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Discovery of oxazoline-triazole based hybrid molecules as DNA gyrase inhibitors: A new class of potential Anti-tubercular agents

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ABSTRACT

A library of novel oxazoline-triazole hybrid analogues (**6a-6 g** and **7a-7 m**)was designed using a molecular hybridization approach and synthesized from commercially available ethyl 2/3/4-hydroxybenzoate. The synthesized compounds were characterized by modern art instrumentation, including IR and NMR (^{1}H, ^{13}C). All the final compounds were evaluated for their *in-vitro* antibacterial (*S. aureus, B. subtilis, E. coli* and *P. aeruginosa*), antifungal (*C. neoformans, C. albicans* and *A. niger*) and anti-tubercular (*Mycobacterium tuberculosis* H_{37}Rv, MDR and XDR strains) activities. Among the series, compound **7a-7i** exhibited excellent activity (**MIC** = 1.6 µM) against H_{37}Rv strain of *M.tuberculosis*. However, antibacterial screening data (in vitro) revealed a moderate inhibition for **6e-6 g** and **7f-7 h** against gram-positive bacteria (*Bacillus subtilis*) and **7a-7i** against gram-negative bacteria with a **MIC** value of 25 µg/ml. While moderate activity was observed against fungal (*C. neoformns* and *C. albicans*) strains with **MIC** value of 25–200 µg/mL. Additionally, five compounds (**7a, 7d-7f** and **7 h**) were further evaluated for their in vitro inhibitory activity against *E-coli* DNA gyrase. These compounds displayed significant inhibitory activity against the DNA gyrase enzyme with an $\mathbf{IC_{50}}$ value of 0.08 – 0.5 µM.

1. Introduction

The research on antimicrobial agents is a continuing process as there are many reasons like prolonged and excessive use of antibiotics resulting in drug resistance. Since the early stages of childhood, antibiotics have often been used to develop new strains of microorganisms having resistance to the antibiotic used. Therefore, continuous research would help for the development of better and more effective antimicrobial drug molecules [1,2].

Tuberculosis is an air-born contagious disease caused by mycobacterium tuberculosis (*Mtb*). In 2012, World Health Organization (WHO) reported 8.6 million infections, and among them, 1.3 million people died because of infectious diseases, and in 2016, 490,000 new cases of multidrug resistance were widely estimated. There is a growing resistance to existing drugs resulting from deadly diseases that become more deadly and difficult to treat. MDR and extensive drug resistance (XDR) *Mtb* are diseases caused by bacteria that don't respond to first-line anti-tubercular drugs. Existing treatment consists of various drugs that need to be taken for more than a year, resulting in numerous side effects and a substantial economic burden. In developing countries, pollution is the primary concern as *Mtb* can be gained through the air. In recent years the death rate was declined, but it is still a significant cause of death after AIDS. Streptomycin, Isoniazid, Ethambutol, Rifampicin, Ethionamide, Cycloserine and Kanamycin, etc., are the drugs used for the treatment. Most of these drugs have been discovered and used for the last 70 years. Hence, there is an alarming concern about the drug-resistant strains of *Mtb* [3–8]. A constant research is underway for understanding the reasons behind the evolution and existence of resistant strains of *Mtb*. Synthesis and high-throughput screenings of different derivatives with a broad spectrum of novel and known scaffolds have been carried out to obtain lead derivatives as anti-TB [9–11].

Fused oxazole and oxazoline were widely distributed in nature, and these attracted much attention due to their diverse pharmaceutical activities. These scaffolds consist of nitrogen and oxygen atoms in an aromatic five-membered ring. These heteroatoms bind with different receptors and enzymes in the body mechanism's biological system through non-covalent interactions. The structure

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shows weak interactions like H-bond, ion-dipole, π-π stacking, and weak hydrophobic character is some of the advantages of oxazole ring in medicinal chemistry.

Oxazole and its derivatives mainly show applications in medicinal chemistry, agrochemicals, supramolecular chemistry, and material science. In medicinal chemistry, the oxazoline building block is primarily used as anti-microbial, poliovirus inhibitors, anti-viral, anti-tubercular, anticancer, anti-inflammatory, anti-obesity, anti-neuropathic, anti-hepatitis virus, and anti-oxidative [12–18]. Oxazolines are not only the fundamental component of many bioactive compounds but are also related to crop protection [19]. Oxazolines showed their versatile nature as they found their applications in material chemistry as 2-oxazolone, which can create sophisticated functional polymers as different chains and functional groups are easily introduced at the initiation or termination ends [20]. On the other hand, triazole is a class of nitrogen heterocyclic compounds showing diversified biological activities like antimicrobial, antiviral, antimalarial and anticancer [21,22].

Oxazoline and triazole derivatives show promising biological activities, considered essential building blocks in medicinal chemistry. These two heterocyclics have been widely studied in the past decade for their diversified activities. As drug resistance is rapidly increasing, there is a constant need to develop new and efficient entities to solve these emerging problems by properly designing, synthesizing, and testing them for biological activities to develop new drug candidates. The diversified activities of several groups in individual or combination encouraged us to design and synthesize these derivatives to get more effective drug molecules. The designed derivatives were tested for their pharmacokinetic properties like absorption, distribution, metabolism and excretion, and toxicity (ADMET), as these play an essential role in the early stages of drug discovery.

2. Experimental section

2.1. Materials and methods

The analytical grade (AR) chemicals and reagents were obtained from Merck and Sigma-Aldrich and used without further purification. The progress of the reactions and the purity of the synthesized compounds were monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel 60 F254 (mesh) (E. Merck), and spots were visualized under UV light (long and short wavelength). Merck silica gel (60–120 mesh) was used for column chromatography. Melting points of all the synthesized compounds were recorded in open capillaries using Thermo Fisher Scientific (IA9000, UK) digital melting point apparatus and are uncorrected. Bruker Alpha FT-IR spectrophotometer (Billerica, MA, USA) with a universal ATR sampling accessory was used to record all FT-IR spectra. ^{1}H and ^{13}C NMR spectra of all the synthesized compounds were recorded on Bruker Avance IV NMR spectrometer at 400 and 101 MHz, using $CDCl_3$ and DMSO–d_6, respectively, with TMS as an internal standard, and signals were reported in parts per million (ppm).

2.2. Synthesis of drug molecules

2.2.1. Step (i) (General procedure for the synthesis of 2a-2c)

To the solution of compound **1a-1c** (1 eq.) in DMF at 0 °C followed by K_2CO_3 (2 eq.) and stirred for 30 min. at room temperature. Then added Proggyl bromide (1.5 eq.) dropwise and heated the reaction mixture at 100 °C for two h. The progress of the reaction was monitored by TLC for the consumption of starting material. The reaction mixture was cool to room temperature and poured in ice-cold water, stirred for 10 min, and extracted with EtOAc. The organic layer was separated and washed with cold brine solution. The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford title crude compounds (**2a-2c**). The crude compounds were used for the following reaction without further purification.

Ethyl 2-(prop–2-yn-1-yloxy)benzoate (**2a**) The reaction was done on 2 g to obtain a pale yellow liquid with a 90% yield.

Ethyl 3-(prop–2-yn-1-yloxy)benzoate (**2b**) The reaction was done on 2 g to obtain pale yellow liquid with 84% yield.

Ethyl 4-(prop-2-yn-1-yloxy)benzoate (**2c**) The reaction was done on 2 g to obtain an off-white solid with 85% yield.

2.2.2. Step (ii) (General procedure for the synthesis of 3a-3c)

To the solution of compounds **2a-2c** (1 eq.) in THF and water (1:1) was added NaOH (2 eq.) and heated the reaction mixture at 100 °C for 2 h. The progress of the reaction was monitored by TLC for the consumption of starting material. The reaction mixture was cool to room temperature, added water, and extracted with ethyl acetate. The aqueous layer was acidified by using 2 N aq. HCl a solid precipitated out from the aqueous layer. The precipitate was filtered and washed with water to obtain the desired compounds **3a-3c** as off-white solids. The crude compounds **3a-3c** were used for the following reaction without further purification.

2-(prop–2-yn-1-yloxy)benzoic acid (3a) The reaction was done on 2 g to obtain a pale yellow solid with a 91% yield.

3-(prop–2-yn-1-yloxy)benzoic acid (3b) The reaction was done on 2 g to obtain an off-white solid with 86% yield.

4-(prop–2-yn-1-yloxy)benzoic acid (3c) The reaction was done on 2 g to obtain a pale yellow solid with 89% yield.

2.2.3. Step (iii) (General procedure for the synthesis of 4a-4c)

To a stirred solution of compounds **3a-3c** (1 eq.) in DCM cooled to 0 °C and added $SOCl_2$ dropwise to the solution and heated at 50 °C for 30 min. The progress of the reaction was monitored by TLC for the consumption of starting material. On consumption of starting material, the reaction mixture was cool to room temperature, and the solvent was removed under reduced pressure with the anhydrous condition. In another RBF, 2-chloroethylamine hydrochloride (1.5 eq.) was dissolved in DCM add TEA (3 eq.) followed by acid chloride crude in amine solution at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The progress of the reaction was monitored by TLC for the completion of starting material. The reaction mixture was poured into cold water on completion of starting material, stirred for 5 min, and extracted with DCM. The organic layer was washed with cold brine solution. The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford desired compounds **4a-4c** as off-white solids. The crude compound **4a-4c was** used for the next reaction without further purification.

