12)

It was prepared as a white crystalline, Chemical formula: $C_{41}H_{53}N_5O_5S$; 85% yield; mp 68-70 ºC; FT-IR data (cm-1): 3174 (NH)3091(C-H aromatic), 2926(C-H aliphatic),1690(C=O group), 1468(C=C aromatic), 1383(N=N group), 1331(asy SO_2 group), 1281(N-N group), 1149(sy SO_2), 906(S-N), 857(C-S)

^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.43 (s, 1H, -NH-), 7.78-7.17 (m, 13H, Ar-H), 6.15 (s, 1H, C-H-methoxazole ring), 7.81 (d, 1H, triazoline ring), 7.80 (d, 1H, triazoline ring), 4.05 (t, J = 6.1 Hz, 2H, -O-CH_2-alkyl chain), 2.33 (s, 3H, methoxazole ring -CH_3), 1.82 (m, 2H, β-CH_2-), 1.37 –1.21 (m, 22H, -$(CH_2)_{11}$), 0.83 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 169.88 (CO), 153.77(CH_3-C-methoxazole ring),151.29 (sulfonamide-C-methoxazole ring),149.76, 146.58, 146.14, 143.24, 141.28, 139.97, 137.43, 129.52, 128.57, 126.33, 125.10, 124.53,122.51,117.11,116.74,113.14(16C, Ar-C), 94.54(CH-methoxazole ring), 63.11 (C4, triazoline ring), 58.11 (C5, triazoline ring), 33.66 (-O-CH_2-, alkyl chain), 31.15,29.87, 29.77, 29.69, 29.61, 29.57, 29.50,29.40,29.39,29.33, 29.30, 26.19,12.12 (13 -CH_2-), 11.23 (methoxazole ring-CH_3).

4-(4-benzoyl-5-(4-(octadecyloxy)phenyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (13)

It was prepared as a white crystalline, Chemical formula: $C_{43}H_{57}N_5O_5S$; 85% yield; mp 68-70 ºC; FT-IR data (cm-1): 3173(NH)3098(C-H aromatic), 2920(C-H aliphatic),1684(C=O group), 1473(C=C aromatic), 1303(N=N group), 1286(asy SO_2 group), 1222(N-N group), 1152(sy SO_2), 965(S-N), 815(C-S)

^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.43 (s, 1H, -NH-), 7.81-7.21 (m, 13H, Ar-H), 6.15 (s, 1H, C-H-methoxazole ring), 7.85 (d, 1H, triazoline ring), 7.83 (d, 1H, triazoline ring), 4.03 (t, J = 6.1 Hz, 2H, -O-CH_2-alkyl chain), 2.33 (s, 3H, methoxazole ring -CH_3), 1.80 (m, 2H, β-CH_2-), 1.36 –1.22 (m, 30H, -$(CH_2)_{15}$), 0.85 (t, J = 6.1 Hz, 3H, -CH_3).^{13}C-NMR (75 MHz, DMSO-d_6) δ 167.88 (CO), 155.20 (CH_3-C-methoxazole ring),153.77 (sulfonamide-C-methoxazole ring),149.76, 147.56, 146.25, 146.12, 144.24, 139.93, 137.43, 129.52, 128.50, 125.12, 124.39, 122.56,122.54,117.13,116.74,113.04(16C, Ar-C), 96.57(CH-methoxazole ring), 63.15 (C4, triazoline ring), 55.15 (C5, triazoline ring), 29.98 (-O-CH_2-, alkyl chain), 29.86, 29.77, 29.69, 29.65, 29.57, 29.54,29.47,29.46,29.39, 29.36, 29.26,26.20,21.77,14.12 (17 -CH_2-), 12.23 (methoxazole ring-CH_3).

4-(4-benzoyl-5-(4-(benzyloxy)phenyl)-4,5-dihydro-1H-1,2,3-triazol-1-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (14)

It was prepared as a white crystalline, Chemical formula: $C_{32}H_{27}N_5O_5S$; 85% yield; mp 68-70 ºC; FT-IR data (cm-1): 3181(NH) 3010(C-H aromatic),2929(C-H aliphatic),1635(C=O group), 1459(C=C aromatic), 1418(N=N group), 1373(asy SO_2 group), 1283(N-N group), 1150(sy SO_2), 979(S-N), 858(C-S)

^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.45 (s, 1H, -NH-), 7.83-7.20 (m, 18H, Ar-H), 6.16 (s, 1H, C-H-methoxazole ring), 7.85 (d, 1H, triazoline ring), 7.84 (d, 1H, triazoline ring), 5.06 (t, J = 6.1

