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SYNTHESIS OF SOME D-GLUCOSE LINKED 1,2,3-TRIAZOLES AS UREASE INHIBITORS

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ABSTRACT

This research include synthesis of some new 1,2,3-triazole from D-glucose .The synthesis was started by preparation of phenyl propargyl ether derivative from the reaction of phenol with propargyl bromide . The second step of synthesis was performed by the click reaction of glycosyl azide of protected glucose with acetylenic ether to form 1,2,3-triazole derivatives. The biological activity of synthesized compounds were tested against urease activity. Some of these compounds (T1, T2,Tr3) show high inhibition action on the enzyme activity , while (T4)do not show any effect on the activity .

Keywords: D-glucose, 1,2,3-triazole, urease, inhibitors.

INTRODUCTION

Heterocyclic compounds containing nitrogen plays important role in agrochemical and Pharmaceuticals, The basic heterocyclic rings present in the various medicinal agents are mainly 1, 2, 3-triazole and 1, 2, 4-triazole [1] The 1,2,3-triazole ring system has been the subject of considerable research mainly due to its usefulness in synthetic organic chemistry and also because of the pharmacological properties shown by some of its derivatives. [2] It exhibit various biological effects [3] e.g., antiviral, antibacterial, antifungal, and anticancer activities [4-7] The 1,2,3-triazole moiety is a constituent part of many modified nucleosides or carbanucleosides with antiviral, anti-HIV or cytostatic activities [8-10]. However, the scope of triazole chemistry is not confined to drug discovery, There are an increasing number of applications in numerous other areas of modern chemical sciences, such as bioconjugation [11], supramolecular chemistry, [12] and polymer sciences [13].

MATERIALS AND METHODS

Chemical reagents and starting materials were obtained from Ajax and Sigma-Aldrich Chemical.

Instrumentations

FT-IR spectra were recorded by using Fourier transformation infrared Shimadzu FT-IR-8400S infrared spectrophotometer by KBr disc, Faculty of Pharmacy University of Kufa. 1H NMR, 13C NMR were recorded by Bruker spectrometer, operating at (400MHZ) with (DMSO-d6). Measurements were made at Faculty of Science, Osmania University, India. TLC plates were used with an aluminum backing (0.2 mm, 60 F254).

Synthesis of phenyl propargyl ether derivatives (E1-E4) [14] .

To a round bottom flask was added the phenol derivative (1equiv.), followed by acetone (20 ml), K_2CO_3 (1.2 equiv.) and propargyl bromide (1.2 equiv.) were added, and the heterogeneous mixture was heated to reflux overnight. The mixture was cooled to room temperature, and then quenched in water, then by the addition of sat. aq. NH4Cl. The resulting mixture was extracted with diethyl ether. The combined organic layers were dried over MgSO4. Concentration under reduced pressure gave the desired target structure. The material was used without further purification.

Synthesis of 1,2,3-triazole derivatives of D-glucose via click chemistry (general procedure) (T1-T4) [15].

A solution of propargyl ether (1.0 eq) in DMF(5mL) was added to the suspension of sodium ascorbate (1.2 eq) and $CuSO_4.5H_2O$ (1.2 eq) in DMF (4mL).The mixture was stirred for (10 min) and to this was added an aryl azides derivatives (1.2 eq). The mixture was heated to 50°C with stirring for (10-48 h.). The reaction mixture was diluted with distilled water (30 mL), extracted with EtOAc (3×30 mL), the combined organic layers were washed with sat. NaCl (2× 20 mL), dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was flash chromatographed (silica gel, *n*-Hexane:Et_2O) to yield the desired compounds as a yellow syrup (T1-T4) . (60-80) % .

Studying the biological activity of synthesized compounds [16]

Reagents for Urease activity

The following reagents which supported form ―biomerieux ®,, urea kit.

1- Phosphate buffer PH=8 with concentration 50mM, sodium salicylate (26mM), sodium nitroprosside (3.35mM), EDTA (1mM)

2- Sodium hydroxide (0.5mM) and sodium hypochloride (24.8mM).

Phosphate Buffer (PH 7)

0.5L of 1M K2HPO4 at 174.18 g mol-1 = 87.09g .

0.5L of 1M KH2PO4 at 136.09 g mol-1 = 68.045g .

Preparation of 0.1 M potassium phosphate buffer at 25°C .

Estimation of Urease Activity:

Urease activity is estimated by using end point method for the formation of ammonia per minutes. Urease catalyzed degradation of urea results in the formation of ammonia, which is determined by the Berthelot method (according to method of urea kit). The assay is simple, sensitive, stable and high-throughput adaptable The steps of the method are as follows:

1- The blank of reaction consist of five hundred microliters of 1.65mg/ml of free or immobilize or dispersion jack bean urease (dispersion is solution from urease at concentration 1.65 was mixed with 2mg/ml of synthesized compounds) were mixed with a of solution which contain phosphate buffer PH 8 with concentration of 50 mM, sodium salicylate 26mM, sodium nitroprusside 3.35mM, EDTA 1mM).

2- Twenty microliters of one concentration of urea (50 mg/dl) were mixed with 1 ml of blank, (enzyme and buffer) and then incubated for 5 minutes at room temperature.

3- Two hundred microliters from alkaline reagent (consist of sodium hydroxide 0.5mM, sodium hypochloride 24.8mM) were added and the mixture incubated for 5 minutes, the absorbance was measured at 580 nm by spectrophotometer.

4- Calibration curve was obtained from the absorbance of different concentration of ammonium sulfate

5- Urease activity was determined through measurement released ammonia per minute at room temperature and PH 7.

RESULTS AND DISCUSSION

Synthesis of 1-O{(3-nitrophenyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (T1)

Compound (T1) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether of phenol (E1) and glycosyl azide(G.A) to produce a very good yield .

FT-IR spectrum fig. (1) of compound (T1) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr): 3149 ($\upsilon_{C-H,}$ triazole), 3103 ($\upsilon_{C-H\ of\ benzene\ )}$ 2929($\upsilon_{C-H,}$ CH_3), 2962($\upsilon_{C-H,}$ CH_2),1753($\upsilon_{C=O\ of\ acetate}$) 1616($\upsilon$ C=C, aromatic),1535 (υ_{NO2}), 1467($\delta_{as.C-H,}$ CH_3), 1359 ($\delta_{s.C-H,}$ CH_3), 1270($\delta_{C-H\ aromatic,}$), 1230, ($\upsilon_{C-O,}$ C–O–C), 1039 (υ_{C-O}).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the bands around υ (2119, 2127,3284,) cm^{-1} which is attributed to (-N3 ,C≡C and $\upsilon_{C-H,}$ alkyne) respectively .

