Synthesis, Characterization, and Antimicrobial Activity of Some New Tetrazole Derivatives from Hydrazones

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ABSTRACT: *In this investigation, a combination of Z_1-Z_5 hydrazone compounds is obtained from the reaction between 4-methylbenzohydrazide and aryl-aldehydes. A subsequent unique synthetic approach to the preparation of di-substituted tetrazoles T_6-T_{10} was achieved through a 1,3- dipolar cycloaddition of sodium azide and prepared hydrazone compounds in ethyl alcohol. Results have been verified by Fourier-Transform InfraRed (FT-IR)spectroscopy, ^{1}H and ^{13}C-Nuclear Magnetic Resonance (NMR) spectroscopy, and mass spectrometry. The activity of anti-microbial screening has shown that (Z_1–Z_6 and T_{10}) presented antifungal activity. The other tested Z_2 compound was found to exhibit good antibacterial activity, while the other tested compounds revealed low antibacterial activity.*

KEYWORDS: *Syntheses; Hydrazides; Tetrazole; Antimicrobial activity; Hydrazones.*

INTRODUCTION

The tetrazole motif is well-known and widely used in compounds dedicated to various features of science and life [1]. The bioisosterism to carboxyl and amide groups, among other things, has piqued the interest of this five-membered aromatic heterocycle in a growing number of studies over the last few years. The most important area of tetrazole research is in medicine in general, followed by coordination chemistry and materials chemistry [2].

Hydrazones are a special group of compounds in the Schiff's base family that are important for drug design due to their broad spectrum of pharmacological action, as possible ligands for metal complexes, organocatalysis, and also for the synthesis of heterocyclic compounds [3]. One of the most important chemical compounds is sodium azide, which has been used in a variety of applications, including its effect on incubation [4]. Due to its significance, it was used in the preparation of compounds known as tetrazoles [5]. For example, Tetrazoles could be produced by reacting substituted amines with triethyl orthoformate and sodium azide in dimethyl sulfoxide [6]. In addition, the 1,3-dipolar cycloaddition reaction was originally used to synthesize the tetrazole ring by imine as a 1,3-dipolarphile reaction with the azide group as a 1,3-dipolar molecule [7, 8]. The synthesis of tetrazole derivatives is clearly a critical task in modern medicinal chemistry [9]. Tetrazoles are a type of heterocycle that has gained popularity due to its wide range of applications. Pharmacologically, because antimicrobial studies are the most effective way to astound microbial resistance and improve effective therapies [10], some potential products must be synthesized. Tetrazole contains compounds that have been shown to have antibacterial [11] and antifungal properties [12]. *Jackman et al.*

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mentioned the robust cytotoxicity and growth-inhibitory action of Tetrazoles-containing drugs [13]. There is not much systematic report on drug candidates that have both antibacterial and antifungal activities. In this study, we report on the synthesis of tetrazole derivatives by reacting prepared hydrazone compounds with sodium azide. Compounds Z_1–Z_6 and T_{10} demonstrated antimicrobial activity against Gram-negative bacteria, Gram-positive bacteria, and fungi. The substitutions on the phenyl ring have an effect on the activity.

EXPERIMENTAL SECTION

Materials

The reagents and solvents included in this work were developed by Aldrich and Merck with no further purification.

Instrumentation

Fourier-Transform InfraRed (FT-IR) spectroscopy was measured using a KBr disc in an FTIR-84005-SHIMADZU. Melting points were measured with a thermo-scientific apparatus.^{1}H and ^{13}C-Nuclear Magnetic Resonance (NMR) spectroscopy was reported using a Bruker-400 MH_Z in dimethyl sulfoxide (DMSO)-d_6 with tetramethyl silane (TMS) as the internal standard. Electron ionization (EI)/mass spectroscopy samples were run at 70 eV using Agilent technologies. The effectiveness of reactions was tracked with Thin-Layer Chromatography (TLC).

Chemistry

General synthetic route of the hydrazones (Z_1-Z_5)

To an ethanolic solution of 2,4-dihydroxybenzaldehyde, 3-hydroxy-4-methoxy benzaldehyde, piperonal, 4-nitrobenzaldehyde, and 4-bromobenzaldehyde (0.003 mol) respectively was added glacial acetic acid (2-3) drops. Each mixture was stirred for 10 minutes and the solution of 4-Methylbenzohydrazide (0.003 mol) in 10 mL of ethyl alcohol was mixed, the reaction was heated to reflux for 5 to 12 hrs. The progress of the reaction is determined by TLC and chloroform/ethanol used as an eluent. After completion of the reaction, the product was precipitated from the reaction mixture by cooling, which was magnetically separated, washed several times by adding ethanol, dried at reduced pressure at room temperature, and then re-crystallized with ethanol to give pure product (Scheme 1). The physical and spectral data for Z_1 – Z_5 are as follows:

N'-(2,4-dihydroxybenzylidene)-4-methylbenzohydrazide (Z_1)

Yield: 70% with an R_f = 0.73 (EtOH: CHCl3, 2:8 v/v), white color, melting point 286 dec. °C, FTIR (cm^{-1}) ν of 3304 (O-H), 3224 (N-H), 3026 (CH aromatic), 1660 (C=O), 1616 (C=N), 1564 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.82 (s, 1H, NH), 11.52 (s, 1H, OH_a), 9.92 (s, 1H, OH_b), 8.48 (s, 1H, CH=N), 6.34-7.84 (m, 7H, Ar-H), 2.36 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 21 (CH_3), 102.70, 107.67, 110.56, 127.55, 129.02, 130.10, 131.40 (Ar-C), 141.86 (C-CH_3), 149.08 (CH=N), 159.53 (C-OH_b), 160.68 (C-OH_a), 162.37 (C=O), and MS: m/z =270 (M^+).

N'-(3-hydroxy-4-methoxybenzylidene)-4-methylbenzohydrazide (Z_2)

Yield: 80% with an R_f = 0.93 (EtOH: $CHCl_3$, 2:8 v/v), yellow color, melting point 246-248°C, FTIR (cm^{-1}) *ν* of 3213 (O-H), 3122 (N-H), 3043 (CH aromatic), 1643 (C=O), 1602 (C=N),1585 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.61 (s, 1H, NH), 9.28 (s, 1H, OH), 8.35 (s, 1H, CH=N), 6.93-7.85 (m, 7H, Ar-H), 3.80 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 20.93 (CH_3), 55.51 (OCH_3), 111.82, 112.38, 120.21, 127.27, 127.53, 128.91, 130.70 (Ar-C), 141.58 (CH_3), 146.89 (CH=N), 147.76 (C-OH), 149.74 (OCH_3), 162.82 (C=O), and MS: *m/z* =284 (M^+).

N'-(benzo[d][1,3] dioxol-5-ylmethylene)-4-methylbenzohydrazide (Z_3)

Yield: 65% with an R_f = 0.9 (EtOH: $CHCl_3$, 2:8 v/v); yellow color, melting point 216-218°C; FTIR (cm^{-1}) *ν* of 3230 (N-H), 3064 (CH aromatic), 1658 (C=O), 1629 (C=N), 1597 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.68 (s, 1H, NH), 8.36 (s, 1H, CH=N), 6.96-7.83 (m, 7H, Ar-H), 6.08 (s, 2H, CH_2), 2.36 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 21 (CH_3), 101.54 ($CH_{2\ ring}$), 105.08, 108.44, 123.26, 127.58, 128.81, 128.95, 130.61 (Ar-C), 141.68 (CH_3), 147.34 (CH=N), 147.98 (C-O_a) , 149.04 (C-O_b), 162.84 (C=O), and MS: *m/z* =282 (M^+).

