SYNTHESIS, DIAGNOSIS, BIOLOGICAL ACTIVITY FOR SOME SUBSTITUTED TETRAZOLE DERIVED FROM METHYL 2-(*p*-TOLYL) BENZOATE

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Abstract: *Drug-resistant bacteria—including multidrug-resistant strains, persistent infections, and emerging pathogens—continue to spread rapidly, posing a serious threat to global public health. This situation highlights the urgent need to develop new, highly effective antimicrobial agents.*
In this study, a series of tetrazole derivatives were synthesized as potential antibiotic candidates. The compounds were prepared via the conventional reaction of hydrazones with sodium azide, using 4'-methyl-[1,1'-biphenyl]-2-carbohydrazide as the core scaffold.
The synthesized compounds demonstrated notable antibacterial activity, particularly against Gram-positive Staphylococcus aureus, with several derivatives surpassing the reference antibiotic amoxicillin in potency. Compounds Li4, Li7, and Li9 showed inhibition zone diameters of 29, 21, and 24 mm, respectively, compared with the control. Against Gram-negative bacteria, compounds Li4 and Li9 also exhibited high activity, with inhibition zones of 26 mm and 24 mm, respectively—again exceeding the performance of the standard antibiotic. In contrast, compounds Li1, Li2, Li3, Li5, Li6, Li7, and Li8 displayed no detectable inhibitory activity against Escherichia coli. Spectroscopic measurements such as FT-IR and ^{1}H and ^{13}C-NMR were used to confirm the formation of the prepared compounds.
Keywords: *Tetrazole, Hydrazones, Hydrazide, Biological activity.*

1. Introduction

The majority of diseases in communities and hospitals are caused by bacteria [1]. Although the significant increase in harmful bacteria that can withstand one or more medicines is a significant danger to world health, antimicrobial drugs are a useful tool for treating bacterial illnesses [2]. The prolonged use and even abuse of antibiotics, together with the dearth of novel antibacterial medicines, make this issue worse [3]. About 30 novel antibacterial chemical medications are now being developed for clinical use [4], but this is still not enough. The identification of bioactive compounds with novel or enhanced pharmacological characteristics has benefited greatly from the development of heterocycles in recent years [5]. Medicinal chemists are particularly interested in nitrogen-containing heterocyclic molecules since they have shown great promise [6]. Tetrazole is enhanced in its physicochemical characteristics and binding ability to biomolecular targets by exerting a variety of non-covalent interactions, including hydrogen bonding and dipole-dipole interactions [7]. Anticancer, antifungal, antiangiogenic, antiviral, antimalarial, and antibacterial actions [8-12] are only a few of the many biological and pharmacological characteristics that tetrazole derivatives possess. Tetrazole derivatives are undoubtedly essential to the creation of novel medications. Molecules can decrease toxicity, improve effectiveness, and overcome medication. Several of the hybrid compounds' resistances are presently undergoing clinical evaluation for the purpose of treatment of different illnesses [13]. Tetrazoles combined with other antibacterial drug molecules may therefore yield novel antibacterial options that are potentially effective against infections that are both drug-sensitive and drug-resistant. Over the past 20 years, a lot of work has been done to create and identify tetrazole molecules as potential antibacterial agents. Tedizolid is the most well-known example of a tetrazole chemical that has already been employed as an antibacterial agent [14].

The goal of the current work is to create novel tetrazole derivatives and evaluate how sensitive they are to bacteria.

2. Experimental part

Chemicals used. The chemicals used were made by Fluka, Merck, BDH Thomas, and Aldrich.

Devices used. The Electrothermal Melting Apparatus 9300 was used to measure melting points. FT-IR 8400S 400–4000 cm^{-1} scale Shimadzu spectrophotometer via KBr disc. At 400 MHz, Bruker equipment generated ^{13}C-NMR and ^{1}H-NMR spectra. Thin Layer Chromatography (TLC) was performed using Fluka silica gel plates that were 0.2 mm thick and activated with fluorescent silica gel G. The findings were seen using UV light. The microbiological medium used in the study was sterilized using the Raypa steam sterilizer (Spain) autoclave at the Advanced Microbiology Research Laboratory of the University of Tikrit. In the same lab, Petri dishes utilized for the microbiological investigation were incubated using a Heraeus D-63450 incubator (Germany). The output and an 808-nanometer wavelength were used in visible laser tests.

2.1. Preparation of 4'-methyl-[1,1'-biphenyl]-2-carbohydrazide (Li_1). 0.001 mol of methyl 2-(p-tolyl) benzoate dissolved in a very small amount of ethanol was mixed with 0.001 mol of 80% aqueous hydrazine solution in a round-bottomed flask. The mixture was heated for 10 h before the solution was concentrated and recrystallized from ethanol [15].

White (87%); mp = 83–85 °C. *Elemental analysis:* $C_{14}H_{14}N_2O$; IR (KBr) ν(cm^{-1})= 3246, 3219 (NH_2), 3151 (NH), 3064 (Ar–CH), 2951 and 2926 (Ar–$CH_{Aliphatic}$), 1631 (C=O), 1577 and 1508 (C=C) cm^{-1}. *^{1}H-NMR (DMSO-d^6, 300 MHz)*: δ(ppm) = 10.37 (1H, s, NH), 8.19-7.27 (8H, m, Ar–H), 6.18 (2H, s, NH_2), 2.73 (3H, s, CH_3). *^{13}C-NMR (DMSO-d^6 , 75 MHz)*: δ(ppm)= 161.52 (C=O), 141.33-123.80 (Ar–C=C), 31.25 (CH_3).

2.2. Preparation of hydrazone (Li_2-Li_5). 0.001 mol of methyl 2-tolyl benzo hydrazide dissolved in a small amount of ethanol was mixed with 0.01 mol of benzaldehyde substitute and heated for 5 hr. The solution was cooled, concentrated; the precipitate was filtered dried and recrystallized from ethanol after crushed ice was added [16].

N'-(4-bromobenzylidene)-4'-methyl-[1,1'-biphenyl]-2-carbohydrazide (Li_2). Yellow (83%); mp = 153–155°C. *Elemental analysis:* $C_{21}H_{17}BrN_2O$; IR (KBr) ν(cm^{-1}) = 3182 (NH), 3049 (Ar–CH), 2943,2901 ($CH_{ALiphatic}$), 1651 (C=O), 1606 (C=N), 1566 and 1485 (C=C), 1280 (C–N), 1087 (N–N), 553 (C–Br)$^{cm-1}$. *^{1}H-NMR (DMSO-d^6, 300 MHz):* δ(ppm) = 9.72 (s, 1H, NH), 8.36 (s, 1H, CH=N), 7.68-6.87 (12H, m, Ar–H), 3.18 (3H, s, CH_3). *^{13}C-NMR (DMSO-d^6, 75 MHz)*: δ(ppm) = 167.10 (C=O), 155.83 (C=N), 144.50-120.11 (Ar–C=C), 31,21 (CH_3).

