Synthesis of Some New Bis-Heterocyclic Derivatives Based on 1,2,3-Triazoline and Study Their Antibacterial Activity

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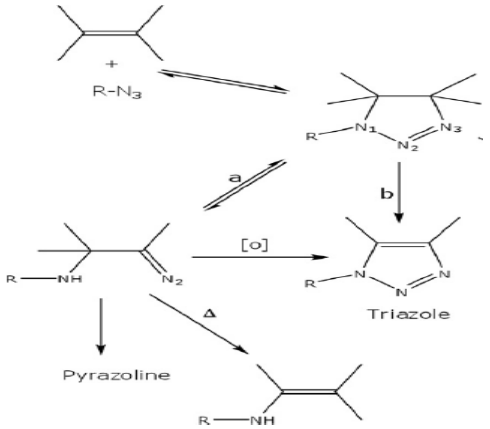
Abstract

Heptanol and octanol were converted into corresponding *n*-alkyl azides (2) using 48%HBr and sodium azide in two subsequent steps. Alkylation of diethyl malonate with allyl iodide and sodium ethoxide gave diethyl 2-allylmalonate (4) in a good yield. The reaction of compound (4) with urea in the presence of sodium ethoxide guide to obtain 5-allyl barbituric acid (5) in an excellent yield, while 4-allylpyrazolidine-3,5-dione (6) was obtained in very good yield by the reaction of compound (4) with hydrazine hydrate. The 1,3-dipolar cycloaddition reaction of compounds (5) and (6) with *n*-alkyl azides (2) afforded the targeted 1,2,3-triazolines (7a, 7b, 8a and 8b) in accepted yields. All the synthesized compounds had been characterized by TLC, FT-IR in addition to ^{1}H NMR (7b and 8a). The antibacterial activity of the final compounds were evaluated against two types of bacteria *Staphylococcus Aureus* and *Escherichia Coli*, the results showed that most of the new triazolines possess high biological activity.

Keywords: 1,2,3-triazolines, 1,3-dipolarcycloaddition, barbituric, pyrazolidine.

Introduction

Triazoline and its derivatives belong to the heterocyclic class of compounds and possess interesting biological characteristics. These compounds can be used in medicine as antibacterial, antiviral, anticancerous, antiasthmatic, analgesic and antiinflammatory drugs because of their pharmaceutical properties [1]. Furthermore, triazolines Interest in 1,3-dipolar cycloadditions involving olefins as dipolarophiles and azides as 1,3-dipoles originates from the synthetic potential of these reactions which lead to the formation of five membered nitrogen containing heterocycles like 1,2,3-triazolines. The synthesis of 1,2,3-triazolines may be carried out by three major routes (Scheme 1) [2].



Scheme (1) Synthesis of triazolines [2].

The first involves the isomerisation of arylazoaziridines [3]. The second synthetic route to triazolines is the 1,3-dipolar cycloaddition of diazoalkenes to Schiff bases (imines) [4]. A third route to 1,2,3-triazoline is the 1,3-dipolar cycloaddition of azides to ethylenic compounds [5]. 1,2,3-triazolines have been used as versatile precursors of *N*-containing heterocycles, E. Erba and D. Sporchia [6] synthesized 4-aminoquinazolines and 6-aminopurines from their corresponding triazolines. Triazoline can be converted into thiadiazole ring under acidic conditions [7], also they used in the synthesis of 1,2,3-triazoles [8]. In our work we synthesized four new 1,2,3-triazoline derivatives starting from olefins and organic azides.

Experimental Methods

Chemicals and Instruments

Chemical reagents and starting materials were obtained from Ajax and Sigma-Aldrich. Infrared spectra were recorded using Testean Shimadzu FT-IR 8000 series, Japan and NMR 300 MHz Bruker DPX spectrometer Aa'l Al-Bait University Jordan. Silica TLC plates were used with an aluminum packing (0.2 mm, 60 F_{254}). The reactions were monitored by TLC and visualized by development of the TLC plates with an alkaline potassium permanganate dip.

Synthesis of n-alkyl azides (2a) and (2b)

Alcohol (5 mmol) was converted into the corresponding *n*-alkyl bromide using an acidic mixture of HBr 48% (7 mL) and H_2SO_4 98% (3.0 mL) at reflux for 3 hrs [9], and the crude alkyl bromide (5 mmol) was dissolved in DMF (30 mL), sodium azide (0.98g, 15 mmol) was added. The suspension was stirred at 70°C overnight. The reaction mixture was diluted with water (100 mL), extracted with diethyl ether (3×50 mL). The combined organic layers were washed with brine (2×25 mL), dried over Na_2SO_4, and evaporated to dryness to give the alkyl azide as colorless oil.

n-heptyl azide (0.55g, 77%), *n*-octyl azide (0.53g, 68%).

