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Synthesis and Characterization of Novel Tetrazole Derivatives and Evaluation of Their Anti-candidal Activity

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ABSTRACT

This research includes synthesis of new heterocycles containing disubstituted Tetrazole derivatives. Imine compounds were synthesized by reaction of primary aromatic amines with various substituted benzaldehydes in the presence of glacial acetic acid as catalyst in absolute ethanol to obtain new imine compounds O_1-O_5. The novel five-membered heterocycles as Tetrazole derivatives O_6-O_{10} were obtained from treatment of each new imine compounds with sodium azide compound. Newly synthesized compounds were identified via spectral methods (FT-IR, ^{1}H-NMR and ^{13}C-NMR) spectra and some physical properties. O_6 is the best derivative that has significantly (p<0.01) recorded a stronger influence to inhibit the growth of *Candida guilliermondii* at an average of the zone of inhibition 14.0 mm. While, O_9 derivative recorded the lowest zone of inhibition 7.3 mm toward the same clinical fungal pathogen. The present work may be helpful in designing more potential antibacterial and antifungal agents for therapeutic use in the future.

Keywords: Tetrazole, *Candida* sp., anti-candidal, imine compounds, sodium azide.

INTRODUCTION

Imine compounds are class of the compounds which contain -HC=N- group, they are usually synthesizing by the condensation of a primary aromatic amino

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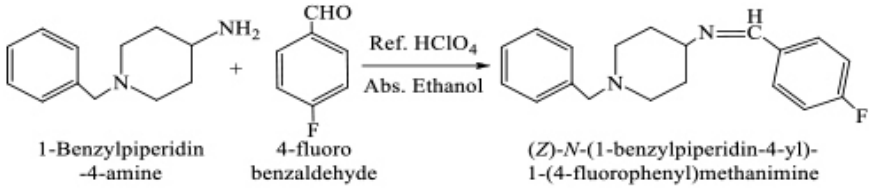
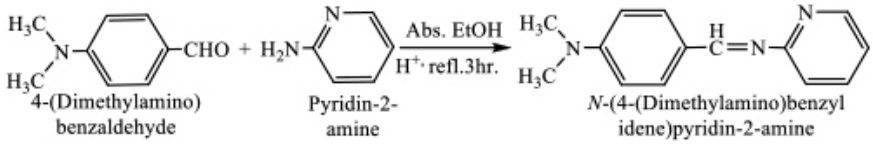
group with an active carbonyl aromatic aldehyde. They are versatile precursors in the synthesis of organic, bio-organic, organometallic and industrial compounds via ring closure, cycloaddition and replacement reactions.[1-4] Imine compounds were discovered by a German chemist, Nobel prize winner, Hugo Schiff in 1864.[5] Imine compounds produced from the reaction between ketone or aldehyde compounds with amine compounds.[6] In the presence of perchloric acid (**Scheme 1**) the reaction of 4-fluorobenzaldehyde with 1-benzylpiperidin-4-amine gives the next product.[7]



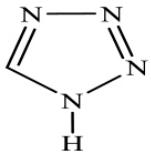
Scheme 1. The effect of perchloric acid on imine compound formation

The reaction of pyridine-2-amine with 4-(dimethyl amino) benzaldehyde (**Scheme 2**) produces the imine compound.[8]



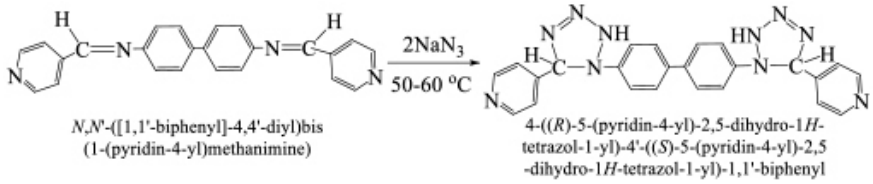
Scheme 2. Using glacial acetic acid to prepare the imine compound

One of the most important chemical compounds is sodium azide, which has been used in many fields including its effect on germination.[9] Due to its great importance, it was used in the preparation of compounds called tetrazoles. Tetrazoles are a class of synthetic organic heterocyclic compounds consist of five-member ring of four nitrogen atoms and one carbon atom **(Scheme 3)**.[10]



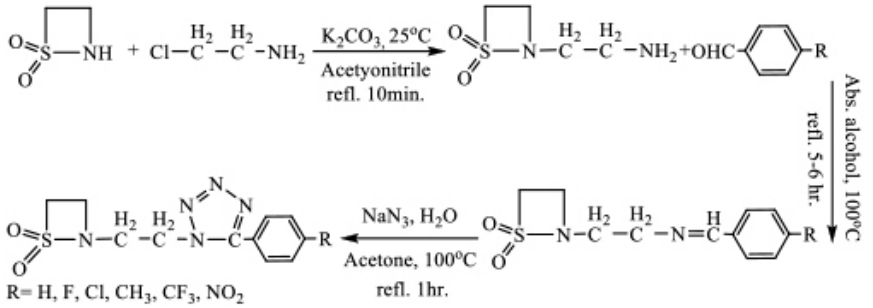
Scheme 3. Structure of tetrazole ring

Synthesis of tetrazole derivatives is an important task in modern medicinal chemistry.[11] Tetrazoles are class of heterocycles that have received attention due to their wide range of applications.[12] Pharmacologically, because of the effect of gram-negative or gram-positive bacteria on the health of human, thus some potential drugs/products must be synthesized.[13,14] Tetrazole contains compounds reported to possess diverse chemotherapeutic activities as antibacterial,[15] and antifungal.[16] Example of one of tetrazole derivatives is the product from the reaction between imine compound (N,N'-([1,1'-biphenyl]-4,4'- diyl) bis (1- (pyridine - 4 - yl) methanimine)) and sodium azide **(Scheme 4)**.[17]



