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Research article

Synthesis of 1,2,3-triazole-thymol derivatives as potential antimicrobial agents

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ABSTRACT

Background: Thymol as a natural biological template can be modified chemically since the hydroxyl group makes it a candidate for structural modification. Thus, this study incorporated the triazole moiety on thymol and the chlorination of thymol moiety to help improve its biological potency.

Materials and methods: A series of ten 1,2,3-triazole-thymol derivatives **1–10** were synthesized from thymol, by a click reaction between O-propargyl terminal alkyne of thymol and its chlorothymol with benzyl azide and substituted benzyl azides. Their structures were confirmed by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, IR, GC-MS-EI/CI and LC-ESI-QTOF-MS). The Well diffusion method using Müeller-Hinton agar plates was used to demonstrate the antimicrobial activities of the synthesized triazole-thymol derivatives on selected bacterial strains; *Escherichia coli ATCC* 25922, *Staphylococcus aureus* ATCC25923, Methicillin resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa* ATCC 29853, *E. coli* ESBL, *Klebsiella pneumoniae* NCTC 13438 and Meropenem Resistant *E. coli.*

Results: All the synthesized triazole-thymol derivatives showed significant but variable antibacterial activity against the seven medically important bacterial strains tested. The compound 4-((4-chloro-2-isopropyl-5-methylphenoxy)methyl)-1-(2-nitrobenzyl)-1*H*-1,2,3triazole **(9)** demonstrated a higher antibacterial activity with a mean zone of inhibition (38.7 mm) compared with ampicillin as the positive control which gave a zone size of 30.0 mm. In addition, the compound showed a three-fold potency than the parent compound, thymol (11.0 mm) against *MRSA* at a concentration of 100 μg/ml.

Conclusion: These results provide additional evidence of the exploitation of natural products like thymol as leads for drug development against medically important bacterial pathogens.

1. Introduction

Antibacterial activities of essential oils from the herbaceous plant thyme (*Thymus vulgaris* L), family Lamiaceae and oregano (*Origanum vulgare*) are linked primarily to carvacrol and thymol present in the oil [1]. Thymol and carvacrol are currently being used as plant-based health products due to the known antimicrobial properties they possess [1]. Thymol, like many safe essential oil components, is mostly applied as food additive and flavour [1]. Studies have demonstrated that both thymol and carvacrol, are potent antibacterial agents against both Gram-positive and Gram-negative bacteria. Additionally, a synergism behaviour of thymol and carvacrol with known antibiotics have considerably exhibited activity against bacteria commonly found in food that shows resistance [1]. In traditional medicine the essential oil of thyme is used as an expectorant, anti-inflammatory, antiviral, antibacterial, and antiseptic agent, mainly in the treatment of the upper respiratory infections [2].

Thymol (2-isopropyl-5-methylphenol), is a major monoterpene in the essential oil of thyme (30–40%) [3] and *Carum copticum* (Ajwan) (35–60%) [4]. Thymol is also present in the essential oil of *Nigella sativa* L. seeds [5]. When released by plants, it plays a role in chemical defence against phytopathogens [5]. Despite its medicinal properties [6, 7, 8, 9, 10, 11, 12] it has poor physico-chemical properties including very low aqueous solubility (0.9 g in 1 L), high sublimation, high photoreactivity

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and poor heat sensitivity [13]. The chemical modification of the hydroxy (–OH) group into its ether and ester derivatives afforded new lead compounds found to be effective against *Helicobacter pylori* infection to prevent the occurrence of severe gastric disease conditions [14]. A recent study also showed that the hydroxyl functional group of thymol was indispensable for its anti-bacterial effect [15]. Modification of the hydroxy group into ester and ether derivatives resulted in low anti-bacterial activities on the following organisms *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative) and *Pseudomonas aeruginosa* (Gram negative) compared to the parent compound, thymol [15].

Triazole derivatives have gained attention in drug design and synthesis recently because of their enormous pharmacological activity, less adverse effects, low toxicity and high bioavailability among others. Previous studies have shown that they are excellent candidates for the treatment of various types of plant and animal diseases [16, 17, 18]. Thus, a range of triazole compounds has been reported as medicinal drugs and good molecular scaffold candidates, Human 17β-hydroxysteroid dehydrogenase type 1(17β-HSD1) inhibitors, antifungal, anticancer, antibacterial, antitubercular, antiviral, anti-inflammatory, analgesic, anticonvulsant, antiparasitic, and other health and related pharmacological and biological conditions [19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30].

In the present study, we hypothesized that incorporation of a triazole moiety into thymol can improve upon its solubility in water and increase its biological potency. The hydroxyl group of thymol makes it an excellent candidate for structural modification by introducing a triazole group comprising three nitrogen hetero-atoms in a five-membered ring system. The structural features of 1,2,3-triazoles enable them to act as a bioisostere of different functional groups such as carboxylic acid (–COOH) and its amide ($–CONH_2$) and ester (–COOR) derivatives. Again, the triazole group is capable to form hydrogen bonds which makes its derivatives more polar and soluble in most systems and their ability to form π−π interactions and also easily coordinate to metal ions serves as a good substrate for biological targets, thus making them good template for the synthesis of medicinal compounds [19]. Here, we report the synthesis of ten 1,2,3-triazole compounds containing thymol moiety, and their antimicrobial activity.

2. Materials and Methods

2.1. Chemicals

All solvents (organic and inorganic), reagents and chemicals used for the research work were of analytical grade and obtained from Sigma-Aldrich, St. Louis, MO.

2.2. Bacterial strains

Bacterial strains were obtained from the Department of Medical Microbiology, University of Ghana Medical School located within Korle-Bu, Accra, Ghana.

2.3. Experimental

Reaction progress was monitored by thin-layer chromatography on silica gel GF254 with ultraviolet detection. Flash column chromatography purification was achieved on silica gel 100–200 mesh, elution; hexane/ethyl acetate gradient. The synthesized compounds were analyzed using a Waters Synapt G2-*Si* LC-ESI-QTOF-MS in full scan mode, mass range *m/z* 50–2000 in an MS^E positive mode. Briefly, 0.2 μL of the sample was automatically injected into a Waters UPLC and separated on a 250 mm × 4.6 mm i.d., 5 μm, ACE C-18 column (Advance Chromatography Technologies, Aberdeen, Scotland) using a gradient program with mobile phases of water (A) and methanol (B) each with 0.01% formic acid. The mass spectrum was generated for every peak and potential assignments done using monoisotopic masses with tolerance of 10 ppm. Library searches were performed using the commercial NIST MS/MS library and online databases (METLIN, ChemSpider, ChemCalc) and literature.

Coupled gas chromatography−mass spectrometry (GC−MS) analysis was carried out on an HP7890A gas chromatograph coupled to an HP5975C inert XL EI/CI mass spectrometer (Agilent, Palo Alto, CA). The column used was a 30 m × 0.25 mm i.d., 0.25 μm HP-5 MS (J&W, Folsom, CA, USA), capillary column. An aliquot (1 μL) of the synthesized samples was injected into the GC in splitless mode (270 °C; 6.83 psi) and helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was held at 35 °C for 5 min, then increased at the rate of 10 °C/min to 280 °C and maintained at this temperature for 10 min. Mass spectral data were obtained using electron ionization (EI) mode and the Chemical ionization (CI) mode at 70 eV. The detected fragments of the synthesized compounds were identified by comparing their mass spectral data with those published by National Institute of Standards and Technology NIST 11 library databases, literature and also the fragmentation pattern of the compounds as provided in the supporting information.

The FTIR data were obtained from a Thermo-Nicolet Avatar 370 Fourier Transform Infra-Red Spectrophotometer over the range of 500–4000 cm^{-1}.

The NMR spectral investigations were performed at Rothamsted Research's facility, UK on a Bruker FT-NMR Spectrometer at 500 and 125 MHz for ^{1}H-NMR and ^{13}C-NMR respectively. The chemical shifts are quoted in parts per million (ppm) relative to the signal of tetramethylsilane (TMS), which was used as an external standard for proton (^{1}H-NMR) and carbon-13 (^{13}C-NMR). Standard and coupling constants (J) values were measured in hertz (Hz). All NMR analyses were run in deuterated chloroform ($CDCl_3$) or deuterated dimethyl sulphoxide (DMSO) as solvent unless stated otherwise.