Synthesis of diethyl 2-allylmalonate (4)

A freshly cut sodium (0.5 g) was washed very well with n-hexane then dissolved in absolute EtOH (25 mL), to this solution diethyl malonate (0.8 g, 5mmol) was added, the yellow solution was stirred at 50°C for (30 min). Allyl iodide (0.92 g, 5.5 mmol) was added dropwise, the solution was kept at the same temperature with stirring for (3 hrs), the solution was left to cool to room temperature, then poured on water (50 mL), extracted with diethyl ether (3x30 mL). The combined organic layers were washed with a solution of 5% HCl (2x25 mL), water (2x25 mL), dried over $MgSO_4$ and evaporated to dryness to obtain compound (4) (0.74 g, 74%) as a colorless liquid. bp=219-221°C.

Synthesis of 5-allyl barbituric acid (5)

A solution of compound (4) (1.00 g, 5 mmol) in a small amount of absolute EtOH (2 mL) was added dropwise to the solution of sodium ethoxide (10 mL) (prepared as shown above). A hot solution of urea (0.45 g, 7.5 mmol) in EtOH (10 mL) was added dropwise to the first. The resulting mixture was refluxed for 2 hrs. During which time the sodium salt of the product precipitated, the mixture was filtered and dissolved in distilled water (25 mL), the resulting solution was acidified with conc. HCl (4 mL) and the crude product was filtered with suction, washed with cold water (25 mL) and dried at 70° C to give compound (5) (0.78 g, 93%) as a white solid, mp 215-217°C.

Synthesis of 4-allylpyrazolidine-3,5-dione (6) [9]

A solution of hydrazine hydrate (0.48 g, 15 mmol) in EtOH (5 mL) was added slowly to the stirred solution of compound (4) (2.00 g, 10 mmol) in EtOH (5 mL), the solution was heated to 60°C for 1h a crystalline deposit separated, the mixture was left to cool to room temperature with further stirring for 30 min then cooled in an ice bath to complete the crystallization, the crystalline solid was filtered and washed with cold EtOH to yield compound (6) (1.20 g, 86%) as needle crystals, mp 223-225°C.

General procedure for synthesis of triazolines (7 and 8)

A solution of alkyl azide (3 mmol) in toluene (5 mL) was added to the hot solution of olefin (1 mmol) in toluene (3 mL). The solution was refluxed in an oil bath for 72 h then cooled to room temperature the solid was precipitated, filtered and washed with cold toluene to give the following triazoline derivatives as white solid:

5-[(1-heptyl-1,2,3-triazolin-4-yl) methyl] barbituric acid (7a) (0.145 g, 48%), mp 266-268°C.

5-[(1-octyl-1,2,3-triazolin-4-yl) methyl] barbituric acid (7b) (0.150 g, 46%), mp 270-272°C.

4-[(1-heptyl-1,2,3-triazolin-4-yl) methyl] pyrazolidine-3,5-dione (8a) (0.120 g, 43%), mp 285-287°C.

4-[(1-octyl-1,2,3-triazolin-4-yl) methyl] pyrazolidine-3,5-dione (8b) (0.130 g, 44%), mp 290-293°C.

Biological Screening; Antibacterial Susceptibility Tests:

The biological activity of some prepared compounds was tested against strains or Gram +ve bacteria ***Staphylococcus Aureus*** (ATCC 25923) and Gram – ve bacteria ***Escherichia Coli*** (ATCC 225922). All procedures were conducted under sterilized conditions; Antibacterial activity was carried out by agar diffusion method.

Kirby-Bauer [10] method was used to determine the antibacterial activity for the final prepared compounds (**7 & 8**); ampicillin was used as a control.

Procedure [11]:

1- The concentrations were prepared from the above products (1 μg/mL).
2- DMSO was used as a solvent.
3- Microbial broth was prepared according McForland technique.
4- (0.01 mL) of loopfull of microbial broth was seeded on a plate of Muller-Hinton agar.
5- Each plate contained one type of microbial tested against product and control.
6- These plates were incubated at 37^{o} C for 24 h.
7- The diameters of the inhibition zone of each compound were measured.