*N'-(4-nitrobenzylidene)- 4-methylbenzohydrazide (**Z_4**)*

Yield: 75% with an R_f = 0.71 (EtOH: $CHCl_3$, 2:8 v/v); yellow color, melting point 263 dec. °C; FT-IR (cm^{-1}) *ν*

of 3213 (N-H), 3078 (CH aromatic), 1651 (C=O), 1610 (C=N), 1587 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 12.06(s, 1H, NH), 8.53 (s, 1H, CH=N), 7.32-8.29 (m, 8H, Ar-H), 2.37 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 21.02 (CH_3), 124.02, 127.75, 127.89, 129, 130.16, 140.71 (Ar-C), 142.11(CH_3), 144.91(CH=N), 147.76 (C-NO_2), 163.17 (C=O), and MS: *m/z* =283 (M^+).

N'-(4-bromobenzylidene)-4-methylbenzohydrazide (Z_5)

Yield: 80% with an R_f = 0.81 (EtOH: $CHCl_3$, 2:8 v/v); white color, melting point 245-247°C; FT-IR (cm^{-1}) *ν* of 3226 (N-H), 3045 (CH aromatic), 1672 (C=O), 1639 (C=N), 1593 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.83 (s, 1H, NH), 8.43(s, 1H, CH =N), 7.30-7.84 (m, 8H, Ar-H), 2.36 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 20.99 (CH_3), 123.15 (C-Br), 127.62, 128.83, 128.95, 130.40, 131.78, 133.66 (Ar-C), 141.82 (CH_3), 146.19 (CH=N), 162.95 (C=O), and MS: *m/z* =317 (M^+).

General synthetic route of the derivatives of tetrazole (T_6-T_{10})

A solution of sodium azide (0.04 g, 0.0006 mol) in 5 mL of ethanol was added to the well and mixed with a solution of (0.0006 mol) hydrazones (Z_1.Z_5) in 10 mL

of ethanol. The reaction was heated under reflux for 14 to 30 h. After completion of the reaction (determined by TLC and hexane/ethyl acetate used as an eluent), the contents were cooled to room temperature, the precipitated was separated by filtration from the reaction mixture, washed consecutively with cold water, dried in reduced pressure at room temperature and then re-crystallized with ethanol to give pure product (Scheme 2). The physical and spectral data for T_6-T_{10} are as follows:

*N-(5-(2,4-dihydroxyphenyl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methylbenzamide (**T_6**)*

Yield: 65% with an R_f = 0.93 (EtOAc: Hex, 4:6 v/v); white color, melting point 293 dec.°C; FT-IR (cm^{-1}) *ν* of 3398 (O-H), 3226 (N-H) tetrazole, 3199 (N-H) amide, 3095 (CH aromatic), 1579 (C=C), 1637 (C=O), 1620 (C=N) ring, 1512 (N=N), 1078 (N-N); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.88 (s, 1H, NH), 11.55 (s, 1H, OH_a), 9.99 (s, 1H, OH_b), 8.53 (s, 1H, N-CH), 6.35-7.87 (m, 7H, Ar-H), 2.41 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 21.51 (CH_3), 103.15, 108.15, 111.03, 128.03, 129.53, 130.56, 131.88 (Ar-C), 142.37 (CH_3), 149.52 (N-CH), 159.98 (C-OH_b), 161.14 (C-OH_a), 162.84 (C=O amide), and MS: *m/z* =313 (M^{+}).

*N-(5-(3-hydroxy-4-methoxyphenyl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methylbenzamide (**T_7**)*

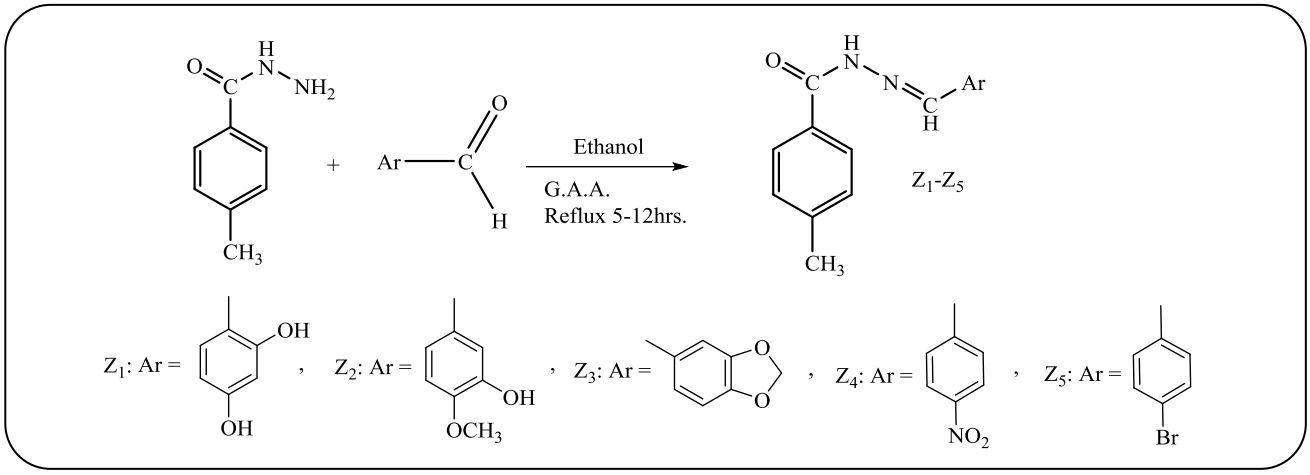
Yield: 80% with an R_f = 0.9 (EtOAc: Hex, 4:6 v/v); white color, melting point 270 dec.°C; FT-IR (cm^{-1}) *ν* of 3300 (O-H), 3213 (N-H) tetrazole, 3124 (N-H) amide, 3099 (CH aromatic), 1583 (C=C), 1643 (C=O), 1604 (C=N) ring, 1525 (N=N), 1066 (N-N); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.59 (s, 1H, NH), 9.28 (s, 1H, OH), 8.31 (s, 1H, N-CH), 6.94-7.83 (m, 7H, Ar-H), 3.80 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 20.96 (CH_3), 55.53 (OCH_3), 111.84, 112.31, 120.17, 127.24, 127.52, 128.91, 130.69 (Ar-C), 141.56 (CH_3), 146.87 (C-OH), 147.69 (OCH_3), 149.72 (N-CH), 162.73 (C=O amide), and MS: *m/z* =327 (M^{+}).

N-(5-(benzo[d][1,3] dioxol-5-yl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methylbenzamide (T_8)

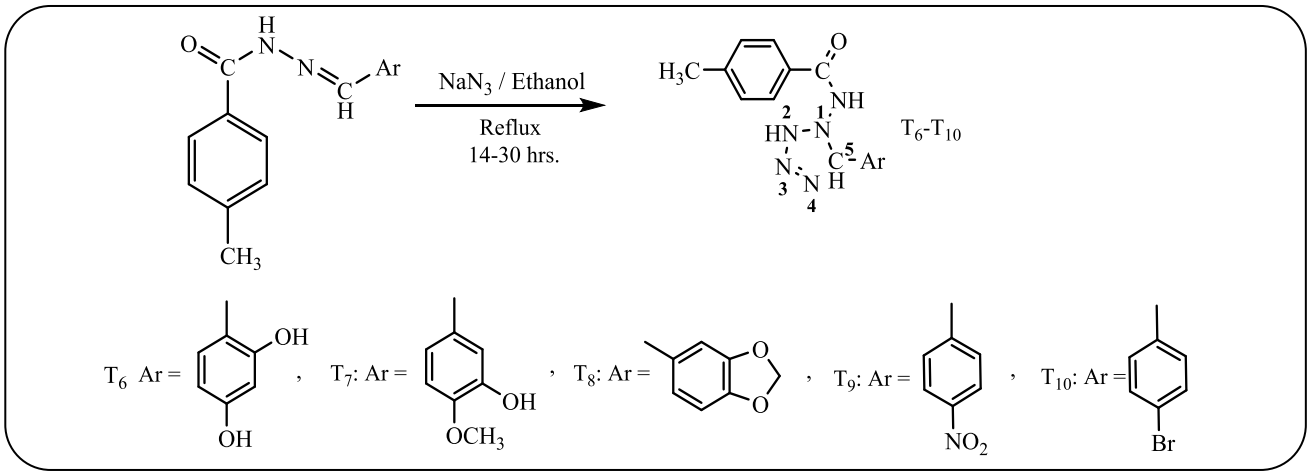
Yield: 60% with an R_f = 0.76 (EtOAc: Hex, 4:6 v/v); white color, melting point 240-242°C; FT-IR (cm^{-1}) *ν* of 3402 (N-H) tetrazole, 3228 (N-H) amide, 3066 (CH aromatic), 1610 (C=C), 1649 (C=O), 1633 (C=N) ring, 1504 (N=N), 1035 (N-N); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 11.70 (s, 1H, NH), 8.39 (s, 1H, N-CH), 7.00-7.85 (m, 7H, Ar-H), 6.12 (s, 2H, CH_2), 2.40 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 21.50 (CH_3), 102.02 ($CH_{2\ ring}$) 105.57, 108.95, 123.75, 128.06, 129.29, 129.45, 131.09 (Ar-C), 142.19 (CH_3), 147.82 (C-O_a), 148.47 (C-O_b), 149.53 (N-CH), 163.31(C=O amide), and MS: *m/z* =325 (M^{+}).