2.4. Synthesis of aromatic terminal alkyne of thymol

The sodium salt of thymol was prepared by dissolving thymol (1.00 g, 1 mmol) in 10% NaOH (10 ml), followed by addition of potassium carbonate (5.00 g, 1.5 mmol) and propargyl bromide (1.90 g, 1.2 mmol) with acetone as solvent. The reaction mixture was refluxed at 60 °C for 4 h under nitrogen. The progress of reaction was monitored by TLC and GC analysis. The reaction mixture was cooled to room temperature and distilled deionized water (50 ml) was added and extracted with ethyl acetate (3 × 40 ml). The organic phase was separated, dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude product was subjected to column chromatography (silica gel 100–200 mesh, elution; hexane/ethyl acetate gradient) to afford a pale yellowish oily liquid. Also, thymol was chlorinated using thionyl chloride ($SOCl_2$) in carbon tetrachloride. This was refluxed over water bath 70–80 °C for 1 h. The aromatic terminal alkyne of the chlorothymol is prepared following the same protocol in the preparation of the aromatic terminal alkyne of thymol.

2.5. Synthesis of aromatic alkyl azides

2.5.1. Preparation of 3-chlorobenzyl azide from 3-chlorobenzyl chloride

3-Chlorobenzyl chloride (0.5 g, 3.1 mmol) was dissolved in 10 ml DMSO. Sodium azide (0.25 g, 3.8 mmol) was added as solid and the reaction was stirred overnight at ambient temperature. Water (50 ml) was added and the reaction product extracted into diethyl ether (3 × 20 ml). The combined ether layer was washed with brine (2 × 20 ml), dried over anhydrous sodium sulphate and concentrated in *vacuo* to afford a clear product. The product was further purified by column chromatography using the solvent system hexane/ethyl acetate gradient to afford the azido compound intermediate. The preparation of the following aromatic azides is carried out using the above experimental procedure: 3-fluorobenzyl azide, benzyl azide and 2-nitrobenzyl azide.

2.6. Synthesis of triazole compounds with thymol moiety

2.6.1. Synthesis of 1-(3-chlorobenzyl)-4-((2-isopropyl-5-methylphenoxy) methyl)-1H-1,2,3-triazole (1)

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of 3-chlorobenzyl azide (0.05g, 1 mmol) in *t*-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and sodium ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 h using a magnetic stirrer. The progress of reaction was monitored by TLC analysis. After completion of the reaction, the mixture was filtered and extracted with ethyl acetate (3 × 20 ml) and water (3 × 20 ml). The ethyl acetate layer was separated and the solvent was evaporated *in vacuo*. The crude product was subjected to column chromatography (silica gel 100–200 mesh, elution; hexane/ethyl acetate gradient) to afford compound **1**, 0.71 g (75%) as pale greenish semi-solid.

IR (KBr, cm^{-1}): 3158.2, 3085.4, 2957.7, 2927.7, 2885.5, 2867.0, 1885.6, 1610.1, 1599.2, 1432.8 and 1241.9. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.67 (s, 1H, triazole H); 7.40 (d, 1H, J = 1.5 Hz, Ar–H); 7.35 (s, 1H, Ar–H); 7.33 (t, 1H, J = 7.2Hz, Ar–H); 7.30 (d, 1H, J = 1.6 Hz, Ar–H); 7.20 (d, 1H, J = 6.8 Hz, Ar–H), 7.13 (d, 1H, J = 7.9 Hz, Ar–H); 6.80 (d, 1H, J = 7.5 Hz, Ar–H); 5.55 (s, 2H, O–CH_2); 5.23 (s, 2H, N–CH_2); 3.29 (m, 1H, CH); 2.34 (s, 3H, Ar–CH_3). 1.20 (d, 3H, J = 6.9 Hz, –CH_3), 1.19 (d, 3H, J = 6.9 Hz, –CH_3) ^{13}CNMR ($CDCl_3$, 126 MHz): δ_C 155.26 (Ar–C), 136.59 (Ar–C), 136.50 (Ar–C), 135.01 (Ar–C), 134.32 (Ar–C), 130.44 (Ar–C), 128.99 (Ar–C), 128.04 (Ar–C), 126.06 (Ar–C), 126.06 (Ar–C), 126.05 (Ar–C), 121.95 (Ar–C), 116.49 (Ar–C), 112.94 (Ar–C), 62.52 (–CH_2), 53.61(–CH_2), 26.57 (–CH), 22.81 (–CH_3), 21.36 (–CH_3), 21.36 (–CH_3). MS (CI): *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ 356.1 and 384.1 respectively. MS (EI): $[M]^+$ 355.1, 312.1, 284.1, 178.0, 125.0, 91.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{23}ClN_3O]$, 356.1530; found 356.1565 and $[M + K]^+$ 394.1696.

The above protocol for the synthesis of compound **1** was followed in the preparation of compounds **2, 3, 4, 6, 7, 8** and **9** using terminal alkyne of thymol or terminal alkyne of chlorothymol. Their spectroscopic data is indicated as shown below:

2.6.2. 1-(3-fluorobenzyl)-4-((2-isopropyl-5-methylphenoxy) methyl)-1H-1,2,3-triazole (2)

Compound **2**, 0.67 g (77%) was synthesized as pale greenish semi-solid.

IR (KBr, cm^{-1}): 3156.4, 3080.8, 2963.4, 2938.2, 2872.9, 1793.0, 1730.7, 1617.3, 1590.9, 1450.2 and 1249.1 ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.34 (s, 1H, triazole H); 7.33 (s, 1H, Ar–H); 7.30 (t, 1H, J = 8.2 Hz, Ar–H), 7.14 (d, 1H, J = 7.9 Hz, Ar–H); 7.11 (d, 1H, J = 8.1 Hz, Ar–H); 6.98 (s, 1H, Ar–H); 6.91 (d, 1H, J = 9.2 Hz, Ar–H); 6.78 (d, 1H, J = 9.2 Hz, Ar–H); 5.56 (s, 2H, O–CH_2); 5.22 (s, 2H, N–CH_2); 3.24 (m, 1H, CH); 2.32 (s, 3H, Ar–CH_3), 1.17 (d, 3H, J = 6.9 Hz, –CH_3); 1.12 (d, 3H, J = 6.8 Hz, –CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 126 MHz): δ_C 163.99 (Ar–C), 155.22 (Ar–C), 136.98 (triazole Ar–C), 136.92 (Ar–C), 136.50 (Ar–C), 134.29 (Ar–C), 130.78 (Ar–C), 129.96 (Ar–C), 126.05 (Ar–C), 123.51 (Ar–C), 121.94(triazole Ar–C), 116.48 (Ar–C), 115.89 (Ar–C), 114.85 (Ar–C), 64.38 (–CH_2), 53.59 (–CH_2), 26.63 (–CH), 22.77 (–CH_3), 21.32 (–CH_3), 21.32 (–CH_3). MS (CI): *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ 340.1 and 368.1 respectively. MS (EI): $[M]^+$ 339.2, 296.1, 268.1, 191.1, 162.1, 109.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{23}FN_3O]$, 340.1825; found 340.1847 and $[M + K]^+$ 378.2007.

2.6.3. 1-Benzyl-4-((2-isopropyl-5-methylphenoxy)methyl)-1H-1,2,3-triazole (3)

Compound **3**, 0.60 g (85%) was synthesized as pale yellowish oily liquid.

IR (KBr, cm^{-1}): 3150.2, 3087.5, 3031.3, 2961.6, 2928.5, 2868.8, 1733.2, 1698.8, 1610.1, 1579.0, 1557.5, 1456.0, and 1256.8. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.54 (s, 1H, trizole H); 7.41 (t, 1H, Ar–H); 7.40 (t, 1H, Ar–H); 7.31 (t, 1H, J = 1.8 Hz, Ar–H); 7.29 (d, 1H, J = 1.2 Hz, Ar–H), 7.12 (d, 1H, J = 8.0 Hz, Ar–H); 6.80 (d, 1H, J = 5.8 Hz, Ar–H); 6.79 (s, 1H, Ar–H); 5.57 (s, 2H, O–CH_2); 5.22 (s, 2H, N–CH_2); 3.25 (m, 1H, CH); 2.34 (s, 3H, Ar–CH_3), 1.18 (d, 3H, J = 6.9 Hz, –CH_3); 1.13 (d, 3H, J = 7.0 Hz, –CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 126 MHz): δ_C 155.34 (Ar–C), 145.34 (Ar–C), 136.47 (Ar–C), 134.65 (Ar–C), 134.33 (Ar–C), 129.14 (Ar–C), 129.04 (Ar–C), 128.78 (Ar–C), 128.01 (Ar–C), 127.80 (Ar–C), 126.03 (Ar–C), 122.34 (Ar–C), 121.88 (Ar–C), 112.95 (Ar–C), 62.58 (–CH_2), 54.21 (–CH_2), 26.54 (–CH), 22.80(–CH_3), 22.76 (–CH_3), 22.76 (–CH_3). MS (CI): *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ 322.1 and 350.1 respectively. MS (EI): $[M]^+$ 321.2, 278.1, 250.1, 173.1, 144.1, 91.1. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{24}N_3O]$, 322.1919; found 322.1942 and $[M + K]^+$ 360.2098.