^{1}H NMR spectrum fig. (2), (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.96-2.02 (s, 12H, 4C$H_{3\ acetate}$), 4.01 (m, 2H, of C6-H), 4.06(m, 1H, of C3-H), 4.09 (m, 1H, of C2-H),),4.35(m,2H of O-CH_2 of triazole),5.16 (m,1H of C4-H), 6.38 (1H, of C1-H),7.21-8.51 (m , of aromatic and triazoles) .

^{13}C NMR spectrum fig. (3), (100 MHz, DMSO-d_6) showed the following signals at δ (ppm): 24.10,25.05(4C of CH_3 of acetate),62.66(1C of C6),64.64 (1C of C4),76.19(1C of C3),78.26 (1C of OCH2C=C of triazole),79.09 (1C of C5),83.65(1C of C2),90.69 (1C of C1), 110.09,112.25,115.54,120.45,130.36,141.61,151.51,162.51 (m C of aromatic carbon of benzene and triazole ring) 170.15 (4C of 4C=O of acetate)

Synthesis of 1-O{(2-nitrophenyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (T2)

Compound (T2) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether of phenol (E2) and glycosyl azide(G.A) to produce a very good yield

FT-IR spectrum fig. (4) of compound (T2) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr): 3149 ($\upsilon_{C-H,}$ triazole), 3109 ($\upsilon_{C-H\ of\ benzene\ )}$ 2951($\upsilon_{C-H.}$ CH_3), 2956($\upsilon_{C-H,}$ CH_2),1751($\upsilon_{C=O\ of\ acetate}$) 1602($\upsilon$ **C=C**, aromatic),1529 (υ_{NO2}), 1452($\delta_{as.C-H,}$ CH_3), 1371 ($\delta_{s.C-H,}$ CH_3), 1240($\delta_{C-H\ aromatic,}$), 1226, ($\upsilon_{C-O,}$ C–O–C), 1047 (υ_{C-O}).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the bands around υ (2119, 2135, 3253,) cm^{-1} which is attributed to (-N3 ,C≡C and $\upsilon_{C-H,}$ alkyne) respectively .

^{1}H NMR spectrum fig. (5), (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.96-2.02 (s, 12H, 4CH_3 $_{isopropylidene}$), 4.05 (m, 2H, of C6-H), 4.09(m, 1H, of C3-H), 4.14 (m, 1H, of C2-H),),4.34(m,2H of O-CH_2 of triazole),5.16 (m,1H of C4-H), 6.37 (1H, of C1-H),7.12-8.55 (m , of aromatic and triazoles) .

^{13}C NMR spectrum fig. (6), (100 MHz, DMSO-d_6) showed the following signals at δ (ppm): 25.25,25.59 (4C of CH_3 of acetate),62.37(1C of C6),64.64 (1C of C4), 76.19(1C of C3),78.26 (1C of OCH2C=C of triazole),79.09 (1C of C5),83.71(1C of C2),90.64 (1C of C1), 110.07,112.25,115.57,122.78,130.38,137.86,138.48, (m C of aromatic carbon of benzene and triazole ring) 171.18 (4C of 4C=O of acetate).

Synthesis of 1-O{(2-al-phenyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (T3)

Compound (T3) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether of phenol (E3) and glycosyl azide(G.A) to produce a very good yield

FT-IR spectrum fig. (7) of compound (T3) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr): 3149 ($\upsilon_{C-H,}$ triazole), 3082 ($\upsilon_{C-H\ of\ benzene\ )}$ 2949($\upsilon_{C-H,}$ CH_3), 2930($\upsilon_{C-H,}$ CH_2),2883(υ_{C-H} of aldehyde), (1753 ($\upsilon_{C=O\ of\ acetate}$) 1683($\upsilon$ **C=C**, aromatic), 1458($\delta_{as.C-H,}$ CH_3), 1373 ($\delta_{s.C-H,}$ CH_3), 1250($\delta_{C-H\ aromatic,}$), 1230, ($\upsilon_{C-O,}$ C–O–C), 1039 (υ_{C-O}).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the bands around υ (2119, 2125, 3271,) cm^{-1} which is attributed to (-N3 ,C≡C and $\upsilon_{C-H,}$ alkyne) respectively .

^{1}H NMR spectrum fig. (8), (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.22-1.35 (s, 12H, 4CH_3 $_{isopropylidene}$), 3.56 (m, 2H, of C6-H), 4.06(m, 1H, of C3-H), 3.68 (m, 1H, of C2-H),),4.49(m,2H of O-CH_2 of triazole),4.58 (m,1H of C4-H), 6.73 (1H, of C1-H),7.28-7.78 (m , of aromatic and triazoles),10.06(s,1H of C-H of aldehyde) ^{13}C NMR spectrum fig. (9), (100 MHz, DMSO-d_6) showed the following signals at δ (ppm): 24.19,25.20(4C of CH_3 of acetate),62.39(1C of C6),64.64 (1C of C4),76.19(1C of C3),78.26 (1C of OCH2C=C of triazole),79.09 (1C of C5),83.95(1C of C2),108.49 (1C of C1), 110.09,114.35 ,130.36,137.96,138.49,141.61,146.55,162.15 (m C of aromatic carbon of benzene and triazole ring) 170. 51 (4C of 4C=O of acetate), 190.15 (1C of C=O of aldehyde).

Synthesis of 1-O{(1-naphthyl)-1H-1,2,3-triazole-4-yl}methyl]- 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (T4)

Compound (T4) was synthesized by 1,3-dipolar cycloaddition reaction catalyzed with Cu(I) of propargyl ether of phenol (E4) and glycosyl azide(G.A) to produce a very good yield

FT-IR spectrum fig. (10) of compound (T4) showed the following bands at $\bar{\upsilon}$ cm^{-1} (KBr): 3130 ($\upsilon_{C-H,}$ triazole), 3089 ($\upsilon_{C-H\ of\ benzene\ )}$ 2962 ($\upsilon_{C-H.}$ CH_3), 2924($\upsilon_{C-H,}$ CH_2),1753($\upsilon_{C=O\ of\ acetate}$) 1629($\upsilon$ **C=C**, aromatic), 1444($\delta_{as.C-H,}$ CH_3), 1369 ($\delta_{s.C-H,}$ CH_3), 1271($\delta_{C-H\ aromatic,}$), 1224, ($\upsilon_{C-O,}$ C–O–C), 1041 (υ_{C-O}).