*N-(5-(4-nitrophenyl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methylbenzamide (**T_9**)*

Yield: 70% with an R_f = 0.93 (EtOAc: Hex, 4:6 v/v); yellow color, melting point 270 dec.°C; FT-IR (cm^{-1}) *ν* of 3302 (N-H) tetrazole, 3192 (N-H) amide, 3034 (CH aromatic), 1558 (C=C), 1651 (C=O), 1610 (C=N) ring, 1409 (N=N), 1072 (N-N); ^{1}H NMR (DMSO-d_6, 400 MHz) yielded δ: 12.11(s, 1H, NH), 8.57 (s, 1H, N-CH), 7.36-8.34 (m, 8H, Ar-H), 2.41 (s, 3H, CH_3), and ^{13}C NMR (DMSO-d_6, 400 MHz) δ: 21.35 (CH_3), 124.02, 128.58, 129.11, 129.97, 130.40, 130.67 (Ar-C), 142.22 (CH_3), 149.47 (N-CH), 154.58 (C-NO_2), 166.21 (C=O amide), and MS: *m/z* =326 (M^{+}).



Scheme 1: Preparation of hydrazones compounds.



Scheme 2: Preparation of 2,5- dihydro-1H-tetrazol derivatives.

*N-(5-(4-bromophenyl)-2,5-dihydro-1H-tetrazol-1-yl)-4-methylbenzamide (**T₁₀**)*

Yield: 80% with an R_f = 0.89 (EtOAc: Hex, 4:6 v/v); white color, melting point 272 dec.°C; FT-IR (cm^{-1}) *v* of 3410 (N-H) tetrazole, 3215 (N-H) amide, 3047 (CH aromatic), 1591 (C=C), 1649 (C=O), 1633 (C=N) ring, 1487 (N=N), 1068 (N-N); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) yielded δ: 11.87 (s, 1H, NH amide), 8.45 (s, 1H, N-CH), 7.34-7.86 (m, 8H, Ar-H), 2.40 (s, 3H, CH_3), and ^{13}C NMR (DMSO-*d*$_6$, 400 MHz) δ: 21.49 (CH_3), 123.75 (C-Br), 128.12, 129.42, 129.53, 130.77, 132.33, 134.09 (Ar-C), 142.50 (CH_3), 146.89 (N-CH), 163.70 (C=O amide), and MS: *m/z* =360 (M^{+}).

The reaction involves a (3+2) cycloaddition reaction, adding 1,3-dipoles like azide to an unsaturated system, such as an imine bond in a dipolarphile. The product is a ring of five-membered as in Fig. 1.

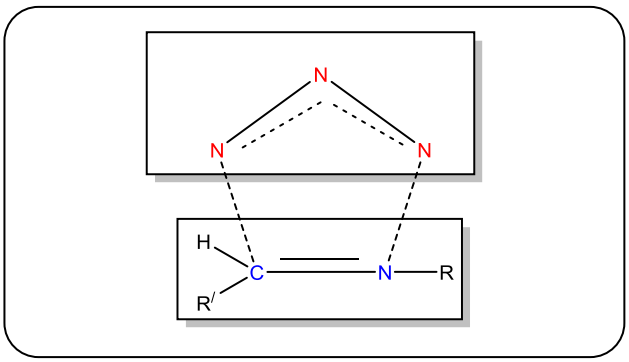
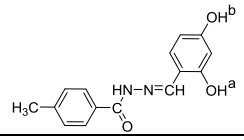
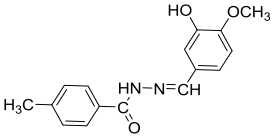
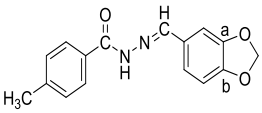
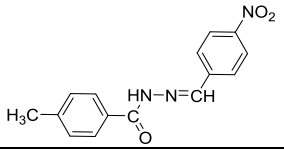
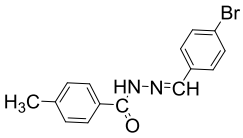
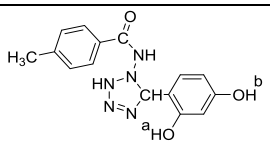
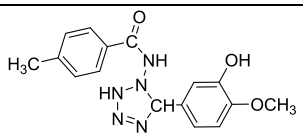
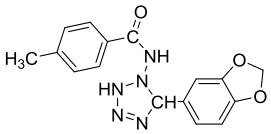
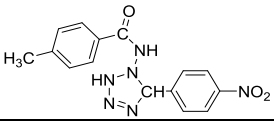
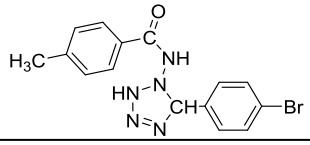


Fig. 1: Tetrazole transition state.

Anti-microbial testing

Prepared compounds were clinically examined for their antimicrobial activities. *In vitro* antibacterial and antifungal assays were performed using an agar well diffusion test [14] against two different species of bacteria:

Table 1: The physical properties of prepared compounds.

Comp No.	Product	Color	Time/h	Yield (%)	M.P. (ºC)	R_f
Z_1		white	8	70	286 dec.	0.73
Z_2		yellow	10	80	246-248	0.93
Z_3		yellow	12	65	216-218	0.9
Z_4		yellow	8	75	263 dec.	0.71
Z_5		white	5	80	245-247	0.81
T_6		white	20	65	293 dec.	0.93
T_7		white	20	80	270 dec.	0.9
T_8		white	24	60	240-242	0.76
T_9		yellow	30	70	270 dec.	0.93
T_{10}		white	30	80	272 dec.	0.89

(1) *Staphylococcus aureus*, Gram (+ve), and (2) *Escherichia coli*, Gram (-ve) in addition to *Candida albicans* as a fungal strain. Fungal and bacterial inocula (0.2 mL of each) were cultivated by streaking samples on the surface of nutrient agar and potato dextrose agar media, respectively, in addition to obtaining 24 h young colonies. Using a cotton bud, each growing organism (1 mL) was spread in three directions on the Petri dish in Mueller Hinton Agar (MHA) for homogeneous growth. Fifty microliters of 100 mg/mL of the prepared compounds were dissolved in DMSO and placed in each well (7 mm diameter holes cut in the agar gel, 20 mm apart from one another).