Scheme 4. Synthesized of 2,5-dihydro-1H-tetrazol derivative

Tetrazole derivatives of type of 5-phenyl-1H-tetrazol-1-yl) thiazetidine dioxide prepared from the next reaction **(Scheme 5)**,[18] below:



Scheme 5. Potassium carbonate in tetrazole derivatives synthesis

This study aims to prepare tetrazole derivatives for first time and investigate their activity against pathogenic fungi, *Candida* spp. *in vitro*.

METHODOLOGY

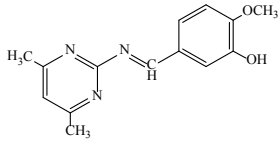
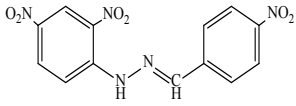
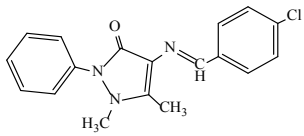
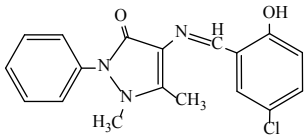
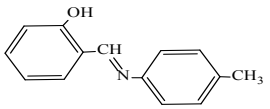
Materials

All chemicals were obtained and purchased from Sigma Aldrich.

General procedure for the synthesis of imine compounds O_1-O_5

Equimolar mixtures 0.02 mole of aldehydes and aromatic amines and trace of glacial acetic acid dissolved in 25 ml absolute ethanol was placed in a 100-ml round-bottom flask equipped with condenser and stirrer bar. The mixture was allowed to react at reflux (at the boiling temperature of absolute ethanol) for 4hr, then allowed to cool down to the room temperature, whereby a crystalline solid was separated out. The solid product was recrystallized twice from absolute ethanol.[19-22] The structural formulae, names, melting points, colors, and percentage of yields for the synthesized imine compounds are recorded in Table 1.

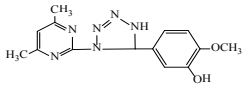
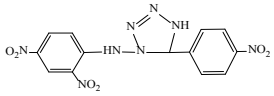
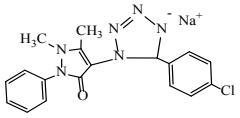
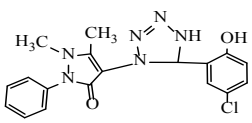
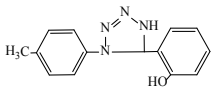
Table 1. Structural formula, nomenclature, melting points, percentages of yield and colors of imine compounds O_1-O_5

Comp. Code	Structural formula	Nomenclature	Yield %	m.p. °C	Color
O_1		(E)-5-((4,6-dimethyl pyrimidin-2-ylimino)methyl)-2-methoxyphenol	68%	78-80	Tan
O_2		(E)-1-(2,4-dinitrophenyl)-2-(4-nitrobenzylidene) hydrazine	81%	291-293	Orange
O_3		(E)-4-(4-ethoxybenzylidene amino)-1,5-dimethyl-2-ph enyl-1H-pyrazol-3(2H)-one	90%	212-214	Bright yellow
O_4		4-(5-chloro-2-hydroxy benzyl ideneamino)-1,5-dimethyl -2-phenyl-1H-pyrazol-3(2H)-one	89%	138-140	Bright pale yellow
O_5		(E)-2-((p-tolylimino) methyl) phenol	87%	94-96	Bright yellow

General procedure for the synthesis of tetrazole derivatives O_6-O_{10}

Equimolar mixtures 0.01 mole of imine compounds and sodium azide dissolved in 20 ml of tetrahydrofuran and 2 ml of distilled water and refluxed the mixture (at the boiling temperature of tetrahydrofuran and distilled water) for 4 hr and left to stand for 24 hr. The solid product was precipitated, filtered off and recrystallized from absolute ethanol.[23,24] The structural formulae, names, melting points, colors, and percentage yields for the synthesized tetrazole derivatives are presented in table 2. Melting points were recorded on electrothermal melting point apparatus (uncorrected). FT-IR spectra were recorded at the room temperature from (4000-400) cm^{-1} with KBr disc by infrared spectrophotometer model tensor 27 Bruker Co., Germany. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded by Bruker Ac-300MHz spectrometer, it making sure from the purity and reaction occur of synthesized derivatives O_6-O_{10} by the comparison between the physical measurements (Table 1) of O_1-O_5 and the physical measurements of O_6-O_{10} (Table 2) and between FT-IR spectra of O_1-O_5 (Table 3) and FT-IR spectra of O_6-O_{10} (Table 4).