FT-IR spectrum is a good evidence that cycloaddition reaction happened successfully through disappearing the bands around υ (2119, 2358,3292) cm^{-1} which is attributed to (-N3 ,C≡C and $\upsilon_{C-H,}$ alkyne) respectively .

^{1}H NMR spectrum fig. (11), (400 MHz, DMSO-d_6) for the compound showed the following signals at δ (ppm): 1.92-2.02 (s, 12H, 4CH_3 $_{isopropylidene}$), 4.01 (m, 2H, of C6-H), 4.06(m, 1H, of C3-H), 4.09 (m, 1H, of C2-H),),4.35(m,2H of O-CH_2 of triazole),5.16 (m,1H of C4-H), 6.38 (1H, of C1-H),7.21-8.71 (m , of aromatic and triazoles) .

^{13}C NMR spectrum fig. (12), (100 MHz, DMSO-d_6) showed the following signals at δ (ppm): 24.55,25.15(4C of CH_3 of acetate),62.35(1C of C6),64.64 (1C of C4),76.15(1C of C3),78.26 (1C of OCH2C=C of triazole),79.59 (1C of C5),83.55(1C of C2),108.24 (1C of C1), 110.09,112.22,115.52,116.45,130.36,137.96,141.61,146.21,162.12 (m C of aromatic carbon of benzene and triazole ring) 171.21 (4C of 4C=O of acetate).

Studying the biological activity of synthesized compounds

The biological activity of the synthesized compounds were tested against the activity of urease enzyme (which is catalyze urea to ammonia and carbon dioxide). The enzyme was immobilize on each one of synthesized compound by taking one concentration of each compound with enzyme and stirring the mixture for 10 min. The triazoles

derivatives show different inhibition action toward the enzyme shown in figure (13).
Some of these compounds (T1,T2,T3) show high inhibition action on the enzyme , while (T4)do not show any effect on the activity .
The activities of enzyme with different compound were calculated from the amount of liberated ammonia per 5 min as shown in table (2).
Table (1) show the amount of liberated ammonia and enzyme activities
* The amount of liberated ammonia were calculate from relationship
(ammonia concentration = Absorbance-0.41/0.091) which is obtain from calibration curve figure (12) .
*The enzyme activity were calculate by relationship
(Urease activity = amount of liberated ammonia /time) . *The time was 5 min

CONCLUSION

1-possibility to synthesis wide range of new triazoles derivatives containing different sugars by different methods .
2-All synthesized compound was prepared with high yield and high stability .
3-most of synthesized compounds have inhibition action towards urease enzyme.

ACKNOWLEDGEMENTS

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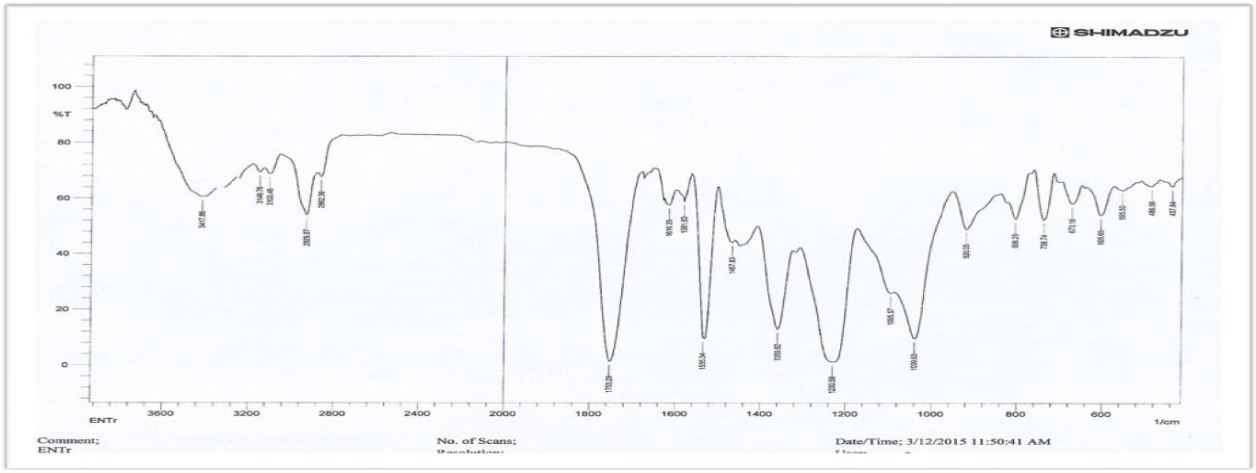


Figure (1)FT-IR spectrum of compound (T1)

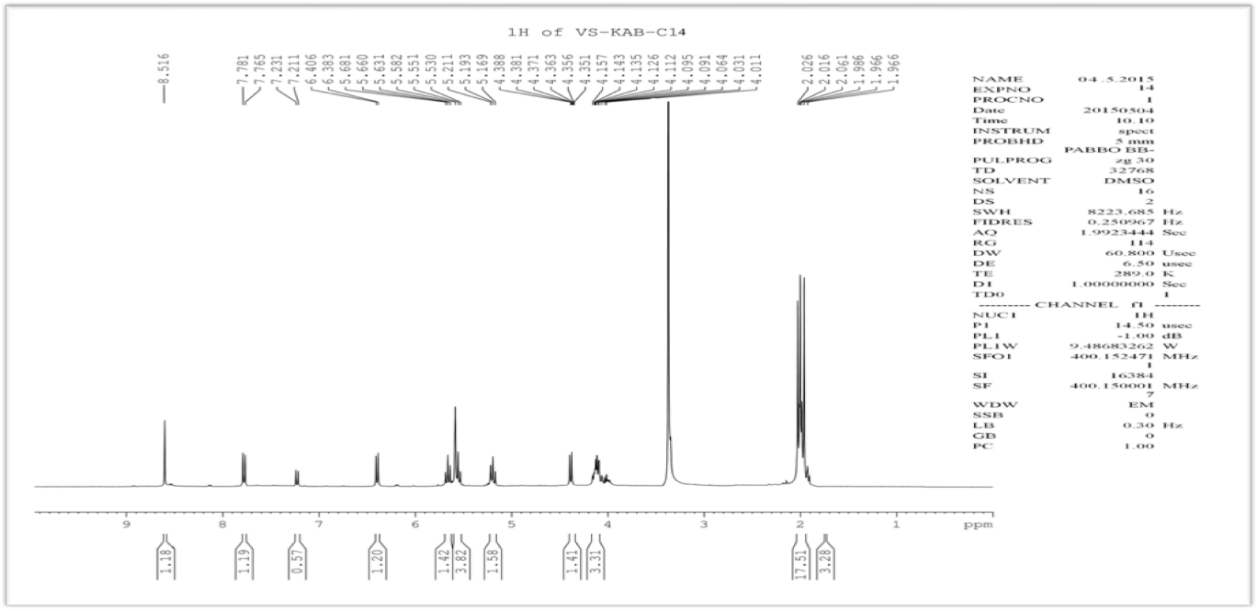


Figure (2) ^{1}H NMR spectrum of compound (T1)

