Synthesis, Characterization and Biological Study of Two Azide and Two bis 1,2,3- Triazol Acyclonucleoside Anaglues of Thimen

Faeza A. Almashal **Hamsa H. Al-hujaj** **Usama H. Ramadhan**[2]

Dept. of Chemistry, College of Education for Pure Sciences University of Basrah – Iraq

[1] *Dept. of Pharmacognosy Chemistry, College of Pharmacy, University of Basrah – Iraq*
Fae71za@tahoo.com

Abstract

Tow new Azide and two new bis-1,2,3-triazole acyclonucleoside compounds were synthensized. The triazole acyclonucleoside compounds have been prepared by 1,3-dipolar cycloaddition reaction of azido sulfaisoxazole and azido sulfaquanidine with propergylthymine in water and ethanol as solvents under reflux condition with Cu(I) as catalyst. The expected structure of the final newly compounds were determined on the basis melting point FT-IR and H-NMR.

Keywords: Azides , 1,2,3-triazole- acyclonucleoside, 1,3-dipolar cycloaddition reactions, click chemistry

Introduction

" Click chemistry " one of the most versatile and modular approaches to couple two reactive partnes in a facile, quick , selective , reliable and high yield reaction under mild conditions (Arti, P.K. *et al.,* 2013; Kolb, C.H. *et al.,* 2001).

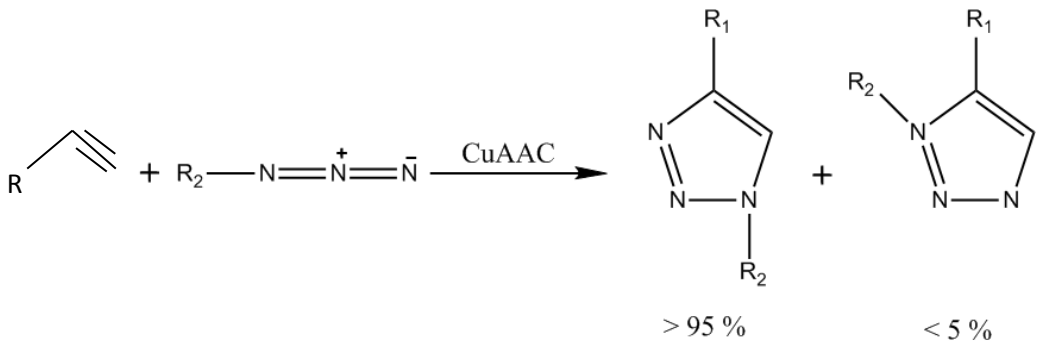
Since then click chemistry has become one of the most common and reliable methods to link molecules covalently, and it finds applications in a variety of disciplines including the chemistry of nanomaterial-s, chemical biology, drug delivery and medicinal chemistry (Binder, W.H. 2008; Hou, J. *et al.,* 2012).

This types of reactions are using to introduced heterocyclic compounds 1,2,3-triazole one more of compounds "click chemistry " (Elayadi, H. *et al*., 2010).

1,2,3- triazole nucleosides are N-heterocyclic compounds which have been the subject of considerable research, mainly due to their value in synthetic organic chemistry (Broggi, *et al.,* 2007; Guezguez *et al.,* 2006).

Based on the sharpless-Meldal modified Huisgen reaction using Cu(I) salts as catalyst (Huigen, R. 1963; Rostovtsev, v.v. 2002). The classical 1,3-dipolar cycloaddition of azids and alkynes discovered by Huisgen (Tornoe, C.W. *er al.,* 2002) often given mixture of regioisomers (1,4 and 1,5-disubstituted triazoles). However, it didn't altractmuch interest until it was demonstrated this high temperature reaction could also be carried out under mild condition using Cu(I) as the catalyst, and with tremendous region-selectivity(scheme 1).

The coordination of Cu (I) to alkynes in an aqueous solution forming a copper-acetylide intermediate is an exothermic reaction. The azide binds to this Cu(I)-acetylide intermediate forming a six membered Cu(III)-metallocycle (Huisgen, R. 1963). Subsequently, the triazole ring formation is very rapid (Himo F, *el al.,* 2005). The advantages of this alkyne-azide coupling reaction include an almost quantitative conversion, the robust nature of the products, biomolecular ligation, in vivo tagging (Beatty, K. E. 2006). And used in the synthesis of linear polymers (Golas, P.L. 2007).



Scheme 1. Copper - Catalyzed alkyne - azide cycloaddition

Experimental

Melting points were determined on thermo scientific apparatus at laboratories chemistry dept. college of

education. All ^{1}H-NMR were recorded on Bruker –Spectrometer at 250 MHZ, faculty of science, micro analytical center Tahrane. TLC is performed on silica gel 60 F_{254} sheet layer (Merck). The materials from Merck, Ridel and Flukal companies.

General method for preparation of Azido compounds 2 and 4.

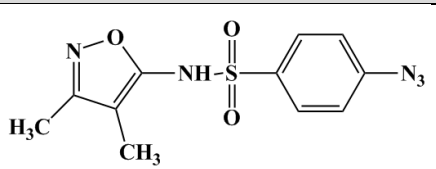
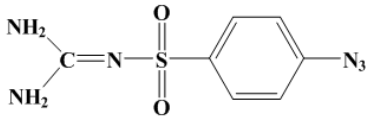
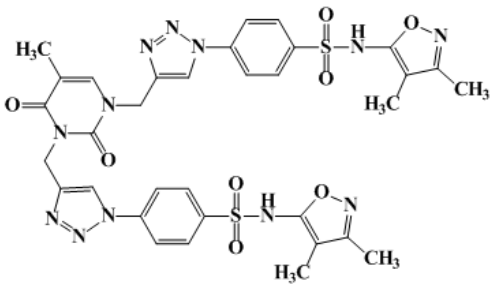
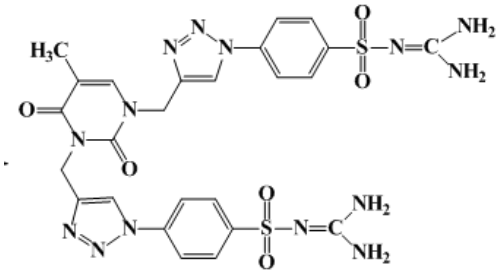
To a stirred solution of 0.001 mol sulfa compounds 2 or 4, (mekni N., *et al.*, 2009) in 0.35 ml conc. hydrochloric acid and 10 ml water at 0°C a solution (of 0.078 gm, 0.001 mol sodium nitrite in 5 ml water), was added drop wise. The mixture were stirred at 0°C for 15 min, then 10 ml water was added slowly with cooling, left tell white would form collected by suction filtration and recrystallised from chloroform to give white needel crystal with melting pint and yield as shown in Table 1.

General method fo preparation of bis 1,2,3- triazole acyclonucleoside compounds T_1 and T_2.

A mixture of 0.002 mol Azido compounds 2 or 4, 0.20gm, 0.001mol propargylthymine, 0.004 gm, 0.00l mol Et_3N and 0.05 gm, 0.001 mol CuI (Hwang, S. *et al.*, 2012; Lewis, W.G. *et al.*, 2004) were dissolved in water / ethanol (1:1 v/v), reaction mixture was reflexed several hours as shown in Table 1. The reaction mixtures were followed by TLC, until the starting material were no more detected, solvent was evaporated.