Table 2. Structural formula, nomenclature, melting points, percentages of yield and colors of tetrazole derivatives O_6-O_{10}

Comp. code	Structural formula	Nomenclature	Yield %	m.p. °C	Color
O_6		5-(1-(4,6-dimethylpyrimidin-2-yl)-4,5-dihydro-1H-tetrazol -5-yl)-2-methoxy phenol	81%	107-109	Bright Pale yellow
O_7		N-(2,4-dinitrophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-tetrazol-1-amine	89%	> 300	Pale Orange
O_8		5-(4-chlorophenyl)-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4,5-dihydrotetrazol-1-ide	93%	242-244	Pale yellow
O_9		4-(5-(5-chloro-2-hydroxyphe nyl)-4,5-dihydro-1H-tetrazol-1-yl)-1,5-dimethyl -2-phenyl-1H-pyrazol-3(2H)-one	85%	169-171	Pale yellow
O_{10}		R)-2-(1-p-tolyl-4,5-dihydro-1H-tetrazol-5-yl) phenol	84%	119-120	Bright Golden

Anti-Candidal activity

This test was achieved *in vitro* to investigate inhibitory effects of the synthesized tetrazole derivatives using well diffusion method on Muller-Hinton agar. This experiment was done as mentioned by Owaid et al.[25,26] Four milligrams of each tetrazole derivative was dissolved in DMSO and applied separately as 4 mg/well (6 mm-well). After 18 hr of incubation at 37 °C, the zone of inhibition was taken using the ruler in millimeters.

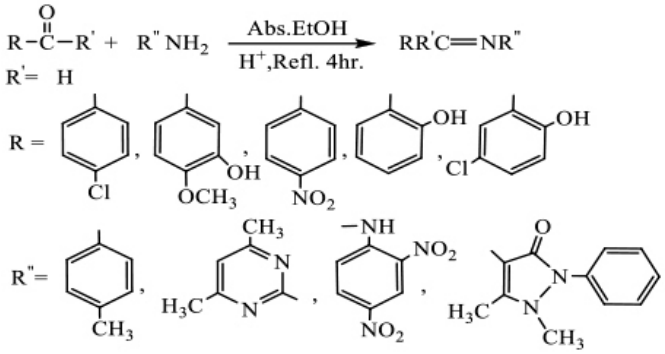
Statistical Analysis

The data (triplicates) were analyzed by one-way analysis of variance using ANOVA table by SAS program for Windows, version 9.0, SAS Institute Inc., USA. The significance of differences was calculated using Duncan's Multiple Range Test (DMRT). Probability value least than 1% was considered to be statistically significant.

RESULTS AND DISCUSSION

Imine compounds O_1-O_5

Imine compounds **(Scheme 6)** were synthesized from commercially available aromatic aldehydes and primary amines and identified by their melting points, and FT-IR. The FT-IR spectra showed the appearance of the stretching absorption bands of azomethine (C=N) at 1591-1669 cm^{-1},[27,28] beside the characteristic bands of the residual groups in the structure Table 3. See Figs. 1 and 2.



Scheme 6. Structure of the synthesized imine compounds

Table 3. FT-IR spectra of imine compounds O_1-O_5

FT-IR, n(cm^{-1})							
Comp. Code	C=N	C=C Aromatic	C-H		C-H Ali.		Others
			Aromatic	Alkene	Asymmetric	symmetric	
O_1	1669	1510	3000	3045	2974	2941	O-H b3309, C=N yrimidine1547
O_2	1610	1572	3042	3089	--	--	NO_2 1505, 1322 N-H 3277
O_3	1591	1569	3044	3067	2983	2875	C=O 1645, C-Cl 829
O_4	1594	1559	3044	3075	2983	2874	C=O 1634, C-Cl 815 O-H b3450
O_5	1614	1566	3046	3079	2980	2867	O-H b3375