N-(2-chloroethyl)–4-(prop–2-yn-1-yloxy)benzamide (**4a**) The reaction was done on 1 g to obtain an off-white solid with an 82% yield.

N-(3-chloroethyl)–4-(prop–2-yn-1-yloxy)benzamide (**4b**) The reaction was done on 1 g to obtain a white solid with an 80% yield.

N-(4-chloroethyl)–4-(prop–2-yn-1-yloxy)benzamide (**4c**) The reaction was done on 1 g to obtain an off-white solid with an 89% yield.

2.2.4. Step (iv) (General Procedure for the synthesis of 5a-c): To a stirred solution of compound **4a-4c** (1 eq.) in DMF was added $NaHCO_3$ (3 eq.) and stirred to the reaction mixture at 80 °C for 4 h. The reaction progress was monitored by TLC for the consumption of starting material. The reaction mixture was cool to room temperature, then poured into ice-cold water and extracted with EtOAc to separate the organic layer from the aqueous layer. Then the organic layer was washed with cold brine solution. The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent removed under reduced pressure to afford desired compounds **5a-**

5c as brown solids. The crude compounds **5a-5c** were used for the next reaction without further purification.

2-(2-(prop–2-yn-1-yloxy)phenyl)–4,5-dihydrooxazole (5a) The reaction was done on 0.5 g to obtain a faint yellow solid with 78% yield.

2-(3-(prop–2-yn-1-yloxy)phenyl)–4,5-dihydrooxazole (5b) The reaction was done on 0.5 g to obtain brown solid with 73% yield.

2-(4-(prop-2-yn-1-yloxy)phenyl)–4,5-dihydrooxazole (5c) The reaction was done on 0.5 g to obtain an off-white solid with 82% yield.

2.2.5. Step (v) (General Procedure for the synthesis of 6a-g and 7a-m): To a stirred solution of compounds **5a-c** (1 eq.) in BuOH (8 Vol.) and water (2 Vol.) was added sodium ascorbic acid (0.4 eq.) and CuSO4 (0.2 eq.) at 0 °C and stirred to the reaction mixture for 10 min. Then add substituted azide compounds (2 eq.) and continue stirring the reaction mixture at room temperature for 14 h. Solid precipitated out in the reaction mixture. Progress of the reaction was monitored by TLC for the consumption of starting material. After completion of starting material, the solvent was evaporated under reduced pressure to obtain crude adhesive material. The adhesive material was diluted by using cold water and extracted with EtOAc to separated the organic layer. The organic layer was washed with cold water brine solution, then dried over anhydrous sodium sulfate and evaporated under reduced pressure to obtain desired compounds **6a-g** and **7a-m**.

2-(4-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6a):

Brown Solid, yield- 67%, m.p.120–123 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.05 (t, J = 8.91 Hz, 3H), 7.75–7.72 (m, 2H), 7.53 (t, J = J8.00 Hz, 2H), 7.48–7.43 (m, 1H), 7.06 (d, J = 8.81 Hz, 2H), 5.36 (s, 2H), 4.59 (t, J = 6.01 Hz, 2H), 3.62 (t, J = J6.05 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.66, 162.14, 144.20, 136.89, 131.93, 129.02, 122.68, 121.13, 120.64, 114.53, 64.02, 62.06, 28.96; IR(υ, cm^{-1}): 3147, 2878, 1710, 1452, 1171, 762, 422.

2-(4-((1-(3-(trifluoromethyl)phenyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6b):

White solid, yield- 70%, m.p.119–123 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.14 (s, 1H), 8.08–7.96 (m, 4H), 7.75–7.66 (m, 2H), 7.06 (d, J = 8.70 Hz, 2H), 5.37 (s, 2H), 4.60 (t, J = 6.35 Hz, 2H), 3.63 (t, J = 6.08 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.64, 162.14, 137.24, 132.72, 132.39, 131.96, 130.66, 125.66, 124.63, 123.67, 122.82, 121.91, 117.56, 114.50, 64.02, 61.98, 28.94; IR(υ, cm^{-1}): 3124, 2953, 1714, 1486, 1120, 903, 465.

2-(4-((1-(4–chloro-3-(trifluoromethyl)phenyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6c):

White Solid, yield- 80%, m.p.132–134 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.11 (s, 1H), 8.10 (d, J = 2.45 Hz, 1H), 8.07–8.03 (m, 2H), 7.91 (dd, J = 2.52 Hz, 1H), 7.70 (d, J = 8.63 Hz, 1H), 7.06 (d, J = 8.92 Hz, 2H), 5.37 (s, 2H), 4.60 (t, J = 6.35 Hz, 2H), 3.63 (t, J = 6.08 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.61, 161.94, 135.35, 133.12, 132.79, 131.97, 130.30, 129.98, 124.40, 123.40, 122.89, 120.86, 120.68, 119.70, 119.65, 114.48, 64.06, 61.92, 28.94; IR(υ, cm^{-1}): 3086, 2923, 1708, 1442, 1114, 916, 470. HRMS (ESI-TOF) (m/z): 326.70 [M + 3]

2-(4-((1-(2–chloro-5-(trifluoromethyl)phenyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6d):

Light Yellow Solid, yield- 69%, m.p.123–125 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.14 (s, 1H), 8.05 (d, J = 8.86 Hz, 2H), 7.95 (s, 1H), 7.74 (s, 2H), 7.07 (d, J = 8.97 Hz, 2H), 5.39 (s, 2H), 4.60 (t, J = 6.35 Hz, 2H), 3.63 (t, J = 6.08 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.65, 162.04, 143.83, 135.17, 132.28, 131.94, 131.68, 131.03, 130.69, 127.56, 127.52, 125.04, 125.01, 124.81, 124.16, 122.81, 121.45, 114.53, 64.04, 61.90, 28.94; IR(υ, cm^{-1}): 3088, 2893, 1732, 1441, 1130, 940, 420.

2-(4-((1-(4–bromo-3-(trifluoromethyl)phenyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6e):

Light Yellow Solid, yield- 66%, m.p.138–140 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.12 (s, 1H), 8.09 (d, J = 2.55 Hz, 1H), 8.05 (d, J = 8.89 Hz, 2H), 7.90 (d, J = 8.60 Hz, 1H), 7.82 (dd, J = 2.38 Hz, 1H), 7.06 (d, J = J8.96 Hz, 2H), 5.37 (s, 2H), 4.60 (t, J = 6.35 Hz, 2H), 3.63 (t, = J J = 6.08 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.61, 161.94, 136.60, 135.92, 132.14, 131.98, 131.69, 124.37, 122.89, 120.78, 119.87, 114.48, 64.06, 61.92, 28.94; IR(υ, cm^{-1}): 2953, 1705, 1420, 1121, 765, 420. HRMS (ESI-TOF) (m/z): 469.75 [M + 2]

2-(4-((1-(4-(trifluoromethyl)phenyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6f):

White Solid, yield- 63%, m.p.150–153 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.14 (s, 1H), 8.05 (d, J = 8.86 Hz, 2H), 7.91 (dd, J = 9.00 Hz, 2H), 7.81 (dd, J = 8.38 Hz, 2H), 7.06 (d, J = 8.97 Hz, 2H), 5.39 (s, 2H), 4.60 (t, J = 6.35 Hz, 2H), 3.63 (t, J = 6.08 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.62, 161.99, 144.84, 139.25, 131.97, 131.68, 131.19, 130.86, 127.21, 127.18, 127.14, 124.83, 122.85, 122.13, 120.87, 120.56, 114.49, 144.32, 64.04, 61.90, 28.94;IR(υ, cm^{-1}): 2986, 1705, 1395, 1162, 766.

2-(4-((1-(2-fluoro-5-(trifluoromethyl)phenyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (6 g):

White Solid, yield- 61%, m.p.123–126 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.09 (s, 1H), 8.07–7.94 (m, 4H), 7.41 (t, J = 9.0 Hz,1H), 7.06 (d, J = 9.0 Hz, 2H), 5.36 (s, 2H), 4.60 (t, J = 6.35 Hz, 2H), 3.63 (t, J = 6.08 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.62, 161.96, 158.08, 144.96, 133.01, 131.97, 126.07, 125.98, 123.08, 122.86, 121.09, 120.14, 119.79, 119.76, 118.87, 118.64, 114.48, 64.04, 61.90, 28.94; IR(υ, cm^{-1}): 2982, 1708, 1235, 1065, 750. HRMS (ESI-TOF) (m/z): 409.87 [M + 3]

2-(4-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (7a):

White Solid, yield- 76%, m.p. 125–128 °C, ^{1}H NMR (DMSO, 400 MHz, δ ppm): 8.31 (s, 1H), 7.92 (d, J = 8.84 Hz, 2H), 7.43–7.28 (m, 5H), 7.17 (d, J = 8.81 Hz, 2H), 5.61 (s, 2H), 5.24 (S, 2H), 4.59 (t, J = 5.54 Hz, 2H), 3.82 (t, J = 5.62 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.42, 162.54, 142.88, 136.41, 131.80, 129.24, 128.65, 128.42, 125.37, 122.26, 115.34, 64.53, 61.82, 53.34, 31.52; IR (υ, cm^{-1}): 3147, 2975, 1719, 1452, 1119, 760, 422. HRMS (ESI-TOF) (m/z): 336.18 [M + 2]

2-(3-((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (7b):

Light Yellow Solid, yield- 74%, m.p. 77–79 °C, ^{1}H NMR (DMSO, 400 MHz, δ ppm): 8.29 (s, 1H), 7.62–7.54 (m, 2H), 7.48 (t, J = 7.8 Hz, 1H), 7.43–7.28 (m, 6H), 5.61 (s, 2H), 5.22 (s, 2H), 4.59 (t, J = 5.54 Hz, 2H), 3.82 (t, J = 5.62 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm) : 165.76, 158.19, 144.06, 134.38, 130.98, 129.66, 129.19, 128.88, 128.15, 122.73, 120.21, 115.50, 77.37, 77.05, 76.73, 64.32, 62.22, 54.34, 28.76; IR (υ, cm^{-1}): 3124, 2950, 1712, 1439, 1112, 747.