N'-(4-chlorobenzylidene)-4'-methyl-[1,1'-biphenyl]-2-carbohydrazide (Li_3). Light Yellow (81%); mp = 151–153 °C. *Elemental analysis:* $C_{21}H_{17}ClN_2O$; IR (KBr) ν(cm^{-1})=3179(NH), 3034 (Ar–CH), 2925 and 2872 ($CH_{ALiphatic}$), 1655 (C=C), 1610 (C=N), 1561 and 1485 (C=C), 1264 (C–N), 1090 (N-N), 721 (C–Cl) cm^{-1}. *^{1}H-NMR (DMSO-d^6, 300 MHz):* δ(ppm) = 9.27 (s, 1H, NH), 8.25 (s, 1H, CH=N), 7.81-7.01 (12H, m, Ar–H), 3.27 (3H, s, CH_3); *^{13}C-NMR (DMSO-d^6,75MHz):* δ(ppm) = 164.04 (C=O), 151.48 (C=N), 148.01-121.03 (Ar–C=C), 28.69 (CH_3).

4'-methyl-N'-(4-nitrobenzylidene)-[1,1'-biphenyl]-2-carbohydrazide (Li_4). Light Brown (79%); mp = 164–166°C. *Elemental analysis*: $C_{21}H_{17}N_3O_3$; IR (KBr) ν(cm)= 3190 (NH),3076 (Ar–CH), 2945,2865 ($CH_{ALiphatic}$), 1660 (C=O), 1601 (C=N) , 1556,1474 (C=C) , 1251 (C–N), 1123 (N–N), 1231,1515 (NO_2) cm^{-1}; ^{1}H-NMR (DMSO-d^6, 300 MHz): δ(ppm) = 9.03 (s, 1H, NH), 8.42 (s, 1H, CH=N), 7.95-7.12 (12H, m, Ar–H), 2.94 (3H, s, CH_3); ^{13}C-NMR (DMSO-d^6, 75 MHz): δ(ppm) = 163.54 (C=O), 150.98 (C=N), 143.76-122.16 (Ar–C=C), 29.92 (CH_3).

4'-methyl-N'-(4-methylbenzylidene)-[1,1'-biphenyl]-2-carbohydrazide (Li_5). Yellow (85%); mp = 159–161 °C. *Elemental analysis:* $C_{22}H_{20}N_2O$; IR (KBr) ν(cm^{-1})= 3210 (NH),3085 (Ar–CH), 2972,2907 ($CH_{ALiphatic}$), 154 (C=O), 1603 (C=N), 1534,1492 (C=C) , 1262 (C–N), 1116 (N–N) cm^{-1}. *^{1}H-NMR(DMSO-d^6,300MHz):* δ(ppm)= 8.88 (s, 1H, NH), 8.19 (s, 1H, CH=N), 7.87-

6.96 (12H, m, Ar–H), 3.36 (3H, s, CH_3), 2.53 (3H, s, CH_3). *^{13}C-NMR (DMSO-d^6, 75 MHz)*: δ(ppm)= 166.01 (C=O), 149.75 (C=N), 140.68-120.61 (Ar–C=C), 28.56 (CH_3), 23.37 (CH_3).

2.4. Preparation of Tetrazole (Li$_6$-Li$_9$). 0.0001 mol of the prepared hydrazone (Li_2-Li_5) was dissolved in 10 mL of THF, and 0.0001 mol of sodium azide (NaN_3) was dissolved in the same solvent. The reaction was kept in an ice bath for 20 min, and then the temperature was increased for 8 hr. Crushed ice was added once the solution had cooled to room temperature, and the precipitate was filtered, dried, and recrystallized in ethanol [17].

N-(5-(4-bromophenyl)-4,5-dihydro-1H-tetrazol-1-yl)-4'-methyl-[1,1'-biphenyl]-2-carboxamide (Li$_6$). White (70%); mp = 198-191 °C. ***Elemental analysis:*** $C_{21}H_{18}BrN_5O$; IR (KBr) ν(cm^{-1})= 3215 (NH), 3065 (Ar–CH), 2921 and 2878 ($CH_{ALiphatic}$), 1664(C=O), 1538 and 1502 (C=C), 1444 (N=N), 1243 (C–N), 1078 (N–N), 915 (C–F), 767 (C–Cl) cm^{-1}. *^{1}H-NMR (DMSO-d^6, 300 MHz)*: δ(ppm)= 8.56 (1H, s, NH), 7.97-6.96 (12H, m, Ar–H), 5.76 (1H, s, $CH_{Tetrazole}$), 2.38 (3H, s, CH_3), 2.71 (1H, s, $NH_{Tetrazole}$). *^{13}C-NMR (DMSO-d^6, 75 MHz): δ(ppm)* = 163.14 (C=O), 149.04 (C=N), 138.63-125.23 (Ar–C=C), 104.39 (C–H), 27.93 (CH_3).

N-(5-(4-chlorophenyl)-4,5-dihydro-1H-tetrazol-1-yl)-4'-methyl-[1,1'-biphenyl]-2-carboxamide (Li$_7$). Yellow (78%); mp = 201-203 °C. ***Elemental analysis:*** $C_{21}H_{18}ClN_5O$; IR(KBr) ν(cm^{-1})= 3211 (NH), 3030 (Ar–CH), 2916 and 2862 ($CH_{Aliphatic}$), 1668 (C=O), 1588,1510 (C=C), 1464 (N=N), 1263 (C–N), 1109 (N–N), 711 (C–Cl) cm^{-1}. *^{1}H-NMR (DMSO-d^6, 300MHz)*: δ(ppm) = 9.27 (1H, s, NH), 8.00-7.01 (12H, m, Ar–H), 6.14 (1H, s, $CH_{Tetrazole}$), 3.69 (3H, s, CH_3), 2.14 (1H, s, $NH_{Tetrazole}$); ^{13}C-NMR (DMSO-d^6, 75 MHz): δ(ppm) = 167.34 (C=O), 142.12-123.77 (Ar–C=C), 106.44 (C–H), 36.24 (CH_3).