Hz, 2H, -O-C<u>H</u>$_2$- Ar), 2.34 (s, 3H, methoxazole ring -CH_3),^{13}C-NMR (75 MHz, DMSO-d_6) δ 169.81 (CO), 153.75 (CH_3-C-methoxazole ring),152.23 (sulfonamide-C-methoxazole ring),149.71, 146.51, 145.28, 143.20, 143.16, 139.95, 137.12, 136.77,133.54,129.65,129.50,128.10,127.15,117 .123.58,123.34,123.12,120.86,117.14,116.75,11 3.04 (18 C, Ar-C), 96.57(CH-methoxazole ring), 72.54 (C4, triazoline ring), 59.16 (C5, triazoline ring), 57.11 (-O-CH_2-,), 14.23 (methoxazole ring-CH_3).

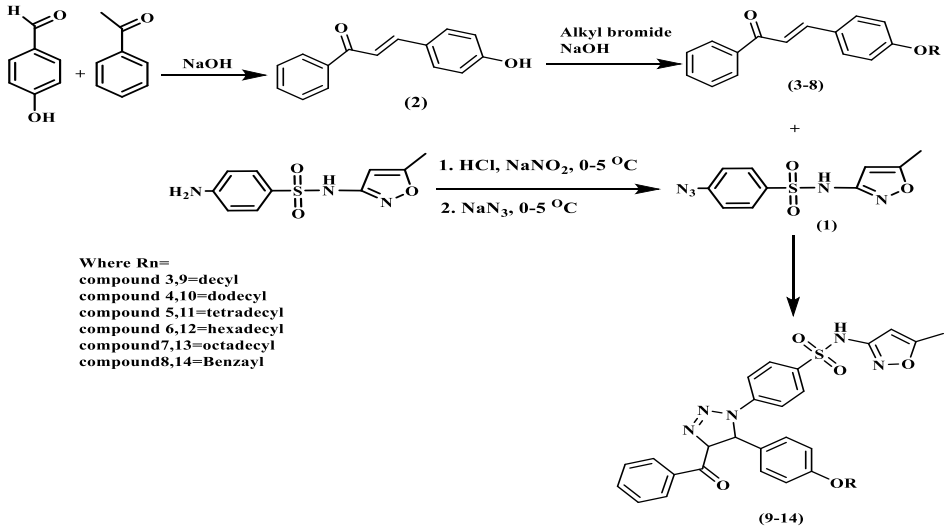
Antibacterial Activity

The in vitro antibacterial activity of the produced compounds against Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria was assessed. After being infused into the nutrient broth (inoculation medium), the bacterial cultures were left to grow overnight at 37°C. A uniform distribution was achieved by adding the inoculated media aseptically to the nutrition medium and thoroughly mixing. A 20 mL solution was placed into sterile culture plates and allowed to warm up to room temperature. Utilizing nutrient agar tubes, sterile agar-disc diffusions were previously bathed in conventional medication sulfamethoxazole and manufactured chemical solutions at known concentrations (12.5, 25, 50, and 100 M) in DMSO. After 24 hours at 37°C, the zones of growth inhibition around the disks were assessed. With a ruler and a comparison to the positive control disk (a disk containing sulfamethoxazole), the inhibition zones were measured and quantified in millimeters [14].

RESULTS AND DISCUSSION

Chemistry

Using our newly published click technique [25], we were able to re-synthesize the targeted 1,2,3-triazolines with sulfa-drug tethers as illustrated in Scheme 1. Sulfamethoxazole has to first be diazotized before being treated with sodium azide to produce the matching sulfamethoxazole azides (1). The O-alkyl chain and benzyl derivatives (3-8) were obtained in high yield by O-alkylation of alkyl halide with the synthesized Schiff base (3) in the presence of strong base (NaOH) in DMSO at 70 °C. Next, under optimized Cu(I)-assisted alkyne-azide cycloaddition conditions ($CuSO_4$, Na-ascorbate; DMSO/H2O (1:1); 6–10 h room temperature), the freshly prepared sulfamethoxazole azides were subsequently ligated to chalcone (2), yielding the desired 1,2,3-triazoline-sulfamethoxazole molecular conjugates (9-14) percent yields.



SCHEME 1: Synthetic Route of heterocyclic compounds

The structures of the synthesized compounds were fully characterized by IR, ^{1}H & ^{13}C NMR and mass analysis. The FTIR spectra of compounds (9-15) showed characteristic bands of 1,2,3- triazoline nucleus in the range of 1500–1300 cm^{-1} corresponding to C=C & N=N bonds.