2-(3-((1-(3-fluorobenzyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (7c):

Yellow Solid, yield:- 80%, m.p. 117–119 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.71–7.61 (m, 3H), 7.39–7.31 (m, 2H), 7.19 (d, J = 2.71 Hz, 1H), 7.09–7.02 (m, 2H), 6.97 (t, J = 1.86 Hz, 1H), 5.55 (s, 2H), 5.25 (s, 2H), 4.61 (t, J = 6.10 Hz, 2H), 3.63(t, J = 6.07 Hz,2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.73, 164.24, 161.78, 158.07, 144.09, 136.60, 136.53, 131.01, 130.93, 130.85, 129.71, 123.68, 123.65, 123.06, 122.83, 120.18, 116.08, 115.88, 115.48, 115.22, 115.00; IR (KBr, υ, cm^{-1}): 3075, 1701, 1596, 1124, 754, 420. HRMS (ESI-TOF) (m/z): 351.73 [M + 1]

2-(4-((1-(3-fluorobenzyl)–1H-1,2,3-triazol-4-yl)methoxy)phenyl)–4,5-dihydrooxazole (7d):

Light Blue Solid, yield- 76%, m.p. 133–136 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.01 (d, =J = 4.02 Hz, 2H), 7.76 (s, 1H), 7.39–7.31 (m, 1H), 7.09–6.93 (m, 5H), 5.55 (s, 2H), 5.26 (s, 2H), 4.59

(t, J = 6.4 Hz, 2H), 3.21 (t, J = 6.07 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.66, 164.24, 162.07, 161.78, 136.69, 136.52, 131.89, 130.94, 130.85, 123.72, 123.69, 122.65, 116.11, 115.90, 115.27, 115.05, 114.54, 64.00, 61.93, 54.08, 28.97; IR(υ, cm^{-1}): 3149, 1704, 1293, 1117, 772.

2-(3-((1-(4-chlorobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7e):

Brown Solid, yield- 81%, m.p. 66–69 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.67 (t, J = 8.13 Hz, 3H), 7.40(m, 4H), 7.23–7.11 (m, 2H),5.52 (s, 2H), 5.24 (s, 2H), 4.61 (t, J = 6.22 Hz, 2H), 3.63 (t, J = 6.14 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.74, 158.13, 136.30, 135.04, 131.00, 130.48, 129.69, 129.10, 128.17, 126.17, 122.78, 120.20, 115.49, 64.33, 62.10, 53.84, 28.82; IR(υ, cm^{-1}): 2986, 1709, 1591, 1273, 116, 747.

2-(4-((1-(4-chlorobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7f): White Solid, yield- 71%, m.p. 116–119 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.02 (d, J = 4.21 Hz, 2H), 7.58 (s, 1H), 7.36–7.23 (m, 3H), 7.15 (d, J = 3.55 Hz, 1H),7.00 (d, J = 4.48 Hz, 2H), 5.51 (s, 2H), 5.25 (s, 2H), 4.58 (t, J = 6.01 Hz, 2H), 3.62 (t, J = 6.05 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.66, 162.11, 136.29, 135.08, 131.88, 130.55, 129.12, 128.15, 126.15, 122.61, 114.50, 64.01, 62.07, 53.61, 28.97; IR(υ, cm^{-1}): 2968, 1701, 1264, 1171, 770.

2-(2-((1-(4-bromobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7 g):

White Solid, yield- 81%, m.p. 82–84 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.86 (dd, J = 1.77 Hz, 1H), 7.70 (s, 1H), 7.51–7.45 (m, 3H), 7.13 (t, J = 8.75 Hz, 3H), 7.02 (t, J = 7.58 Hz, 1H), 5.48 (s, 2H), 5.30 (s, 2H), 4.54 (t, J = 6.11 Hz, 2H) 3.56 (t, J = 6.12 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.2, 158.01, 144.79, 134.07, 133.56, 132.30, 132.02, 139.70, 122.98, 122.95, 121.07, 119.87, 114.08, 64.10, 63.40, 53.56, 28.99; IR(υ, cm^{-1}): 2972, 1706, 1263, 1116, 764.

2-(4-((1-(4-bromobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7 h):

White Solid, yield- 73%, m.p. 147–150 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.00 (d, J = 8.85 Hz, 2H), 7.55 (s, 1H), 7.50 (d, J = 8.40 Hz, 2H), 7.14 (d, J = 8.28 Hz, 2H), 7.0 (d, J = 8.90 Hz, 2H), 5.48 (s, 2H), 5.24 (s, 2H), 4.59 (t, J = 6.11 Hz, 2H), 3.62 (t, J = 6.12 Hz, 2H); ^{13}C NMR($CDCl_3$, 100 MHz, δ ppm): 165.66, 162.12, 114.06, 133.38, 132.37, 131.86, 129.73, 123.08, 122.71, 122.61, 114.49, 64.01, 62.07, 53.60, 28.97; IR(υ, cm^{-1}): 2982, 1704, 1235, 1065, 750.

2-(3-((1-(4-bromobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7i):

Brown Solid, yield- 67%, m.p.149–151 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.93 (s,1H), 7.66 (t, J = 7.40 Hz, 2H), 7.48 (d, J = 3.96 Hz, 2H), 7.35 (s, 1H), 7.24–7.07 (m,3H), 5.50 (s, 2H), 5.21 (s, 2H), 4.59 (t, J = 5.56 Hz, 2H), 3.62 (t, J = 5.40 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.75, 158.12, 133.33, 132.42, 131.39, 131.02, 130.32,130.11,129.81, 123.13, 122.83, 120.45, 115.64, 66.98, 64.47, 62.50, 54.84; IR(υ, cm^{-1}): 3070, 1706, 1440, 1287, 1119, 751.

2-(3-((1-(4-nitrobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)4,5dihydrooxazole (7j):

White Solid, yield- 70%, m.p.114–115 °C, ^{1}H NMR (DMSO, 400 MHz, δ ppm): 8.40 (s, 1H), 8.24 (d, J = 8.25 Hz, 2H), 7.94 (d, J = 8.40 Hz, 2H), 7.54 (d, J = 7.66 Hz, 2H), 7.18 (d, J = 8.40 Hz, 2H), 5.81 (s, 2H), 5.27 (s, 2H), 4.56 (t, J = 6.11 Hz, 2H), 3.81 (t, J = 6.12 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz, δ ppm): 165.40, 162.51, 147.74, 143.81, 143.06, 131.82, 129.53, 125.81, 124.40, 122.30, 115.36, 64.53, 61.80, 52.42, 31.53; IR(υ, cm^{-1}): 2967, 1703, 1328, 1121, 740. HRMS (ESI-TOF) (m/z): 376.77 [M-2]

2-(4-((1-(2,4-difluorobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7k):

White Solid, yield – 71%, m.p.120–123 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.01 (d, J = 8.86 Hz, 2H), 7.65 (s, 1H), 7.34–7.27 (m, 1H), 7.0 (d, J = 8.88 Hz, 2H), 6.92–6.85 (m, 2H), 5.55 (s, 2H), 5.24 (s, 2H), 4.59 (t, J = 6.01 Hz, 2H), 3.62 (t, J = 6.05 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.66, 164.71, 164.59, 162.14, 162.07, 159.46, 144.01, 131.86, 131.78, 131.73, 131.69, 120.79, 122.60, 117.88, 117.73, 114.49, 112.38, 112.34, 112.16, 112.12, 104.72, 104.47, 104.22, 64.01, 62.04, 47.03, 47.27, 28.96; IR(υ, cm^{-1}): 2927, 1703, 1348, 1172, 767.

2-(4-((1-(3-(trifluoromethyl)benzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7l):

Light Brown Solid, yield- 80%, m.p.112–115 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.01 (d, J = 8.55 Hz, 2H), 7.63 (d, J = 7.52 Hz, 2H), 7.57–7.48 (m, 2H), 7.45 (d, J = 8.17 Hz, 1H), 7.00 (d, J = 8.50 Hz, 2H), 5.60 (s, 2H), 5.26 (s, 2H), 4.59 (t, J = 6.01 Hz, 2H), 3.62 (t, J = 6.05 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.66, 162.08, 135.4, 131.88, 131.48, 131.34, 129.84, 125.84, 124.74, 122.76, 114.49, 64.02, 62.05, 53.75, 28.94; IR(υ, cm^{-1}): 2918, 1707, 1288, 1143, 760.

2-(4-((1-(4-fluorobenzyl)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (7 m):

Light Yellow Solid, yield- 76%, m.p.135–137 °C, ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.01 (d, J = 8.49 Hz, 2H), 7.53 (s, 1H), 7.30–7.24 (m, 2H), 7.07 (t, J = 8.64 Hz, 2H), 7.00 (dd, J = 2.10 Hz, 2H), 5.51 (s, 2H), 5.24 (s, 2H), 4.59 (t, J = 6.01 Hz, 2H), 3.62 (t, J = 6.05 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 165.66, 164.17, 162.14, 161.70, 144.01, 131.87, 130.23, 131.20, 131.08, 130.00, 122.60, 116.33, 114.49, 64.01, 62.10, 53.57, 28.96; IR(υ, cm^{-1}): 2948, 1707, 1278, 1150, 750.