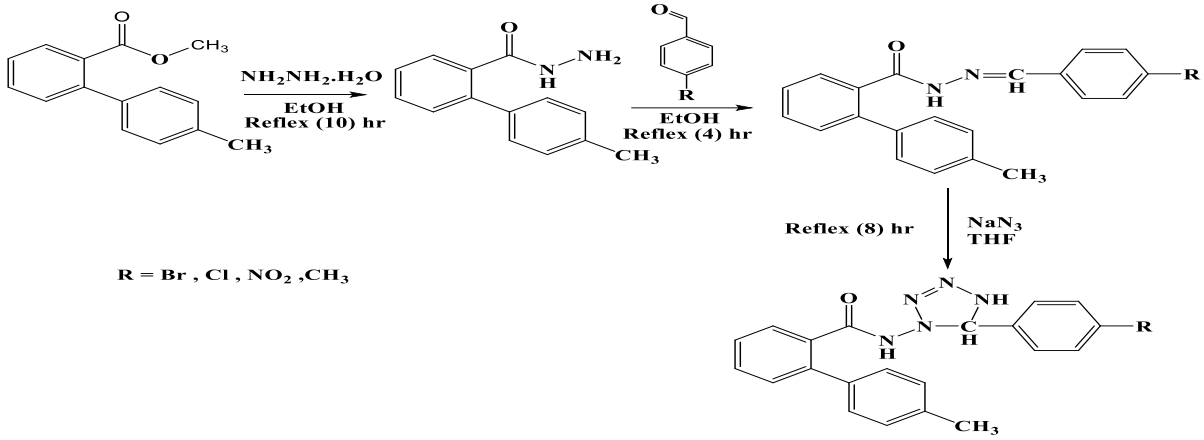
4'-methyl-N-(5-(4-nitrophenyl)-4,5-dihydro-1H-tetrazol-1-yl)-[1,1'-biphenyl]-2-carboxamide (Li$_8$). Brown(76%); mp = 193-196 °C. ***Elemental analysis:*** $C_{21}H_{18}N_6O_3$; IR(KBr) ν(cm^{-1})= 3232 (NH), 3066 (Ar–CH), 2924 and 2847 ($CH_{Aliphatic}$), 1668 (C=O), 1521 and 1494 (C=C), 1446 (N=N), 1290 (C–N), 1107 (N-N) cm^{-1}. *^{1}H-NMR (DMSO-d^6,300MHz)*: δ(ppm) = 8.86 (1H, s, NH), 7.74-6.99 (12H, m, Ar–H), *5.8*4 (1H, s, $CH_{Tetrazole}$), *2.8*9 (3H, s, CH_3), 2.*06* (1H, s, $NH_{Tetrazole}$). *^{13}C-NMR (DMSO-d^6, 75 MHz)*: δ(ppm) = 168.10 (C=O), 145.74-120.27 (Ar–C=C), 102.88 (C–H), 36.74 (CH_3).

4'-methyl-N-(5-(p-tolyl)-4,5-dihydro-1H-tetrazol-1-yl)-[1,1'-biphenyl]-2-carboxamideformideformazan (Li$_9$). White (74%); mp = 207-209 °C. ***Elemental analysis:*** $C_{22}H_{21}N_5O$; IR (KBr) ν(cm-1)= 3230 (NH),*30*55 (Ar–CH), 2916 and 2864 ($CH_{Aliphatic}$), 1660 (C=O), 1500 and 1477 (C=C), 1450 (N=N), 1286 (C–N), 1080(N–N) cm^{-1}; *^{1}H-NMR(DMSOd^6, 300MHz)*: δ(ppm)= 9.10 (1H, s, NH), 8.02-7.08 (12H, m, Ar–H), 5.94 (1H, s, $CH_{Tetrazole}$), 3.09 (3H, s, CH_3),*2.8*8 (3H, s, CH_3), 2.15 (1H, s, $NH_{Tetrazole}$). *^{13}C-NMR (DMSO-d^6, 75 MHz)*: δ(ppm) = 168.73 (C=O), 146.11-125.45 (Ar–C=C), 99.69 (C–H), 42.14 (CH_3), 31.85 (CH_3).

2.5. Biological activity study: Two types of bacteria, Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, were taken from the central laboratory of Tikrit University. 20 g of each was dissolved in half a liter of water to prepare Mueller-Hinton agar medium, stirred until dissolved, and then autoclaved at 1.5 bar at 120°C for 14 minutes. After cooling, the mixture was poured into a Petri dish and dried at 25°C [18]. Solutions of the compounds were then prepared in DMSO at three concentrations (25%, 50%, and 100%) mg/ml of each compound. Petri dishes containing the culture medium were inoculated by streaking the dried bacterial suspension in three directions to ensure uniform distribution. Three wells, each 6 mm in diameter, were then punched into the agar using a sterile cork borer, and the test solutions were introduced into these wells. The inoculated plates were incubated at 37°C for 24 hours in a controlled container, after which the zones of inhibition were measured using a millimeter ruler. Amoxicillin, an antibiotic, served as the control sample [19, 20].

3. Results and discussions

Scheme 1 shows the method of preparing the series of compounds using methyl 4'-methyl-[1,1'-biphenyl]-2-carboxylate as a nucleus in its preparation, where it was reacted with aqueous hydrazine to form hydrazide, which is considered the basis for preparing many heterocyclic compounds, and then reacting the hydrazide with benzaldehyde substitutes to form hydrazones in the presence of ethanol as a solvent, and reacting it with sodium azide to form new five-membered rings called tetrazoles in the presence of THF as a solvent.



Scheme 1. Series of prepared compounds (Li_1-Li_9)

3.1. Characterization prepared compounds (Li_1-Li_9). *In the first step*, 4'-methyl-[1,1'-biphenyl]-2-carbohydrazide (Li_1) was synthesized by nucleophilic attack of the electron pair on hydrazine hydrate at the carbonyl carbon of the corresponding ester. FT-IR analysis showed a decrease in the stretching frequency of the carbonyl group (C=O), appearing at 1631 cm^{-1}, confirming its interaction with the amino group, which reduces the bond force constant. Additional diagnostic bands appeared at 3246 and 3219 cm^{-1}, corresponding to the (NH_2) group, and at 3151 cm^{-1}, corresponding to the (NH) group—clear spectral evidence of hydrazide formation.

^{1}H-NMR spectroscopy revealed the disappearance of the ester methyl (CH_3) resonance and the appearance of two new singlets characteristic of hydrazide: one at 10.37 ppm corresponding to the (NH) proton and another at 6.18 ppm corresponding to the (NH_2) protons. In the ^{13}C-NMR spectrum, the carbonyl carbon signal appeared downfield at 163.14 ppm, reflecting reduced bond strength due to the electron-donating amine group [14]. (Fig. s 1–3).

In the second step, hydrazone derivatives (Li_2–Li_5) were synthesized by condensation of the hydrazide with substituted benzaldehydes via nucleophilic attack of the hydrazide nitrogen on the aldehyde carbonyl, followed by water elimination to form a characteristic azomethine (C=N) linkage. FT-IR spectra confirmed the transformation, showing the disappearance of the (NH_2) bands at 3246 and 3219 cm^{-1} and the emergence of a new azomethine band (C=N) in the range 1601–1610 cm^{-1}, consistent with hydrazone formation.

^{1}H-NMR spectra showed the disappearance of the (NH_2) resonance previously observed at 6.18 ppm and the appearance of a new signal in the range 8.19–8.42 ppm corresponding to the azomethine proton. Similarly, the ^{13}C-NMR spectra revealed new signals at 149.75–155.83 ppm attributable to the azomethine carbon, further confirming the successful synthesis of hydrazones [21] (Fig. s 4–6).

The third step involves the synthesis of tetrazole derivatives (Li_6–Li_9) by cleavage of the azomethine bond (C=N) and subsequent linkage with an azide group. In this process, the sodium ion is expelled, leading to the formation of a heterocyclic five-membered ring composed of four nitrogen atoms and one carbon atom.