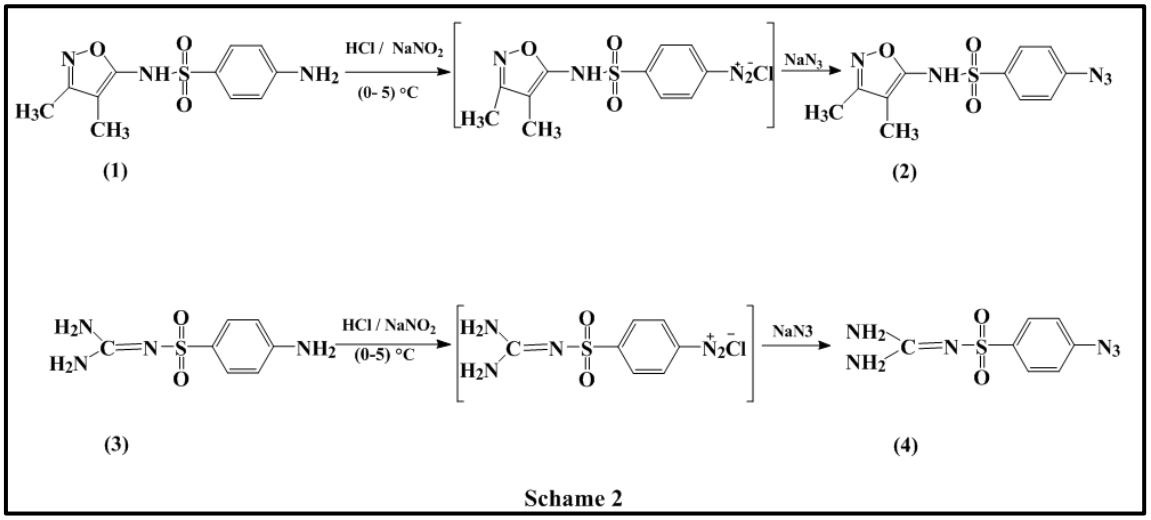
The crude product were purified by columm chromatography silicagel mesh (100-200) then recrystallization from THF: hexane (8:2) to get pure substance as shown in Table 1.

Table 1 the physical properties for the prepared compounds.

Symb.	Structure	Name of IUPAC system	eluent	mp °C	Yield %	coluor	Tim of reactr
2		4-azido-N-(3,4-di methylisoxazol -5-yl) benzene sulfonamide	9.5:0.5 dichloro methan: methanol	131-133	90	White crystal	5 min
4		4-azido-N-(di aminomethylene) benzene sulfonamide	9.5:0.5 dichloro methan: methanol	178-179	80	White crystal	5 min
T_1		4, $\bar{4}$-(4, $\bar{4}$-(5-methyl-2,4- dioxo pyrimidin -1,3(2H, 4H)-diyl) bis (methylene) bis (1H-, 1,2,3-triazol -4,1-diyl)) bis (N-(3,4-dimethyl isoxazol-5-yl) benzene sulfonamide	8:2 chloroform: ethanol	113-116	30	Yellow powder	120 hr
T_2		4, $\bar{4}$-(4, $\bar{4}$-(5-methyl-2,4- dioxo pyrimidin -1,3(2H, 4H)-diyl) bis (methylene) bis (1H-, 1,2,3-triazol -4,1-diyl)) bis (N-(diaminomethylene) benzene sulfonamide	8:2 chloroform: ethanol	183 dec-	20	White powder	15 hr

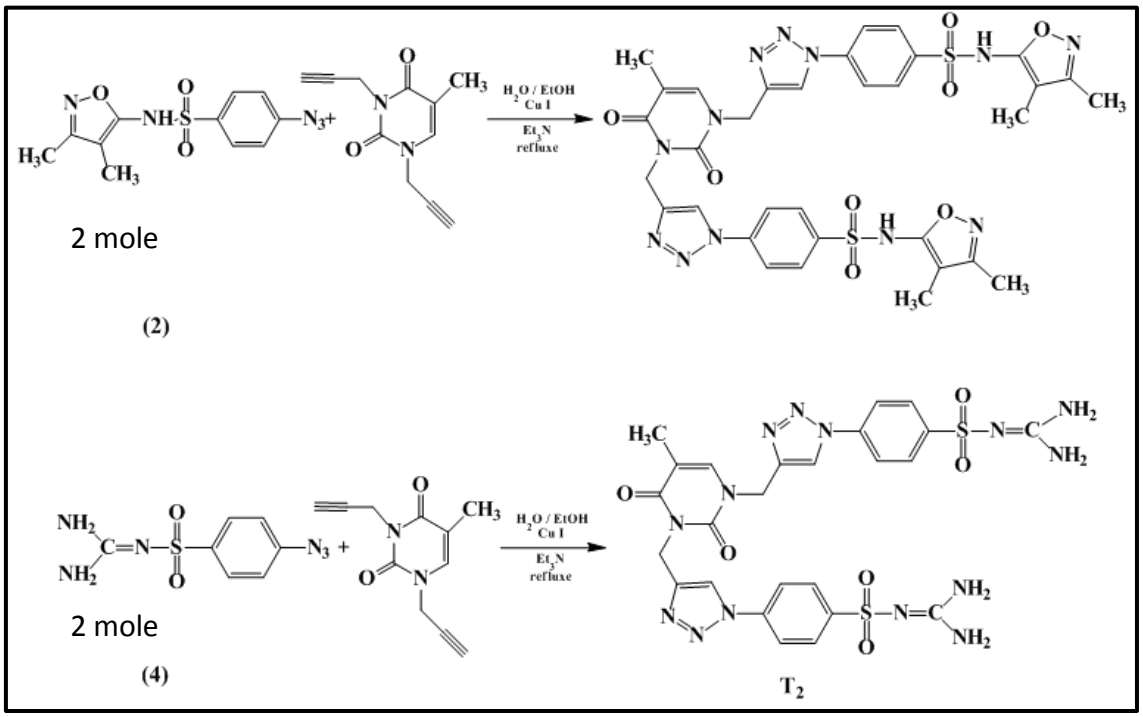
Results and discussion

4-azido –N-(3,4-dimethyl isoxazol-5-yl) benzene sulfonamide 2 and 4- azido –N- (diamino methylene) ben zene sulfonamide 4 can be prepared from diazonium salts according to published method (Nilan, R.A. *et al.* 1973), as shown in Scheme 2.



Schame 2

4-azido –N- (3,4-dimethyl isoxazol-5-yl) benzene sulfonamide 2 and 4-azido –N-(diamino methylene) benzene sulfonamide 4 react with propargylthymine by 1,3-dipolar cycloaddition reaction to give 1,2,3-triazole derivatives T_1 and T_2 (Negron, G.E. 2013; Krim, J. *et al.*, 2012) as shown in Scheme 3 .



Schame 3

1- FT-IR Spectro (Pavia, L. D, 3rd Ed.)

All selected bands of diagnostic importance are collected in Table 2. IR spectra of the 4-azido –N-(3,4-dimethylis oxazol -5- yl) benzene

sulfonamide (2) and 4-azido-N-(diamino methylene) benzene sulfonamide (4) show the presence of strong band at (2135 and 2123) cm^{-1} attributed to stretching vibration of ($-N_3$), give, strong and give bonds attributed to ($-So_2$) stretching vibration within the range (1178 – 1284 and 1249) cm^{-1}, while the IR- spectra of

Triazole compounds (T_1 and T_2) were noted the band for ($-N_3$) group was disappear which related to azide compound and appearance of new absorption bands related to triazole derivatives as shown in Table 2 and figure (1-4). The suggest structures indicate that the cycloaddition reaction was take place the cycloaddition.