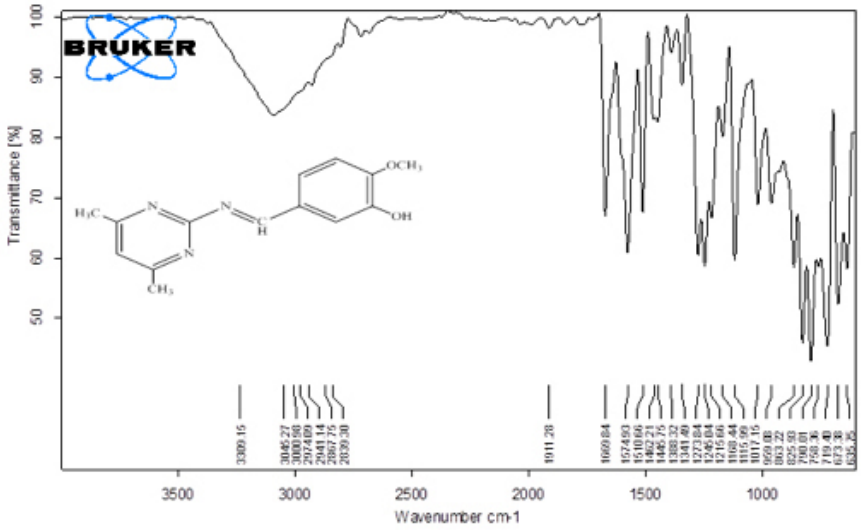


Figure 1. FT-IR spectra of O_1

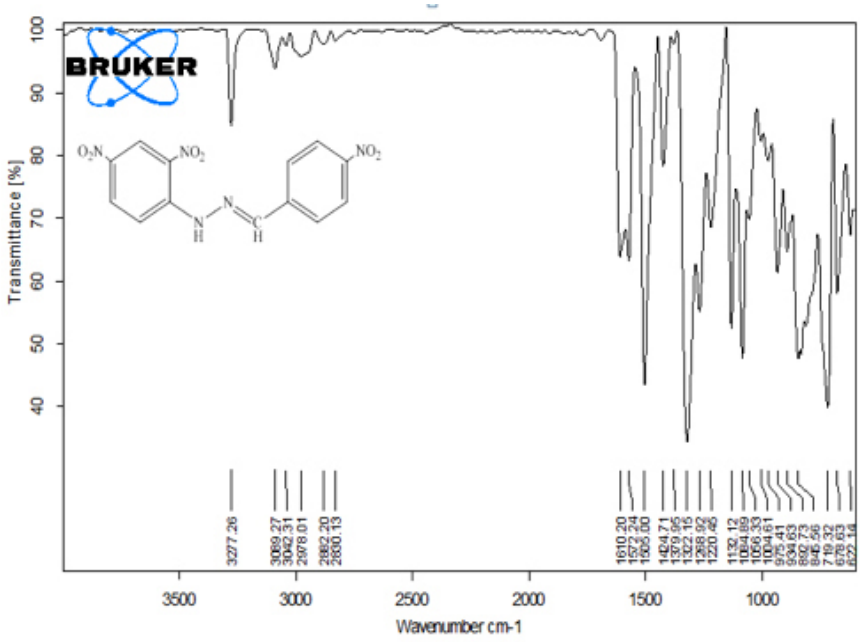
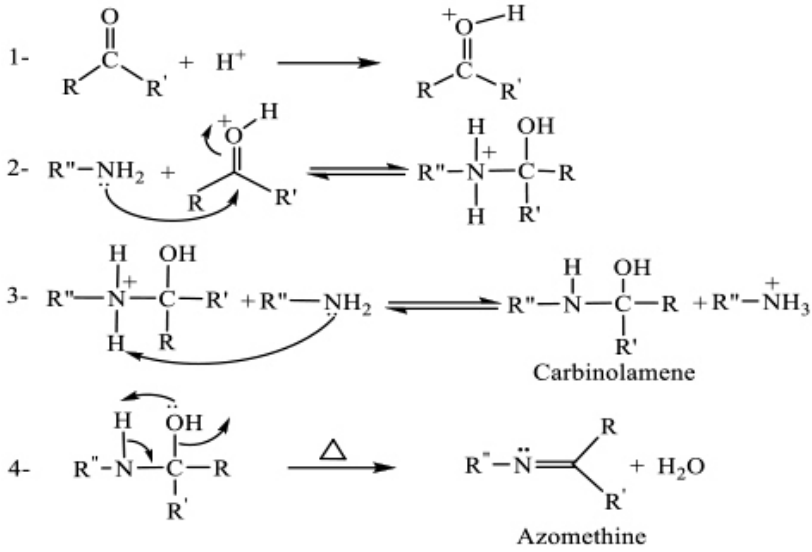


Figure 2. FT-IR spectra of O_2

The physical properties and FT-IR spectra of imine compounds O_1-O_5 prove the synthesis processes, Mechanism of imine compounds formation represented in the following reaction.[29,30] See **scheme 7**.



Scheme 7. Mechanism of imine compounds formation

Tetrazole derivatives O_6-O_{10}

The synthesis of tetrazole derivatives was achieved by the reaction of imine and sodium azide. Their melting points identified the resulted products. FT-IR spectra of the products (table 4) showed characteristic absorption band at 1272-1301, 1022-1089 and 1484-1509 cm^{-1} as an indicative of C-N, N-N and N=N bonds of tetrazole rings formation beside the characteristic bands of the residual groups in the structure as presented in Figs. 3 and 4.[28]

Table 4. FT-IR spectra of tetrazole derivatives O_6-O_{10}

Comp. code	FT-IR n(cm^{-1})								
	N-H	N-N	N=N	C-N	C=C Aromatic	C-H Aromatic	C-H Ali.		Others
							Asymmetric	Symmetric	
O_6	3229	1022	1512	1278	1578	3082	2937	2875	O-H b 3627
O_7	3279	1089	1509	1272	1594	3091	2968	2877	$NO_2$1575,1331
O_8	--	1086	1484	1301	1594	3060	2941	2865	C=O 1650 C-Cl 768
O_9	3280	1087	1484	1273	1564	3055	2954	2874	O-H b 3491 C=O1638 C-Cl 772
O_{10}	3320	1033	1499	1283	1598	3053	2920	2855	O-H b 3446