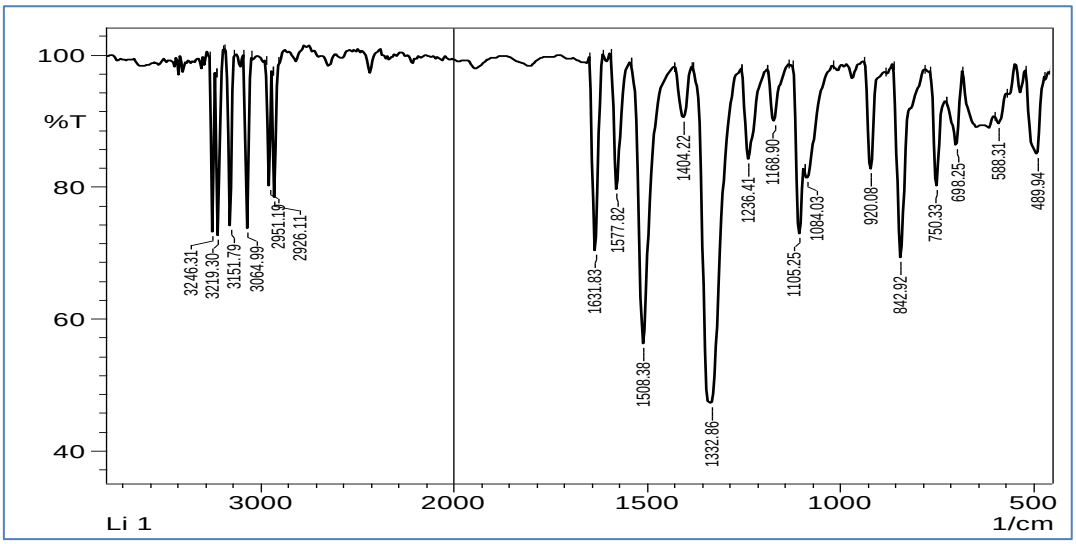


Fig. 1. FT-IR spectrum of (Li $_1$)

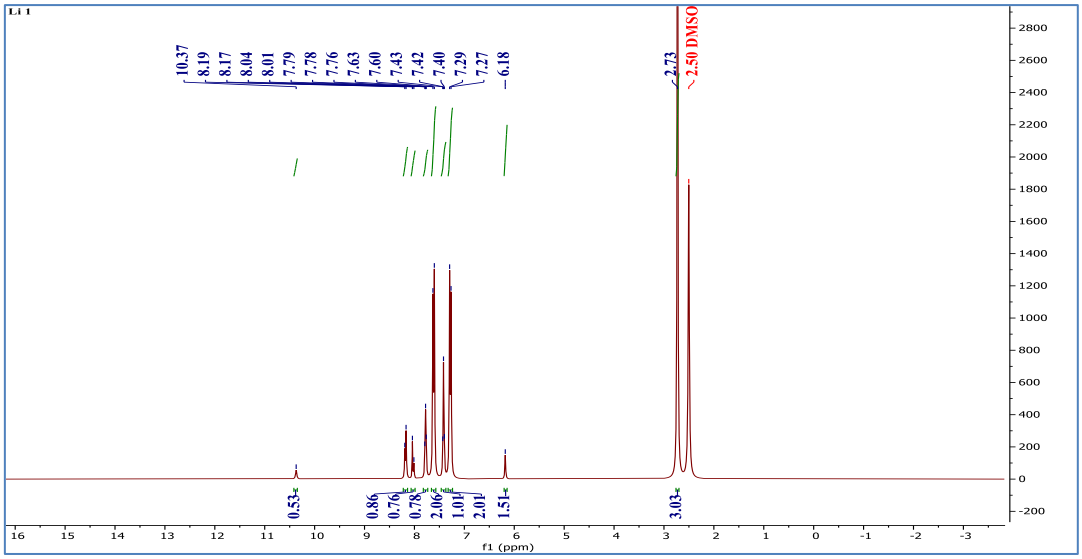


Fig. 2. ^{1}H-NMR spectrum of (Li $_1$)

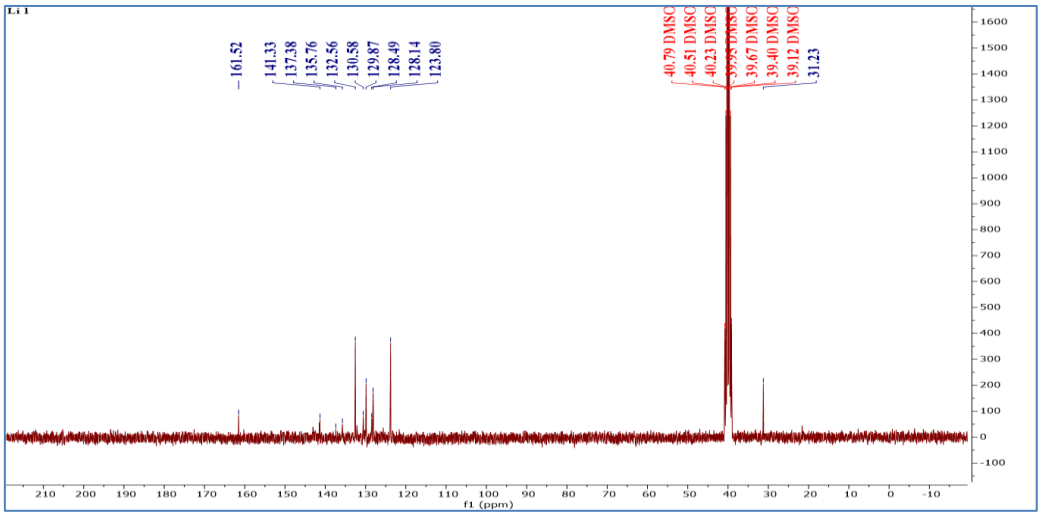


Fig. 3. ^{13}C-NMR spectrum of (Li $_1$)

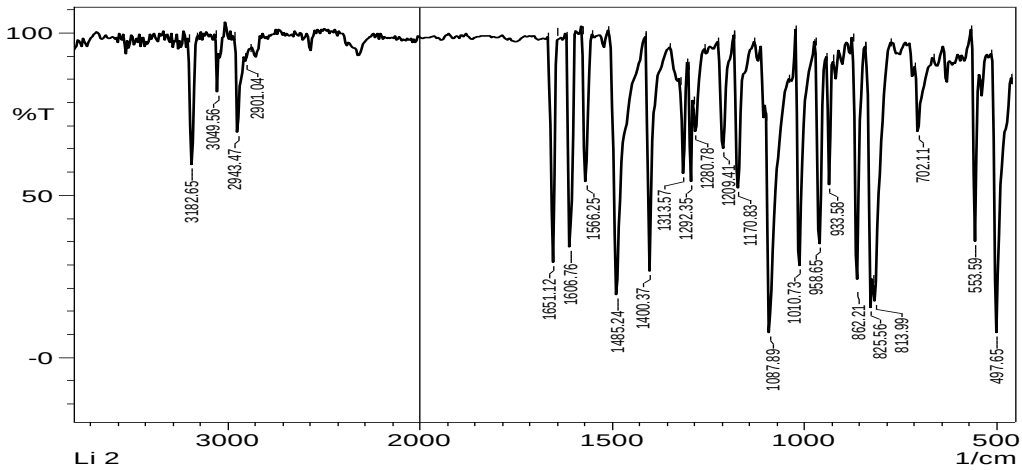


Fig. 4. FT-IR spectrum of (Li_2)