Table 2: Data of IR spectra for the prepared compounds

Symb	N-H	NH_2 cm^{-1}	C-H Arom cm^{-1}	C-H Alif cm^{-1}	N_3 cm^{-1}	C=O cm^{-1}	SO_2 cm^{-1}	C=C trazole C=C ar N=N trizole cm^{-1}
2	3460	-	3095	2989	2135	-	1178-1286	-
4	-	3498-3441	3228	-	2123	-	1176-1249	-
T_1	3261	-	3078	2929	-	1666-1639	1163-1342	1463-1508
T_2	-	3435-3338	3056	2927	-	1627-1662	1172-1348	1465-1504

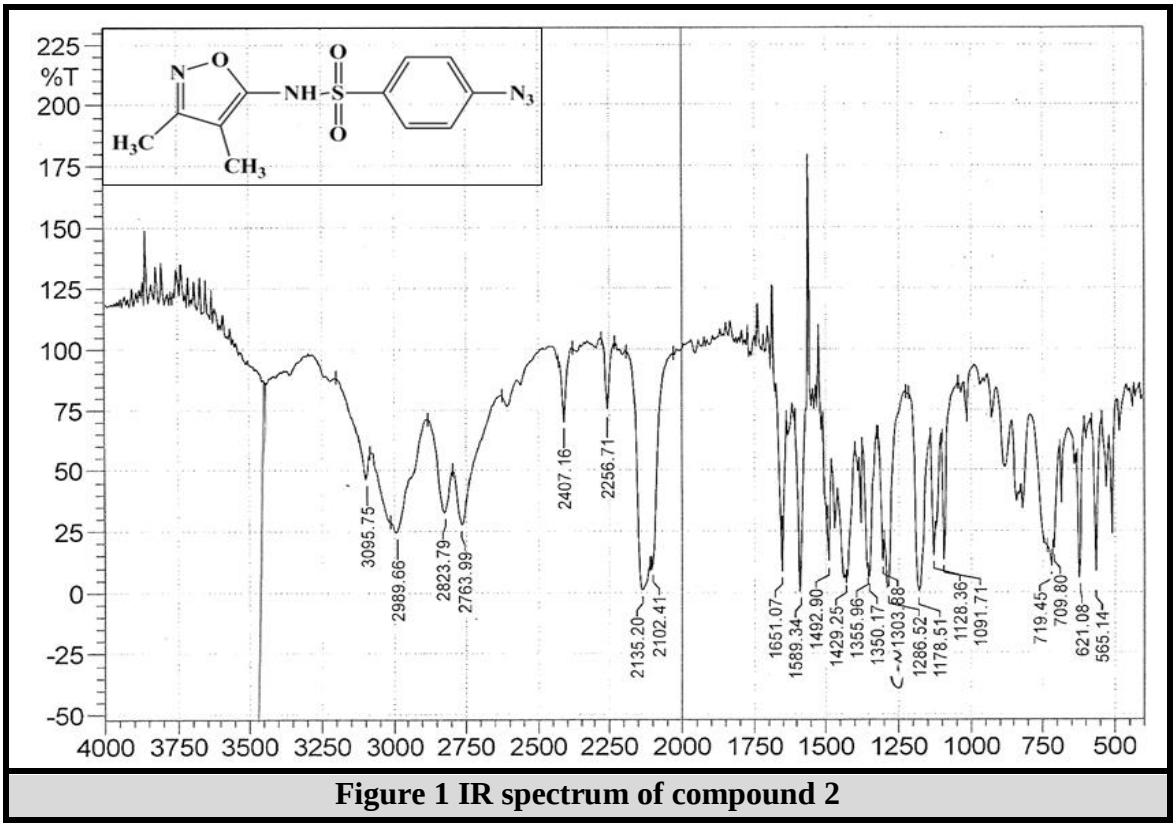


Figure 1 IR spectrum of compound 2

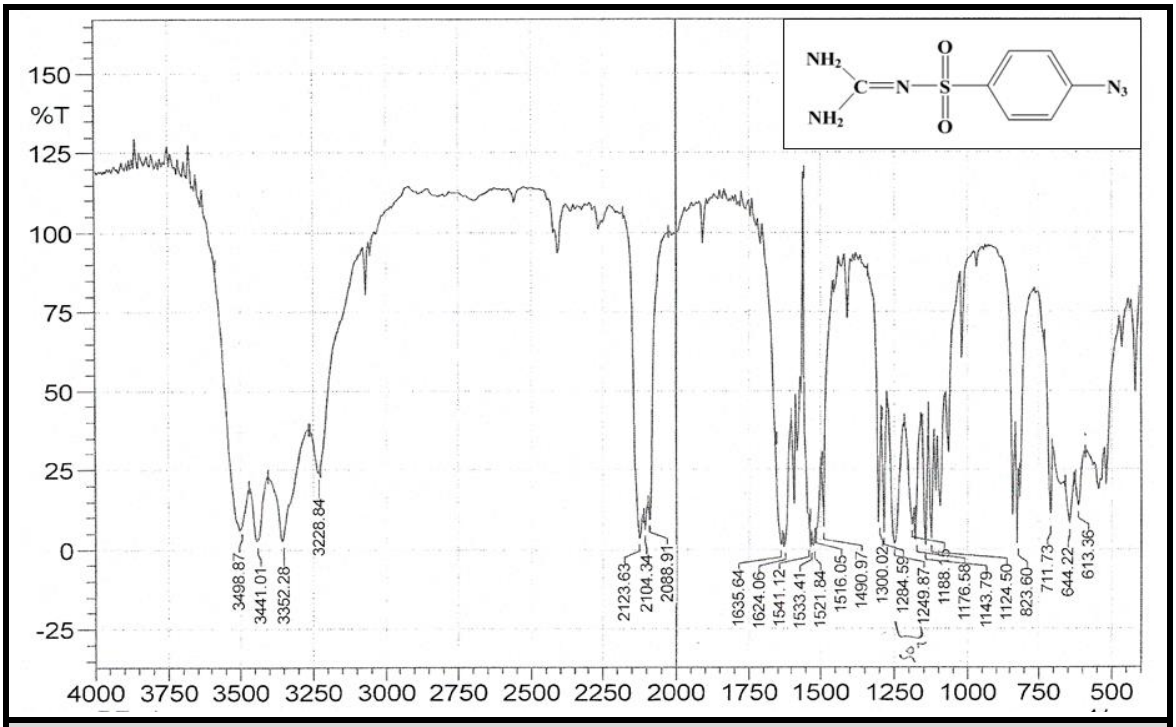


Figure 2 IR spectrum of compound 4

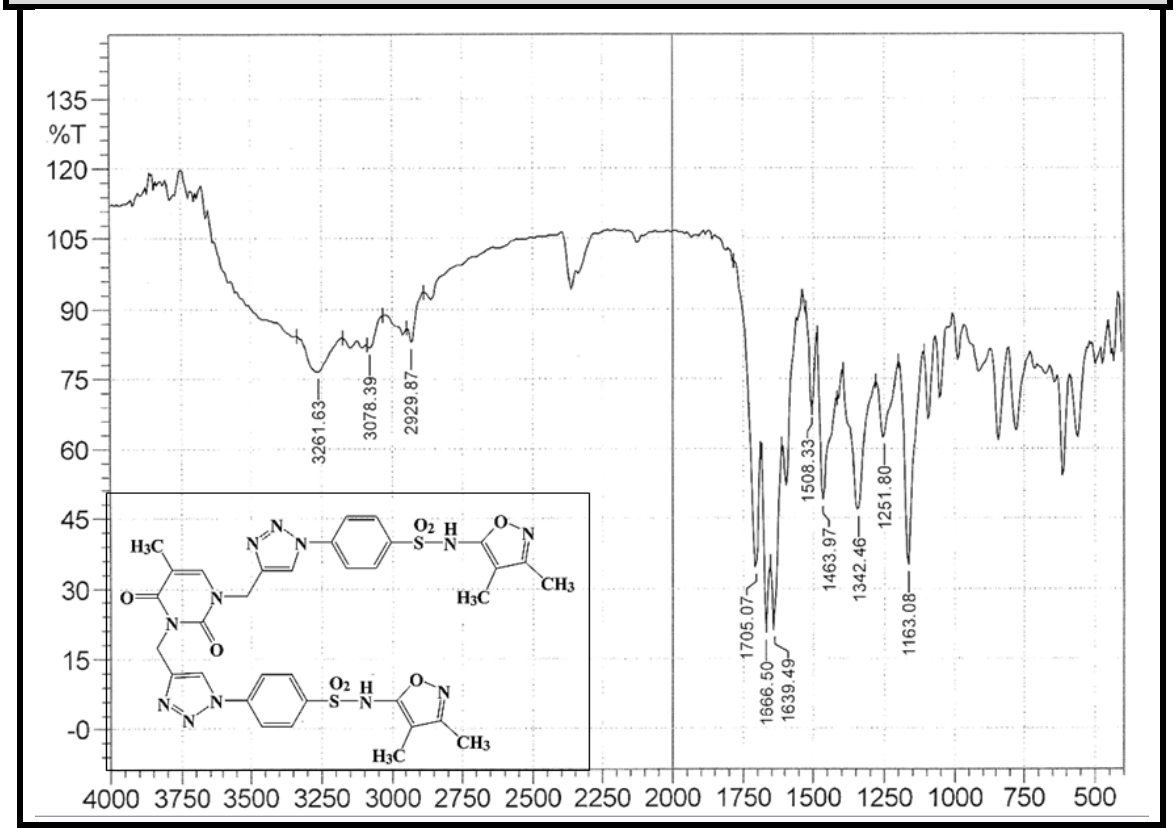


Figure 3 IR spectrum of compound T_1

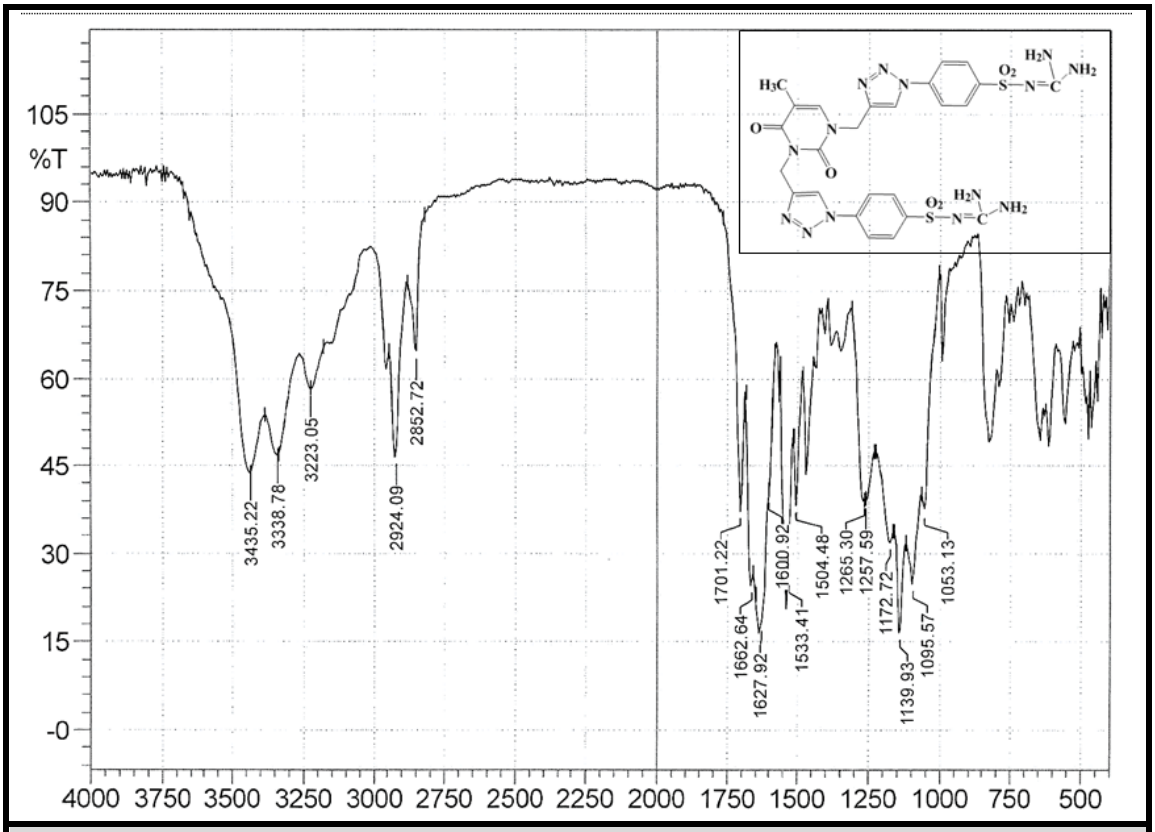


Figure 4 IR spectrum of compound T_2

2- H. NMR spectra [Krim, J. *et al* 2012]

^{1}H. NMR spectra of prepare comounds shows several signals as well two signals for deuterated DMSO. d_6 at 2.5 , 3.5 ppm.

The H-NMR spectrum of compound 2-

1- Doublet signals at 7.74 ppm attribute to the $H_{4,\overline{4}}$ protons.
2- Doublet signals at 7.31 ppm which assigned to $H_{\overline{5},\overline{5}}$ protons.
3- Singlet signal at 11.05 ppm related H_3 protons.
4- Singlet signal at 2.06 ppm to $3H_1$ protons.
5- Singlet signal at 1.62 ppm to $3H_1$ protons.

The H-NMR spectrum of compound 4.

1- Doublet signals at 7.71 ppm attribute to the $H_{4,\overline{2}}$ protons.
2- Doublet signals at 7.22 ppm which assigned to $H_{3,\overline{3}}$ protons.
3- Singlet signal at 6.68 ppm related to $H_{1,\overline{1}}$ protons.