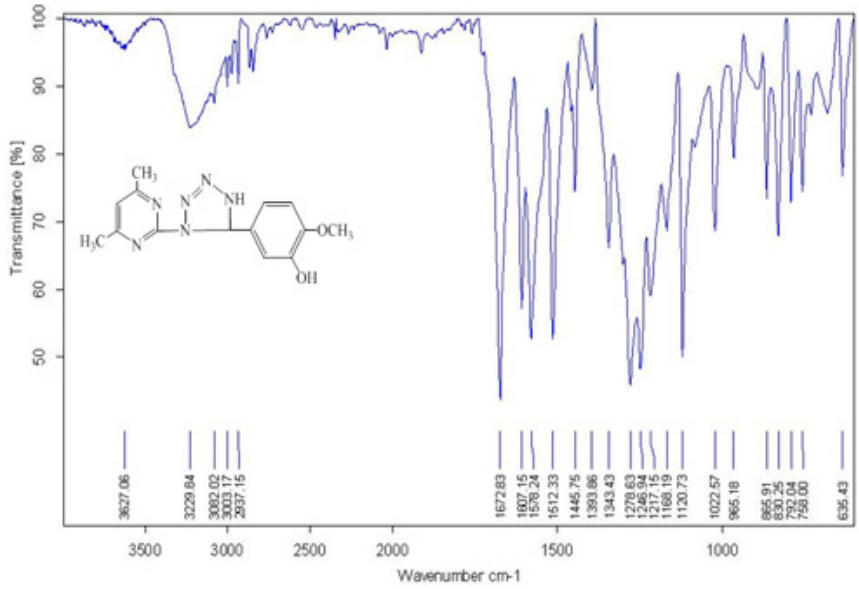


Figure 3. FT-IR spectra of O_6

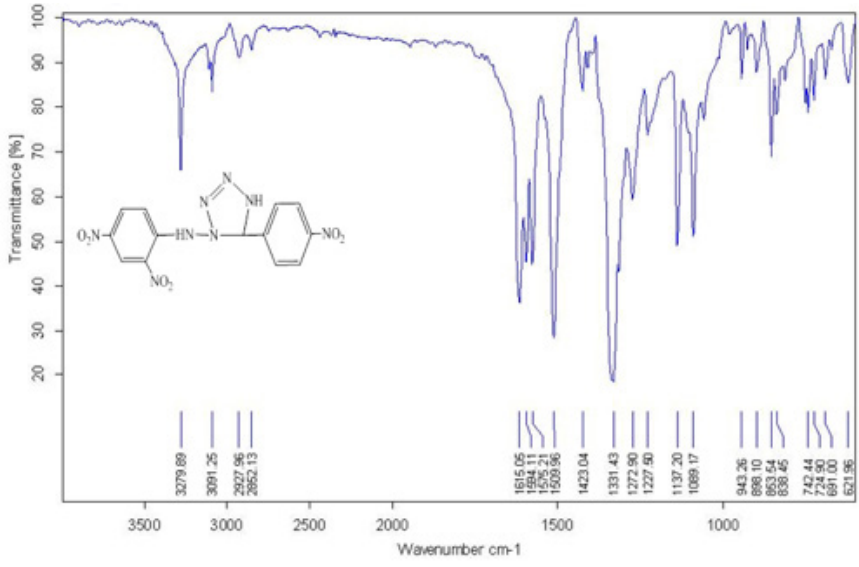


Figure 4. FT-IR spectra of O_7

The ^{1}H-NMR spectrum of compound O_8 in DMSO solvent (Fig. 5) showed chemical shifts, δ(ppm), singlet in 2.46 indicates the presence 3H of the (N-$\underline{CH_3}$) group, singlet in 3.20 indicates the presence 3H of the (=C-$\underline{CH_3}$) group, singlet in 9.57 indicates the presence 1H of the (N-$\underline{CH}$) group, multiplet and doublet of doublet in 7.85-7.36 indicates the presence 9H of the aromatic pro-

tons. Spectrum of compound O_9 (Fig. 6) showed chemical shifts, δ(ppm) at: singlet in 2.42 indicates the presence 3H of the (N-CH_3) group, singlet in 3.23 indicates the presence 3H of the (=C-CH_3) group, singlet in 6.78 indicates the presence 1H of the (-NH) group, singlet in 9.67 indicates the presence 1H (N-CH) group, singlet in 12.73 indicates the presence 1H of the (-OH) group, multiplet in 7.64-6.93 indicates the presence 8H of the aromatic protons.[31] Other chemical shifts of O_6, O_7 and O_{10}, δ(ppm) are presented in table 5.

Table 5. The ^{1}H-NMR Spectra of tetrazole derivatives O_6-O_{10} in DMSO

Comp. code	Chemical Shift δ ppm
O_6	Singlet in 2.40 (6H, 2 CH_3), singlet in 3.34 (3H, O-CH_3), singlet in 7.11 (1H, -NH), singlet in 9.58 (1H, N-CH), singlet in 9.77 (1H, -OH), multiplet and singlet in 7.42-7.11 (4H, aromatic protons)
O_7	Singlet in 3.57 (1H, NH out), singlet in 8.89 (1H, NH in), singlet in 11.86 (1H, N-CH) and multiplet and doublet of doublet in 8.82-8.05 (7H,aromatic protons)
O_8	Singlet in 2.46 (3H, N-CH_3), singlet in 3.20 (3H, =C-CH_3), singlet in 9.57 (1H, N-CH), multiplet and doublet of doublet in 7.85-7.36 (9H, aromatic protons)
O_9	Singlet in 2.42 (3H, N-CH_3), singlet in 3.23 (3H, =C-CH_3), singlet in 6.78 (1H, -NH), singlet in 9.67 (1H, N-CH), singlet in 12.73 (1H, -OH), multiplet in 7.64-6.93 (8H, aromatic protons)
O_{10}	Singlet in 2.34 (3H, CH_3), singlet in 6.80 (1H, -NH), singlet in 8.67 (H, N-CH), singlet in 13.25 (1H, -OH), multiplet and doublet of doublet in 7.66-6.95 (8H, aromatic protons)