2.3. Biological activity protocols

2.3.1. In vitro evaluation of antimicrobial activity

The synthesized title compounds (**6a – 6 g** and **7a –7 m**) were assessed for antimicrobial activity against a panel of bacterial and fungal strains by following the earlier reported MIC assay method using resazurin dye [48].

2.3.2. Microorganisms used

Standard cultures of two Gram-positive [*S. aureus* (ATCC 25,923), *B. subtilis* (ATCC 6051)], two Gram negative [*E. coli* (ATCC 35,218), *P. aeruginosa* (ATCC 27,853)], fungal strains [*C. albicans* (ATCC 90,028), *C. neoformans* (ATCC 66,031), and *A. niger* (ATCC 16,404)] were used for the antibacterial and antifungal activity, respectively. Culturing and sub-culturing (one day before testing) of these microorganisms was carried out at the Department of Microbiology, Inkosi Albert Luthuli Hospital, Durban, South Africa.

2.3.3. Preparation of medium

The nutrient medium was prepared by dissolving 22 g of Muller-Hinton Broth (MHB) containing

(Acid Hydrolysate of Casein, Beef Extract, and Starch) in 1 L of double-distilled water. The pH of this medium was adjusted to 7.4 ± 0.1 and sterilized by autoclave for 15 min at 121 °C. The solution was allowed to cool and stored at a temperature of 4 °C. Sterility check was performed by incubating un-inoculated media in an aerobic incubator at 37 °C for 18–24 h. RPMI 1640 medium with L-glutamine and 0.165 M MOPS and without sodium bicarbonate (Lonza) was used for antifungal activity.

2.3.4. Preparation of test compounds (stock solution and working standard)

An accurately weighed quantity (4.000 mg) of the synthesized compounds and standard drugs were dissolved in 1 mL of DMSO to give stock solution (4000 μg/mL). Further, 100 μL of stock solution was diluted with 900 μL of DMSO to afford a standard working solution (400 μg/mL).

2.3.5. Preparation of inoculums

One day before testing one or more identical colonies of microorganisms, they were suspended in a 4.5 ml sterile saline solution. Inoculates were adjusted to 0.5 McFarland standard (1.5×10^8 cfu/ml). A density check turbidimeter was used to ensure that the inoculum was a 0.5 McFarland standard [49].

2.3.6. Broth micro-dilution method

The preliminary in vitro antimicrobial activity for the newly synthesized compounds (**6a**-6 **g** and **7a-7 m**) was evaluated using the broth micro-dilution method. 200 μL of sterile double distilled water was added to all outer-perimeter wells of a 96-well microliter plate to minimize evaporation of the medium in the test wells during incubation. To the remaining test wells, 100 μL of MHB was added. Two-fold serial dilutions of the test compounds and standard drugs (Amoxicillin and Amphotericin B) were made directly on the microplate using MHB. The compounds were tested at final concentration of (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 μg/mL). Finally, freshly prepared bacterial or fungal inoculum was added to the wells. The microliter plates were covered and sealed with parafilm and incubated at 37 ± 1 ºC for 24 h. After this, 0.5% of freshly prepared resazurin was added to the test wells and incubated further for 5 h. **MIC** was determined as a blue color in the test well was interpreted as no bacterial growth, and a pink color was scored as growth. Thus, the MIC was defined at the lowest drug concentration, preventing a color change from blue to pink. Thus, the **MIC** values in μg/mL were determined.

2.3.7. In vitro evaluation of anti-tubercular activity

Culturing and sub-culturing *Mtb* strains were carried out at the Department of Microbiology, Inkosi Albert Luthuli Hospital, Durban, South Africa. Briefly, *M. tuberculosis* (ATCC H_{37}Rv), MDR (UKQC strain), XDR (UKQC strain) were maintained on 7H11 agar plates at 37 °C in an atmosphere of 5% CO_2. Inoculums of strains were prepared by scraping and re-suspending a loopful of colonies into Middlebrook 7H9 broth, supplemented with 10% ADC and 0.04% tween 80 to avoid clump formation and incubated at 37 °C in 5% CO_2. The inoculum turbidity was adjusted to a McFarland number 1 standard and further diluted 1:10 in Middlebrook 7H9 broth before addition (100 μL) to each test sample and drug-free wells. Growth control and a sterile control were also included for each isolate. Each synthesized test compound and standard drugs were weighed accordingly, dissolved in the appropriate solvent and filter, and sterilized using a 0.2-micron polycarbonate filter. Further, an amount, 100 μL from the stock solution was diluted with 900 μL of DMSO (some protocols used broth, to overcome solubility concern, we have used DMSO with the final well on the plate having a concentration of less than 1% of DMSO) to afford working standard solution (50 μg/mL). A serially diluted drug-free control (DMSO) was used to check the activity on each strain. The preliminary in vitro anti-mycobacterial activity for the newly synthesized test compounds (**6a-6 g** and **7a-7 m**) was evaluated using the colorimetric resazurin microplate assay method. The outer-perimeter wells of a 96-well microliter plate were filled with 200 µL of sterile double distilled water to minimize evaporation of the medium in the test wells [50].

2.3.8. Screening of inhibitory activity of drug molecules on E. coli *DNA gyrase and determination of IC_{50}*

The most active compounds were re-evaluated for their inhibitory activity against E. coli DNA gyrase enzyme on supercoiling of relaxed DNA. These assays were performed based on established protocols attained from the supplier, TopoGEN, Inc [47]. All of the reactions (DNA gyrase and Topoisomerase IV activities) were loaded on 1% agarose, TAE (40 mM Tris-acetate, 0.01 M EDTA, pH 8.3) gel and ran for 3–4 hr at 50 V. The gel was stained with 0.5 µg mL^{-1} ethidium bromide in TAE for 30 min while rocking, then destained for 10 min in deionized water. The images were captured on a Gel doc XR imaging system from BIO-RAD at a wavelength of 300 nm. The intensity of the reaction products was quantitated using Image Lab software, and IC_{50} values were determined by nonlinear regression analysis in Graph Pad Prism-8.

2.3.9. In vitro inhibitory activities of drug molecules on E. coli *DNA gyrase (supercoiling)*

Supercoiling of relaxed plasmid DNA (pHOT1) was examined in a reaction volume of 20 µL contains of assay buffer (35 mM Tris pH 7, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1.8 mM spermidine, 6.5% glycerol, 0.1 mg/ml acetylated BSA, 1 mM ATP), and 0.2 mg of relaxed DNA (pHOT1 a derivative of pBR322 < 3 KB) substrate. Drug compounds with different concentrations (10, 1, 0.5 µM) in DMSO were added, and after that, the reactions were initiated with 2 units of *E. coli* gyrase (TopoGEN). The optimum concentration of ciprofloxacin (0.1 µM) was used as a standard to compare the results. The reaction proceeded for incubation with shaking on a mini-orbital shaker for 1 h at 37 °C. After the incubation, added 0.1volume of 10% SDS and 10 µL of proteinase K (20 mg/mL) and digested for 30 min. at 37 °C. Then add 0.1volume of 10x loading dye (0.25% bromophenol and 50% glycerol), followed by organic extraction using chloroform: isoamyl alcohol mixture (24:1 ratio), vortex briefly, separate blue coloured upper aqueous phase, and load onto 1% agarose gel. The loaded DNA was stained with 0.5 µg mL^{-1} ethidium bromide for 30 min and disdain with distilled water for 10 min at room temperature. The photo was visualized and documented with Gel doc XR imaging system from BIO-RAD. Quantification of the intensity of the bands was performed using Image Lab software provided by BIO-RAD.com, and IC_{50} was determined using Graph Pad Prism 8.

3. Result and discussion

3.1. Rationale

Molecular hybridization is the often-used strategy for synthesizing new therapeutic agents by combining active pharmacophores. The other approach is to take advantage of various available literature works and develop new synthetic organic chemistry pathways to synthesize different targets with a constant active core. We employed the second strategy in our work by keeping the oxazoline and triazole nuclei constant, and for designing, we have taken advantage of available literature as depicted in Fig. 1. The (**A)** and (**C)** bearing oxazoline nuclei having benzyl ester show promising activity with **MIC** of < 1 µg than simple alkyl esters against *Mtb* H_{37} Rv [17].

The compound (**B)** has a 2,4-disubstituted oxazoline compound, and it is most active for *Mtb* H_{37} Rv strain with **MIC** value of 6.8 µg/mL [12]. The compound (**D)** has 1, 4 di-substituted 1, 2, 3- triazole analog as it shows promising anti-mycobacterial activity against *Mtb* H_{37} Ra (ATCC 25,177 strain) with a minimum inhibitory concentration value of 0.78 µg/mL [23]. The compound (**E)** has a triazole analogue derived from carvacrol bearing carboxylic acid functional groups showing potent anti-bacterial activity against *S. pneumonia, E. faecalis* and *E. coli* with IC_{50} of 62.53 µg/mL, 36.66 µg/mL and 15.28 µg/mL, respectively [24]. The compound (**F)** bearing non substituted amidine and phenyl rings shows a strong inhibition effect on ESBL-producing *E. coli* strain with potency better than the reference antibiotics Ceftazidime and Ciprofloxacin [25].