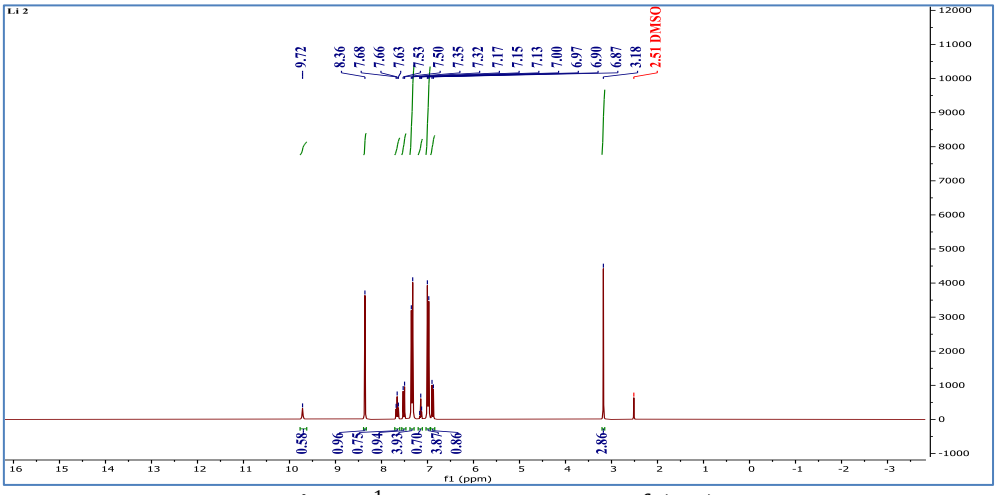


Fig. 5. ^{1}H-NMR spectrum of (Li_2)

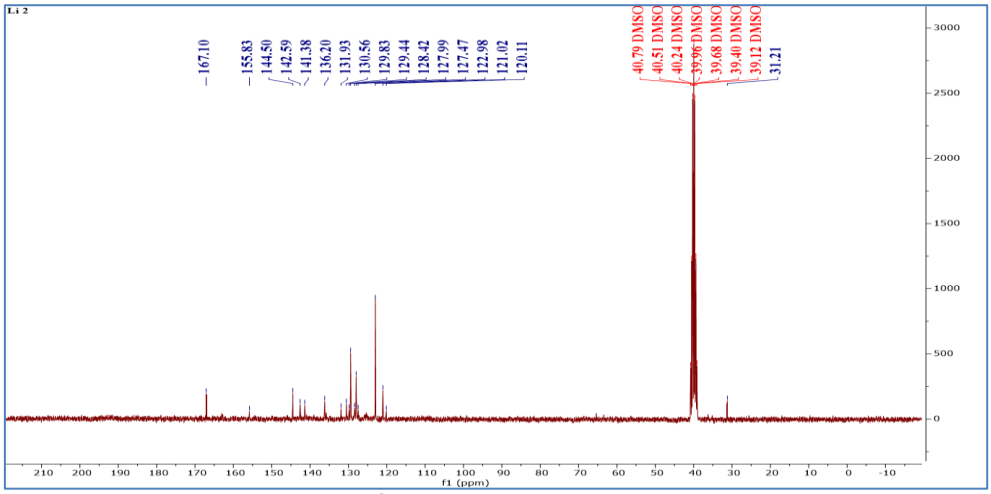


Fig. 6. ^{13}C-NMR spectrum of (Li_2)

The formation of the target compounds was confirmed by several spectroscopic techniques:

- **FT-IR spectroscopy** showed the disappearance of the azomethine group signal characteristic of hydrazine, together with the appearance of a distinctive tetrazole absorption band in the range of 1464–1444 cm^{-1}.
- **^{1}H-NMR spectroscopy** indicated the disappearance of the azomethine proton signal (8.19–8.42 ppm) and the appearance of two new signals: one corresponding to the CH proton of the

tetrazole ring (6.14–5.74 ppm) and another corresponding to the NH group of the tetrazole ring (2.06–2.71 ppm).

- **^{13}C-NMR spectroscopy** confirmed the absence of the C=N carbon resonance and the appearance of a new signal in the range of 99.69–106.44 ppm, attributable to the CH carbon in the tetrazole ring.

Taken together, these diagnostic features provide strong evidence for the successful synthesis of the tetrazole derivatives [22], as illustrated in Fig. s 7–15.

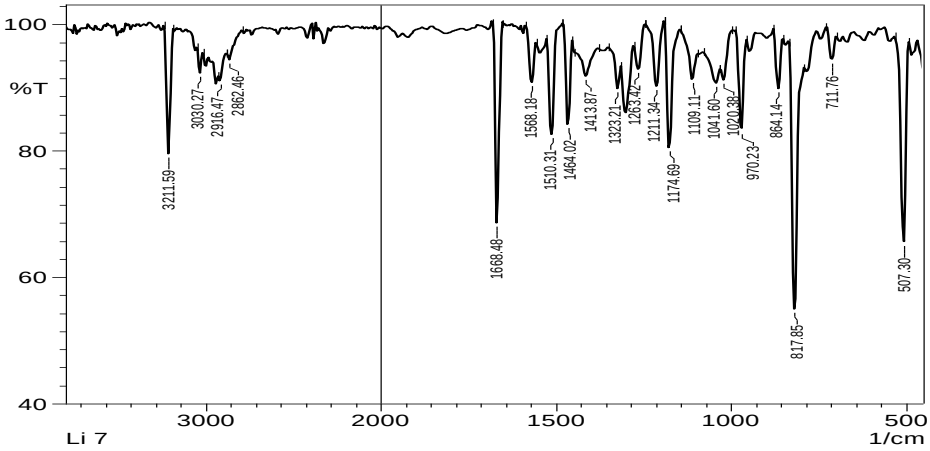


Fig. 7. FT-IR spectrum of (Li_7)

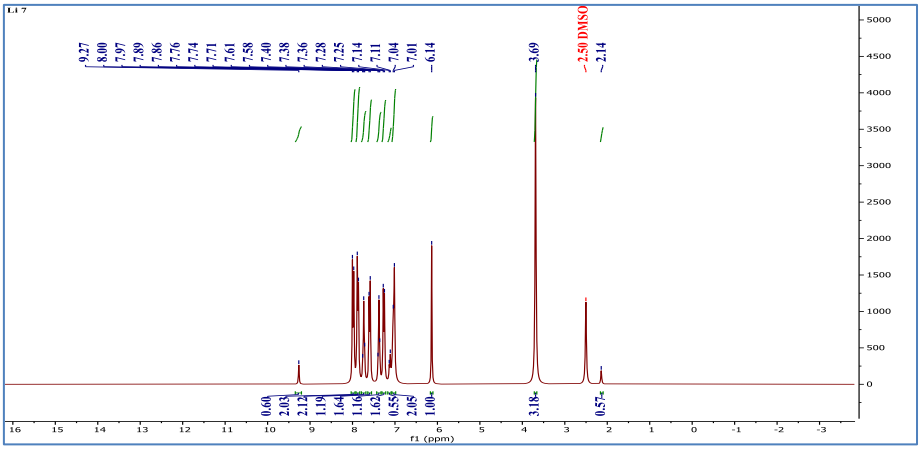


Fig. 8. ^{1}H-NMR Spectrum of (Li_7)