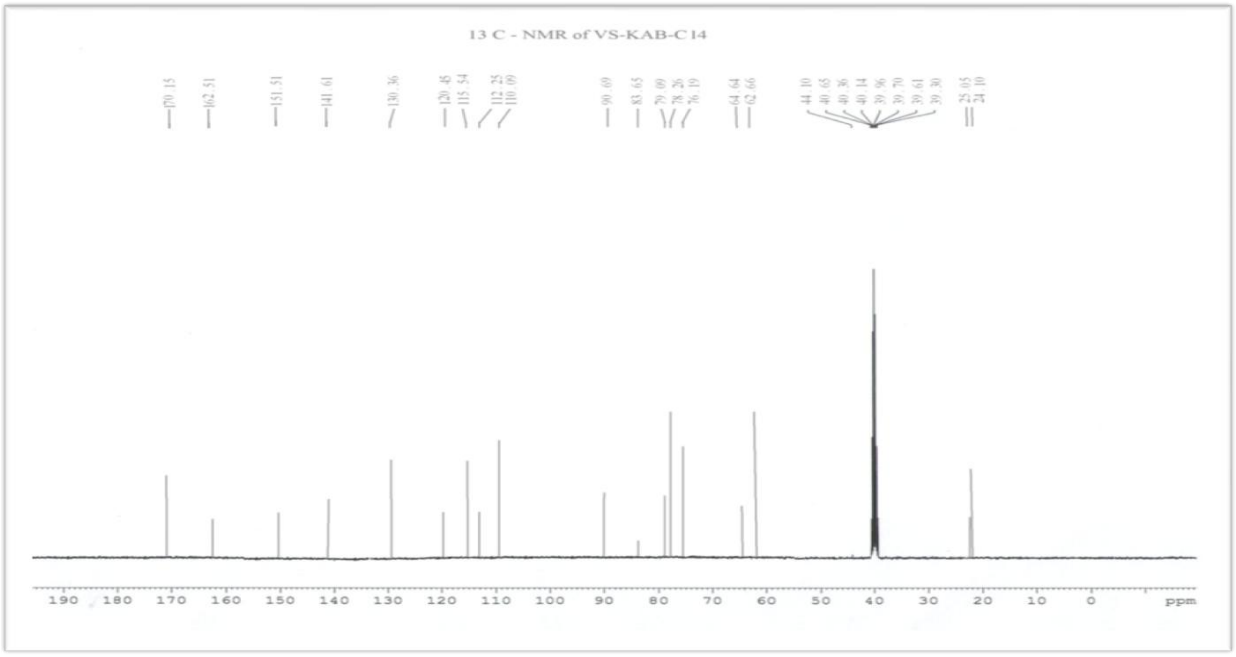


Figure (3) ^{13}C NMR spectrum of compound (T1)

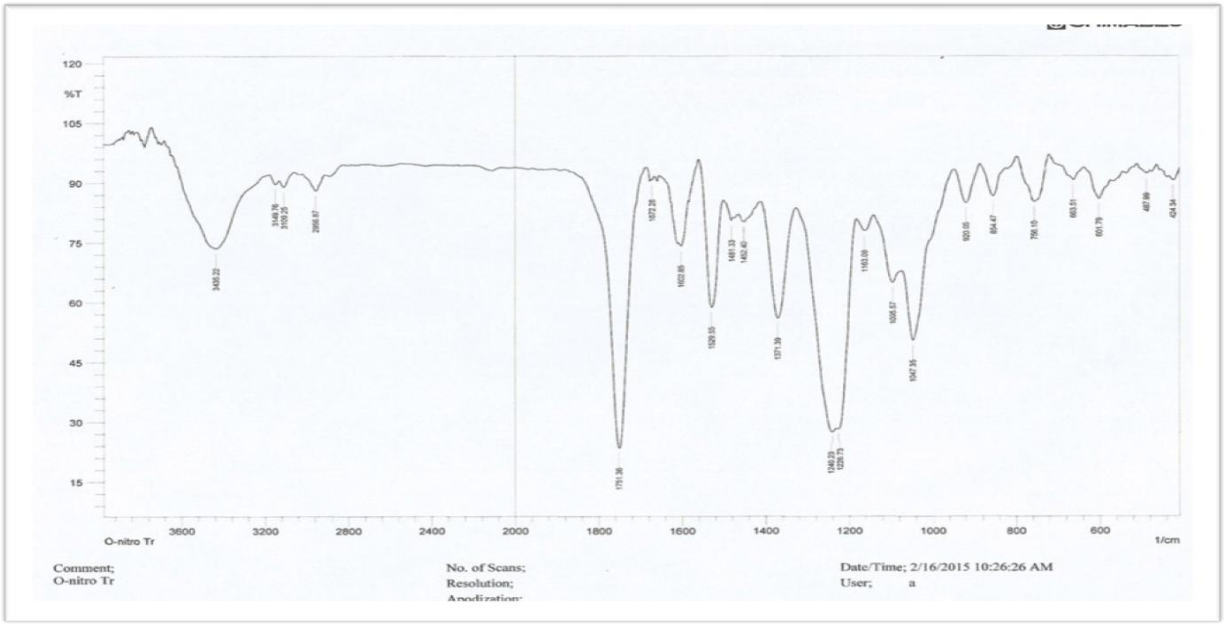


Figure (4) FT-IR spectrum of compound (T2)