2.6.4. 4-((2-Isopropyl-5-methylphenoxy)methyl)-1-(2-nitrobenzyl)-1H-1,2,3-triazole (4)

Compound **4**, 1.6 g (88%) was synthesized as pale yellowish solid.

IR (KBr, cm^{-1}): 3132.4, 3088.8, 2981.0, 2951.3, 2921.7, 2886.6, 2867.2, 1733.3, 1609.7, 1577.3, 1520.1, 1504.1, 1454.4, 1444.5 and 1251.9 ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.78 (s, 1H, triazole H); 7.64 (d, 1H, Ar–H); 7.63 (t, 1H, J = 6.7 Hz, Ar–H); 7.58 (t, 1H, J = 7.2 Hz, Ar–H), 7.29 (s, 1H, Ar–H); 7.12 (d, 2H, J = 7.5 Hz, Ar–H); 7.08 (d, 2H, J = 7.6 Hz, Ar–H); 6.80 (d, 1H, J = 7.3 Hz, Ar–H); 5.29 (s, 2H, O–CH_2); 5.99 (s, 2H, N–CH_2); 3.28 (m, 1H, CH); 2.34 (s, 3H, Ar–CH_3), 1.20 (d, 3H, J = 7.0 Hz, –CH_3); 1.19 (d, 3H, J = 7.0 Hz, –CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, 126 MHz): δ_C 155.21 (Ar–C), 147.40 (Ar–C), 136.49 (triazole Ar–C), 134.43 (Ar–C), 134.32 (Ar–C), 130.71 (Ar–C), 130.40 (Ar–C), 130.25 (Ar–C), 129.70 (Ar–C), 126.09 (Ar–C), 125.43 (Ar–C), 123.51 (Ar–C), 121.95 (triazole Ar–C), 112.88 (Ar–C), 62.48 (–CH_2), 50.97 (–CH_2), 26.62 (–CH), 22.97 (–CH_3), 21.36 (–CH_3), 21.36 (–CH_3). MS (CI): *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ 367.1 and 395.1 respectively. MS (EI): $[M]^+$ 366.2, 218.1, 189.0, 159.1, 136.1, 105.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{23}N_4O_3]$, 367.1770; found 367.1791 and $[M + K]^+$ 405.1907.

2.6.5. 4-((4-Chloro-2-isopropyl-5-methylphenoxy) methyl)-1-(3-chlorobenzyl)-1H-1,2,3 triazole (6)

Compound **6,** 0.56 g (55%) was synthesized as pale greenish waxy liquid.

IR (KBr, cm^{-1}): 3155.7, 3073.7, 2982.5, 2959.5, 2927.8, 2868.3, 1738.8, 1601.9, 1581.3, 1572.4, 1513.9, 1497.2, 1460.1, 1431.1 and 1248.2 ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.63 (s, 1H, triazole H); 7.33 (t, 1H, J = 7.9 Hz, Ar–H); 7.28 (d, 1H, J = 9.4 Hz Ar–H); 7.16 (d, 1H, J = 8.9 Hz, Ar–H); 6.84 (s, 1H, Ar–H); 5.55 (s, 2H, O–CH_2); 5.20 (s, 2H, N–CH_2); 3.23 (m, 1H, CH); 2.33 (s, 3H, Ar–CH_3). 1.17 (d, 3H, J = 6.6 Hz, –CH_3), 1.15 (d, 3H, J = 6.6 Hz, –CH_3); ^{13}C{^{1}H} NMR ($CDCl_3$, 500 MHz): δ_C 153.80 (Ar–C), 136.69 (triazole Ar–C), 136.50 (Ar–C), 135.03 (Ar–C), 133.80 (Ar–C), 133.80 (Ar–C), 130.46 (Ar–C), 129.03 (Ar–C), 128.03 (Ar–C), 126.75 (Ar–C), 126.35 (Ar–C), 126.04(Ar–C), 118.03 triazole (Ar–C), 114.66 (Ar–C), 62.72 (–CH_2), 53.62(–CH_2), 26.61 (–CH), 22.61 (–CH_3), 22.61 (–CH_3), 20.06 (–CH_3). MS (CI): *m/z* $[M]^+$, $[M + H]^+$, $[M + CH_3]^+$ and $[M + C_2H_5]^+$ 389.0, 390.0, 404.0 and 418.1 respectively. MS (EI): $[M]^+$ 389.1, 354.1, 318.0, 207.0, 178.0, 125.0, 91.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{22}Cl_2N_3O]$, 390.1140; found 390.1145 and $[M + K]^+$ 428.1304.

2.6.6. 4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1-(3-fluorobenzyl)-1H-1,2,3 triazole (7)

Compound **7,** 0.53 g (70%) was synthesized as pale brownish waxy liquid.

IR (KBr, cm^{-1}): 3117.1, 3075.5, 2967.7, 2924.9, 2872.2, 1729.3, 1603.4, 1590.2, 1513.3, 1489.5, 1451.0 and 1245.4 ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.63 (s, 1H, triazole H); 7.38 (d, 1H, J = 5.8 Hz, Ar–H); 7.34 (d, 1H, J = 6.2 Hz Ar–H); 7.29 (s, 1H, Ar–H); 7.06 (t, 1H, J = 8.0 Hz Ar–H) 7.00 (s, 1H, Ar–H); 6.95 (s, 1H, Ar–H); 5.67 (s, 2H, O–CH_2); 5.63 (s, 2H, N–CH_2); 3.26 (m, 1H, CH); 2.35 (s, 3H, Ar–CH_3). 1.17 (d, 3H, J = 6.6 Hz, –CH_3), 1.17 (d, 3H, J = 6.6 Hz, –CH_3); ^{13}C{^{1}H} NMR ($CDCl_3$, 500 MHz): δ_C 163.93 (Ar–C), 153.68 (Ar–C), 136.69 (triazole Ar–C; Ring), 133.85

(Ar–C), 130.84(Ar–C), 130.77 (Ar–C), 130.10 (Ar–C), 126.75 (Ar–C), 126.49 (Ar–C), 123.73 (Ar–C), 122.22 (triazole Ar–C), 116.12 (Ar–C), 114.78 (Ar–C), 113.78 (Ar–C), 64.60 (–CH_2), 54.80 (–CH_2), 26.60 (–CH), 22.72 (–CH_3), 22.63 (–CH_3), 20.33 (–CH_3). MS (CI): *m/z* $[M]^+$, $[M + H]^+$, $[M + CH_3]^+$ and $[M + C_2H_5]^+$ 373.0, 374.1, 388.0 and 402.1 respectively. MS (EI): $[M]^+$ 373.1, 356.1, 338.1, 302.1, 184.0, 162.1, 109.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{22}ClFN_3O]$, 374.1435; found 374.1472 and $[M + K]^+$ 412.1640.

2.6.7. 1-Benzyl-4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1H-1,2,3 triazole **(8)**

Compound **8,** 0.78 g (92%) was synthesized as pale yellowish semi-solid.

IR (KBr, cm^{-1}): 3124.4, 3076.5, 3041.6, 2962.2, 2926.6, 2875.0, 1762.8, 1603.4, 1560.4, 1495.8, 1457.4, 1438.5 and 1243.9 ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.56 (s, 1H, triazole H); 7.39 (t, 1H, $J = 3.5$ Hz, Ar–H); 7.37 (t, 1H, $J = 7.0$ Hz, Ar–H); 7.35 (d, 1H, $J = 1.4$ Hz, Ar–H); 7.28 (t, 1H, $J = 5.6$ Hz, Ar–H); 7.26 (d, 1H, $J = 1.5$ Hz, Ar–H); 7.13 (s, 1H, Ar–H); 6.84 (s, 1H, Ar–H); 5.54 (s, 2H, O–CH_2); 5.16 (s, 2H, N–CH_2); 3.21 (m, 1H, CH); 2.31 (s, 3H, Ar–CH_3). 1.15 (d, 3H, $J = 7.0$ Hz, –CH_3), 1.13 (d, 3H, $J = 7.0$ Hz, –CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 153.90(Ar–C), 136.73(triazole Ar–C), 134.65(Ar–C), 133.74(Ar–C), 133.74(Ar–C), 129.12(Ar–C), 129.12 (Ar–C), 128.77 (Ar–C), 128.77 (Ar–C), 126.68 (Ar–C), 126.24 (Ar–C), 122.67 (Ar–C), 126.67 (triazole Ar–C), 114.74 (Ar–C), 62.72 (–CH_2), 54.16 (–CH_2), 26.58 (–CH), 22.59 (–CH_3), 22.59 (–CH_3), 20.02 (–CH_3). MS (CI): *m/z* $[M]^+$, $[M + H]^+$, $[M + CH_3]^+$ and $[M + C_2H_5]^+$ 355.0, 356.1, 370.0 and 384.1 respectively. MS (EI): $[M]^+$ 355.1, 320.2, 284.1, 169.0, 144.1, 91.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{23}ClN_3O]$, 356.1530; found 356.1533 and $[M + K]^+$ 394.1688.