Results and Discussion

The experiments started by synthesis of two crude *n*-alkyl azides (2) and alkylation of diethyl malonate (4) as starting materials for this work. Initially, aliphatic alcohols were converted into corresponding alkyl bromide using an acidic medium of conc. HBr and conc. H_2SO_4, and then reacted with sodium azide to give the alkyl azides (2 a) and (2 b) in good yield. FT-IR spectra of compounds (2 a-b) showed the characteristic (-N_3) stretching band at 2096 cm^{1-} which is the excellent indicator of formation of *n*-alkyl azides (2).

Diethyl malonate was alkylated using sodium ethoxide in ethanol and allyl iodide as alkylating agent to afford compound (4) in a good yield, FT-IR spectrum of compound (4) showed the following bands at cm^{1-}: 3080 (C-H ν olefinic), 1734 (C=O ν ester) and 1643 (C=C ν olefinic), the mentioned olefinic bands are good evidence of formation of compound (4).

The total synthesis of compounds (7) and (8) summarized in Scheme (2). Table (2) shows the: physical states, melting points, R_f values, eluents and FT-IR characteristic band in cm^{1-} for all the prepared compounds.

The reaction of urea with compound (4) in EtOH gave 5-allyl barbituric acid (5) in an excellent yield, FT-IR spectrum of compound (5) Fig.(1) showed the following bands at cm^{1-}: 3436 -NH ν amide, 2943& 2842 -CH ν aliphatic, 1683 C=O ν amide, and 1593 -NH δ amide, the appearance of the amide bands and the shifting of the carbonyl band to lower frequency at 1683 cm^{1-} are very good signs to formation of compound (5).

While the reaction of hydrazine hydrate with compound (4) in EtOH afforded 4-allylpyrazolidine-3,5-dione (6) in a very good yield, FT-IR spectrum of compound (6) Fig.(2) showed the following bands at cm^{1-}: 3300 -NH ν amide, 3033 –CH- ν olefinic, 1683 C=O ν amide, 1622 C=C ν olefinic and 1367 -CH δ aliphatic, again the appearance of the amide bands and the shifting of the carbonyl band to lower frequency at 1683 cm^{1-} are very good signs to formation of compound (6).

5-[(1-heptyl-1,2,3-triazolin-4-yl) methyl] barbituric acid (7a) and 5-[(1-octyl-1,2,3-triazolin-4-yl)methyl] barbituric acid (7b) were prepared by the 1,3-dipolarcycloaddition of compounds (2a) and (2b) respectively with compound (5). FT-IR spectrum of compound (7a) showed the following bands at cm^{1-}: 3191 -NH ν amide, 2950 and 2825 -CH ν aliphatic, 1670 C=O ν amide, 1602 -NH δ amide and 1365 -CH δ aliphatic the disappearance of the azide band at 2096 cm^{1-} is a very good signs to formation of compound (7a). FT-IR spectrum of compound (7b) Fig.(3) showed approximately the same bands in addition to the –N=N- ν band of triazoline at 1427 cm^{1-} and also the same changes.

^{1}H NMR spectrum of compound (7b) Fig.(4) showed the following signals (DMSO-d6) at δ ppm: 0.95 (t, 3H, CH_3), 1.25 (m, 10H, 5-CH_2-), 1.45 (m, 2H, -CH_2-), 2.35 (m, 2H, -CH_2-), 2.75 (broad s, 1H, H barbituric ring), 3.33 (water in DMSO-d6) [12], 4.80 (m, 1H, H-4 triazoline ring), 5.56 (broad m, 4H, H-5 triazoline ring and –CH_2-), 8.75 and 8.99 (broad s, 2H, NH).

4-[(1-heptyl-1,2,3-triazolin-4-yl) methyl] pyrazolidine-3,5-dione (8a) and 4-[(1-octyl-1,2,3-triazolin-4-yl)methyl]pyrazolidine-3,5-dione (8b) were synthesized using the 1,3-dipolarcycloaddition of compounds (2a) and (2b) respectively with compound (6).

FT-IR spectrum of compound (8a) Fig.(5) showed the following bands at cm^{1-}: 3300 -NH ν amide, 2965 -CH ν aliphatic, 1685 C=O ν amide, 1602 -NH δ

amide and 1425 –N=N- ν triazoline the disappearance of the azide band at 2096 cm^{1-} is a very good signs to formation of compound (8a).

^{1}H NMR spectrum of compound (8a) Fig.(6) showed the following signal (DMSO-d6) at δ ppm: 0.57, 1.00, 1.25 and 2.33 (t, m, m, m, 15H, heptyl), 2.95 (broad s, 1H, H pyrazolidine-3,5-dione ring), 3.75 (t, 2H, -CH_2-), 4.26 (broad m, 2H, H-5 triazoline ring), 5.00 (m, 1H, H-4 triazoline ring), 8.87 (broad s, 2H, NH).).