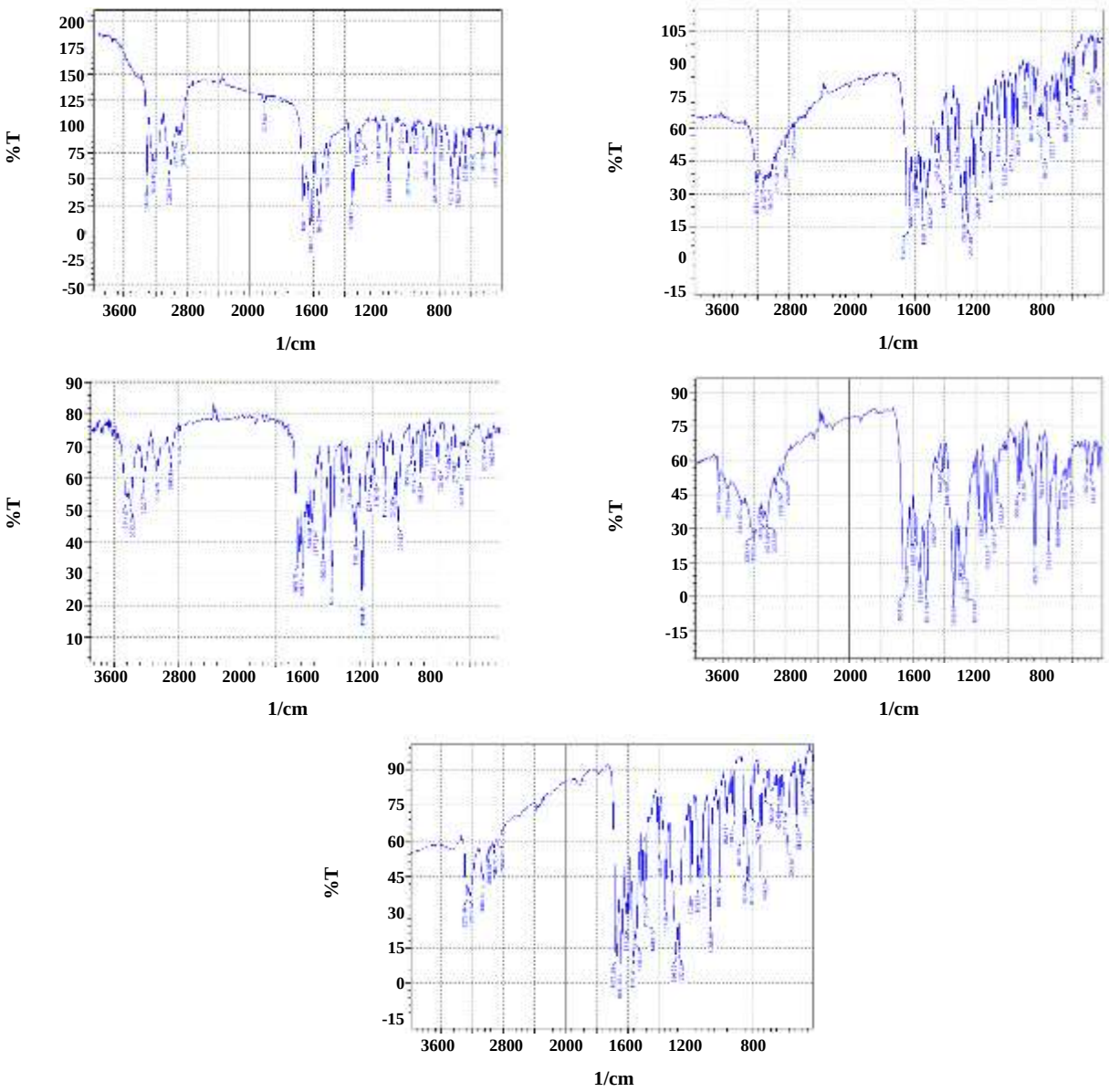


Fig. 2: FT-IR spectra of Z_1-Z_5.

The bacterial plates were kept for 24 h at 36 ± 1 °C, while the fungal plates were kept at 28 ± 2 °C for 24 h under aerobic conditions. Following the confluence of bacterial and fungal growth, inhibition of bacterial and fungal growth was recorded in mm [15, 16].

RESULTS AND DISCUSSION

Hydrazones Z_1-Z_5 may be produced by reacting between 4-Methylbenzohydrazide and commercially available aromatic aldehydes [17], (Scheme 1). The tetrazole compounds T_6-T_{10} were designed via reactions between prepared hydrazones Z_1-Z_5 and sodium azide [18], (Scheme 2). The results are summarized in Table 1. From the course of the reaction and the mechanism proposed, it can be inferred that the reaction takes place by means of a determined mechanism (Huisgen 1,3- cycloaddition dipolar) [19], (Fig.1). The IR spectra of Z_1-Z_5 displayed the appearance of characteristic bands at (3122-3230), (1643-1672) and (1602-1639) cm^{-1} indicating NH, C=O and C=N groups, respectively [20], (Fig.2). In addition, the IR spectra of T_6-T_{10} showed the characteristic bands at (3213-3410), (1604-1633), (1282-1307), (1035-1078) and

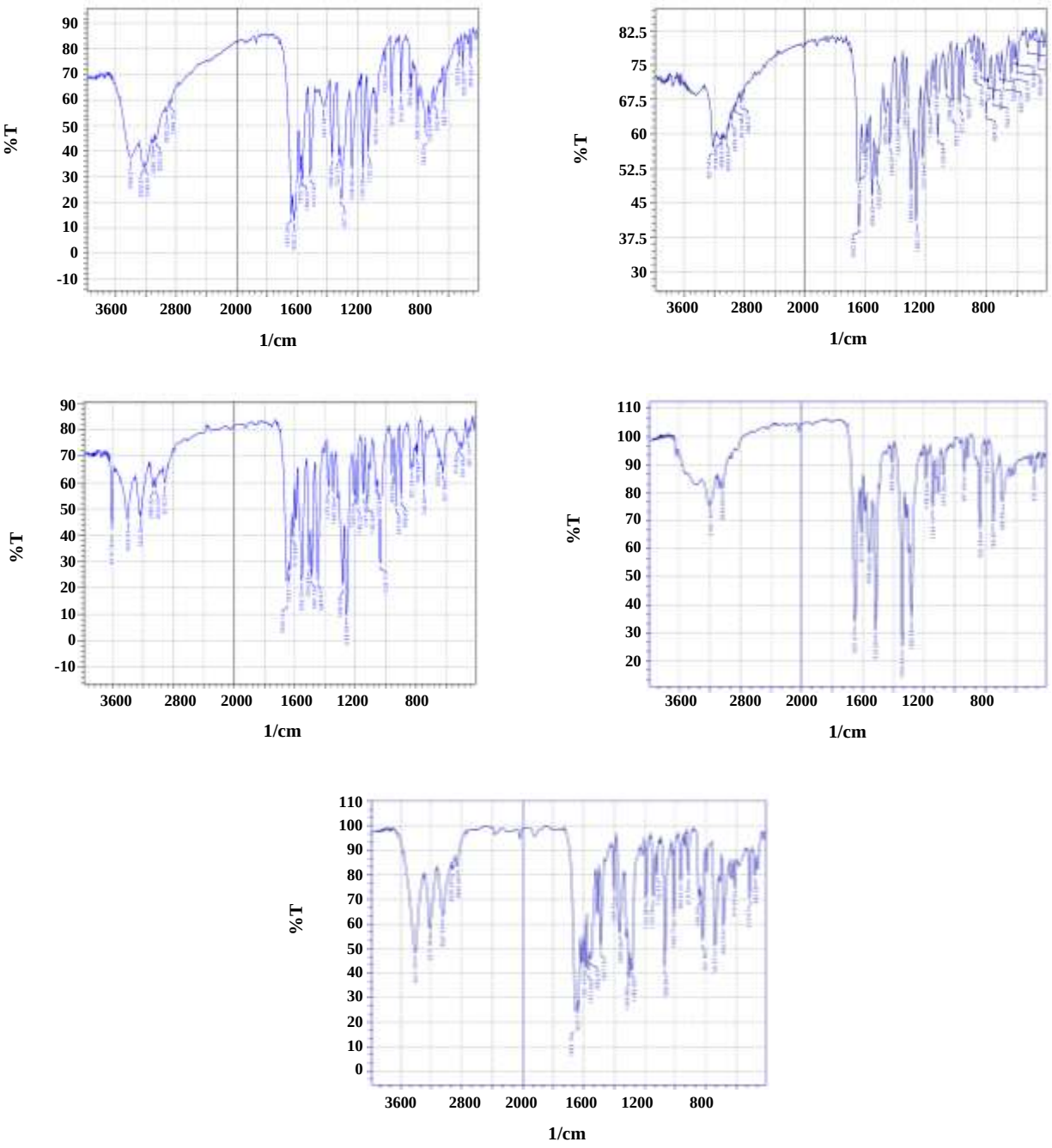


Fig. 3: FT-IR spectra of T_6-T_{10}.