2.6.8. 4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1-(2-nitrobenzyl)-1H-1,2,3 triazole **(9)**

Compound **9,** 0.95 g (88%) was synthesized as white solid.

IR (KBr, cm^{-1}): 3152.8, 3068.7, 3037.4, 3011.8, 2980.9, 2960.2, 2918.5, 2864.2, 1615.5, 1607.2, 1568.0, 1535.5, 1501.3, 1491.3, 1463.7, 1436.5 and 1248.7 ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.73 (s, 1H, triazole H); 7.56 (d, 1H, $J = 6.8$ Hz, Ar–H); 7.49 (t, 1H, $J = 7.4$ Hz, Ar–H), 7.29 (s, 1H, Ar–H); 7.04 (s, 1H, Ar–H); 6.96 (t, 1H, $J = 7.7$ Hz, Ar–H); 6.80 (d, 1H, Ar–H); 5.89 (s, 2H, O–CH_2); 5.12 (s, 2H, N–CH_2); 3.14 (m, 1H, CH); 1.96 (s, 3H, Ar–CH_3), 1.08 (d, 3H, $J = 6.9$ Hz, –CH_3); 1.07 (d, 3H, $J = 6.9$ Hz, –CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 153.82(Ar–C), 147.42(Ar–C), 144.53 (triazole Ar–C), 136.69 (Ar–C), 134.35 (Ar–C), 130.71 (Ar–C), 133.64 (Ar–C), 130.62 (Ar–C), 130.14 (Ar–C), 129.67 (Ar–C), 126.56 (Ar–C), 126.06 (Ar–C), 125.29 (Ar–C), 123.96 (triazole Ar–C), 114.72 (Ar–C), 62.48 (–CH_2), 50.87 (–CH_2), 26.53 (–CH), 20.91 (–CH_3), 20.91 (–CH_3), 14.07 (–CH_3). MS (CI): *m/z* $[M]^+$, $[M + H]^+$, $[M + CH_3]^+$ and $[M + C_2H_5]^+$ 400.0, 401.0, 415.0 and 429.0 respectively. MS (EI): $[M]^+$ 400.1, 357.1, 329.1, 218.1, 189.0, 169.0, 136.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{20}H_{22}ClN_4O_3]$, 401.1380; found 401.1382 and $[M + K]^+$ 439.1548.

2.7. Synthesis of triazole compounds with two thymol groups

2.7.1. Synthesis of 2-isopropyl-5-methylphenoxy chloroethane from thymol

The sodium salt of thymol was prepared by dissolving thymol (1.00 g, 1.0 mmol) in 10% NaOH (10 ml) for 10 min with continuous stirring. DMSO (10 ml) was then added. The required stoichiometric amount of 1,2-dichloroethane (1.0 mmol) was added slowly while stirring. The reaction mixture was then refluxed for 5 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature and the oily product was extracted with dichloromethane (DCM) (3 × 10 ml). The combined DCM extract was transferred into a separatory funnel and washed with saturated, $NaHCO_3$ solution, (2 × 10 ml) and distilled water, (2 × 10 ml). The recovered DCM layer was dried over anhydrous sodium sulphate. This was then filtered and concentrated *in vacuo* to obtain the ether derivative of thymol. The crude product was purified by column chromatography (silica gel 100–200) mesh with hexane/ethyl acetate (21:1 v/v) as eluent in an increasing polarity of the solvent system to afford product. Similarly, 4-chloro-2-isopropyl-5-methylphenoxy chloroethane was prepared from chlorothymol using the same protocol as above.

2.7.2. Preparation of 2-isopropyl-5-methylphenoxyazidoethane from 2-isopropyl-5-methylphenoxy chloroethane

2-Isopropyl-5-methylphenoxychloroethane (0.5 g, 2.4 mmol) was dissolved in 10 ml DMSO. Sodium azide (0.25 g, 3.8 mmol) was added as solid and the reaction was stirred overnight at ambient temperature. Water (50 ml) was added and the reaction product extracted into diethyl ether (3 × 10ml). The combined ether layer was washed with brine (2 × 10 ml), dried over anhydrous sodium sulphate and the solvent removed *in vacuo* to afford a clear product. The product was further purified by column chromatography with silica gel of dimension 100–200 mesh and solvent gradient of hexane/ethyl acetate in an increasing polarity. The preparation of 4-chloro-2-isopropyl-5-methylphenoxyazidoethane was carried out following the above experimental procedure from the prepared 4-chloro-2-isopropyl-5-methylphenoxychloroethane.

2.7.3. Synthesis of 1-(2-(2-isopropyl-5-methylphenoxy)ethyl)-4-((2-isopropyl-5-methylphenoxy) methyl)-1H-1,2,3-triazole **(5)**

The terminal alkyne of thymol (0.10 g, 1.2 mmol) was added to a solution of 2-isopropyl-5-methylphenoxyazidoethane (0.05 g, 1 mmol) in *t*-BuOH: H_2O solvent system taken in 2:1 proportion. $CuSO_4.5H_2O$ (0.12 g, 2 mmol) and sodium ascorbate (0.10 g, 2 mmol) were added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 h s. The progress of reaction was monitored through TLC analysis. After completion, the reaction mixture was filtered and extracted with ethyl acetate (3 × 20 ml) and water (3 × 20 ml). The ethyl acetate layer was separated and the solvent was evaporated *in vacuo*. The crude product was subjected to column chromatography (silica gel 100–200 mesh, elution; hexane/ethyl acetate gradient) to afford compound **5,** 0.22g (65%) as yellowish viscous oily liquid.

IR (KBr, cm^{-1}): 3159.2, 3031.5, 2958.6, 2923.6, 2868.9, 1880.4, 1698.9, 1611.4, 1578.0, 1504.6, 1455.5, 1413.3 and 1252.6. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.80 (s, 1H, triazole-H); 7.29 (s, 1H, Ar–H); 7.13 (d, 1H, Ar–H); 7.11 (q, 2H, $J = 7.8$ Hz Ar–H); 6.81 (d, 1H, $J = 9.8$ Hz, Ar–H); 6.63 (s, 1H, Ar–H), 5.54 (s, 2H, O–CH_2); 5.32 (t, 2H, $J = 4.9$ Hz, –CH_2-O); 5.23 (t, 2H, $J = 5.0$ Hz, N–CH_2-); 3.30 (m, 1H, –CH); 3.20 (m, 1H, –CH); 2.34 (s, 3H, Ar–CH_3); 2.33 (s, 3H, Ar–CH_3); 1.20 (d, 6H, $J = 7.0$ Hz, –CH_3); 1.20 (d, 6H, $J = 7.0$ Hz, –CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 126 MHz): δ_C 155.33 (Ar–C), 154.80 (Ar–C), 144.97 (Ar–C; triazole ring), 136.59 (Ar–C), 136.50(Ar–C), 134.24 (Ar–C), 133.98 (Ar–C), 126.12 (Ar–C), 126.01 (Ar–C), 123.45 (Ar–C), 122.22(Ar–C; triazole ring), 121.82 (Ar–C), 112.71 (Ar–C), 112.30 (Ar–C), 66.49 (–CH_2) 62.48 (–CH_2), 53.47 (–CH_2), 26.52 (–CH), 26.48 (–CH), 22.81 (–CH_3), 22.74 (–CH_3), 21.36 (-CH_3), 21.36 (–CH_3), 21.29 (–CH_3), 21.29 (–CH_3). MS (CI): *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ 408.1 and 436.2 respectively. MS (EI): $[M]^+$ 407.3, 364.2, 336.2, 258.1, 230.1, 188.1, 163.1, 135.0. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{25}H_{34}N_3O_2]$, 408.2651; found 408.2653 and $[M + K]^+$ 446.2797. The above protocol for the synthesis of compound **5** was followed in the preparation of compound **10** using terminal alkyne of chlorothymol instead of thymol.