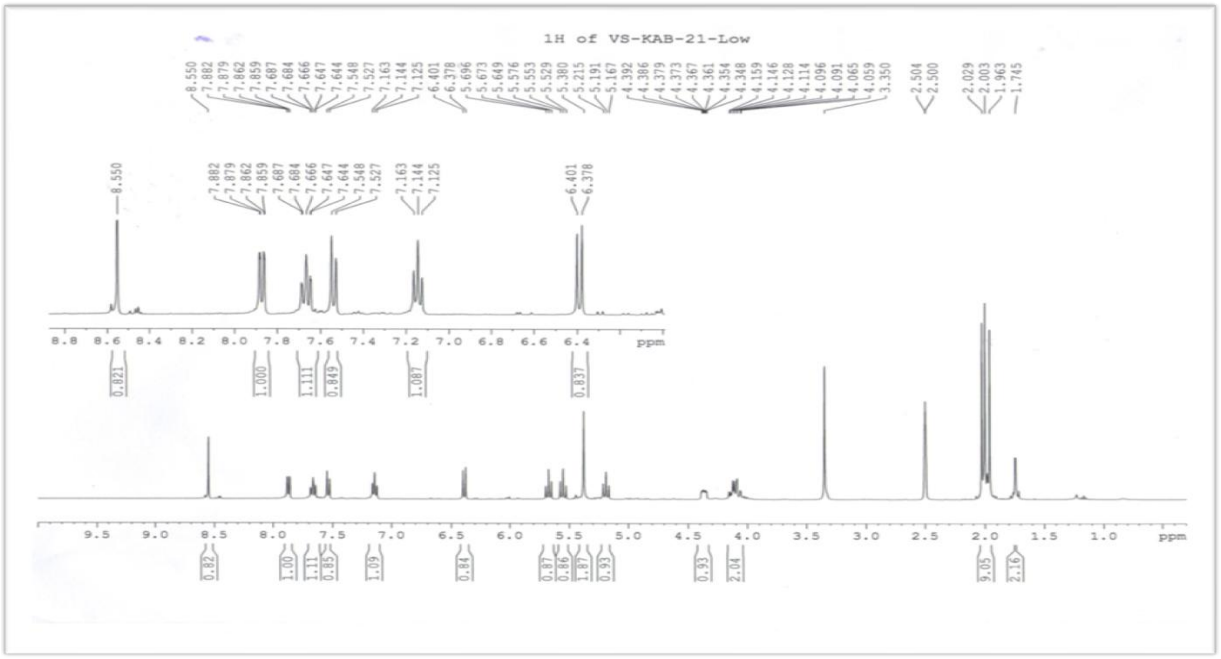


Figure (5) ^{1}H NMR spectrum of compound (T2)

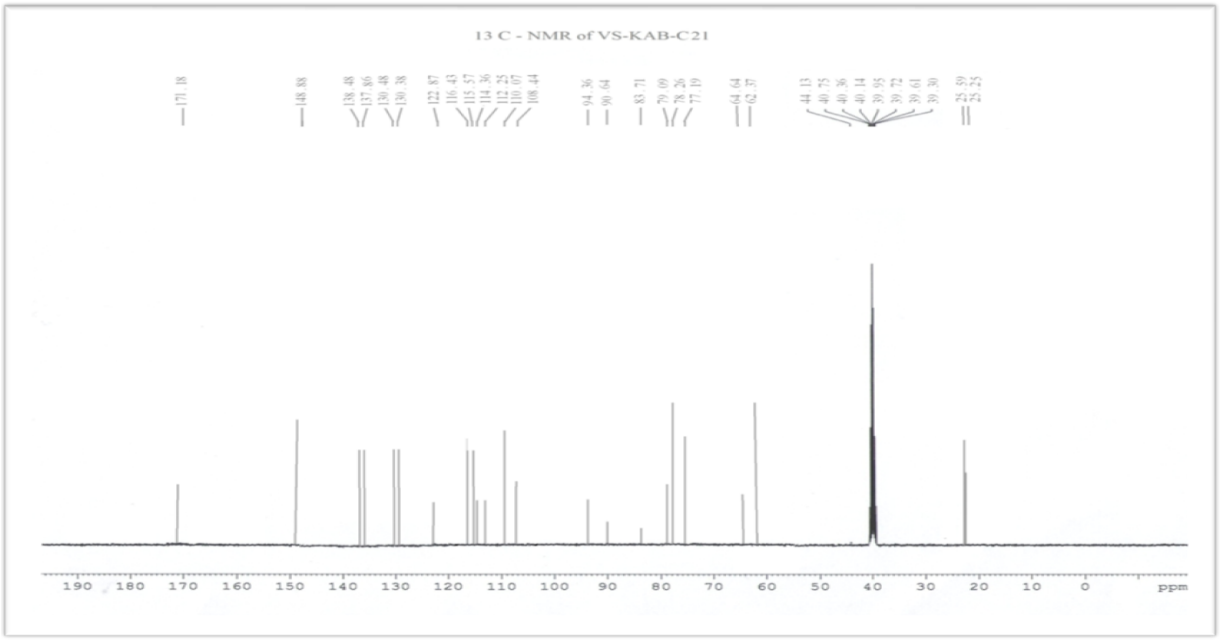


Figure (6) ^{13}C NMR spectrum of compound (T2)

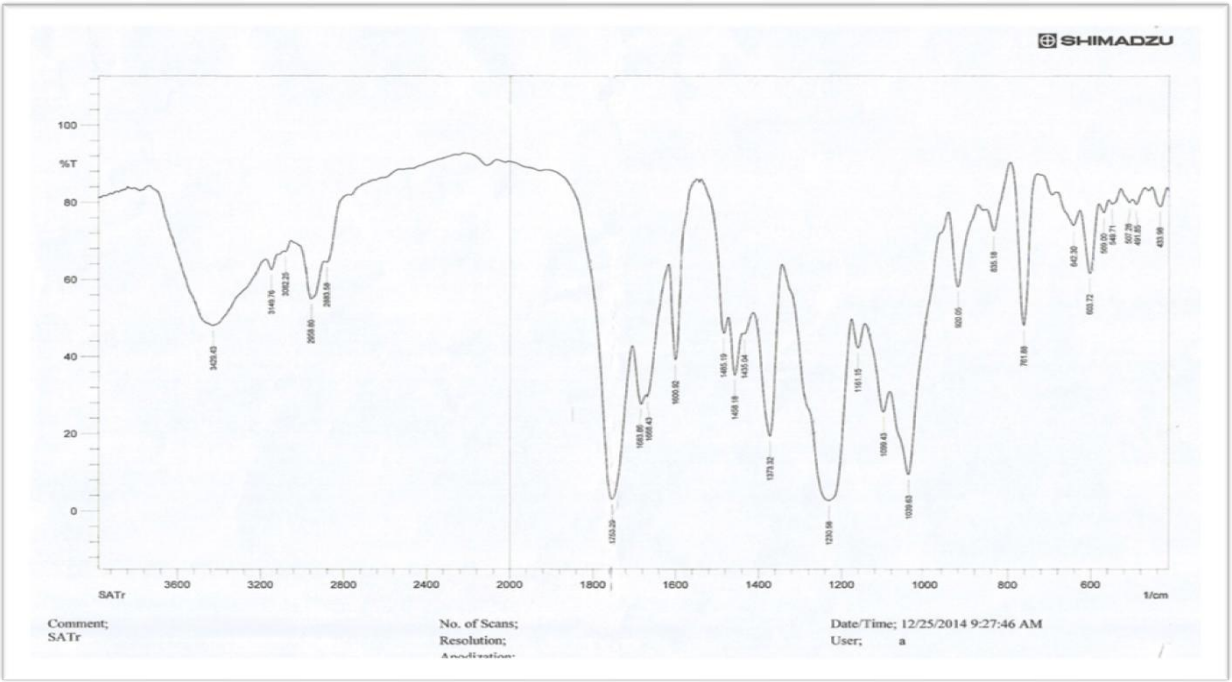


Figure (7) FT-IR spectrum of compound (T3)