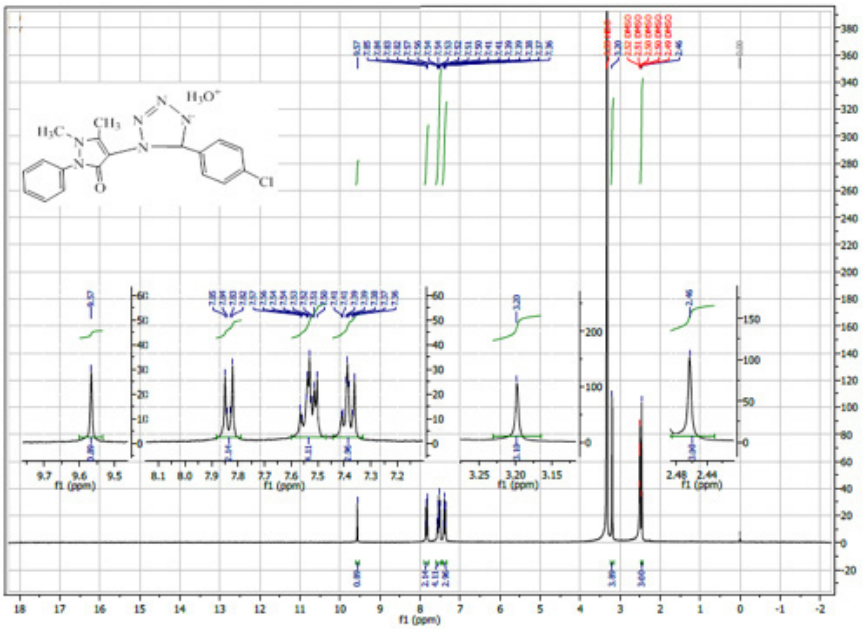


Figure 5. ^{1}H-NMR Spectra of O_8

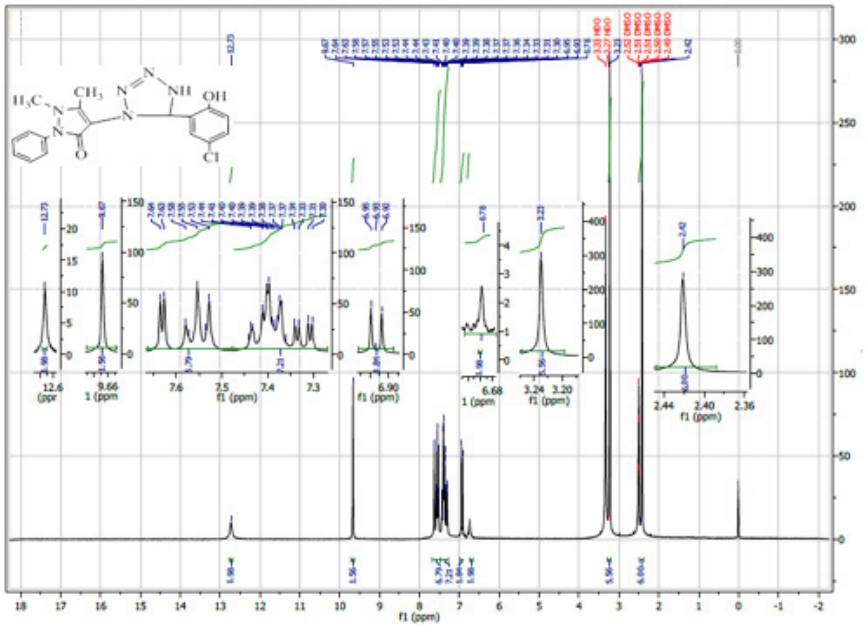


Figure 6. ^{1}H-NMR Spectra of O_9

The ^{13}C-NMR spectrum of compound O_6 in DMSO solvent (Fig. 7) showed chemical shifts, δ(ppm), 37.47 indicates the presence two groups of ($\underline{C}H_3$), 56.27 indicates the presence one group of (O-$\underline{C}H_3$) group, 191.91 indicates the presence one group of (N-$\underline{C}$H), 112.04-124.94 indicates the presence of aromatic carbons, 130.29-153.80 indicates the presence of pyrimidine carbons. While the spectrum of compound O_9 (Fig. 8) exhibited chemical shifts, δ(ppm), 9.79 indicates the presence one group of (N-$\underline{C}H_3$) group, 35.01 indicates the presence one group of (=C-$\underline{C}H_3$), 150.46 indicates the presence one group of (CH_3-$\underline{C}$=), 154.78 indicates the presence one group of (CO-$\underline{C}$=), 157.80 indicates the presence one group of (N-$\underline{C}$H), 158.59 indicates the presence one group of (N-$\underline{C}$O), 113.96-134.10 indicates the presence of aromatic carbons.[32] Other chemical Shifts of O_7, O_8, O_{10}, δ(ppm) are displayed in table 6.

Table 6. The ^{13}C-NMR spectra of tetrazole derivatives O_6-O_{10} in DMSO

Comp. code	Chemical Shift δ ppm
O_6	37.47 (2 $\underline{C}H_3$), 56.27 (O-$\underline{C}H_3$), 191.91 (N-$\underline{C}$H), 112.04-124.94 (Aromatic Carbons), 130.29-153.80 (Pyrimidine Carbons)
O_7	182.49 (N-$\underline{C}$H), 118.96-125.56 (Aromatic Carbons)
O_8	10.33 (N-$\underline{C}H_3$), 35.83 (=C-$\underline{C}H_3$), 144.11 (CH_3-$\underline{C}$=), 152.34 (CO-$\underline{C}$=), 159.99 (N-$\underline{C}$H), 162.47 (N-$\underline{C}$O), 115.08-128.22 (Aromatic Carbons)
O_9	9.79 (N-$\underline{C}H_3$), 35.01 (=C-$\underline{C}H_3$), 150.46 (CH_3-$\underline{C}$=), 154.78 (CO-$\underline{C}$=), 157.80 (N-$\underline{C}$H), 158.59 (N-$\underline{C}$O), 113.96-134.10 (Aromatic Carbons)
O_{10}	21.07 ($\underline{C}H_3$), 163.00 (N-$\underline{C}$H), 117.00-136.95 (Aromatic Carbons)