2.7.4. 4-((4-Chloro-2-isopropyl-5-methylphenoxy)methyl)-1-(2-(2-isopropyl-5methylphenoxy)ethyl)-1H-1,2,3-triazole **(10)**

Compound **10,** 0.25g (68%) was synthesized as yellowish viscous liquid.

IR (KBr, cm^{-1}): 3160.3, 3033.7, 2959.3, 2924.1, 2868.3, 1742.7, 1704.1, 1610.8, 1579.2, 1451.5 and 1245.1. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.80 (s, 1H, triazole-H); 7.29 (s, 1H, Ar–H); 7.11 (d, 1H, Ar–H); 6.81 (d, 1H, $J = 7.7$ Hz, Thymol-Ar-H); 6.63 (s, 1H, Ar–H); 5.32 (s, 1H, Ar–H); 5.21 (s, 2H, O–CH_2); 4.84 (t, 2H, $J = 4.9$ Hz, –CH_2-O); 4.40 (t, 2H, $J = 5.1$

Hz, N–CH_2-); 3.30 (m, 1H, –CH); 3.20 (m, 1H, –CH); 2.34 (s, 3H, Ar–CH_3); 2.33 (s, 3H, Ar–CH_3); 1.30 (d, 6H, J = 7.0 Hz, –CH_3); 1.20 (d, 6H, J = 7.3 Hz, –CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 500 MHz): δ_C 154.77(Ar–C), 153.89(Ar–C), 136.62 (triazole Ar–C), 133.94 (Ar–C), 133.94 (Ar–C), 133.79(Ar–C), 133.79 (Ar–C), 126.73(Ar–C), 126.27 (Ar–C), 126.15(Ar–C), 123.55 (triazole Ar–C), 122.27(Ar–C), 114.44 (Ar–C), 112.30 (Ar–C), 66.47(–CH_2) 62.71 (–CH_2), 53.45 (–CH_2), 26.58 (–CH), 26.48(–CH), 22.74 (-CH_3), 22.74 (—CH_3), 22.62 (–CH_3), 22.62 (–CH_3), 21.29 (–CH_3), 20.06 (–CH_3). MS (CI): m/z $[M]^+$, $[M + H]^+$, $[M + CH_3]^+$ and $[M + C_2H_5]^+$ 441.1, 442.1, 456.0 and 470.0 respectively. MS (EI): $[M]^+$ 441.2, 406.2, 370.2, 258.1, 230.1, 186.1, 135.1, 105.1. LC-ESI-QTOF-MS, m/z $[M + H]^+$ Calculated for $[C_{25}H_{33}ClN_3O_2]$, 442.2261; found 442.2267 and $[M + CH_3]^+$ 456.2060.

2.8. Antimicrobial assay

The Well diffusion method using Müeller-Hinton agar plates was used to demonstrate the antimicrobial properties of the synthesized thymol derivatives [31]. A suspension of the bacteria compared to 0.5 Macfarland standard was seeded on the Mueller-Hinton agar plates. Wells of 6 mm in diameter and 2 cm apart were punctured in the culture media using sterile cork borers. About 80 µl of thymol and the synthesized thymol derivatives at concentrations of 100, 50 and 10 µg/ml were administered to fullness in each well, the plates were incubated overnight at 37 °C. Growth was determined by measuring the diameter of the zone of inhibition. The solvent used for the dissolution, 5% DMSO served as the negative control, while 10 µg ampicillin disc (Oxoid) was used as the positive control. The experiments were carried out in triplicates and results were calculated as mean ± SD.

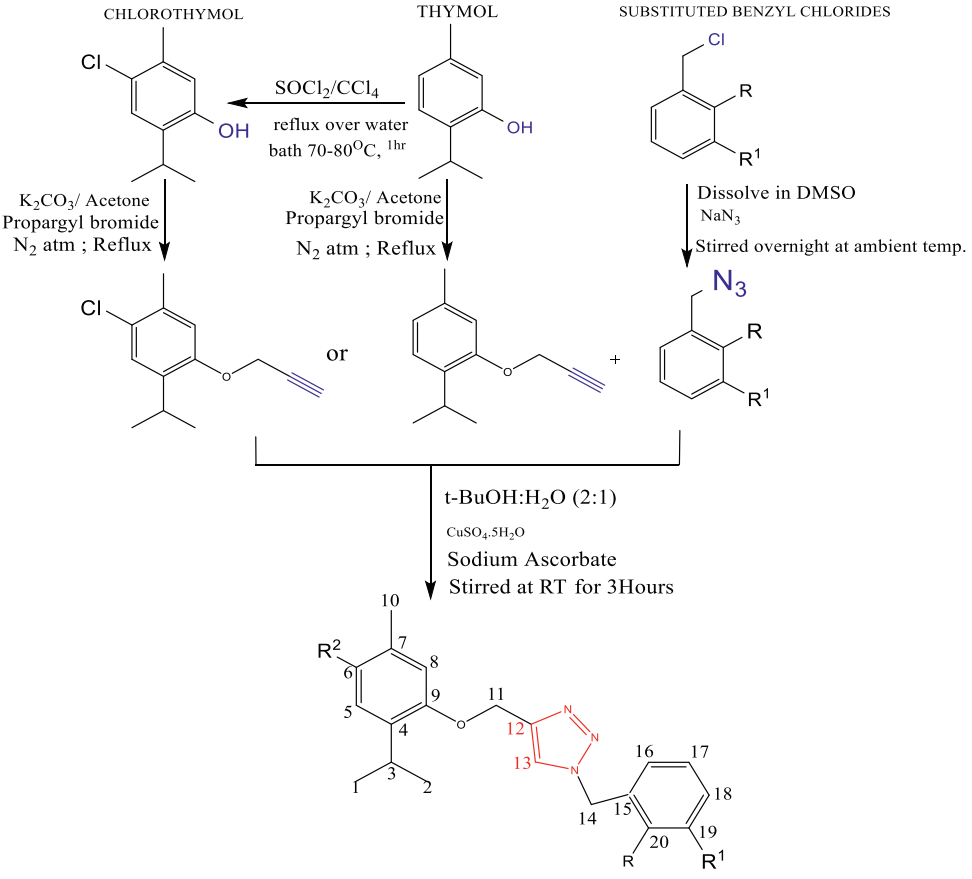
2.9. Statistical analysis

Microsoft excel (2016) was used for data entry and the graph creation. Data was then exported to GraphPad Prism (Version 8) for further analysis. The measured diameter of inhibition for each microorganism was analysed using a two-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Test. A p-value < 0.05 was considered significant.