The characteristic bands of (NH) of sulfonamide was detected in the range 3181–3120 cm^{-1}, whereas alkyl chain appear with high intensity in the range 2900-2800 cm^{-1}. The ^{1}H NMR spectra of compounds (9-15) showed characteristic signals of the protons corresponding to C4 and C5 in the 1,2,3-triazoline ring between 7.83 ppm and 7.80 ppm, in addition to aromatic protons in the region of 7.83–7.20 ppm. We also noticed the presence of signal at 6.16 ppm corresponded to methylene groups attached to oxazole ring and triplet signal at region 0.85 ppm due to methyl proton in alkyl chain and other signals of alkyl chain protons. On the other handn singlet signal at range 11.41-11.46 ppm assigned to sulfonamide proton. The signals of methyl carbons between 12 ppm and 15 ppm and signals of aromatic carbons at 63 ppm and 58 ppm belonging to the 1,2,3-triazoline nucleus were the most prominent carbon signals in 13C NMR spectra that were expected to correspond to sulfamethoxazole-1,2,3-triazoline derivatives.

Antibacterial activity

The synthesised compounds (9–14) were tested for their antibacterial efficacy against the gram-positive Staphyloccocus aureus and the gram-negative Escherichia coli bacterium. Using Mueller Hinton Broth (MBH) medium, the disk-diffusion method has been used to examine the antibacterial activity largely in terms of the growth inhibition zones that were detected. The synthesized compounds' Minimum Inhibitory Concentrations (MIC) were then established. The bactericidal activity of DMSO was tested negatively. The synthesized compounds' antibacterial minimum inhibitory concentration (MIC) data (9–14). The antibacterial activity results showed that both the Gram-positive and Gram-negative bacteria examined were found to be the sensitive bacteria, with the tested chemicals showing varying degrees of inhibition against both types of bacteria. All prepared substances get stronger as their concentration goes up. Except for compound (10), which exhibited no antibacterial activity against the chosen bacteria Staphyloccocus aureus, compounds (9–14) with alkyl or phenyl groups on the sulfamethoxazole-1,2,3-triazoline skeleton exhibited the best antibacterial activity against Staphyloccocus aureus. In addition, compound 12 had a MIC value of 22 µg/mL and shown the most antibacterial activity against Staphyloccocus aureus. Additionally, chemicals (11 and 14) showed no action against the chosen bacterium Escherichia coli. The best antibacterial activity against Escherichia coli was demonstrated by end products (10,11,12,14,15) having alkyl or phenyl groups on the sulfamethoxazole-1,2,3-triazoline skeleton, according to MIC values. In addition, compounds 9 and 12 had MIC values of 18 µg/mL and had the strongest antibacterial activity against Escherichia coli. Table 1 lists the synthesized compounds' observed minimum inhibitory concentration (MIC) antibacterial data (9–14).

TABLE 1: The biological activity of end products (9-14) against Escherichia coli and staphylococcus aureus

Compound.	Concentration	Escherichia coli	staphylococcus aureus
9	100 µg/mL	18	16
	50 µg/mL	15	14
	25 µg/mL	12	11
	12.5 µg/Ml	10	8
10	100 µg/mL	14	0
	50 µg/mL	13	0
	25 µg/mL	11	0
	12.5 µg/mL	9	0
11	100 µg/mL	0	16
	50 µg/mL	0	15
	25 µg/mL	0	13
	12.5 µg/mL	0	12
12	100 µg/mL	18	22
	50 µg/mL	16	19
	25 µg/mL	13	18
	12.5 µg/mL	11	17
13	100 µg/mL	14	19

	50 µg/mL 25 µg/mL 12.5 µg/mL	13 12 11	18 16 14
14	100 µg/mL 50 µg/mL 25 µg/mL 12.5 µg/mL	0 0 0 0	20 18 16 15

CONCLUSION

With a 72–81% yield, 1,2,3-triazoline derivatives were effectively produced using a click reaction. Using melting point and spectroscopic methods (1H and 13C NMR), the produced compounds were thoroughly analyzed. Compounds (9 and 12) showed the greatest efficacy against E. coli with an inhibitory zone of 18 g/mL when the in vitro antibacterial activities of synthesized compounds were assessed against two bacterial strains, S. aureus and E. coli. In addition, compound (12) with a MIC value of 22 g/mL had the strongest antibacterial activity against Staphyloccocus aureus.

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