From these observations, the oxazoline and triazole nuclei play a key role in biological activity as anti-tubercular, anti-microbial, so we tried to modify the oxazoline nuclei in combination with triazole in a single core with the hope to get better activity.

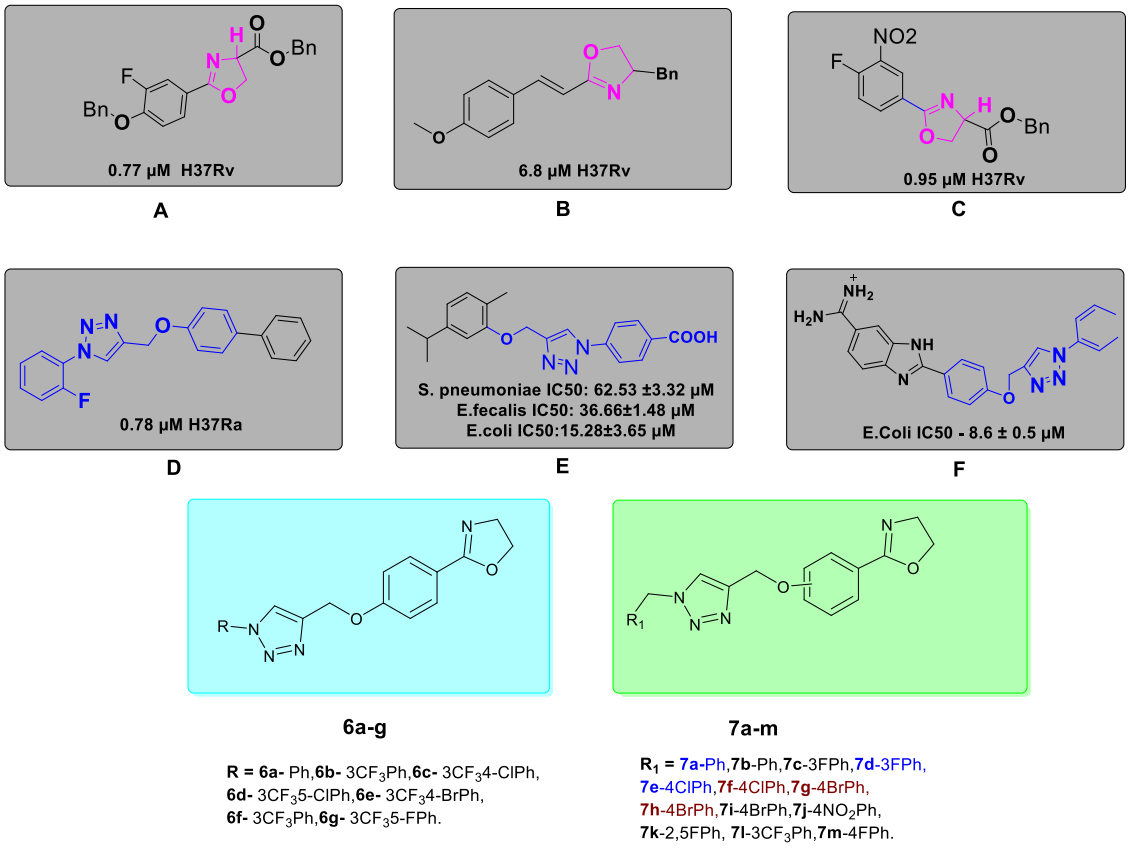


Fig. 1. Structures of some biologically active moieties bearing oxazoline and triazole: (A-F) from the literature and (**6a-6 g** and **7a-7 m**) the compounds under study.

3.2. Chemistry

The synthesis of 2-(3-((1-(substituted)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (**6a-6 g** and **7a-7 m**) was done through a series of reactions like alkylation, hydrolysis, coupling and cyclization. We have synthesized final derivatives by optimizing the reaction steps by considering the yield of the reaction, reaction time, cost of raw material and reagents, less hazardous/mild conditions, and neat reaction profiles. All the derivatives were either purified by crystallization or, in some cases, by Combi-flash.

The synthesized compounds, **6a-6 g** and **7a-7 m,** were screened for their ADME properties study before synthesis. ADME properties play an important role in optimizing the synthesized derivatives' pharmacokinetics, ultimately increasing the success rate for oral drug-like properties. The Lipinski rule and others explained the importance of physicochemical property studies in the early stages of drug discovery. The rule state that molecule with molecular mass less than 500, log P value less than 5, hydrogen bond donors less than 5, hydrogen bond acceptors less than 10, polar surface areas less than 140 and the number of rotatable bonds less than 10 are the ideal parameters for molecules to become a drug [26–29].

The absorption, distribution, metabolism and excretion (ADME) properties of compounds were predicted using QikProp v3.5 (Schrödinger LLC, New York, NY, USA). We studied molecular volume (MV), molecular weight (MW), Predicted octanol-water partition coefficient (log Po/w), number of hydrogen bond acceptors (n-ON), number of hydrogen bonds donors (n-OHNH), percentage human oral absorption (% ABS) and polar surface area (PSA). These properties study helps in understanding the drug-likeness of synthesized compounds. The ADME property data of designed compounds were presented in table 1. All the designed compounds show very good drug-likeness model scores compared with the available data.

The detailed reaction sequences for the synthesis of **6a-6 g** and **7a-7 m** are depicted in scheme 1. In scheme 1**,** we have synthesized key intermediate **4a-4c** with ortho, meta and para positions, respectively**.** The intermediates were then used to synthesize **6a-6 g** and **7a-7 m** to obtain the final derivatives via click reaction.

Reagents and conditions: (**i**) Propargyl bromide, K_2CO_3, DMF, 100 °C, 2 h; (**ii**) NaOH, THF, H_2O, 100 °C, 2 h; (**iii**): $SOCl_2$, DCM, 50 °C, 0.5 h; 2-chloroethylamine hydrochloride, TEA, DCM rt, 1 h; **(iv)** $NaHCO_3$, DMF, 80 °C, 4 h; **(v)** Substituted azidobenzene, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, BuOH, H_2O, rt, 16 h. **(vi)** Substituted (azidomethyl)benzene, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, BuOH, H_2O, rt, 16 h.

In step (**i**), we carried out O-alkylation using propargyl bromide and base by varying different conditions. Previous research show different bases and conditions researchers used for the synthesis of propargyl ethers [30–33]. We reacted ethyl 2-hydroxybenzoate (**1a**), ethyl 3-hydroxybenzoate (**1b**) and ethyl 4-hydroxybenzoate (**1c**) reacted with propargyl bromide by using K_2CO_3 as base and acetone as solvent to obtain ethyl 2-(prop–2-yn-1-yloxy) benzoate (**2a**), ethyl 3-(prop–2-yn-1-yloxy)benzoate (**2b**) and ethyl 4-(prop–2-yn-1-yloxy)benzoate (**2c**) with 75%, 69% and 85% yields respectively, after 18 h at room temperature. We repeated the same reaction conditions using DMF as solvents to obtain 80%, 78%, and 79% yields, respectively. The obtained yields are analogues in both the solvents, so further, we have tried to push the reaction for completion in less time by heating. When both the solvents were heated at 60 °C temperature for 10 h resulted in 60–70% yields in acetone and 65–75% in DMF. We further, increasing the temperature for acetone to reflux and 100 °C for DMF and results were observed after 2 h. The reactions in acetone gave 65–75% yields, and that of

Table 1
ADME properties of synthesized targets (**6a-6 g** and **7a-7 m**).

Entry	% ABS[a]	TPSA[b] (A^2)	n-ROTB[c]	MV[d]	MW[e]	miLog[f]	n-ON[g]	n-OH NH[h]	Lipinski violations[i]	Drug likeness model score
	–	–	–	–	<500	≤ 5	<10	<5	≤ 1	0–1.5
6a	87.76	61.55	5	284.71	320.35	2.76	6	0	0	0.20
6b	87.76	61.55	6	316.01	388.35	3.84	6	0	0	−0.08
6c	87.76	61.55	6	329.54	422.79	4.47	6	0	0	0.12
6d	87.76	61.55	6	329.54	422.79	4.68	6	0	0	0.10
6e	87.76	61.55	6	333.89	467.25	4.60	6	0	0	−0.23
6f	87.76	61.55	6	316.01	388.35	3.65	6	0	0	−0.40
6 g	87.76	61.55	6	320.94	406.34	4.17	6	0	0	0.0
7a	87.76	61.55	6	301.51	334.38	3.08	6	0	0	0.35
7b	87.76	61.55	6	301.51	334.38	3.05	6	0	0	0.21
7c	87.76	61.55	6	306.44	352.37	3.19	6	0	0	0.45
7d	87.76	61.55	6	306.44	352.37	3.22	6	0	0	0.64
7e	87.76	61.55	6	315.05	368.82	3.71	6	0	0	0.56
7f	87.76	61.55	6	315.05	368.82	3.73	6	0	0	0.81
7 g	87.76	61.55	6	319.40	413.27	3.84	6	0	0	0.80
7h	87.76	61.55	6	319.40	413.27	3.89	6	0	0	0.36
7i	87.76	61.55	6	319.40	413.27	3.86	6	0	0	0.26
7j	71.95	107.37	7	324.84	379.38	3.04	9	0	0	0.19
7k	87.76	61.55	7	332.81	402.38	3.95	6	0	0	0.40
7l	87.76	61.55	6	311.37	370.36	3.33	6	0	0	0.74
7m	87.76	61.55	6	306.44	352.37	3.24	6	0	0	0.51

[a] Percentage Absorption;.
[b] Topographical polar surface area;.
[c] Number of rotatable bonds;.
[d] Molecular volume;.
[e] Molecular Weight;.
[f] Lipophilicity;.
[g] No. of hydrogen bond acceptors;.
[h] No. of hydrogen bond acceptors;.
[i] Number of violations.