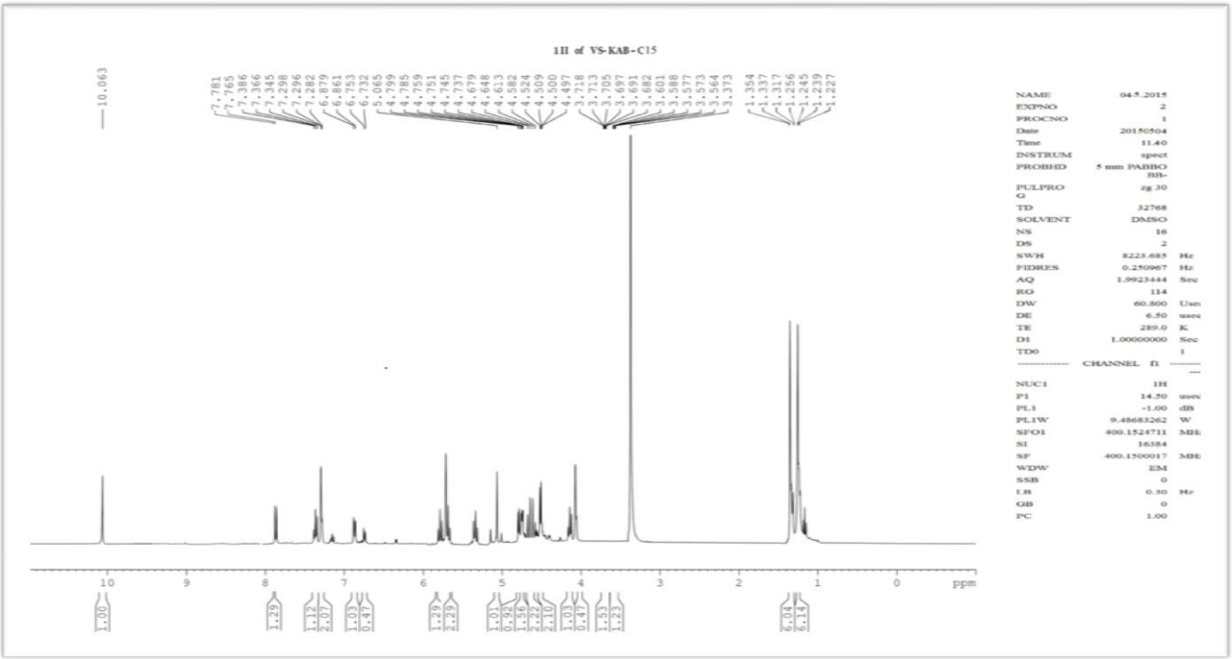


Figure (8) ^{1}H NMR spectrum of compound (T3)

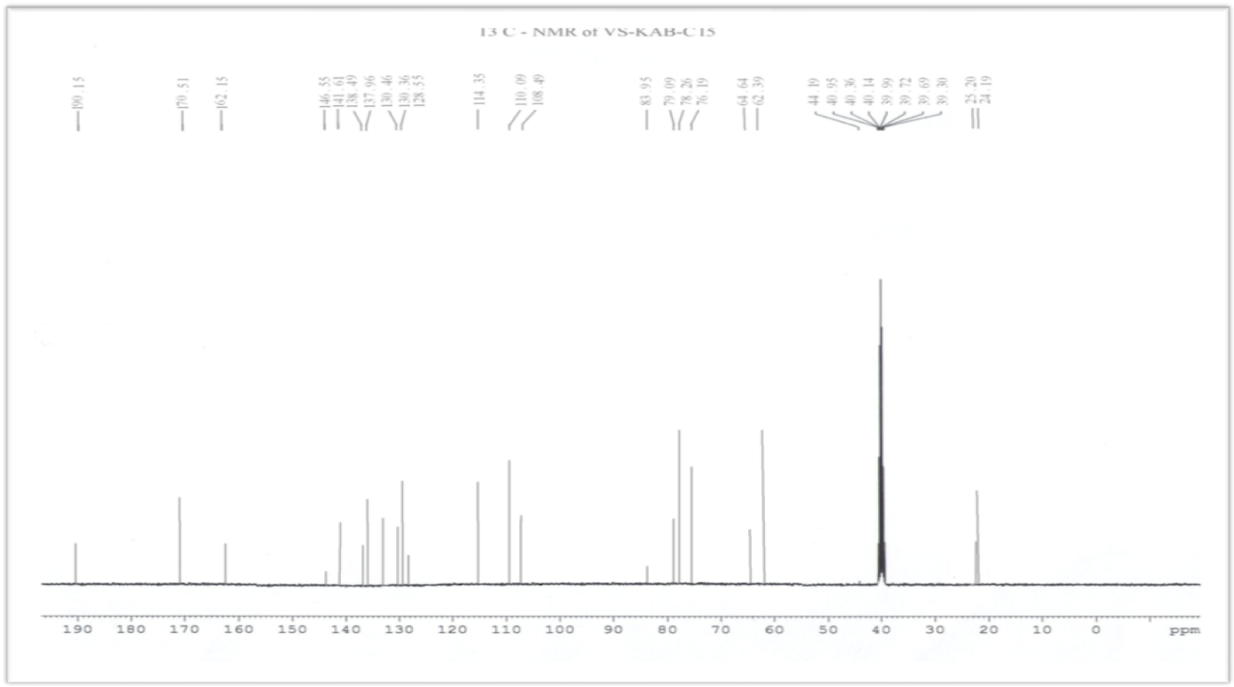


Figure (9) ^{13}C NMR spectrum of compound (T3)