3. Results and discussion

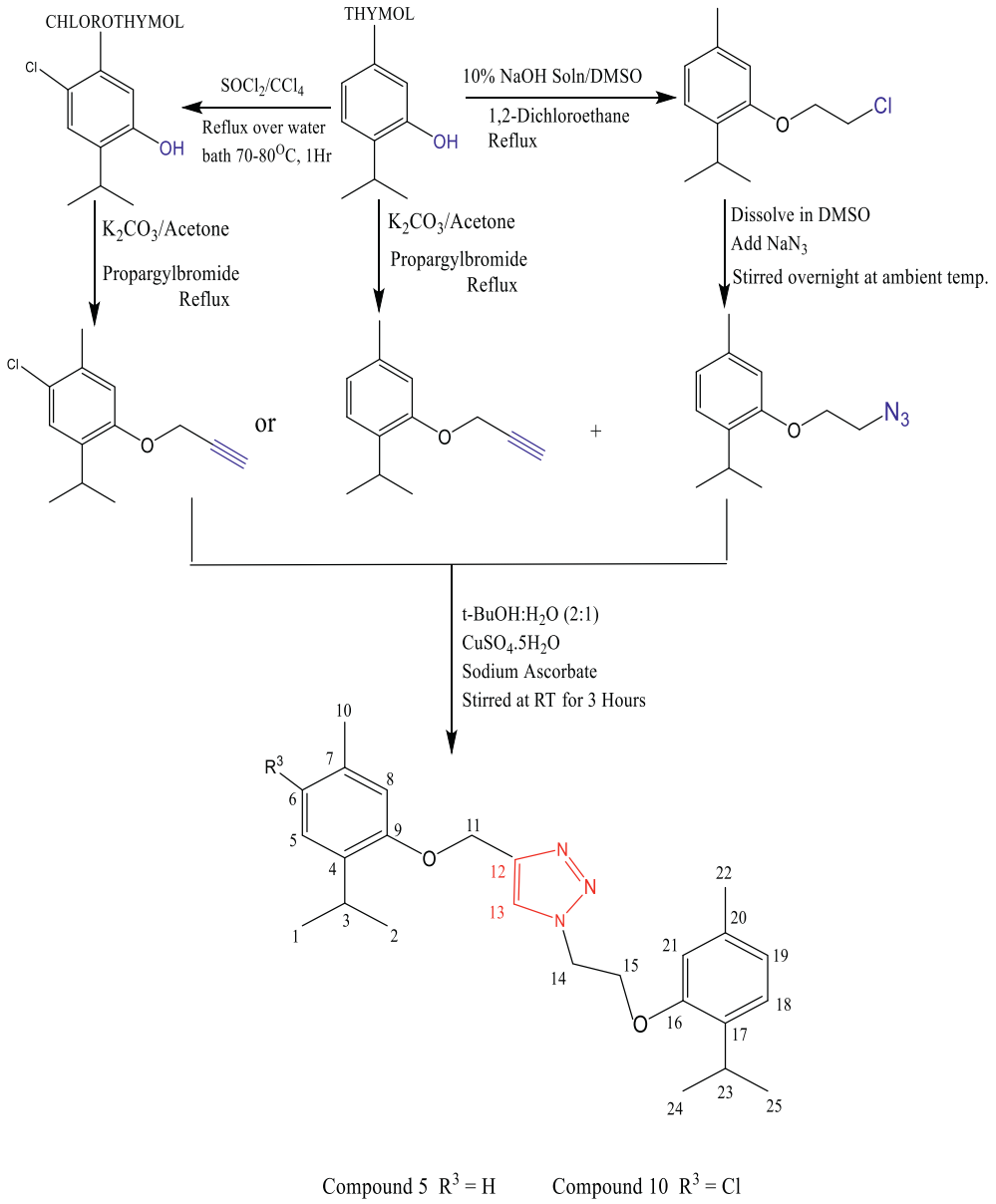
3.1. Chemistry

Compounds **1–10** were synthesized following a very efficient and straightforward synthetic route outlined in Schemes 1 and 2. The chloro-substituted ether derivative of thymol was obtained by reaction of thymol with 1,2-dichloroethane in the presence of K_2CO_3 in acetone. Thymol was chlorinated to Chlorothymol using $SOCl_2$ in CCl_4 and refluxed over water bath for an hour. The *O*-alkylation reaction of thymol and chlorothymol were effectively done in the presence of K_2CO_3 and propargyl bromide in acetone at low temperature to obtain the O-propargyl terminal alkyne intermediate of thymol and chlorothymol. The substituted benzyl azide intermediates and an azido ether derivative of



Compound 1 R = H ; R^1 = Cl ; R^2= H
Compound 2 R = H ; R^1 = F ; R^2= H
Compound 3 R = H ; R^1 = H ; R^2= H
Compound 4 R = NO_2 ; R^1 = H ; R^2= H
Compound 6 R = H ; R^1 = Cl ; R^2= Cl
Compound 7 R = H ; R^1 = F ; R^2= Cl
Compound 8 R = H ; R^1 = H ; R^2= Cl
Compound 9 R = NO_2 ; R^1 = H ; R^2= Cl

Scheme 1. Synthesis of 1-substituted 1,2,3-triazole derivatives of thymol moiety.



Scheme 2. Synthesis of 1-substituted 1,2,3-triazole derivatives with two thymol groups.

thymol were prepared from various substituted benzyl chlorides and the synthesized chloro-substituted ether derivative of thymol and chlorothymol. The target compounds were achieved by using a click reaction [32] whereby the O-propargyl terminal alkyne of thymol and chlorothymol were coupled with the various substituted benzyl azides as well as the azido ether derivative of thymol and chlorothymol. The synthesized products were characterized based on analysis of their FTIR, GC-MS-EI/CI, LC-ESI-QTOF/MS, ^{1}H-NMR and ^{13}C-NMR data.

The FTIR spectrum of compound **1** showed absorption bands at 3158.2 and 3085.2 cm^{-1} (Ar–H), 2957.7 and 2927.7 cm^{-1} (C–H stretching in alkyl region), 2885.5 and 2867.0 cm^{-1} (–CH_2 and –CH_3), 1885.6 cm^{-1} (–N=N–) and 1241.9 cm^{-1} (–C–O–). The absence of the representative absorption peak for azido group at 2100 cm^{-1} confirmed the formation of the triazole moiety. The ^{1}H-NMR spectrum in chloroform-*d* of compound **1** showed characteristic signals at 6.80–7.40 ppm which were assigned to the aromatic protons with δ_H 6.80 (1H, d, J = 7.5Hz, 6-H), δ_H 7.13 (1H, d, J = 7.9Hz, 5-H), δ_H 7.20 (1H, d, J = 6.8Hz, 16-H), δ_H 7.30 (1H, d, J = 1.6Hz, 18-H), δ_H 7.33 (1H, d, J = 7.2Hz, 17-H), δ_H 7.35 (1H, s, 8-H), and δ_H 7.40 (1H, d, J = 1.5Hz, 20-H). There are two methylene proton signals in the alkyl region at δ_H 5.55 (2H, s, H-11) and δ_H 5.23(2H, s, H-14). Again, two methyl protons on the isopropyl substituent appeared at δ_H 1.19 (3H, d, J = 6.9Hz, 1-H) and δ_H 1.20 (3H, d, J = 6.9Hz, 2-H), whilst an aromatic methyl proton was observed at δ_H 2.34 (3H, s, 10-H) in the alkyl region. A characteristic triazolyl singlet proton was observed at δ_H 7.67 (1H, s, 13-H), indicative of the formation of the triazole moiety. The ^{13}C-NMR spectrum of compound **1** exhibited a total of 20 carbons with characteristic peaks at δ_C 112.94–δ_C 155.26 which were assigned to the aromatic carbon atoms. The methyl carbons were observed at δ_C 21.36 (t) and δ_C 22.81(t) as well as the methine carbon of the isopropyl group was at δ_C 26.57 (d). The peaks at δ_C 121.95 (d) and δ_C 136.59 (d) were assigned to the two carbon atoms of the triazole moiety. The peaks at δ_C 53.61(d) and δ_C 62.52(d) were assigned to the two methylene carbon atoms. The ^{1}H-NMR and ^{13}C-NMR spectra signals listed are in agreement with the proposed structure of compound **1** and the same explanation given to support the proposed structures of compounds **2, 3, 4, 6,7, 8 and 9**. The mass spectrum (GC-MS-EI) of compound **1** which led to a confirmation of the structure gave the molecular ion $[M]^+$ peak at m/z 355 and a corresponding base peak observed at m/z 125. A characteristic tropylium ion peak was observed at m/z 91. The other prominent mass