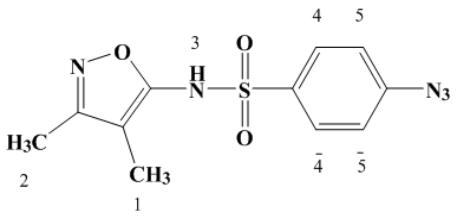
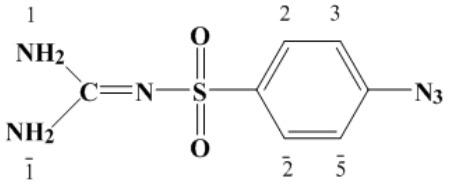
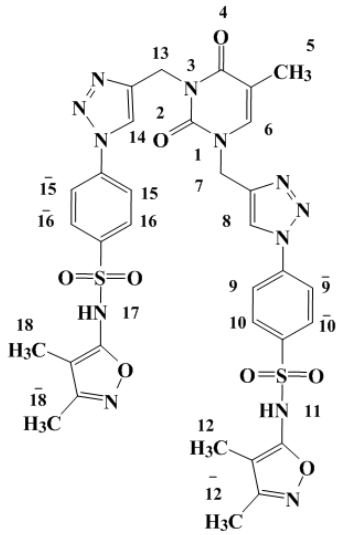
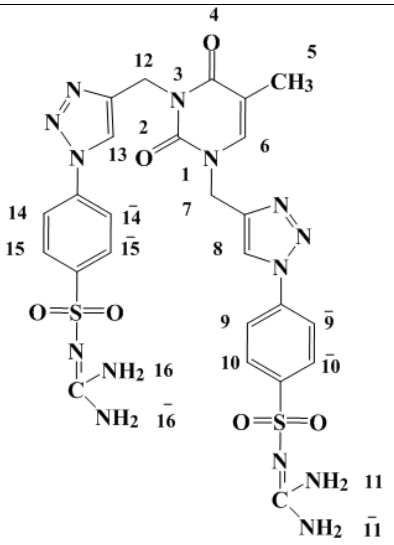
The ^{1}H-NMR spectrum of compound T_1

1- Singlet signal at 1.82 ppm related to H_5 protons.
2- Singlet signal at 1.60 ppm attribute to $H_{12,\overline{12},18,\overline{18}}$ protons.
3- Singlet signal at 4.52 ppm which assigned to $H_{11,\overline{17}}$ protons.

4- Tow singlet signal at 5.07 ppm and 5.15 ppm related to $4H_{7,\ 13}$ protons.
5- The multiplet signals within the rang (7.67 -8.07) ppm assigned to aromatic protons.
6- Two singlet signal at 8.77 ppm and 8.88 ppm related to $H_{4,\overline{14},}$ protons.
7- Singlet signal at 7.51 ppm related to H_6 protons.

The ^{1}H-NMR spectrum of compound T_2

1- Singlet signal at 1.84 ppm related H_5 protons.
2- Tow signal at 5.09 ppm and 5.15 ppm related to $4H_{7,12}$ protons.
3- Singlet signal at 6.76 ppm related to $8H_{11,\overline{11},16,\overline{16}}$ protons.
4- The multiplet signals signals within the rang (8.03-7.87) ppm assigned to aromatic protons.
5- Two singlet signal at 8.68 ppm and 8.85 ppm related to $H_{8,13}$ protons.
6- Singlet signal at 7.80 ppm related to H_6 proton.

Comp.	Structure	^{1}H-NMR
2		H_1(3H, δ=2.06 ppm), H_2 (3H. δ=1.62 ppm), H_3(1H, δ=11.05ppm) $H_{4,\bar{4}}$ (2H, δ=7.74 ppm, J=8.62 Hz), $H_{5,\bar{5}}$ (2H, δ=7.31 ppm,J =8.62 Hz)
4		$H_{1,\bar{1}}$(4H, δ=6.68ppm), $H_{2,\bar{2}}$(2H. δ=7,71ppm, J=2.07 Hz), $H_{3,\bar{3}}$ (2H, δ=7.22 ppm, J=2.31Hz)
T_1		H_5 (3H, δ=1.82 ppm), $H_{12,\overline{12},18,\overline{18}}$ (12H, δ=1.60ppm) $H_{11,\ 17}$(2H, δ=4.52 ppm), $H_{7,\ 13}$(4H, δ=5.07 ppm, δ=5.15 ppm), H_6 (1H,δ=7.51 ppm),$H_{8,\ 14}$(2H, δ=8.72 ppm , δ=8.88 ppm), the aromatic rings appears in (8.07-7.67) ppm
T_2		H_5 (3H, δ=1.84 ppm), $H_{7,12,}$ (4H, δ=5.09ppm δ=5.15 ppm) $H_{11.\overline{11},16.\overline{16}}$ (8H, δ=6.76 ppm),H_6 (1H, δ=7.80 ppm), $H_{8,13}$(2H, δ=8.68ppm, δ=8.85 ppm), the aromatic rings a pears in (8.03-7.87) ppm