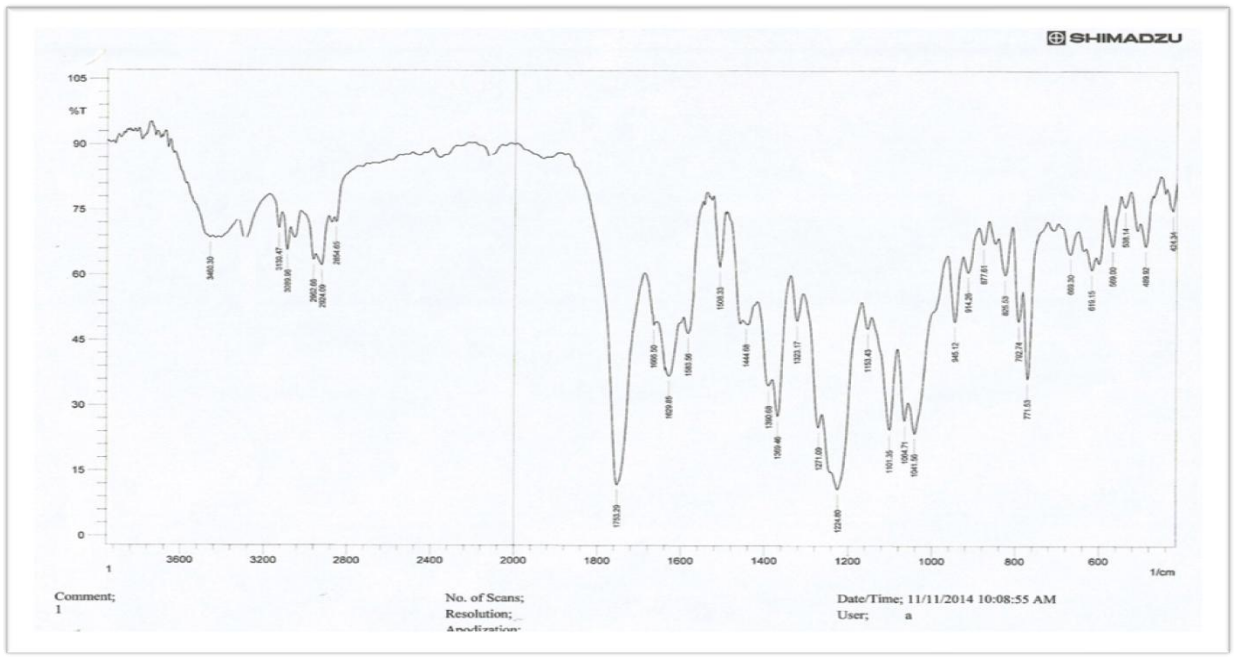


Figure (10)FT-IR spectrum of compound (T4)

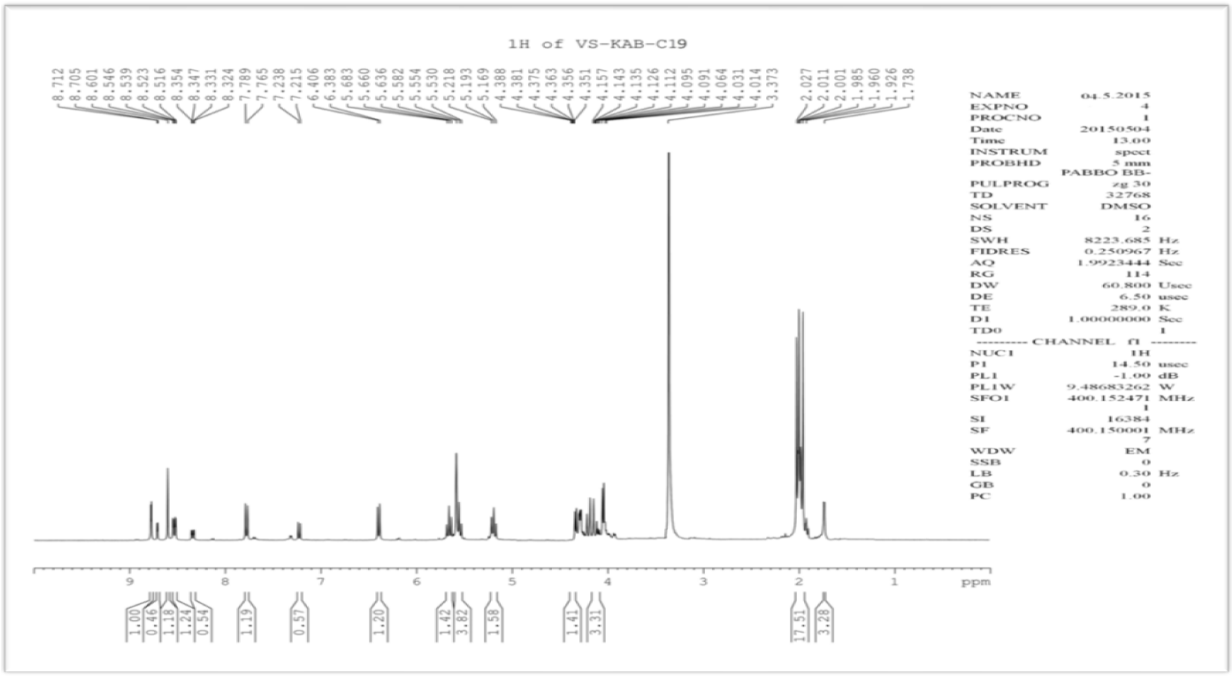


Figure (11) ^{1}H NMR spectrum of compound (T4)