fragments for this compound are 312, 284, and 178.0 as accounted for in the fragmentation pattern of the compound (supporting information). The mass spectrum (CI) gave the *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ as 356 and 384 respectively. The LC-ESI-QTOF-MS, m/z $[M + H]^+$ calculated for $[C_{20}H_{23}ClN_3O]$, was 356.1530; found 356.1565. Also, there was a potassium adducts *m/z* $[M + K]^+$ 394.1696 and a characteristic dimeric peak of the $[M + K]^+$ ion at 788.1669. Similarly, the structural elucidation of the other synthesized compounds **2, 3, 4, 6,7, 8 and 9** were determined. The infrared spectrum of compound **5** revealed all the major functional groups expected including a weak absorption at 3159.2 and 3031.5 cm^{-1} (Ar–H), 2958.6 and 2923.6 cm^{-1} (C–H stretching in alkyl region), 2868.9 cm^{-1} (–CH_2 and –CH_3), 1880.4 and 1698.9 cm^{-1} (–N=N–) and 1252.6 cm^{-1} (–C–O–). The absence of the representative absorption peak for the azido group at 2100.0 cm^{-1} confirmed the formation of the triazole moiety in the compound. The ^{1}H-NMR spectrum in chloroform-*d* of compound **5** indicated the presence of fifteen major signals. A characteristic triazolyl proton signal was observed at δ_H 7.80 (1H, s, H-13). The signals at δ_H 3.30 (1H, m, H-3) and δ_H 3.20 (1H, m, H-23) for compound **5** included a multiplet, indicative of the two methine protons of the isopropyl groups with two adjacent methyl protons. Two signals appearing at δ_H 2.33 (3H, s, H-22) and δ_H 2.34 (3H, s, H-10) in the alkyl region are indicative of protons on the methyl groups attached to the aromatic nuclei. Three other signals were seen at δ_H 5.54 (2H, s, H-11), δ_H 5.32 (2H, t, $J = 4.9$Hz, H-15) and δ_H 5.23 (2H, t, $J = 5.0$Hz, H-14) in the alkyl region which represents the three methylene protons in the compound. Six signals were observed in the aromatic region which were characterized by four doublet signals at δ_H 6.81 (1H, d, $J = 9.8$Hz, H-19), δ_H 7.13 (1H, d, $J = 9.8$Hz, H-5), δ_H 7.11 (1H, dd, $J = 7.8$Hz, H-18) and δ_H 7.11 (1H, dd, $J = 7.8$Hz, H-6) as well as two singlet signals at δ_H 6.63 (1H, s, H-21) and δ_H 7.29 (1H, s, H-8). The ^{13}C-NMR revealed a total of twenty-five carbon environments for the compound. The alkyl region showed the isopropyl methyl carbons at δ_C 21.29(t) and δ_C 21.36(t). The methyl carbons bonded to the aromatic ring were observed at δ_C 22.74(t) and δ_C 22.81(t). The methine carbons of the two isopropyl groups were at δ_C 26.48(d) and δ_C 26.52(d). The methylene carbons closer to the oxygen of the ether linkage were both observed further downfield at δ_C 62.48(d) and δ_C 66.49(d) with the third methylene carbon further away from the oxygen observed at δ_C 53.47(d). The triazole ring carbons were observed at δ_C 144.97(d) and δ_C 123.45(d). The mass spectrum (GC-MS-EI) of the compound which led to a confirmation of the structure gave the molecular ion $[M]^+$ peak at m/z 407 and a corresponding base peak was observed at m/z 135.0. The other prominent mass fragments for the compound are 364, 336, 258, 230 and 163 as accounted for in the fragmentation pattern of the compounds (supporting information). The mass spectrum (CI) gave the *m/z* $[M + H]^+$ and $[M + C_2H_5]^+$ as 408 and 436 respectively. The LC-ESI-QTOF-MS, m/z $[M + H]^+$ calculated for $[C_{25}H_{34}N_3O_2]$, 408.2651; found 408.2653. There was a potassium adduct $[M + K]^+$ 446.2797. Similarly, the structure of compound **10** was elucidated.

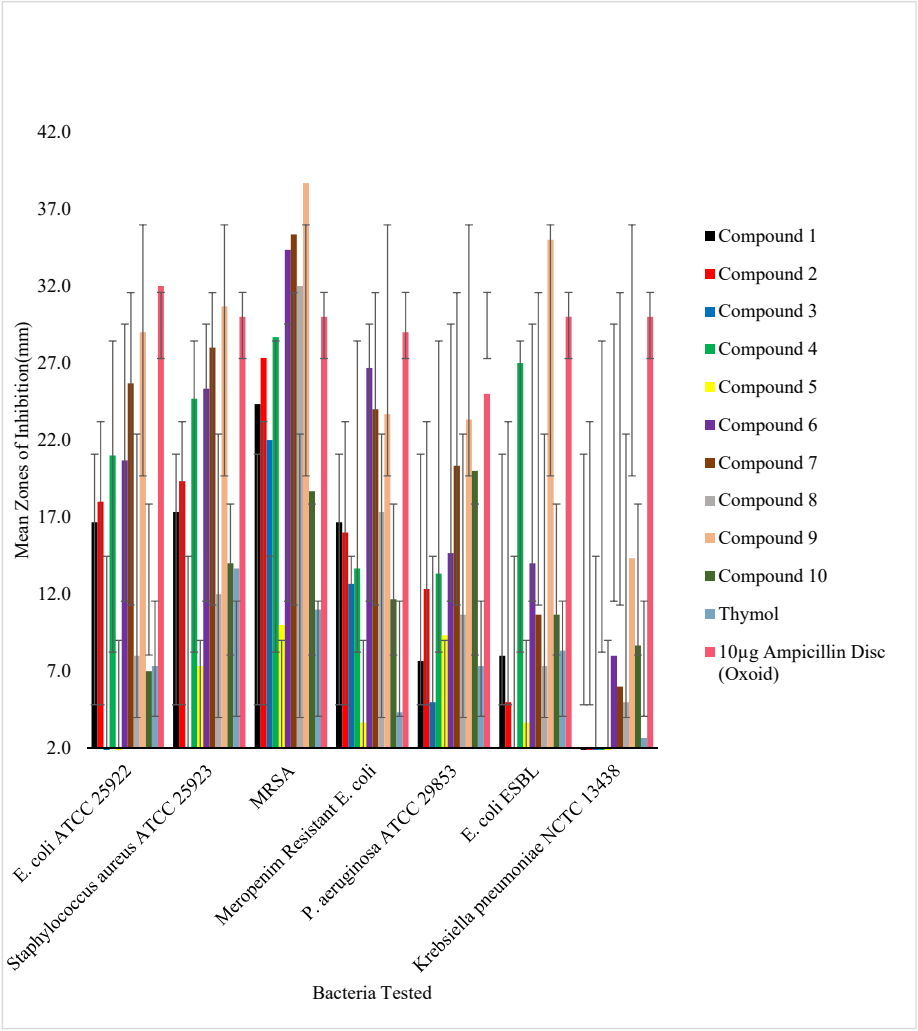


Figure 1. Mean zones of inhibition (mm) of compounds **1–10** (100 µg/ml) against tested bacteria.

3.2. Antimicrobial activity

The antimicrobial activities of the ten triazole-thymol derivatives, thymol as the parent compound and a standard drug 10 μg Ampicillin Disc (OXOID) are shown in Figures 1, 2 and 3 at concentrations of 100, 50 and 10 μg/ml respectively. This is summarized in Table S4 (supporting information). The ten compounds showed variable antibacterial activity against the seven bacterial strains tested; *E. coli* ATCC 25922, *S. aureus* ATCC25923, *Methicillin resistant S. aureus* (MRSA), *P. aeruginosa* ATCC 29853, *E. coli* ESBL, *Klebsiella pneumoniae* NCTC 13438 and *Meropenem Resistant E. coli*. Of the bacterial strains tested, *K. pneumoniae* NCTC 13438 was only susceptible to the derivatives with chlorinated thymol nucleus (compounds **6, 7, 8, 9** and **10**) showing mean zone of inhibitions 8.0, 5.0, 5.0, 14.3 and 8.7 mm respectively, thymol (2.7 mm) and the standard drug 10 μg Ampicillin Disc (OXOID) (30 mm) at a concentration of 100 μg/ml. On the contrary, this bacteria strain was resistant to the triazole-thymol derivatives (compounds **1**, **2**, **3**, **4** and **5**). Thus, this activity could be attributed to the substituted chlorine atom on position-4 of the thymol nucleus. The compound 4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1-(2-nitrobenzyl)-1*H*-1,2,3 triazole **(9)** exhibited the highest mean zone of inhibition of 38.7 mm and 24.7 mm at concentrations of 100 μg/ml and 10 μg/ml respectively compared to 30.0 mm for the standard drug 10 μg Ampicillin Disc (OXOID) against *Methicillin resistant S. aureus (MRSA)*. Thus, compound **9** can be compared favourably to the standard drug (ampicillin) in the management of infections caused by staphylococci and other bacterial strains tested. Again, the antibacterial activity of compound **9** at concentrations 100 μg/ml and 10 μg/ml was approximately three-fold and fifteen-fold more potent than the parent compound, thymol with mean zones of inhibition 11.0 mm and 1.7 mm respectively. Compound1-(2-(2-isopropyl-5-methylphenoxy)ethyl)-4-((2-isopropyl-5-methylphenoxy)methyl)-1H-1,2,3-triazole **(5)** recorded the least inhibition activity of 10.0 mm at a concentration of 100 μg/mL against *Methicillin resistant S. aureus (MRSA)*. The antibacterial activity of compounds 4-((2-isopropyl-5-methylphenoxy) methyl)-1-(2-nitrobenzyl)-1H-1,2,3 triazole **(4)** (28.7 mm), 4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1-(3-chlorobenzyl)-1*H*-1,2,3 triazole **(6)** (34.3 mm), 4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1-(3-fluorobenzyl)-1*H*-1,2,3 triazole **(7)** (35.3 mm) and 1-benzyl-4-((4-chloro-2-isopropyl-5-methylphenoxy) methyl)-1*H*-1,2,3 triazole **(8)** (32.0 mm) relatively showed the same or a superior activity compared to the standard drug 10 μg Ampicillin Disc (OXOID) (30.0 mm) against *Methicillin resistant S. aureus (MRSA)* at a concentration of 100 μg/ml. Generally, the varied antibacterial activity of the synthesized triazole-thymol derivatives **1–10,** present them as potential antimicrobial agents to be used against multidrug resistant bacteria. The varied bioactivity among the triazole derivatives of thymol could be attributed to the introduction of the triazole functional group and the different substitution of chlorine (–Cl), fluorine (–F) and nitro ($–NO_2$) groups on the aromatic nucleus of compounds **1, 2 and 4**. The substitution of chlorine on the carbon-4 positions of the thymol nucleus played vital role that afforded the superior activity of compounds **6,7, 8, 9** and **10** over the other compounds and the parent compound, thymol. The