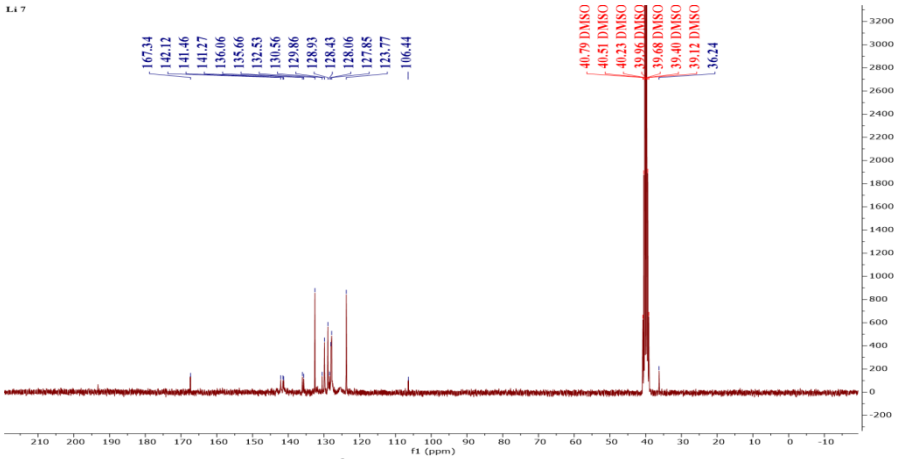


Fig. 9. ^{13}C-NMR Spectrum of (Li_7)

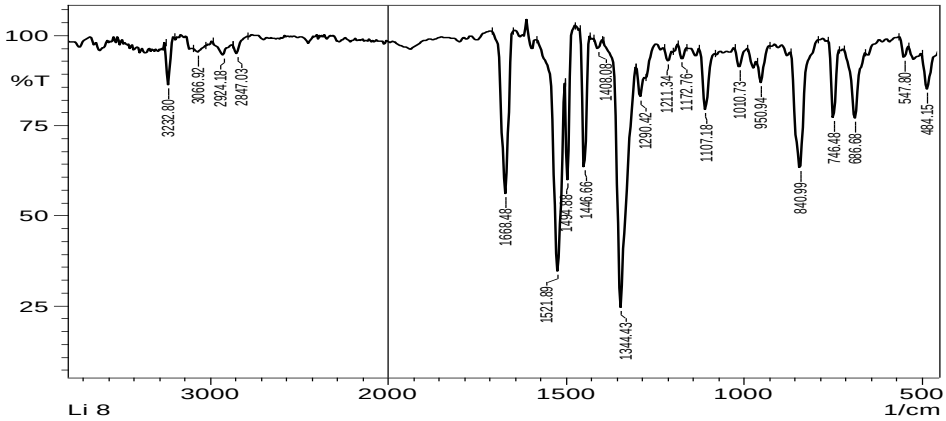


Fig. 10. FT-IR spectrum of (Li_8)

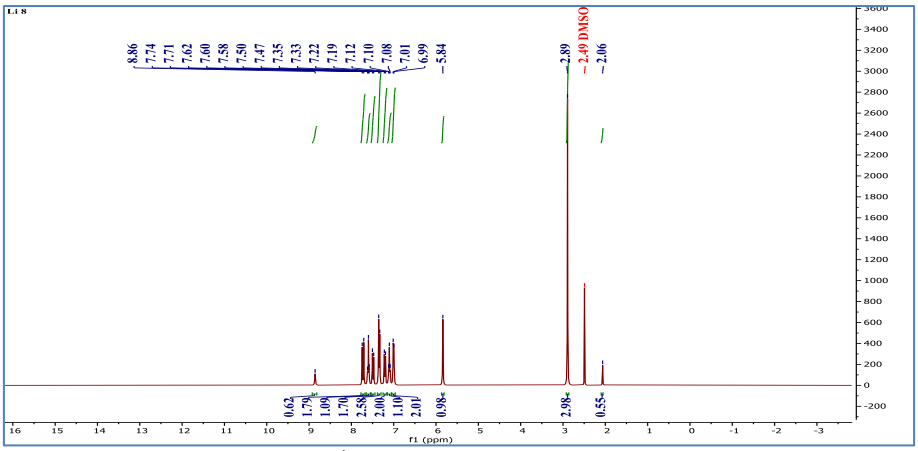


Fig. 11. ^{1}H-NMR Spectrum of (Li_8)

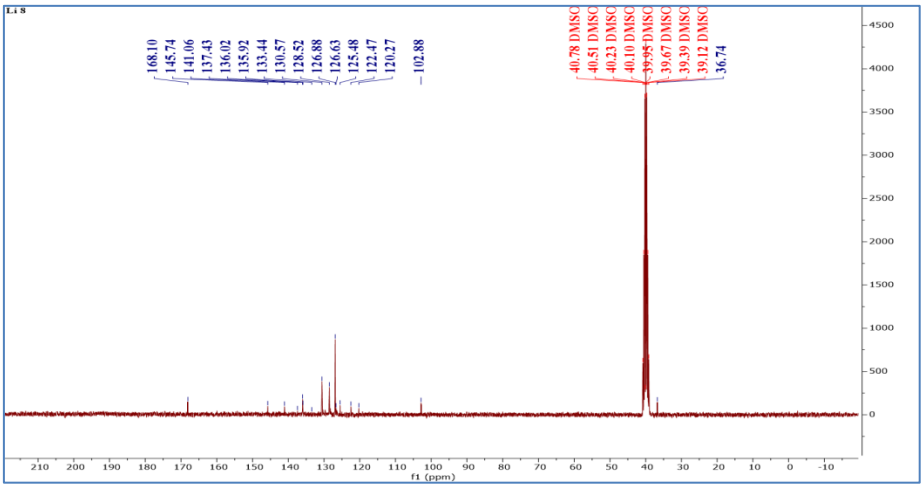


Fig. 12. ^{13}C-NMR Spectrum of (Li_8)