(1409-1525) cm^{-1} representing NH, C=N, C–N, N–N, and N=N, respectively [21] and the disappearance of the CH=N group (Fig.3), (see supporting information).

The ^{1}H-NMR spectra of Z_1-Z_5 displayed singlet signals at δ (11.61-12.06) ppm corresponding to the NH group, signals as a singlet at δ (8.53-8.35) ppm of CH=N group [22, 23] and multiplet signals at δ (6.34-8.29) ppm due to protons in the rings (Fig.4). In addition, the ^{1}H-NMR spectra of T_6- T_{10} exhibited singlet signals at δ (11.59-12.11) ppm representing the NH group (NH outside of the tetrazole ring), signals as a singlet at δ (8.31-8.57) of the N-CH group [24], and multiple signals at δ (6.35-8.34) ppm due to protons in the rings (Fig.5), (see supporting information).

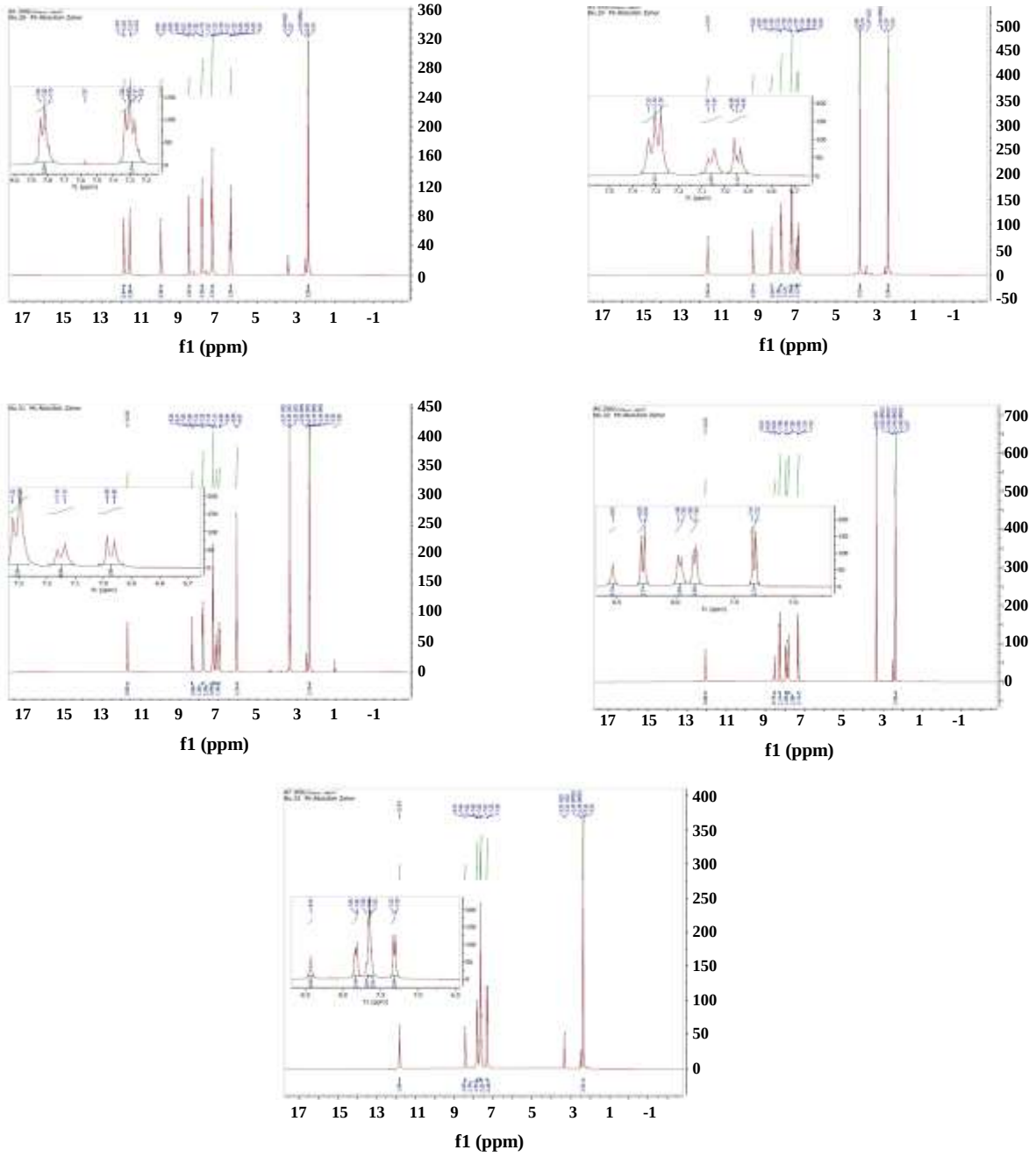


Fig. 4: ^{1}H nuclear magnetic resonance (NMR) spectra of Z_1-Z_5.

The ^{13}C-NMR spectra of Z_1-Z_5 showed signals at (144.91-149.08) ppm due to carbon of the C=N group and signals at (162.37-163.17) ppm corresponding to C=O group (Fig.6). In addition, ^{13}C-NMR spectra of T_6- T_{10} demonstrated signals at (146.89-149.52) ppm due to carbon of the N-CH group and signals at (162.73-166.21) ppm corresponding to the C=O group, other signals appeared at (103.15-134.09) ppm due to rings carbons [25, 26] (Fig.7).

Mass spectra of hydrazones Z_1-Z_5 showed the presence of molecular ion peaks at m/z 270 (M^+), 284 (M^+), 282 (M^+), 283 (M^+), and 317 (M^+), respectively. However, the tetrazoles T_6-T_{10} mass spectra gave molecular ion peaks at m/z 313 ($M^{+\cdot}$), 327 ($M^{+\cdot}$), 325 ($M^{+\cdot}$), 326 ($M^{+\cdot}$), and 360 ($M^{+\cdot}$), respectively [27] (Fig.8), (see supporting information).