DMF gives 80–90% yields. The workup of reaction in DMF was easy as aqueous workup gave reasonably pure derivatives. We have used this condition to synthesize scale-up batches for all the derivatives to get compounds **2a-2c**.

In step (**ii),** we have done hydrolysis of compounds **2a-2c** using different bases like $Li(OH)H_2O$ and NaOH. We have tried to modify the reaction condition that earlier researchers used [34–37]. The compounds **2a-2c** were treated with $Li(OH)H_2O$ to obtain desired compounds **3a-3c** with 85 to 95% yields, respectively, after 18 h. We tried to reduce the reaction time, so we have used NaOH as the base, and after 12 h, we got desired yields of 85–90% at room temperature. Further, we have tried heating conditions at 50 °C and 100 °C and monitored the reaction completion. At 50 °C, we got 70% yield after 4 h, and that at 100 °C, we got 95% yield after 2 h. For all the synthesized derivatives **3a-3c,** we got yields of more than 90% after 2 h, and all the compounds were isolated from an aqueous workup. This condition was used for the scale-up batches.

In step (**iii),** the compounds **3a-3c** were converted to amide **4a-4c,** which was achieved using different conditions [38–40]. We first synthesized the benzyl chlorides by using $SOCl_2$ in DCM, and later, these were treated with 2-chloroethylamine hydrochloride in the presence of TEA in DCM to obtain the desired compounds **4a-4c** with 82%, 80% and 89% yields, respectively in 2 h. The desired compounds were obtained with good purity and used for the next step without purifications.

The synthesis of oxazoline nuclei was achieved using chlorides and amino alcohol to form hydroxyl–amide and later base-catalyzed cyclization through an inversion of configuration. We can achieve this in other ways by using hydroxyl–amide and PPh_3-CCl_4 as a Mitsunobu approach. Some researchers used dehydrating reagents like carbodiimide, Et_2NSF_3, $(MeOCH_2CH_2)_2NSF_3$ and PPH_3-DDQ etc., for the synthesis of oxazoline nuclei. Oxazoline synthesis was achieved by using boronic acid-catalyzed condensation- cyclodehydration of the carboxylic acid with amino alcohols. Some researchers used metal-catalyzed reactions involving different acids, esters and lactones to get oxazoline nuclei [41–44]. All these approaches are harsh, time-consuming and therefore, there is the need to develop conditions with milder approaches.

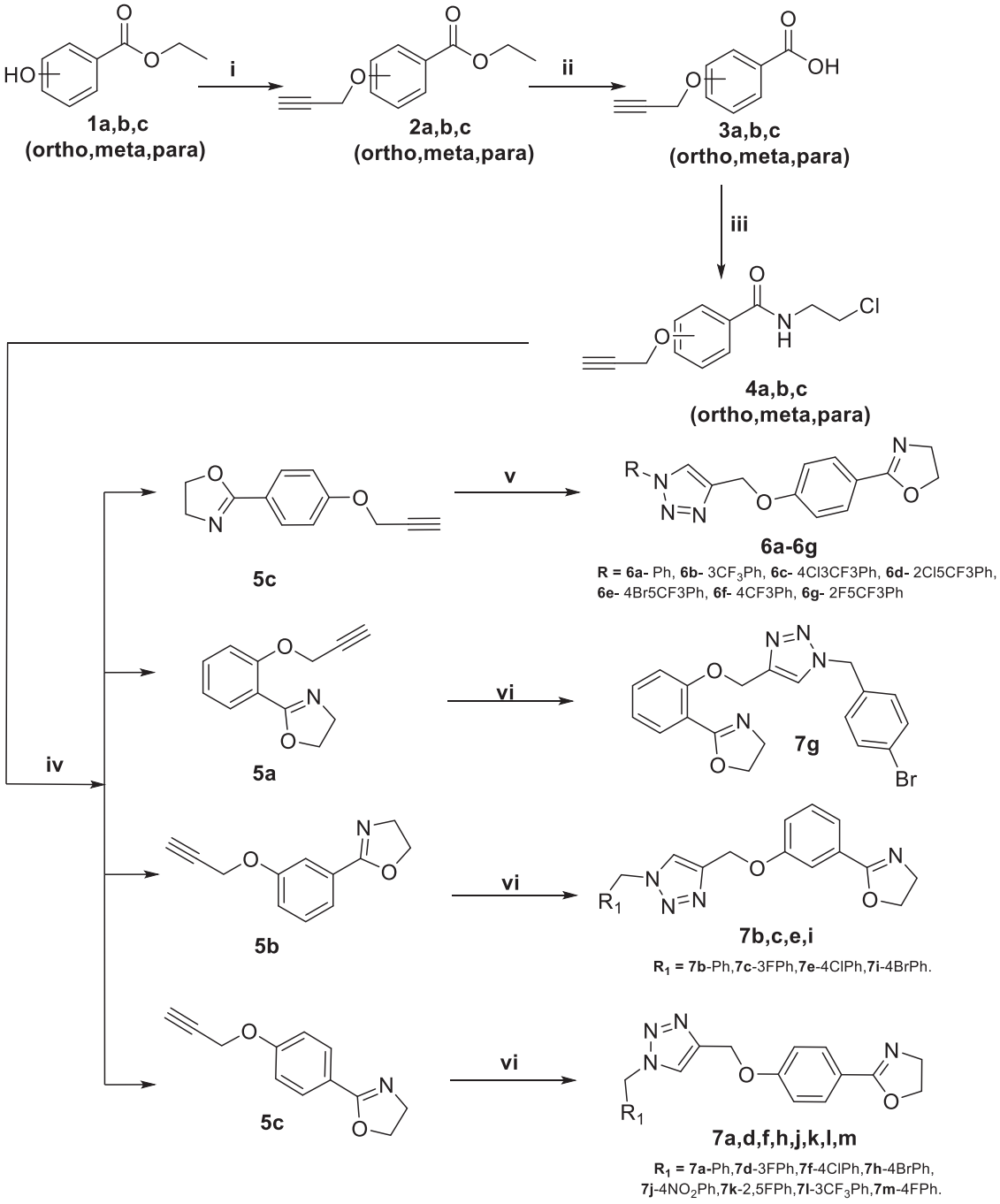
In step (**iv),** the compounds **4a-4c** were reacted with $NaHCO_3$ in DMF at 80 °C for 4 h with the formation of compounds **5a-5c** with 78%, 73% and 82%, respectively [45].

We have optimized the base for the cyclization to achieve a good yield and less reaction time. We used $NaHCO_3$, Na_2CO_3 and K_2CO_3 as bases and monitored reaction at room temperature and heating conditions. The reaction at room temperature failed to give the desired product in all the bases after prolong time. On heating conditions at 80 °C and 100 °C for all the bases after 4 h, we could obtain a 75% of the product in $NaHCO_3$, 30% product in Na_2CO_3 and 20% product in K_2CO_3 as a base. At the temperature of 100 °C, the yields were 20%, 10% and no reaction, respectively, after 4 h in all the three bases. The mild base and mild heating conditions have helped to form the oxazoline ring. These conditions observed were used for the scale-up purpose to synthesize **5a-5c** [45].

In step (**v**), we have used the Click reaction with different substituted azidobenzene and substituted (azidomethyl)benzene to get the final targets **6a-6 g** and **7a-7 m**. We have used *t*-butanol and water as combined solvents, sodium ascorbate and $CuSO_4{\cdot}5H_2O$ in molar quantities [46]. The reaction was completed in 16 h and at room temperature. The compound **5c** was converted to **6a-6 g,** and these compounds show good drug-likeness model scores, which are depicted in table 1. The compounds **7a-7 m** were synthesized from **5a-5c,** having different positions of substitutions as displayed in **scheme-1**. All the final derivatives were obtained as solids. The detailed experimental procedures are given in the experimental section.

3.3. Biology

The synthesized derivatives **6a-6 g** and **7a-7 m** were tested for their antibacterial, antifungal and anti-tubercular activity. The screened compounds belong to



Scheme 1. Synthesis of 2-(substituted-((1-(substituted)−1H-1,2,3-triazol-4-yl)methoxy) phenyl)−4,5-dihydrooxazole **(6a-6 g)** and 2-(substituted -((1-(substituted)−1H-1,2,3-triazol-4-yl)methoxy) phenyl)−4,5-dihydrooxazole **(7a-7 m)**.

two types of linkages 2-(substituted-((1-(substituted)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (**6a-6 g**) and 2-(substituted -((1-(substituted)−1H-1,2,3-triazol-4-yl)methoxy)phenyl)−4,5-dihydrooxazole (**7a-7 m**). From the tested oxazoline-triazole hybrid, nine compounds showed moderate inhibition against *M. tuberculosis* $H_{37}Rv$ with **MIC** 1.6 μM, and the remaining compounds showed less inhibitions against UKQC and XDP strains. The anti-microbial and anti-tuberculosis activity data of the synthesized derivatives are presented in tables 2 and 3.