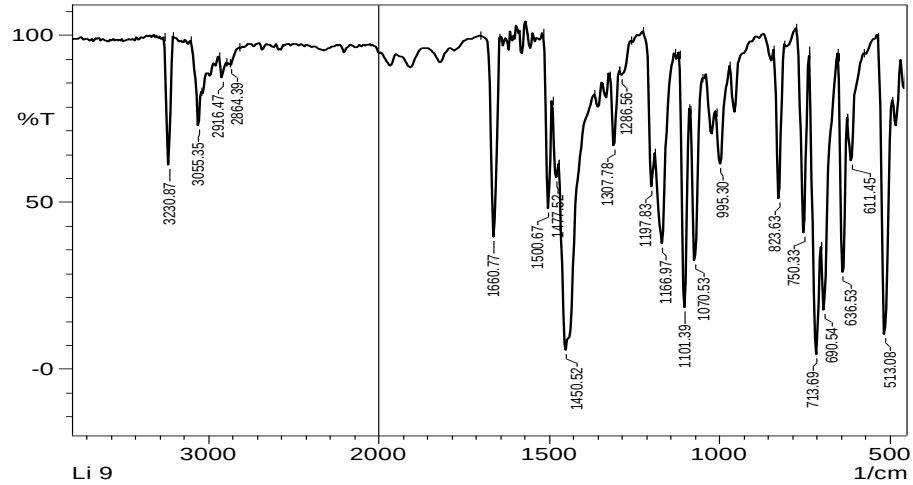


Fig. 13. FT-IR spectrum of (Li_9)

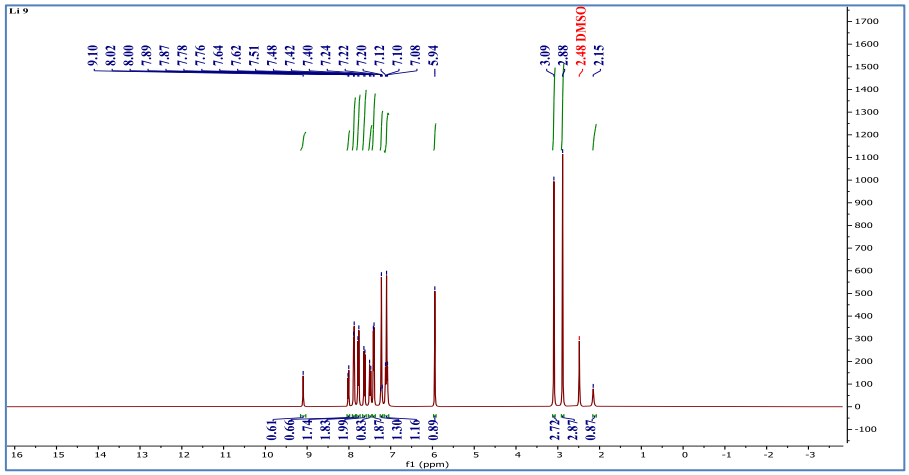


Fig. 14. ^{1}H-NMR spectrum of (Li_9)

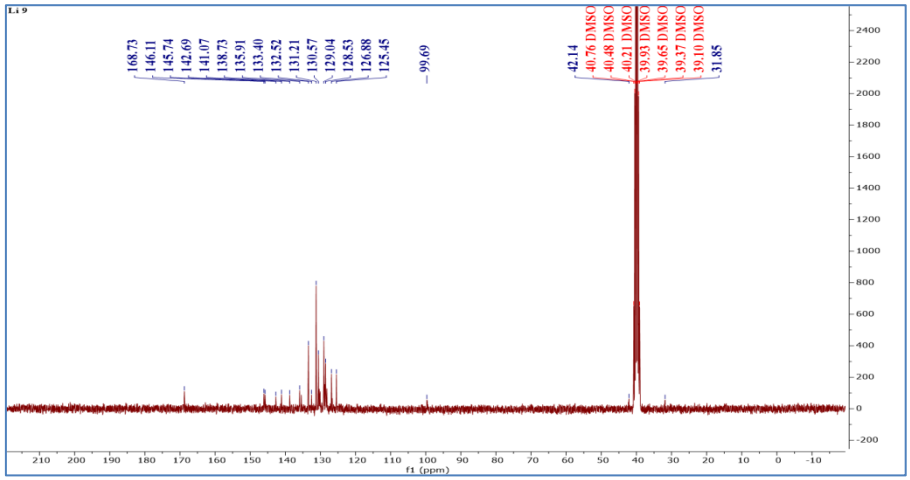


Fig. 15. ^{13}C-NMR Spectrum of (Li_9)

3.2. Analysis of certain produced chemicals via Biological Activity. The inhibition diameters of the synthesized compounds were measured in millimeters. The compounds exhibited pronounced activity against *Staphylococcus aureus* (Gram-positive) but were largely ineffective against *Escherichia coli* (Gram-negative). The only exceptions were compounds (Li_4) and (Li_9), which produced inhibition zones of approximately 24 mm and 26 mm, respectively, against *E. coli*.

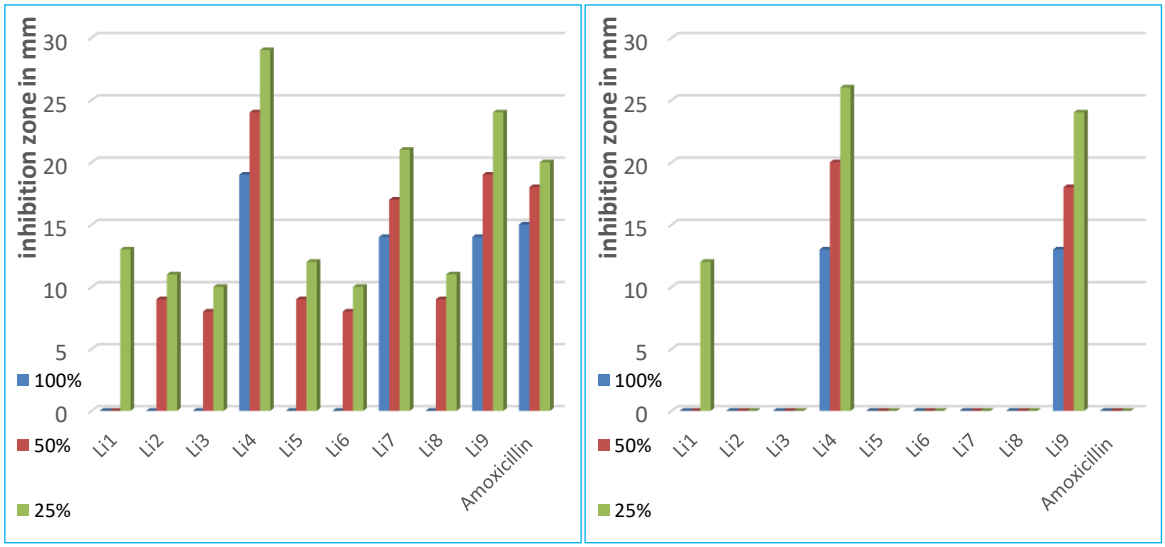
This limited activity suggests either intrinsic ineffectiveness of most compounds against Gram-negative bacteria or insufficient compound concentrations [23, 24].

Against *S. aureus*, nearly all compounds demonstrated significant antibacterial activity. Compound (Li_4) exhibited the strongest effect at all tested concentrations, reaching an inhibition diameter of 29 mm at 25% concentration. Compounds (Li_9) and (Li_7) also showed substantial activity, with inhibition zones of 21 mm and 24 mm, respectively. These results indicate that (Li_4, Li_7, and Li_9) could serve as potential candidates for future antibiotic development [25, 26].