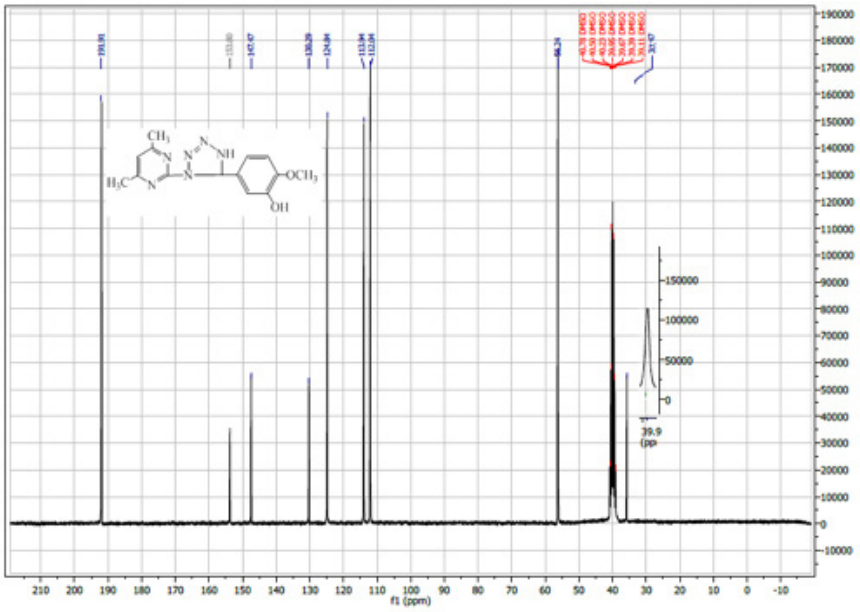


Figure 7. ^{13}C-NMR Spectra of O_6

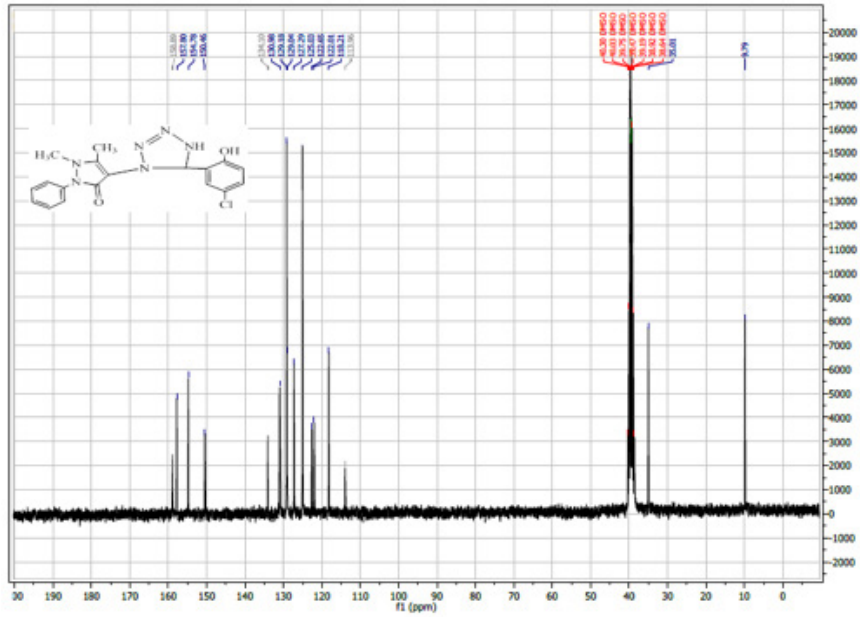
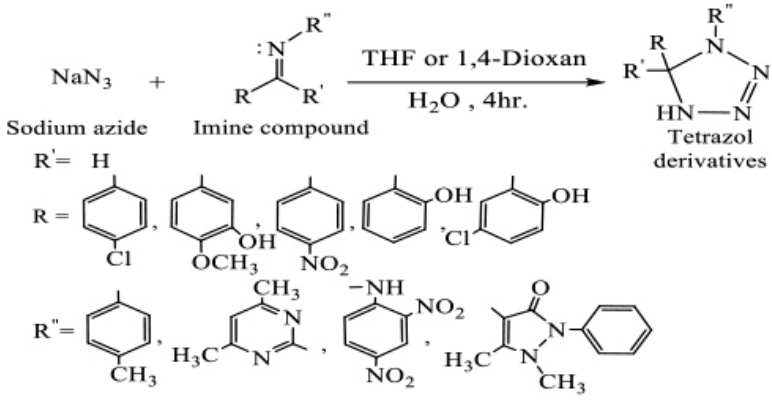


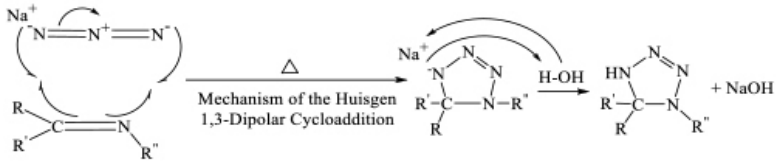
Figure 8. ^{13}C-NMR Spectra of O_9

Products of the reaction of the synthesized imine compounds with sodium azide compound are given in the following equation (**Scheme 8**):



Scheme 8. Structure of the synthesized tetrazole derivatives

It may be concluded that the reaction takes place via the concerted mechanism of the Huisgen 1,3-dipolar cycloaddition mechanism as represented in the following reaction.[33] See **scheme 9**.