The results of in vitro antibacterial screening were summarized in table 2. From the two series, the compounds **6e-6 g** and **7f-7 h** showed moderate activity (**MIC** 50 μg/mL) against gram-positive bacterial strains. The remaining compounds showed less inhibitions. For gram-negative bacterial strains, the compounds **7a-7i** showed moderate inhibitions with (**MIC** 50 μg/mL), the rest of the compounds showed less inhibition than the standard amoxicillin (**MIC** 0.39 μg\mL). None of the compounds in these series presented any significant inhibition against *staphylococcus aureus* and *pseudomonas aeruginosa* bacterial strains.

The results of antifungal screening were given in table 2. The derivatives of oxazoline-triazole hybrid showed moderate activity of (**MIC** 50 μg/mL) against *C. albicans*. The compounds **6a, 6b, 6 g, 7a, 7d, 7f**, and **7 g** showed less inhibitions for *C. albicans*, and the remaining derivatives showed good activity with (**MIC** 50 μg/mL) compare to standard. For the remaining antifungal strains, most of the synthesized compounds showed less inhibition than the stan-

Table 2
Antimicrobial evaluation of synthesized oxazoline-triazole hybride (**6a-6 g** and **7a-7 m**).

Entry	R/R1	Gram Positive Staphylococcus aureus(ATCC 25,923)	Bacillus subtilis (ATCC 6051)	Gram Negative E. coli (ATCC 35,218)	Pseudomonas aeruginosa (ATCC 27,853)	Candida albicans (ATCC 90,028)	Anti Fungal C. Neoformans(ATCC 66,031);	Aspergillus Niger (ATCC 16,404);
6a	pH	>200	>200	>200	>200	>200	25	50
6b	$3CF_3Ph$	>200	>200	>200	>200	>200	25	50
6c	$4Cl3CF_3Ph$	>200	>200	>200	>200	50	25	200
6d	$2Cl5CF_3Ph$	>200	>200	>200	>200	50	25	50
6e	$4Br5CF_3Ph$	>200	**50**	>200	>200	50	25	50
6f	$4CF_3Ph$	>200	**50**	>200	>200	50	25	50
6 g	$2F5CF_3Ph$	>200	**50**	>200	>200	50	25	50
7a	pH	>200	>200	**50**	>200	>200	25	50
7b	pH	>200	>200	**50**	>200	50	25	50
7c	3FPh	>200	>200	**50**	>200	50	25	50
7d	3FPh	>200	>200	**50**	>200	>200	25	50
7e	4ClPh	>200	>200	**50**	>200	50	25	50
7f	4ClPh	>200	**50**	**50**	>200	>200	25	50
7 g	4BrPh	>200	**50**	**50**	>200	>200	25	50
7h	4BrPh	>200	**50**	**50**	>200	50	25	50
7i	4BrPh	>200	>200	**50**	>200	50	25	50
7j	$4NO_2Ph$	>200	>200	>200	>200	50	25	50
7k	2,4-FPh	>200	>200	>200	>200	50	25	50
7l	$3CF_3Ph$	>200	>200	>200	>200	50	25	50
7m	4FPh	>200	>200	>200	>200	50	25	50
Amoxicillin		<0.39	<0.39	<0.39	<0.39	–	–	–
Amphotericin		–	–	–	–	< 0.39	1.5	< 0.39

* = Values in bold represents more active compounds.

Table 3
Anti-tubercular evaluation and *E-coli* DNA gyrase inhibition of synthesized oxazoline-triazole hybride (**6a-6 g** and **7a-7 m**).

entry	R/R1	$H_{37}Rv$(ATCC $H_{37}Rv$)	MDR(UKQC strain)	XDR (patient)	(*E-coli* DNA gyrase)IC_{50}, μM
6a	pH	>1.6	>1.6	>1.6	–
6b	$3CF_3Ph$	>1.6	>1.6	>1.6	–
6c	$4Cl3CF_3Ph$	>1.6	>1.6	>1.6	–
6d	$2Cl5CF_3Ph$	>1.6	>1.6	>1.6	–
6e	$4Br5CF_3Ph$	>1.6	>1.6	>1.6	–
6f	$4CF_3Ph$	>1.6	>1.6	>1.6	–
6 g	$2F5CF_3Ph$	>1.6	>1.6	>1.6	–
7a	pH	**1.6**	>1.6	>1.6	**0.4781**
7b	pH	**1.6**	>1.6	>1.6	–
7c	3FPh	**1.6**	>1.6	>1.6	–
7d	3FPh	**1.6**	>1.6	>1.6	**0.0915**
7e	4ClPh	**1.6**	>1.6	>1.6	**0.5071**
7f	4ClPh	**1.6**	>1.6	>1.6	**0.0825**
7 g	4BrPh	**1.6**	>1.6	>1.6	–
7h	4BrPh	**1.6**	>1.6	>1.6	**0.4902**
7i	4BrPh	**1.6**	>1.6	>1.6	–
7j	$4NO_2Ph$	>1.6	>1.6	>1.6	–
7k	2,4-FPh	>1.6	>1.6	>1.6	–
7l	$3CF_3Ph$	>1.6	>1.6	>1.6	–
7m	4FPh	>1.6	>1.6	>1.6	–
INH		0.1	0.1	0.1	–
RIF		0.05	0.05	0.05	–
Moxifloxacin		0.2	0.2	0.2	–
Ciprofloxacin		–	–	–	0.1856

* = Values in bold represents more active compounds, all values are in μg/mL.

dard. The methyl's linkage on the triazole ring does not significantly impact antibacterial, antifungal, and anti-tubercular activities.

The active compounds **7a, 7d, 7e, 7f** and **7 h** were further tested for their DNA gyrase activity. The results showed promising DNA cleavage tendency. The results of DNA gyrase were mentioned in table 3**.**

3.3.1. Antibacterial results

The antibacterial inhibition results for strain *S. aureus* show that all the derivatives bearing electron-donating and electron-withdrawing substitutions show less inhibitions in the series. The inhibition results for the strain *B. subtilis* showed similar results except for the derivatives **6e-6 g** and **7f-7 h**. These showed promising inhibitions with **MIC** 50 µg/mL for those bearing trifluoro methyl substitutions along with halo substitutions. The electron-withdrawing effect of the substitutions increases its inhibitions for the *B. subtilis* strain in the series. For gram-negative strain *P. aeruginosa* all the derivatives show less inhibitions. For the strain *E. coli*, the derivatives **7a-7i** show promising inhibitions with **MIC** 50 µg/mL, and the remaining derivatives show less inhibitions. The derivatives with benzylic substitution with halo substitution showed promising inhibitions. The halo substituted derivatives in the series were sensitive for strains *B. subtilis* and *E. coli* showing good inhibitions compared to the remaining strains *S. aureus* and *P. aeruginosa*.

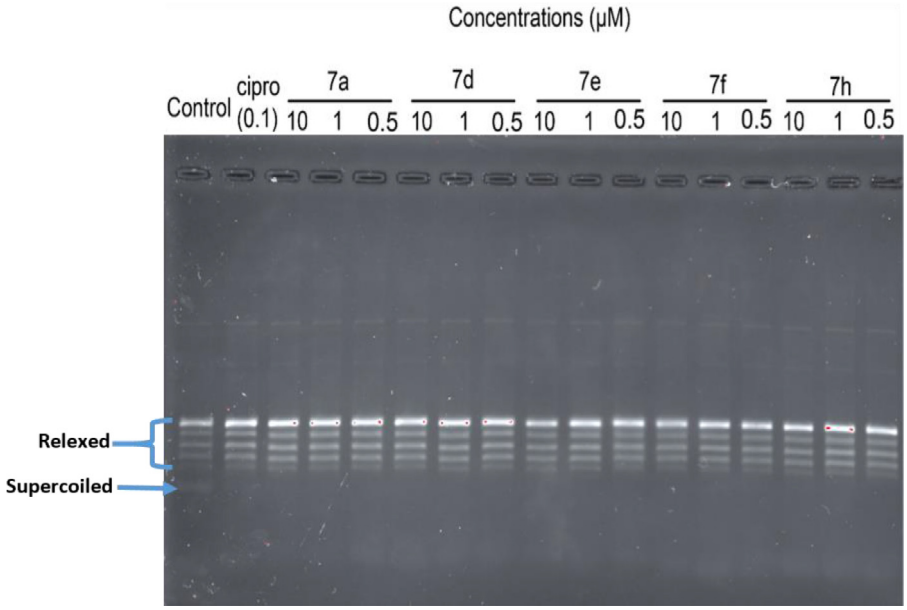


Fig. 2. Gel image after the inhibition of *E.coli* DNA gyrase activity by drug molecules **7a, 7d, 7e, 7f** and **7 h**. Relaxed pBR322 DNA was incubated with a unit of gyrase enzyme in the absence (control) or the presence of drug molecules.

3.3.2. Antifungal results

The inhibitions results for the strains *C. Neoformans* show promising inhibitions for all the synthesized derivatives **6a-g** and **7a-m** with **MIC** 25 μg/mL, revealing that there is less effect of substitution on the strain. For the strain *A. Niger*, similar results were obtained for all the derivatives with **MIC** 50 μg/mL, indicating significantly less effect of substitutions on the inhibitions. In the series, the inhibitory activity varies to less extent for both the tested strains. For the strain, *C. albicans*, the derivatives **6a, 6b, 7a, 7d, 7f** and **7 g** showed less inhibitions with **MIC** 200 μg/mL, and for the remaining derivatives show **MIC** 50 μg/mL, inhibitions. The results reveal this strain is sensitive for the synthesized derivatives but, those derivatives bearing phenyl and benzylic ring without any substitution show less inhibitions than halo substitutions at meta position in the ring through the series.