A clear dose–response relationship was observed: antibacterial effectiveness increased with compound concentration, peaking at 25%. Most compounds were more active against Gram-positive than Gram-negative bacteria, which is consistent with structural differences in their cell walls. Gram-negative bacteria possess an additional lipid-rich outer membrane that hinders penetration of many chemical agents, making them inherently more resistant than Gram-positive species [27–31]. These findings are summarized in Table 1 and Scheme 2, and illustrated in Fig. s 16 and 17.

Table 1. Antibacterial activity of the synthesized compounds (inhibition zone in *mm*)

Comp. No	***Staphylococcus aureus* +*ve***			***Escherichia coli* – *ve***		
	100%	50%	25%	100%	50%	25%
Li_1	N. a	N. a	13	N. a	N. a	12
Li_2	N. a	9	11	N. a	N. a	N. a
Li_3	N. a	8	10	N. a	N. a	N. a
Li_4	19	24	29	13	20	26
Li_5	N. a	9	12	N. a	N. a	N. a
Li_6	N. a	8	10	N. a	N. a	N. a
Li_7	14	17	21	N. a	N. a	N. a
Li_8	N. a	9	11	N. a	N. a	N. a
Li_9	14	19	24	13	18	24
Amoxicillin	15	18	20	N. a	N. a	N. a



Scheme 2. Inhibitory activity of (Li_1-Li_9) for *Staphylococcus aureus* and *Escherichia coli*

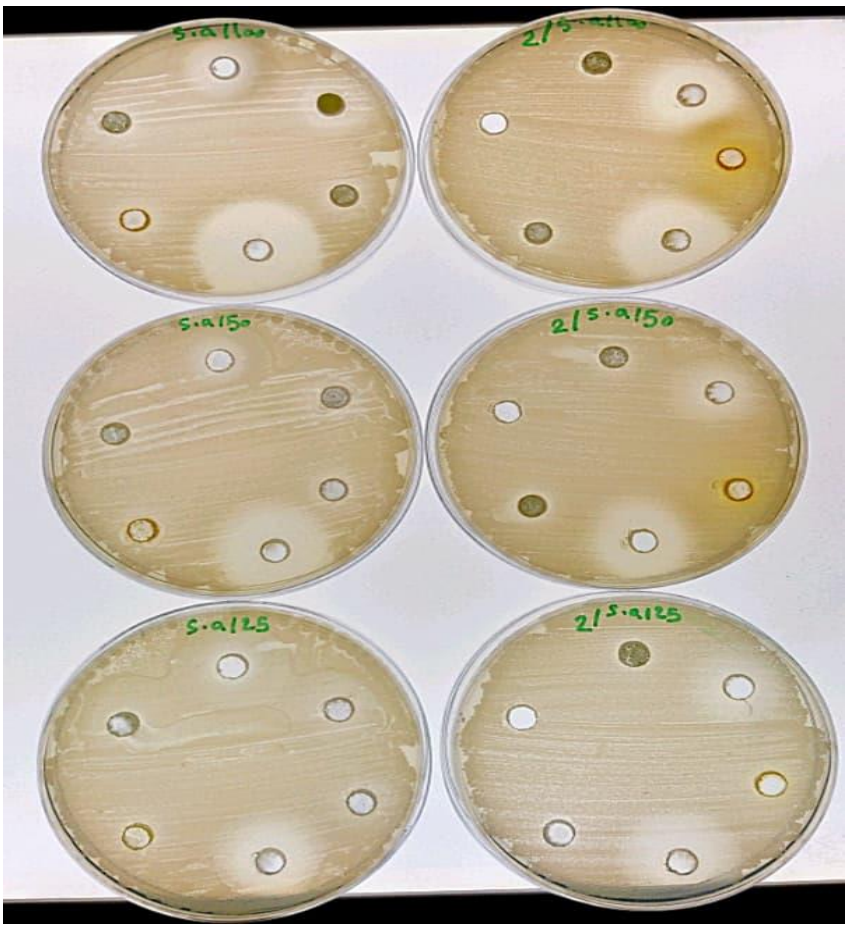


Fig. 16. Activity of the prepared compounds against *Staphylococcus aureus* bacteria

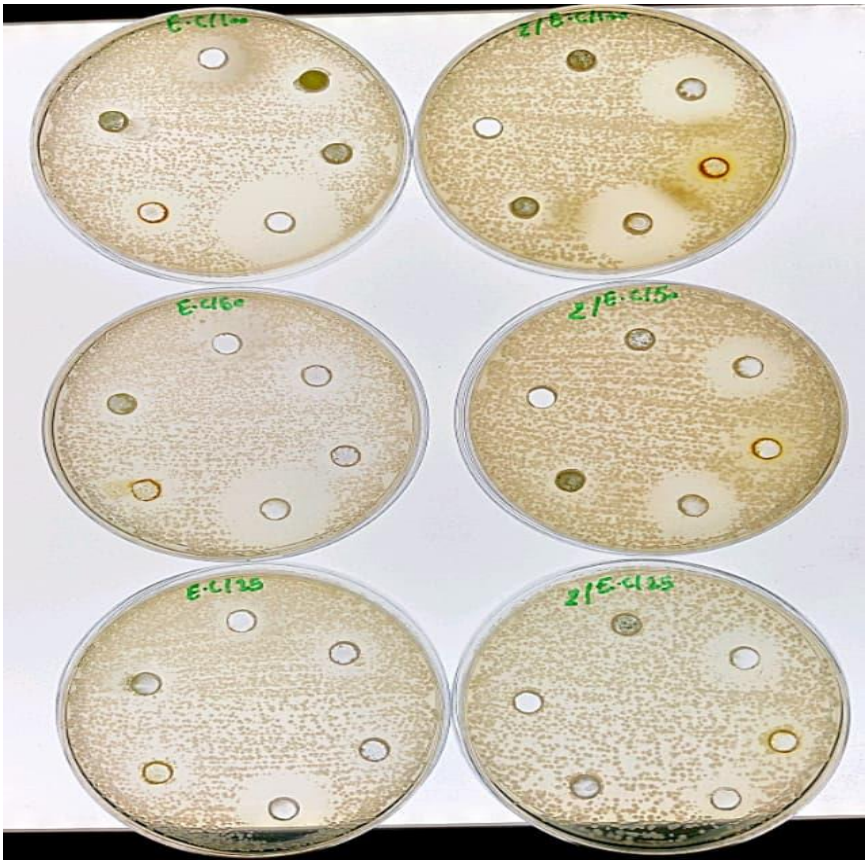


Fig. 16. Activity of the prepared compounds against *Escherichia coli* bacteria

4. Conclusions

The reaction of azomethine groups with functionalized solvents leads to the formation of five-membered tetrazole rings, which are well known for their antibacterial activity. The synthesized compounds exhibited higher inhibitory efficiency than both their parent nuclei and the reference antibiotic used as a control. This highlights their potential as future antibacterial agents.

The compounds demonstrated stronger activity against Gram-positive bacteria, with significant inhibition observed in several cases.

Spectroscopic analyses—including infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), and carbon-13 nuclear magnetic resonance (^{13}C-NMR)—confirmed the structural accuracy and validity of the synthesized products.

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