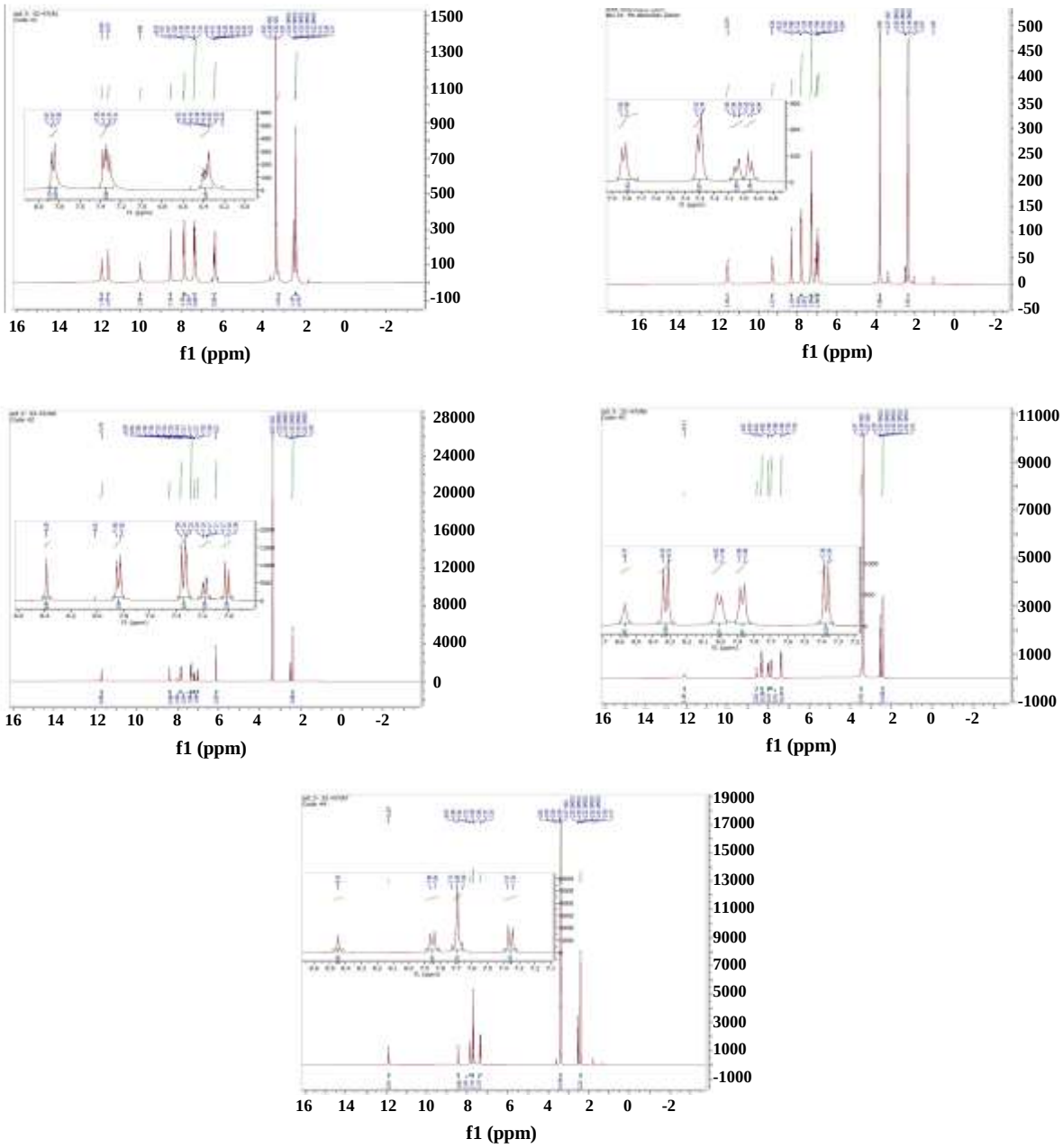


Fig. 5: ^{1}H nuclear magnetic resonance (NMR) spectra of T_6-T_{10}.

Anti-microbial testing

Table 2 shows the compounds that were synthesized as well as the zones of inhibition that were measured. The desired products were tested *in vitro* for antibacterial activity against S. aureus and E. coli, as well as antifungal activity against Candida albicans. The tested compounds' concentrations (100 mg/mL) were used in the agar well diffusion method. Six of the prepared compounds (Z_1–Z_6 and T_{10}) exhibited potent antifungal bioactivity. Electron withdrawing substituents such as (*p*-bromo and *p*-nitro), which have function instead of phenyl groups for the derivatives Z_4, Z_5, T_9, and T_{10} exerted poor antibacterial activities. The compound Z_2 was found to exhibit good antibacterial activity, and become potent by the incorporation of the methoxy group at the para position and hydroxy group at the ortho position of the phenyl moiety, compared

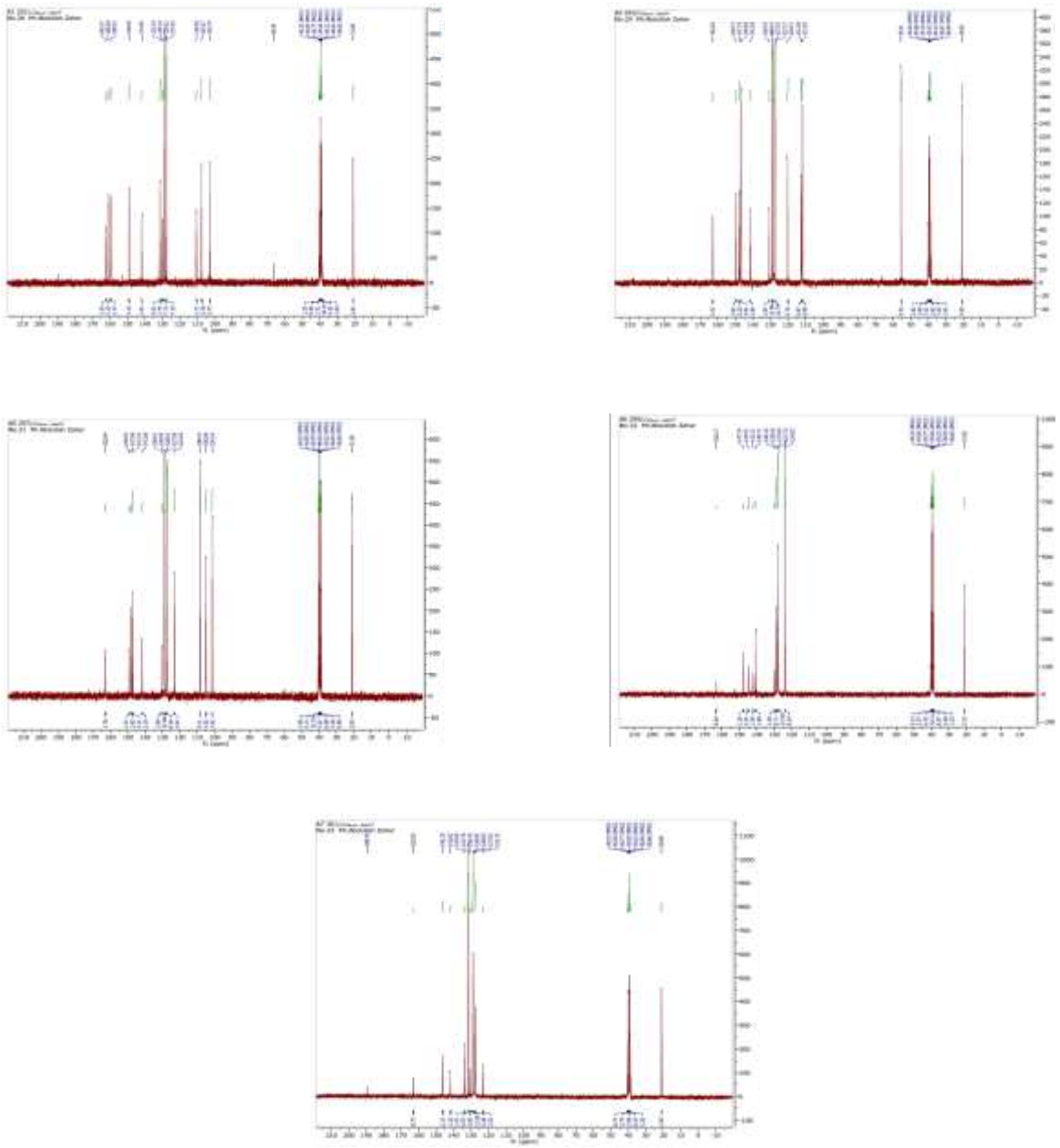


Fig. 6: ^{13}C nuclear magnetic resonance (NMR) spectra of Z_1-Z_5.

with other substituents (Figs. 9, 10, and 11), (See supporting information). The standard drugs can be used ciprofloxacin for antibacterial and fluconazole for antifungal, under similar conditions [28].

CONCLUSIONS

The structures of the synthesized hydrazone compounds (Z_1-Z_5) and tetrazole derivatives (T_6-T_{10}) were confirmed by their melting point, FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra, Mass spectral studies, and antimicrobial evaluation. Antimicrobial activity of compounds Z_1–Z_6 and T_{10} against *S. aureus* and *E. coli* in addition to antifungal activity against *C. albicans* was found to be better compared with other compounds screened.