3.3.3. Anti-tubercular results

The results reveal the derivatives showed promising activity among all the tested strains. All the derivatives show inhibitions of **MIC** >1.6 μM, compared with the standard Moxifloxacin. The derivatives **7a-7i** show **MIC** 1.6 μM for *M. tuberculosis* H_{37}Rv strain. The derivatives are benzylic and substituted with mono halo substitutions like F, Cl and Br. The derivatives bearing di halo substitutions and strong electron-withdrawing groups like NO_2 show reduced inhibitions among the series. In the strains MDR and XDR, there was less effect of substitutions of derivatives on the activity among the series.

3.4. DNA gyrase inhibition study of drug molecules

To further examine the antimicrobial activity of the highly active oxazoline triazole derivatives, invitro inhibition against *E. coli* DNA gyrase was tested using the electrophoresis gel technique. These analyses were performed based on established protocols attained from the supplier, TopoGEN, Inc [47]. Ciprofloxacin was used as a standard reference for *E. coli* DNA gyrase enzyme shown in Fig. 2. DNA gyrase enzyme plays a significant role in the supercoiling of relaxed DNA. Herein reaction mixture, supercoiling of relaxed plasmid DNA (pHOT1) was examined in the presence or absence of drug molecules. Here Fig. 2 represents the band of reflexed and supercoiled DNA after the incubation of the reaction. The band's intensity has been used to calculate IC_{50} from the graph pad prism software.

The active compounds **7a, 7d, 7e, 7f**, and **7 h** were tested on *E. coli* DNA gyrase enzyme to study the inhibition effect of these compounds. The results are shown in table 3. It shows all these derivatives also inhibited *E. coli* gyrase potentially with the IC_{50} from 0.5- 0.08 μM. These results show a good correlation between the **MIC** and IC_{50} (table 3), signifying that these derivatives successfully quash bacterial cell growth. As shown in table 3, derivatives **7d** and **7f** exhibited higher inhibitory activity against *E. coli* DNA gyrase in comparison with ciprofloxacin.

The antimicrobial data of synthesized compounds **(6a-6 g)** and **(7a-7 m)** showed moderate anti-microbial activity. For antibacterial strains against gram-positive bacterial strains *Staphylococcus aureus*, most compounds showed moderate activity compared to standard amoxicillin. For *Escherichia coli,* the compounds **7a-7j** were most active, and the remaining compounds were moderately active. For gram-negative bacterial strains like *Pseudomonas aeruginosa,* all the compounds showed moderate activity. For *Bacillus subtilis,* the compounds **7 g, 7 h, 7i, 6e, 6f** and **6 g** were more active than the remaining compounds. The compound **6e, 6f, 6 g** showed good activity against bacillus subtilis due to the presence of 4Br5CF3Ph, 4CF3Ph, 2CF5CF3Ph functional groups respectively. While, compound no. **7a, 7c, 7e** showed poor activity against bacillus subtilis due to their respective functional groups pH,3- FPh, 4ClPh. The compounds **7a-7i** showing good activity against E.*coli* due to presence of pH, 3FPh, 4ClPh, and 4BrPh functional groups. The functional groups $4NO_2$Ph, 2,4-FPh, $3CF_3$Ph and 4FPh in the compounds **7j, 7k, 7l, 7 m** seems responsible for their poor activity against E.*coli*.

From the series, some of the compounds were active against *E. coli* and *Bacillus subtilis*. In contrast, for the *Staphylococcus aureus* and *Pseudomonas aeruginosa,* the synthesized compounds were moderately active compared to the standard.

For antifungal strains, most of the compounds were moderately active for *the Candida albicans* strain. The compounds **7b, 7c, 7e, 7h-m** and **7c-7 g** were moderately active compared to the standard, and the remaining compounds were less active. For *C. neo-*

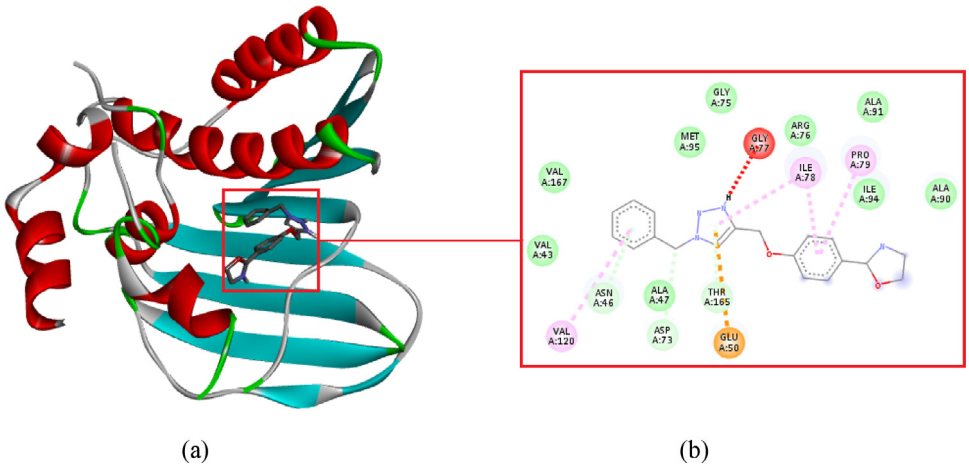


Fig. 3. 3D (a) and 2D (b) structures of Ligand **7a** docked in the active site of E. coli DNA gyrase B kinase (PDB code :1AJ6) showing amino acids interaction.

Table 4
Docking scores of reference drug and compounds (7a,7d, 7e, 7f and 7 h) with E. coli DNA gyrase B kinase.

Sr. No.	Compd. No.	Docked Scores(kcal/mol)
1	Ciprofloxacin	−7.5
2	7a	−8.4
3	7d	−8.3
4	7e	−7.7
5	7f	−7.7
6	7h	−7.4

formans and *Aspergillus niger* strains, most of the synthesized compounds were moderate to less active than the standard.

The synthesized compounds showed moderate inhibitions for M. tuberculosis $H_{37}Rv$ with **MIC** 1.6 μM, and the rest compounds show more than **MIC** 1.6 μM. Most compounds showed moderate to less inhibitions for MDR and XDR Mtb strains than the standard drugs INH, RIF, and Moxifloxacin.

3.5. Molecular docking studies of the synthesized products

The X-ray crystal structures of the E. coli DNA gyrase B kinase (PDB code: 1AJ6) were obtained from the RSCB Protein Data Bank [51]. The two proteins' structures were then prepared on the UCSF Chimera software package [52]. The structures of the proteins were prepared by removing water molecules, nonstandard naming, protein residue connectivity. The missing atoms of side chains and protein backbone were added to the protein structure before the molecular docking. The standard drugs Ciprofloxacin were accessed from PubChem [53].

The 3-D structures of the compounds **7a,7d, 7e, 7f,** and **7 h** and the standard drugs Ciprofloxacin were prepared on the Avogadro software package. Molecular docking validations were done to predict the binding energy between active sites in the E. coli DNA gyrase B kinase 1AJ6 and the synthesized products **7a,7d, 7e, 7f,** and **7 h** in comparisim to the standard Ciprofloxacin as common drug which used as a reference. From the obtained results in Table 4, compounds **7a,7d, 7e, 7f,** and **7 h**, showed promising docking results which presented good fit with the selected receptor compared with Ciprofloxacin. This has validated the antimicrobial result as highligted in Table 3 above. The compounds **7a 7d, 7e** and **7f** showed very promising docking results except for compound **7 h** which show lesser score to the reference by −0.1(kcal/mol). This lower score could be atributed to the less electronegative bromo atom attached to the compound.

4. Conclusion

In summary, herein, we established the synthesis of a series of terminal oxazoline-triazol hybrids synthesized from readily available hydroxyl ethyl benzoate for their in vitro anti-TB activity. We report here a total of 20 derivatives with oxazoline-triazole hybrids directly linked to benzene and phenyl substitutions. Due to the readily available reactants, reagents, simple reaction conditions, less harsh conditions, less time, we were able to obtain a good yield (70–95%) for all the steps and derivatives. In vitro, anti-tubercular activity data against *M. tuberculosis* $H_{37}Rv$ showed moderate inhibition (**MIC_{50}** 1.6 μM) for compounds **7a-7i**. The active compounds **7a, 7d, 7e, 7f**, and **7 h** were further tested for their DNA gyrase activity. The results showed promising DNA gyrase tendency. In vitro antimicrobial activity data reveals moderate inhibition (**MIC** 50 μg/mL) for **6e, 6f, 6 g, 7f, 7 g** and **7 h** exclusively against antibacterial strains and very less inhibitions for antifungal strains. Thus these findings can help future researchers develop novel oxazoline bases to help future researchers develop novel oxazoline-based libraries for antimicrobial and antitubercular drugs.

5. Credit author statement

Suraj R. Shinde: Design of the experiments, synthesis, isolation and draft of manuscript

Shaukatali N. Inamdar: manuscript corrections, evaluation of the studies, docking study help

Mahadev Shinde: characterization of the compounds, NMR and other results analysis

Chandrakant Pawar: support in synthesis

Babita Kushwaha: support in writing the manuscript

Vincent A. Obakachi: docking, separation of compounds

Afsana Kajee: Anti-tuberculosis studies

Ruchika Chauhan: Antimicrobial studies

Rajshekhar Karpoormath: Guidance in all respect starting from design to communication

Declaration of Competing Interest

Financial contributions to the work being reported are clearly acknowledged and there are no potential conflicts of interest.

Data Availability

No data was used for the research described in the article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2022.134243.

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