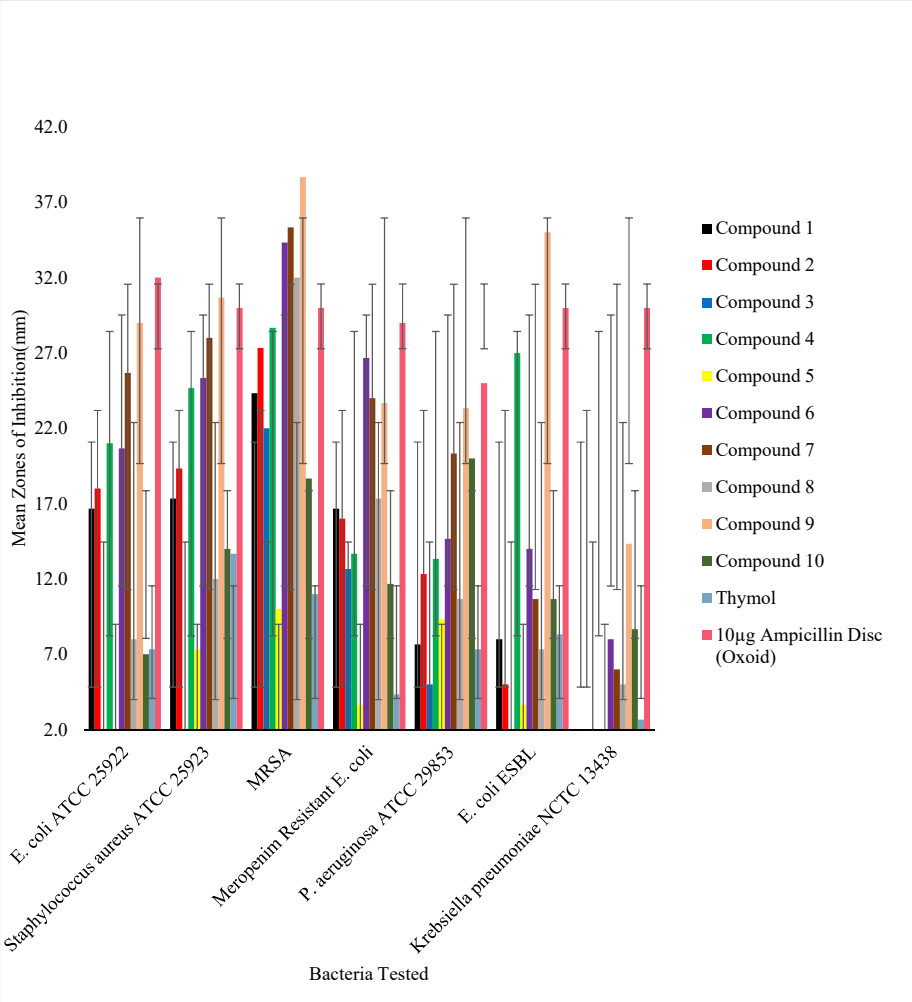


Figure 2. Mean zones of inhibition (mm) of compounds **1–10** (50 μg/ml) against tested bacteria.

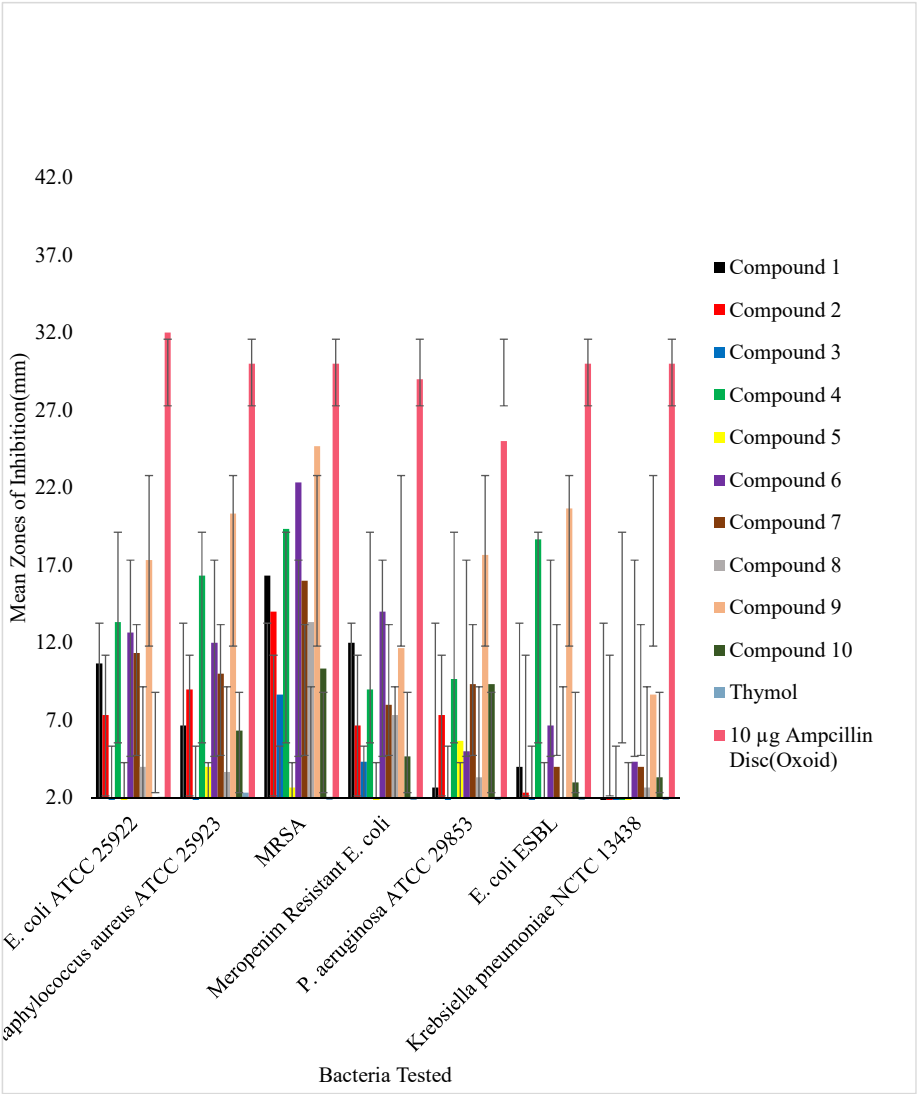


Figure 3. Mean zones of inhibition (mm) of compounds **1–10** (10 µg/ml) against tested bacteria.

hydroxyl moiety on thymol also did confer an activity as thymol showed a mean zone of inhibition against all the bacteria with mean zone of inhibition ranging between 13.7 and 2.7 mm which confirms earlier results that [15], the hydroxyl group of thymol is indispensable for its antimicrobial activity.

4. Conclusion

In summary, ten triazole compounds containing thymol moieties have been synthesized by chemical methods. These compounds were obtained in moderate to high yield and of high purity. We recommend the exploration of the use of potassium iodide which might be fast and provide better yield in future reactions. The structures of the synthesized derivatives were confirmed by infrared, ^{1}H- and ^{13}C-NMR and mass spectrometric analysis. The antimicrobial activity shows that the triazole moiety incorporated into the thymol nucleus as well as the substitution of chlorine (–Cl) on the thymol nucleus and substitution of chlorine (–Cl), fluorine (–F) and nitro (–NO_2) groups on the aromatic nucleus of some of the synthesized compounds significantly contributed to the broad-spectrum antimicrobial activity compared to the parent compound, thymol. These results provide additional evidence of the exploitation of natural products as leads for drug development against medically important bacterial pathogens.

Declarations

Author contribution statement

Justice Kwaku Addo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ernest Owusu-Ansah: Conceived and designed the experiments; Contributed reagents, materials and analysis tools.

Nicholas T.K.D. Dayie: Performed the experiments; Analyzed and interpreted the data.

Xavier Cheseto: Analyzed and interpreted the data; Wrote the paper.

Baldwyn Torto: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials and analysis tools; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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