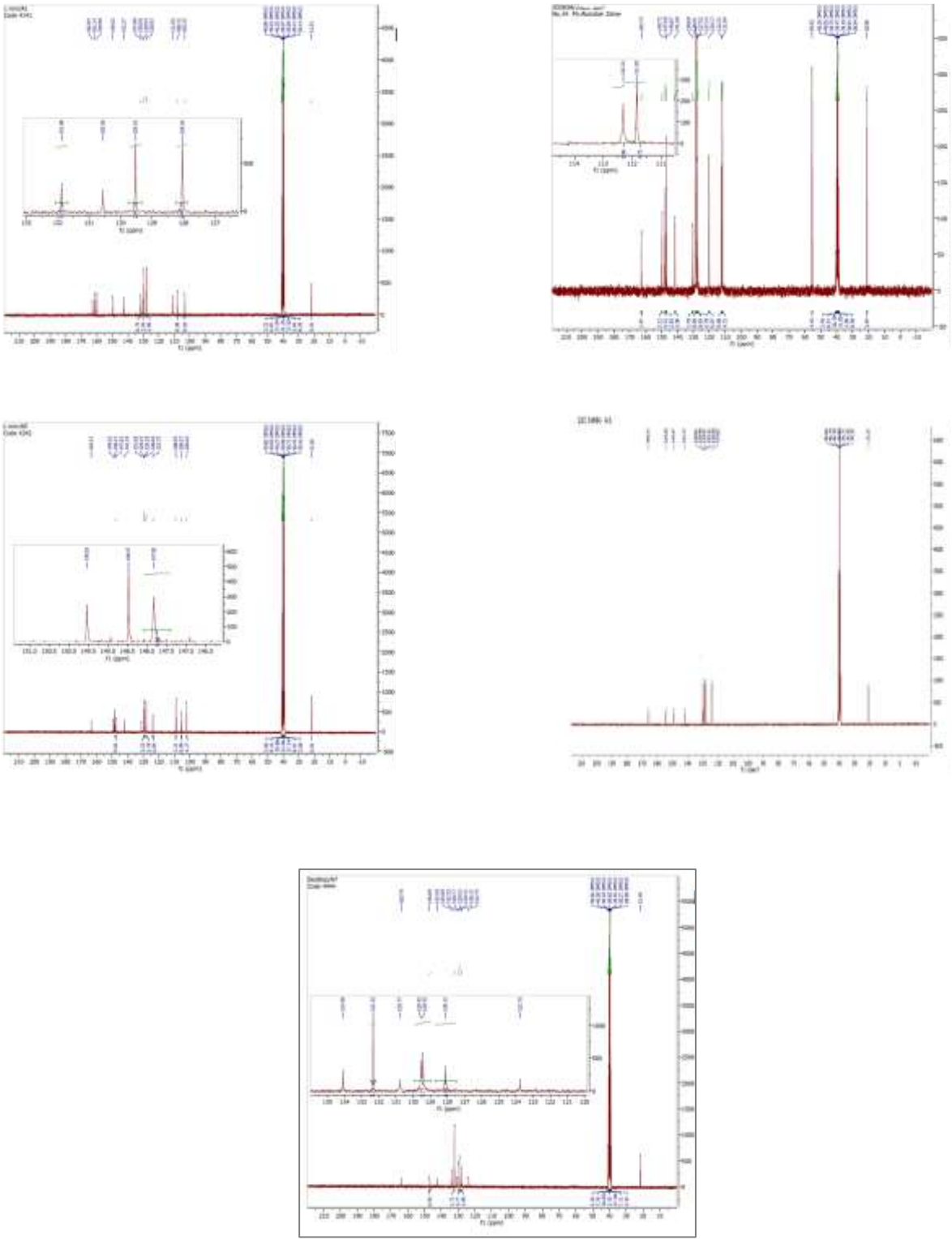


Fig. 7: ^{13}C nuclear magnetic resonance (NMR) spectra of T_6-T_{10}.

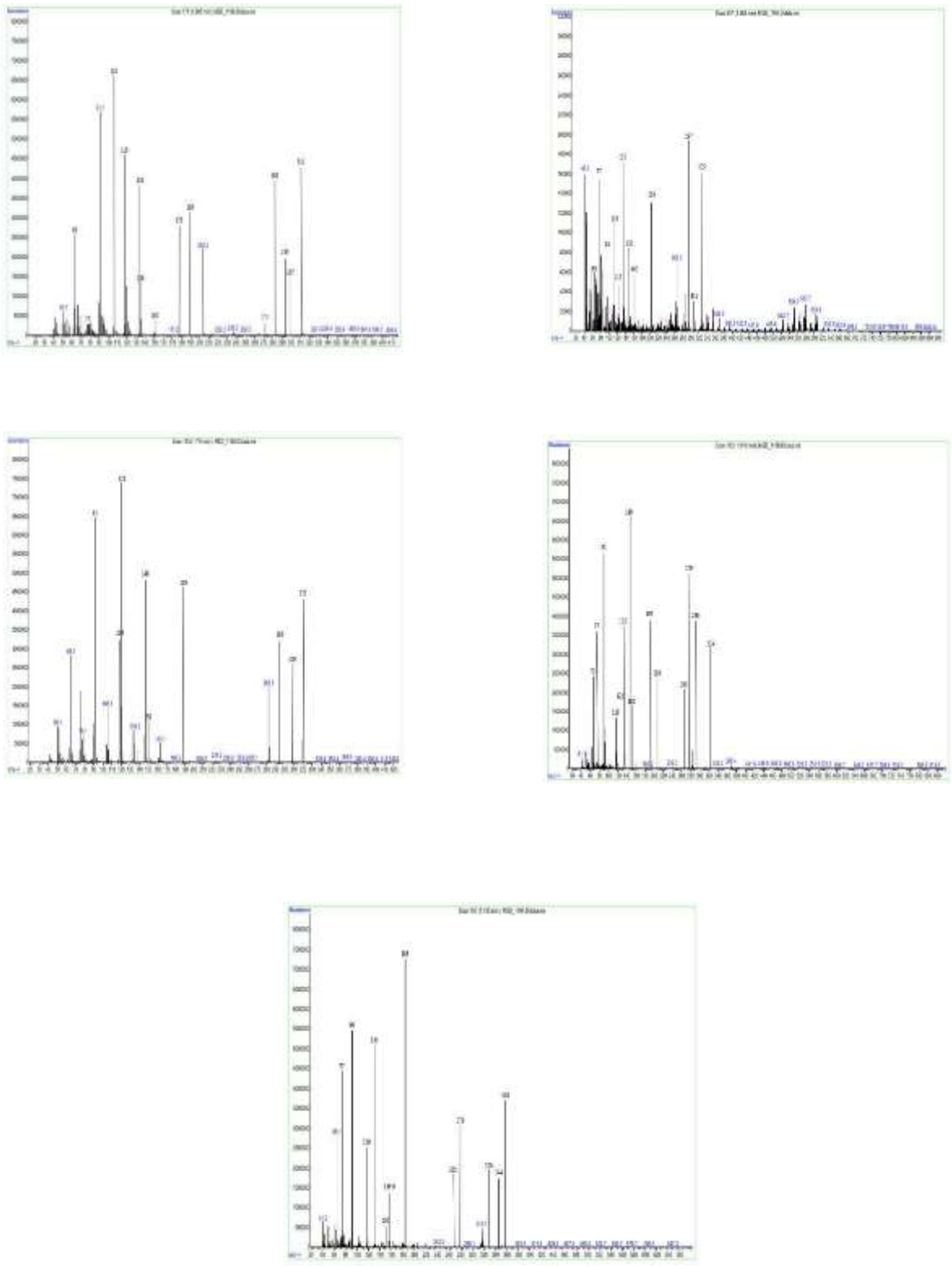


Fig. 8: Electron ionization (EI)-mass spectra of T_6-T_{10}.

Table 2: Anti-microbial activity of the synthesized compounds.

Comp. No.	Inhibition zone diameter, (mm)		
	Gram +ve bacteria	Gram -ve bacteria	Fungi
	S. aureus	*E. coli*	*Candida. albicans*
Z_1	13	13	15
Z_2	25	25	40
Z_3	15	0	15
Z_4	0	0	0
Z_5	0	0	15
T_6	7	0	11
T_7	0	0	0
T_8	0	8	0
T_9	0	0	0
T_{10}	0	0	16

*Highly active (inhibition zone ≥ 20 mm); moderately active (inhibition zone 16–19 mm); slightly active (inhibition zone 10–15 mm).