Scheme 9. Mechanism of tetrazole derivatives formation

The results of FT-IR ^{13}C-NMR and ^{1}H-NMR showed that the five-ringed compounds were the least obstructed in all preparation processes. Because of the complete clarity in infrared beams and clear signals separated from each another by the resonance spectrum nuclear magnetic of hydrogen and carbon, this is the basis of organic preparation processes.

Anti-Candidal activity

Zone of inhibition of some human pathogenic fungi was done well-diffusion method to test the potential of the tetrazole derivatives O_6-O_{10} as shown in Figs. 8 and 9. O_6 is the best derivative that has significantly (p<0.01) recorded a stronger influence to inhibit the growth of *Candida guilliermondii* at an average of the zone of inhibition 14.0 mm. However, O_9 derivative recorded the lowest zone of inhibition 7.3 mm toward the same clinical fungal pathogen. From another hand, O_6 showed zone of inhibition 12.0 mm against *Candida zeylanoides*. Furthermore, O_6 derivative recorded zone of inhibition 11.3 mm against *Candida krusei* and *Candida albicans*. O_{10} did not inhibit the growth of *Candida albicans* as shown in Fig. 9. The resistance mechanisms depend on which specific pathways are inhibited by the drugs and the alternative ways available for those pathways that the organisms can modify to get a way around to survive.[34] Many new metal complexes and new 1,3-oxazepine derivatives had good antibacterial activity. Tetrazole derivatives are important to synthesize inflammatory agents. [35]

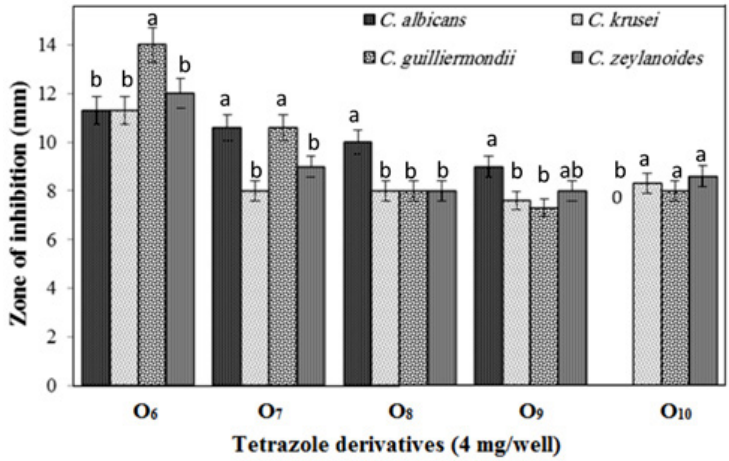


Figure 8. Zone of inhibition of *Candida* sp. using the synthesized tetrazole derivatives O_6-O_{10}, LDS (p<0.01)

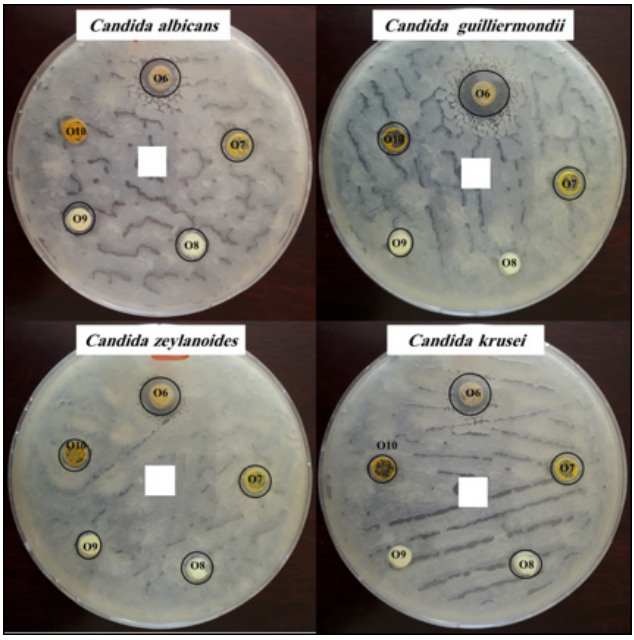


Figure 9. Anti-Candidal activity of the synthesized tetrazole derivatives O_6-O_{10}

This study referees to that preparing derivatives of tetrazole are possible. The results of FT-IR, ^{13}C-NMR and ^{1}H-NMR showed that the five-ringed compounds were the least obstructed in all preparation processes. Because of the complete clarity in infrared beams and clear signals separated from each by

the resonance spectrum nuclear magnetic of hydrogen and carbon, this is the basis of organic preparation processes. O_6 is the best derivative that has significantly ($p<0.01$) recorded a stronger influence to inhibit the growth of *Candida guilliermondii* at an average of the zone of inhibition 14.0 mm. However, O_9 derivative recorded the lowest zone of inhibition 7.3 mm against *Candida guilliermondii*. The present work may be helpful in designing more potential antifungal agents for the therapeutic use in the future.

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