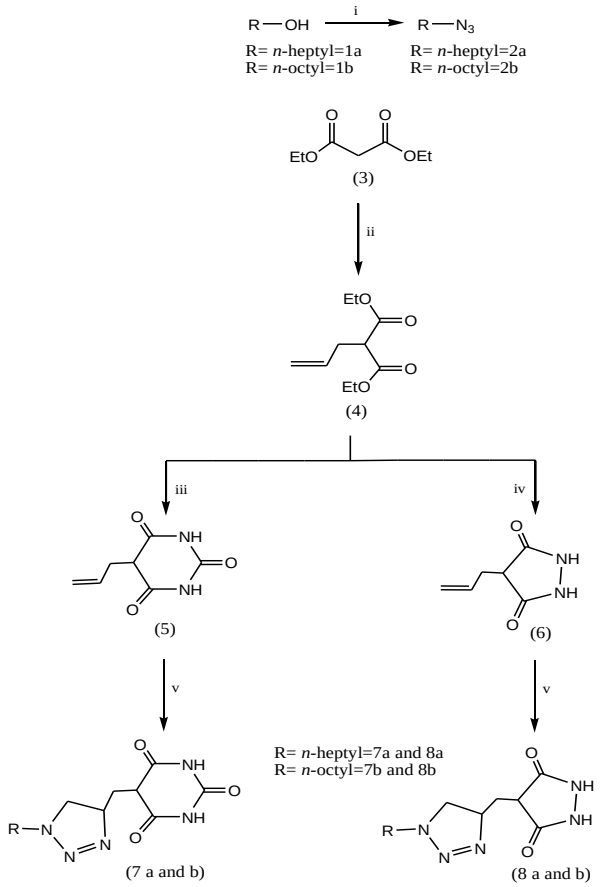
FT-IR spectrum of compound (8b) showed approximately the same bands that attributed to compound (8a) and also the same changes.

In this work, the prepared triazolines were expected to possess biological activity because they were constructed from biologically active moiety. Antibacterial activity results of final compounds shown in Table (1), compounds (7a & 7b) appeared moderate to high activity against both types of bacteria because the mentioned compounds contain the barbiuric moiety in addition to triazoline ring while compounds (8a & 8b) showed moderate activity against *E. Coli*. Compound (8a) showed slightly activity against *S. Aureus* while compound (8b) appeared no activity against the same species.

Table (1)
Antibacterial Activity of Compounds (7 & 8).

Compound No.	***Gram –ve bacteria***	***Gram +ve bacteria***
	Escherichia Coli	***Staphylococcus Aureus***
7a	+++	++
7b	+++	+++
8a	++	+
8b	++	-

Key symbols:
Inactive = - (inhibition zone < 6 mm)
Slightly active = + (inhibition zone 6-9 mm)
Moderately active=++ (inhibition zone 9-12)
Highly active=+++ (inhibition zone 13-17mm)



Reagents and conditions; (i) HBr 48%, H_2SO_4, reflux 3 hrs then NaN_3, DMF, 70°C overnight; (ii) allyl iodide, NaOEt, EtOH, reflux 3 hrs; (iii) urea, NaOEt, EtOH reflux 2 hrs; (iv) hydrazine hydrate 98%, EtOH 60°C-rt 1.5 hr; (v) alkyl azide, toluene, reflux 72 hr.

Scheme (2) Synthesis of triazolines (7) and (8).

Table (2)
Physical properties and FT-IR characteristic band of the prepared compounds