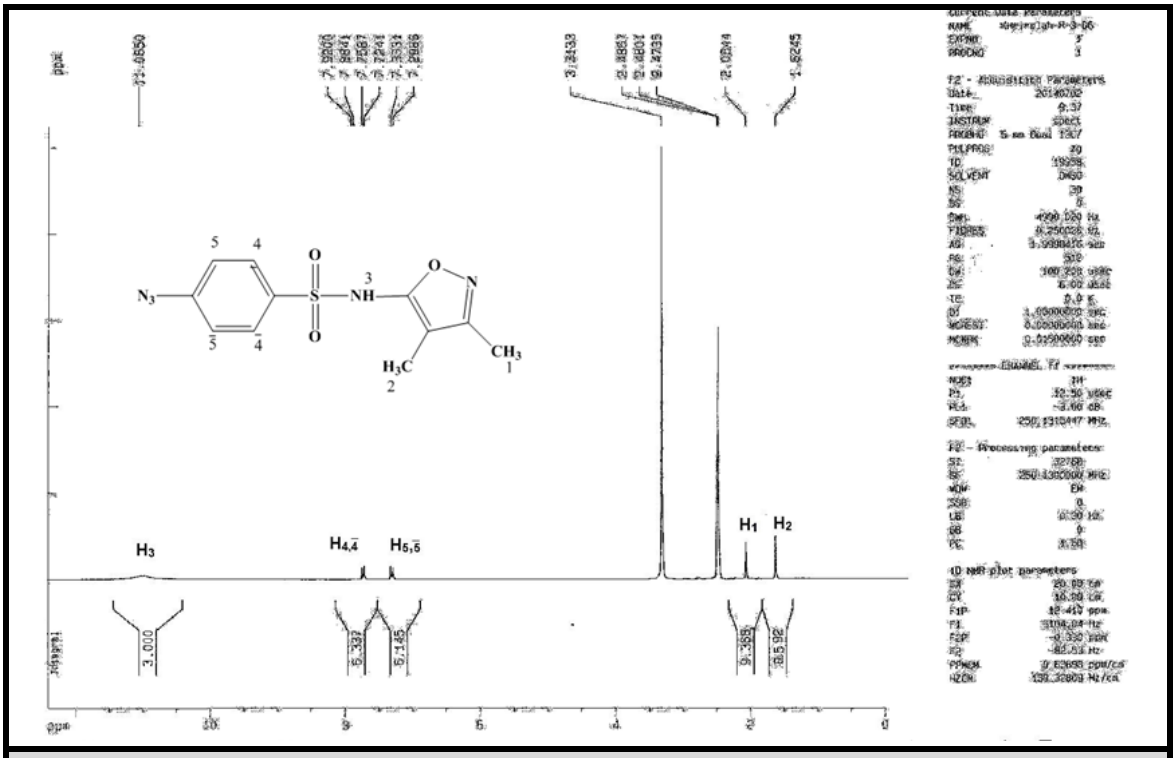


Figure 1 H NMR spectrum of compound 2

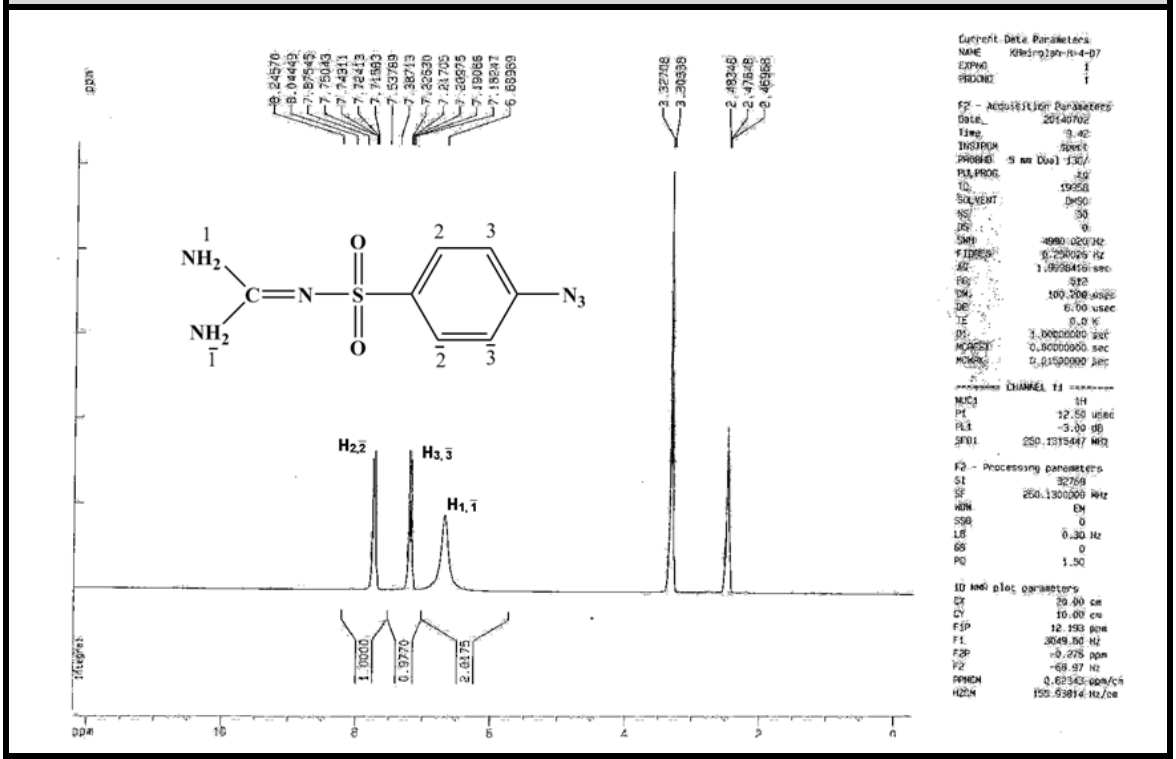


Figure 2 H NMR spectrum of compound 4

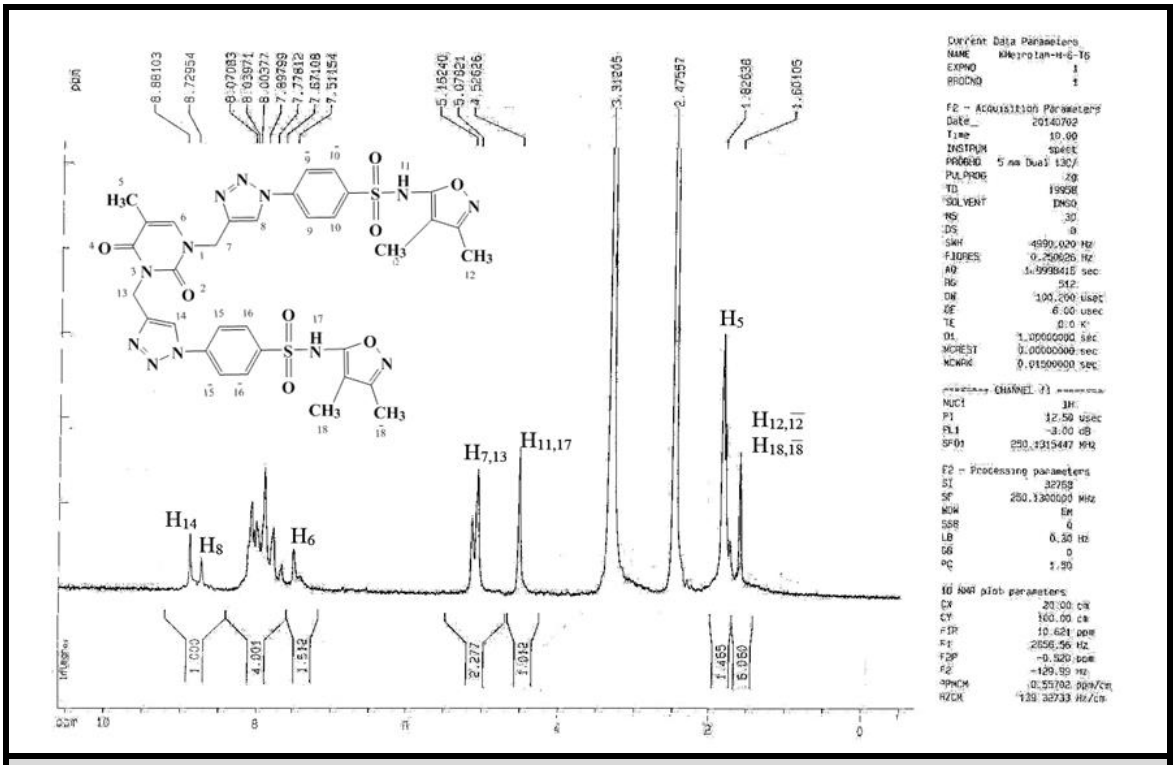


Figure 3 H NMR spectrum of compound T_1

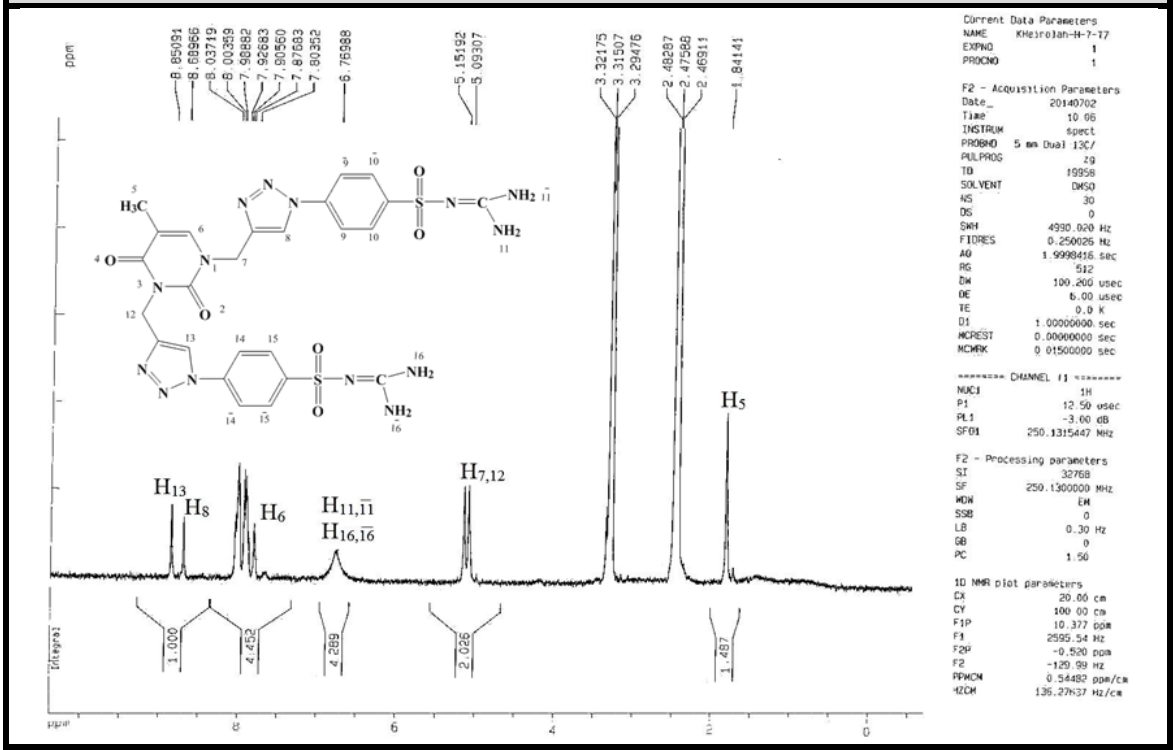


Figure 4 H NMR spectrum of compound T_2

Biological activity

Animals

Male and female Swiss albino mic e of weight range 25-35g were used in t his study. The animals were obtained from animal house of University of Basrah, c ollege of education. They were maintain ed under standard laboratory conditions. The animals were allowed free access to standard dry pellet diet and given water.

Methods (Nada, N. *et al.*, 2012)

The determination of anti-inflam matory activities were carried out by ind uced inflammation by sub-planter injection of fresh hen egg albumin (25 µL) in mice. The compounds were given to mice as a single dose 100 mg / kg orally, one hour before induced inflammation. The aspiri n was used for positive control group. T he negative control group received 0.2 ml distilled water only. The paw size m easured by digital micrometer divice.