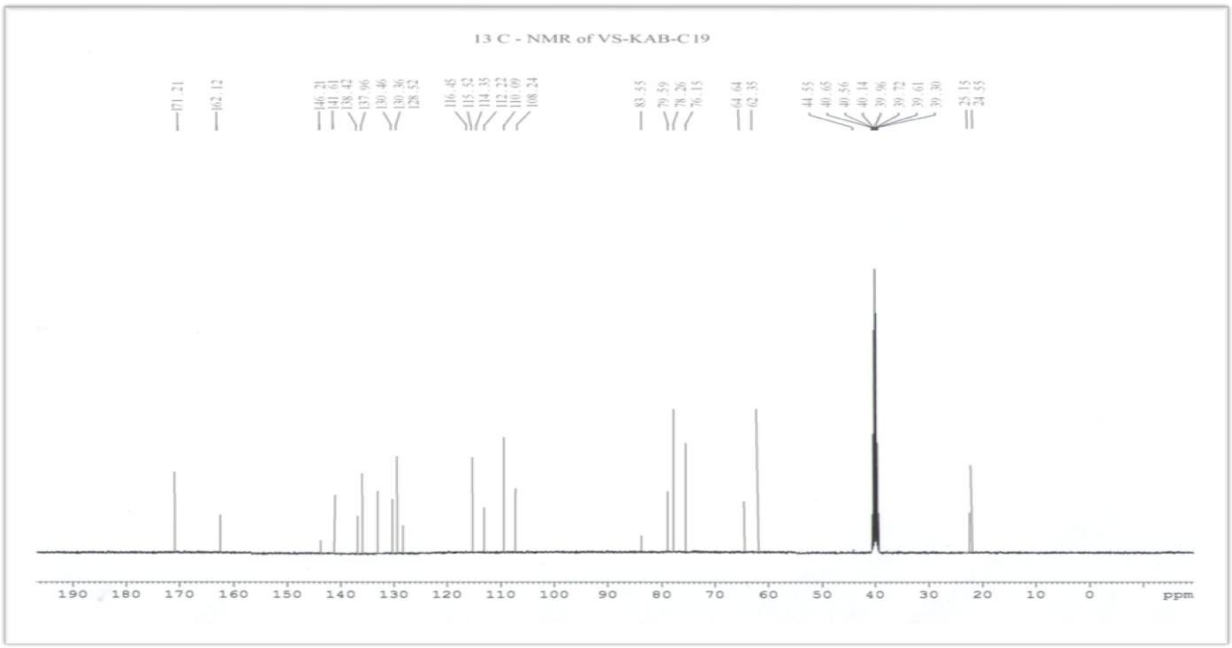
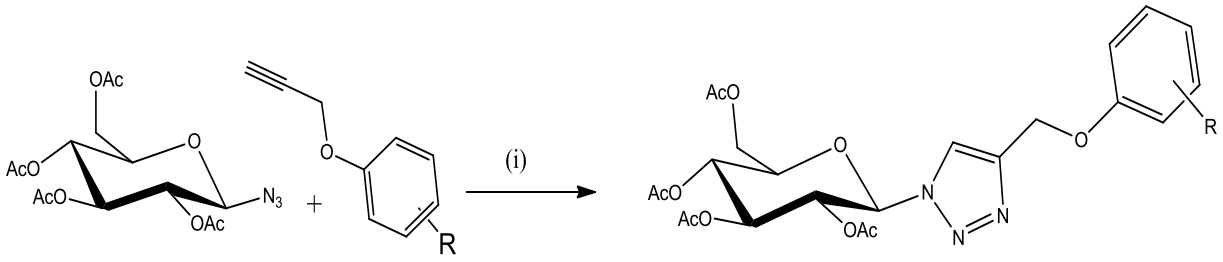


Figure (12) ^{13}C NMR spectrum of compound (T4)



glycosyl azide (G.A) (E1-E4) (T1-T4)

R1=3-NO_2,R2=2-NO_2,R3=2-CHO,R4=phenyl

(i) Na Ascorbate , $CuSO_4$.$5H_2O$, DMSO , 5 ^{0}C ,(10 - 48) hr

Scheme (1) Synthesis of 1,2,3-triazole derivatives of D-glucose

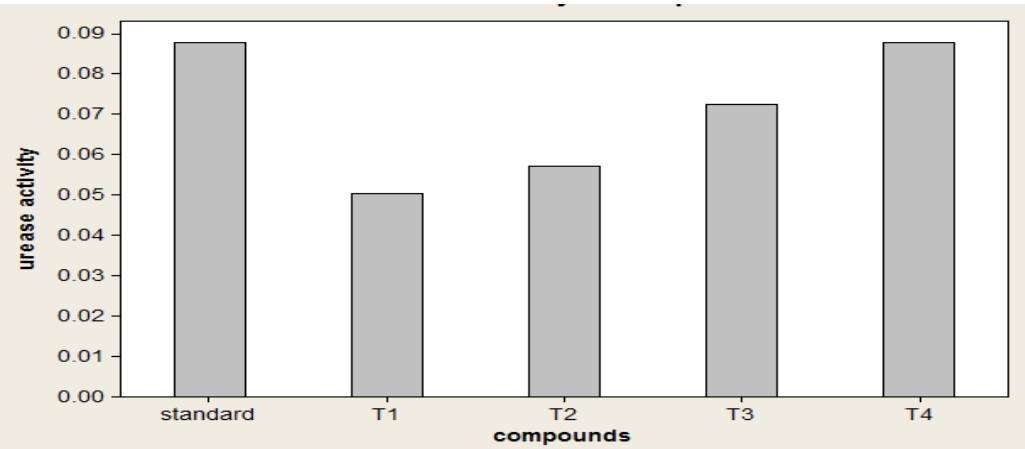


Figure (13) the effect of synthesized compounds on urease activity

Table (1) show the amount of liberated ammonia and enzyme activities

	Name of compound	Absorbance	Ammonia concentration	Urease activity
1	**standard**	**0.180**	**0.439**	**0.0878**
2	**T1**	**0.171**	**0.340**	**0.0680**
3	**T2**	**0.166**	**0.285**	**0.0570**
4	**T3**	**0.173**	**0.362**	**0.724**
5	**T4**	**0.180**	**0.439**	**0.0878**

* The amount of liberated ammonia were calculate from relationship
(ammonia concentration = Absorbance-0.41/0.091) which is obtain from calibration curve figure (12) .
*The enzyme activity were calculate by relationship
(Urease activity = amount of liberated ammonia /time) .
*The time was 5 min

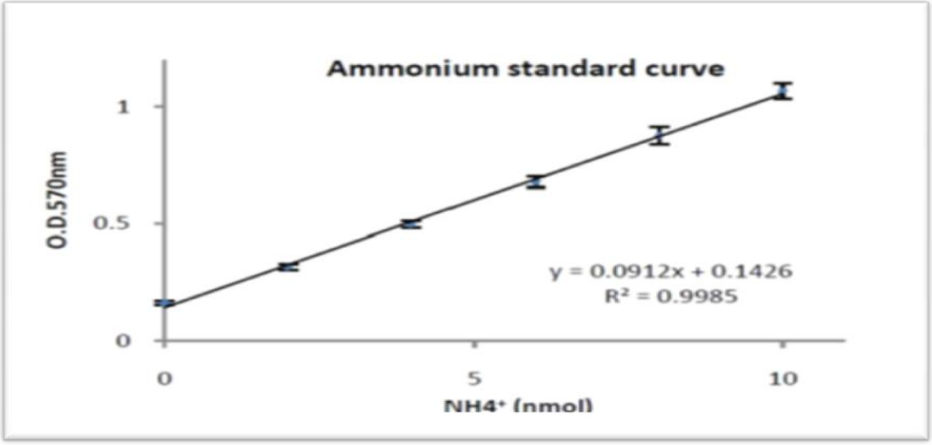


Figure (14) calibration curve for librated ammonia

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