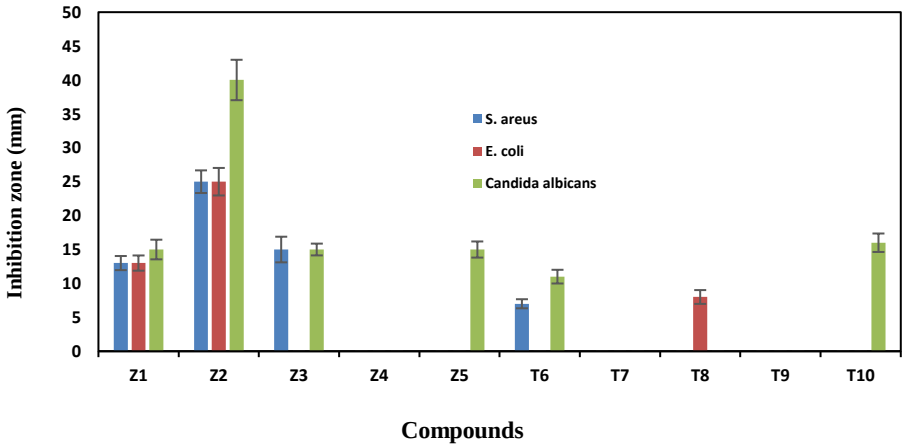


Fig. 9: Zone of inhibition of S. aureus, E. coli, and Candida. albicans using the prepared derivatives.

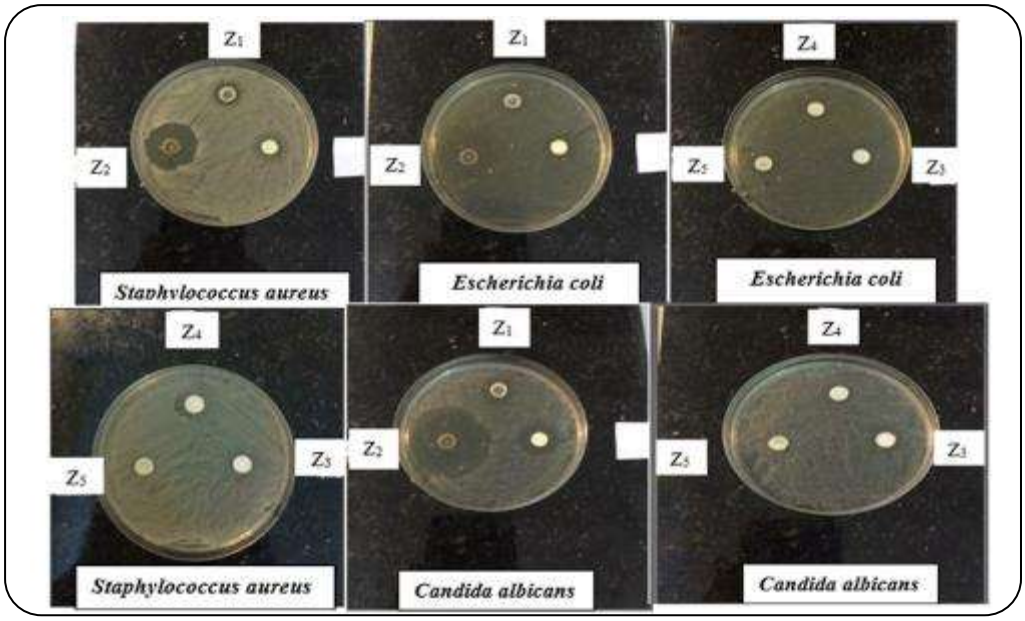


Fig. 10: Anti-microbial activity of the synthesized hydrazone compounds Z_1- Z_5.

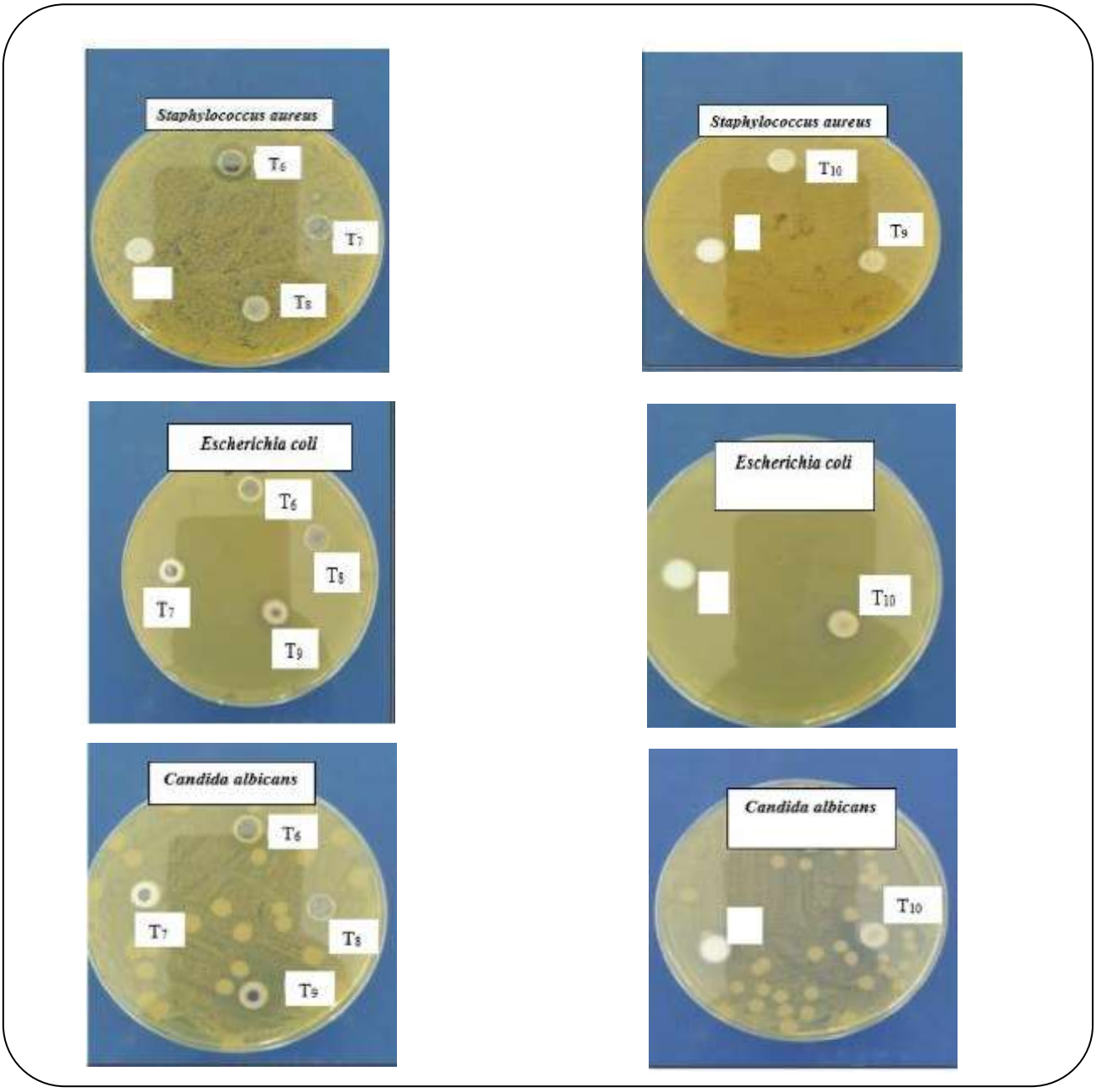


Fig. 11: Anti-microbial activity of the synthesized tetrazole derivatives T_6- T_{10}.

All the synthesized compounds were screened for their antibacterial and antifungal activities by the disc diffusion method.

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