Results and Discussion

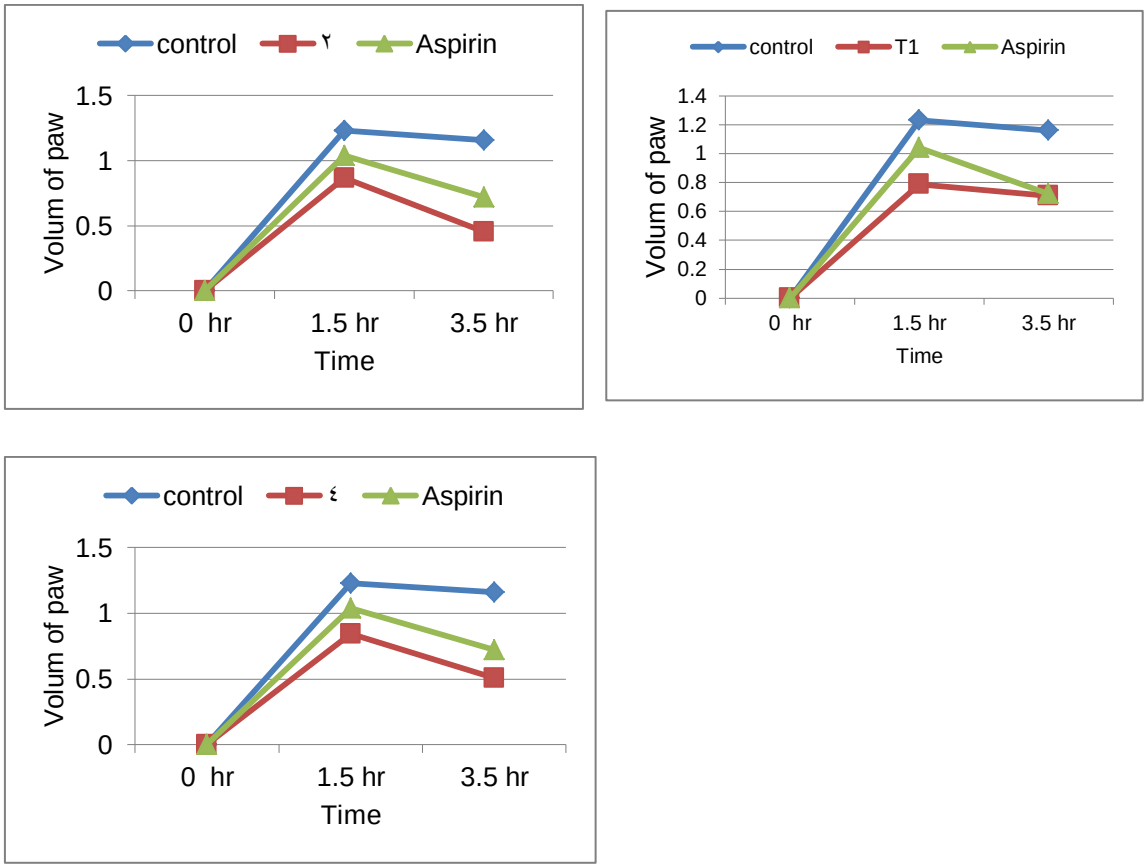


Figure (1) The anti-inflammatory activities of compounds

Compounds symbol	Size of paw in (mm)		
	0 hr	1.5 hr	3.5 hr
T_1	0 ± 0.11	0.79 ± 0.085	0.706 ± 0.12
2	0 ± 0.21	0.867 ± 0.14	0.452 ± 0.19
T_2	0 ± 0.15	0.763 ± 0.13	0.29 ± 0.17
4	0 ± 0.24	0.842 ± 0.20	0.507 ± 0.6
Control -	0 ± 0.17	1.23 ± 0.09	1.16 ± 0.13
Control +	0 ± 0.09	1.04 ± 0.16	0.72 ± 0.55

*** P < 0.05 , ** = P < 0.01 , ANOVA**

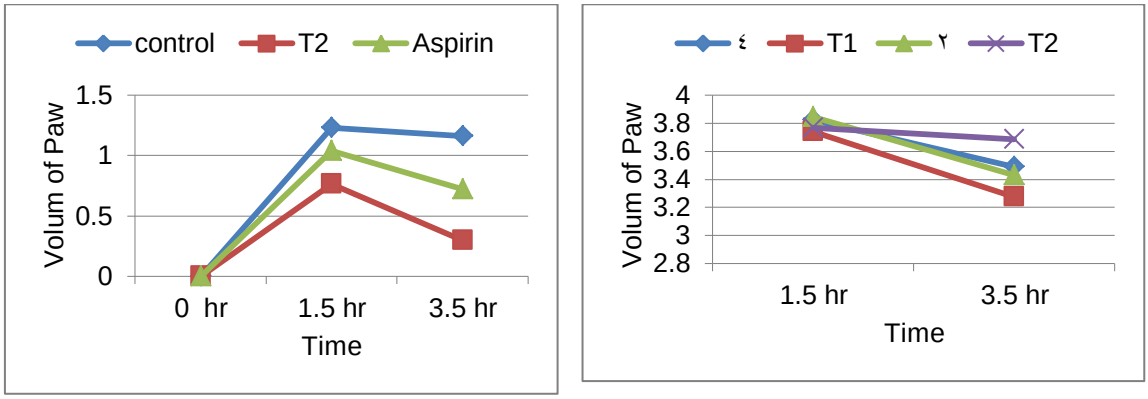


Figure (2) Comparison between compounds activities

Table (2) comparison between compounds activities

Compounds symbol	**Size of paw in (mm)**	
	1.5 hr	**3.5 hr**
T_1	3.77 ± 0.21	3.686 ± 0.23
2	3.847 ± 1.2	3.432 ± 0.8
T_2	3.74 ± 1.9	3.275 ± 1.3
4	3.822 ± 1.7	3.487 ± 1.8

The compound T_2 was more active than other compounds, compounds(2, 4) were equal in their activities, and the compound (T_1) was less active than others.

The change in activity changed with the size of molecules of the compounds, depending on the size of active site of cyclooxygenase enzymes 2 and 4 compounds are similar in their size then we observed there was equivalent their activity. While compounds T_1 and T_2 very different in their molecular size so there were a different in there activity. The compound T_1 decrease his activity, while compound T_2 increase his activity.

References

- **Beatty, K. E.; Liu, J. C.; Xie, F.; Dieterich, D. C.; Schuman, E.M.; Wang, Q.; Tirrell, D. A** (2006). Fluorescence Visualization of newly synthesized proteins in mammatian cells. Angew. Chem. Int. Edit 45, 7364-7367.

- **Binder, W. H.** (2008) "Click" chemistry in polymer and material science: the update , Macromol. Rapid commun , 24, 951 – 951.

- **Broggi, J. ; Joubert, N., Aucagne, V.; Berteina- Rabion, S.; Diez- Gonzales, S.; Nolan, S. P.; Topalis, D.; Deville- Bonne-D.; Balzarini, J.;** Neyts, J.; *et al*. (2007). Alkyne – azide click chemistry mediated carbanucleosides synthesis. Nucleos. Nucleot, Nucl, 26, 1391-1394.

- **Elayadi, H.; Smietana, M.; Pannecouque, C.; Leyssen, P.; Neyts, J.; Vasseur, J.J; Lazrek, H. B**. (2010) Straight forward synthesis of triazolozcylo nucleotide phosphonate as potential HCV inhibitors. Bioorg. Med. Chem. Lett, 20, 7365-7368.

- **Golas, P.L.; Matyjaszewski, K.** (2007) click chemistry and ATRP: A Beneficial bunion of the preparation of functional materials. QSAR Comb. Sci. 26, 1116-1134.

- **Guezguez, R.; Bougrin, K.; El Akri, K.; Benhida, R.** (2006) A highly efficient
microwave – assisted solvent –free synthesis of □- and □-2 – deoxy-1,2,3-triazoly –nucleosides. Tetrahedron lett. 47, 4807 – 4811.

- **Himo, F.; Lovell, T., Hilgraf, R.; Rostovtsev, V.V.; Noodleman. L.; sharpless, K, B.; Fokin, V.V.** (2005) copper (I)- catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates. J. Am. Chem. Soc. 127, 210-216.

- **Hou, J.; Liu, X.; Shen, J.; Zhao, G.; wang, G.** (2012). The impact of click chemistry in medicinal chemistry. Expert Opin. Drug Discovery, 7, 489-501
.

- **Huisgen, R.** (1963) kinetics and Mechanism of 1,3-dipolar cycloaddition
.
Angew. Chem. Int. Ed. 2,633-696.

- **Hwang, S.; Bae, H.; Kim, S.** (2012). An efficient and high – yielding one-pot synthesis of 4-acyl-1,2,3-triazoles via triisopropylsilyl-protected ynones. Tetrahedron, 68,1460-1465.

- **Kolb, H.C.; Finn, M,G.; Sharpless, K.B.** (2001) Click chemistry: Diverse chemical function a few good reaction. Angew. Chem. Int. Edit 40, 2004 – 2-2021.

- **Krim, J.; Taouirte, M., Engeles, J.W.** (2012)Synthesis of 1,4-Disubstituted Mono and Bis-triazolocaboacyclo nucleoside Analogues of 9-(4-Hydroxybutyl) guanine by Cu (I) – Catalyzed Click Azide – Alkyne Cycloadalition. Molecules, 17, 179-190.

- **Lewis, W. G. Magallon, F. G. Fokin V.V. Finn, J. M. G.(**2004). Discovery and characterization of catalysts for azide – alkyne cycloaddition by fluorescence

quenching. J. Am. Chem. Soc. 126, 9152-9153.

- **Mekni, N.; Baklouti, A.** (2009) Journal de al societe chimique de Tunisie, 11, 15-20.
- **Nada, N. Al- Shawi; Ahmed, H. Mahmood, K.; Haider, A**. (2012) possible anti-inflammatory effect of aqueous green tea extract against An inflammation induced by egg white in rats, the experiment , September Vol. 2 (3), 102-108.
- **Negron – Silva, G. E.; Gonzalez – Olvera, R.; Angeles – Beltran, D. (2013) synthesis of new 1,2,3-Trizaole Derivatives of Uracil an**d Thymine with Potential Inhibitory Activity against Acidic Corrosion of Steels. Molecules, 18, 4613-4627.
- **Nilan, R. A.; Sideris, E. G.; Klenihofs, A.** (1973) Mut. Res, 17, 142.
- **Pavia, D.L.; Lampman, G.M.; Kriz, G.S.** (2001) "Introduction to Spectroscopy" Thomas Learning, 3rd Ed.
- **Pramod, K. A.; Dusica, M. and Ashok, K.,** (2013) Al Kyne- Azide "Cli ck" Chemistry in Designing Nanocarrie rs for Applications in Biology , Molecul es, 18, 9531 – 9549.
- **Rostovtsev, V.V.; L. Green, L. G.; Fokin V.V.; Sharpless, K. B.** (2002) Astepwise Huisgen Cycloaddition process: Copper (I)-Catalyzed regioselective ligation of azides and terminal alkynes Angew. Chem. Int. Ed, 41, 2596-2599.
- **Tornoe, C.W.; Christensen, C.; Meldel, M. J.** (2002) Peptidotriazoles on Solid phase: [1,2,3] – Triazoles by Rregiospecific copper (I)- Catalyzed 1,3-Dipolar Cycloaddition of Terminal Alkynes to Azides. J. Org. Chem, 67, 3057-3064.

تحضير وتشخيص، ودراسة بايولوجية لاثنين من الازايدات واتنين من ثنائي 1، 2، 3- ترايازول نظائر النيوكليوسيدات الحلقية غير المتجانسة للثايمين

فائزة عبدالكريم

قسم الكيمياء / كلية التربية للعلوم الصرفه / جامعة البصرة

الخلاصة

حضرت اثنين من مركبات الازايدات وانين من مركبات ثنائي 1، 2، 3- ترايازول النيوكليوسيدات الحلقية غير المتجانسة بواسطة الاضافة الحلقية 1، 3- ثنائية القطب وذلك بتفاعل ازوكزول وازايد وسلفاكوانيدين مع بروباكايل الثايمين باستخدام الماء والايثانول كمذيب تحت شروط التصعيد باستخدام ايون النحاسوز كعامل مساعد وشخصت المركبات الجديدة بقياس درجة الانصهار بمطيافية FT-IR و ^{1}H-NMR.