Compound	*Physical state*	*bp or mp ^{o}C*	*R_f*	*Eluent*	*Sample state*	*FT-IR Characteristic Bands cm^{1-}*				
2a	Colorless oil	-	0.75	*n*-hexane/ether 9:1	neat	2955& 2894 -CH ν aliphatic	2109 $-N_3$ ν	1393 -CH δ aliphatic	-	-
2b	Colorless oil	-	0.76	*n*-hexane/ether 9:1	neat	2955& 2891 -CH ν aliphatic	2108 $-N_3$ ν	1393 -CH δ aliphatic	1357 -CH δ aliphatic	-
4	Colorless liquid	219-221	0.67	*n*-hexane/ether 5:1	neat	3080 C-H ν olefinic	1734 C=O ν ester	1643 C=C ν olefinic	1238 C-O ν ester	-
5	White solid	215-217	0.56	*n*-hexane/ether 3:1	KBr disc	3436 -NH ν amide	2943& 2842 -CH ν aliphatic	1683 C=O ν amide	1593 -NH δ amide	1384 -CH δ aliphatic
6	Needle crystals	223-225	0.47	*n*-hexane/ether 3:1	KBr disc	3300 -NH ν amide	3033 -CH ν olefinic	1683 C=O ν amide	1622 C=C ν olefinic	1367 -CH δ aliphatic
7a	White solid	266-268	0.37	*n*-hexane/EtOAc 3:1	KBr disc	3191 -NH ν amide	2950& 2825 -CH ν aliphatic	1670 C=O ν amide	1602 -NH δ amide	1365 -CH δ aliphatic
7b	White solid	270-272	0.38	*n*-hexane/EtOAc 3:1	KBr disc	3166 -NH ν amide	2955& 2826 -CH ν aliphatic	1670 C=O ν amide	1602 -NH δ amide	1427 -N=N ν triazoline
8a	White solid	285-287	0.32	*n*-hexane/EtOAc 3:1	KBr disc	3300 -NH ν amide	2965 -CH ν aliphatic	1685 C=O ν amide	1602 -NH δ amide	1425 -N=N ν triazoline
8b	White solid	290-293	0.32	*n*-hexane/EtOAc 3:1	KBr disc	3303 -NH ν amide	2826 -CH ν aliphatic	1685 C=O ν amide	1604 -NH δ amide	2826 -CH ν aliphatic

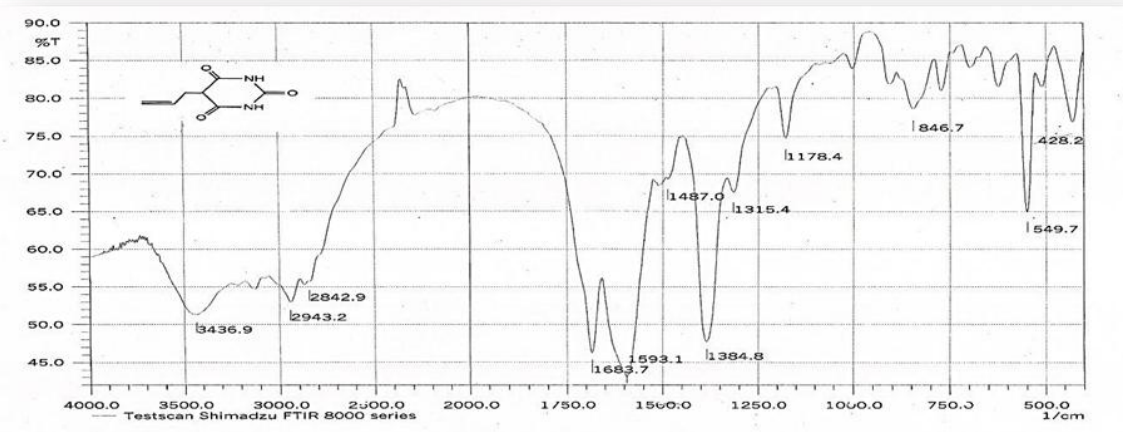


Fig.(1) FT-IR spectrum of compound (5).

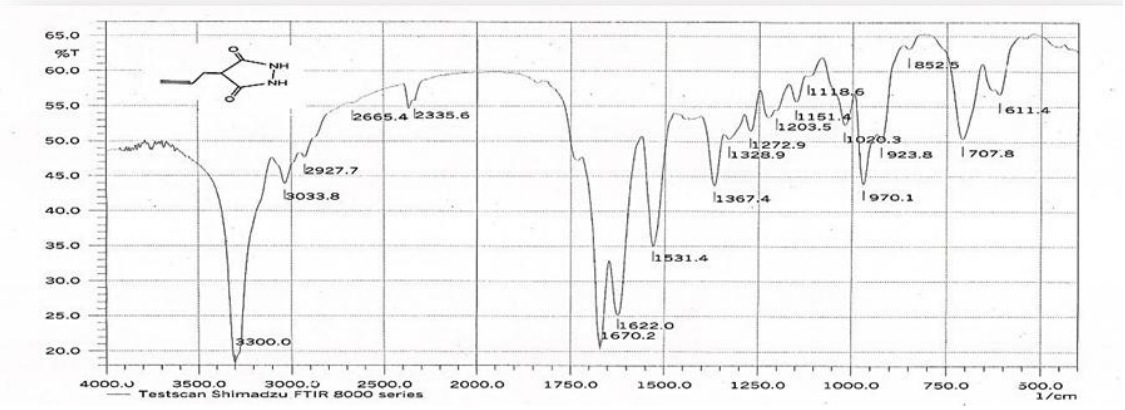


Fig.(2) FT-IR spectrum of compound (6).

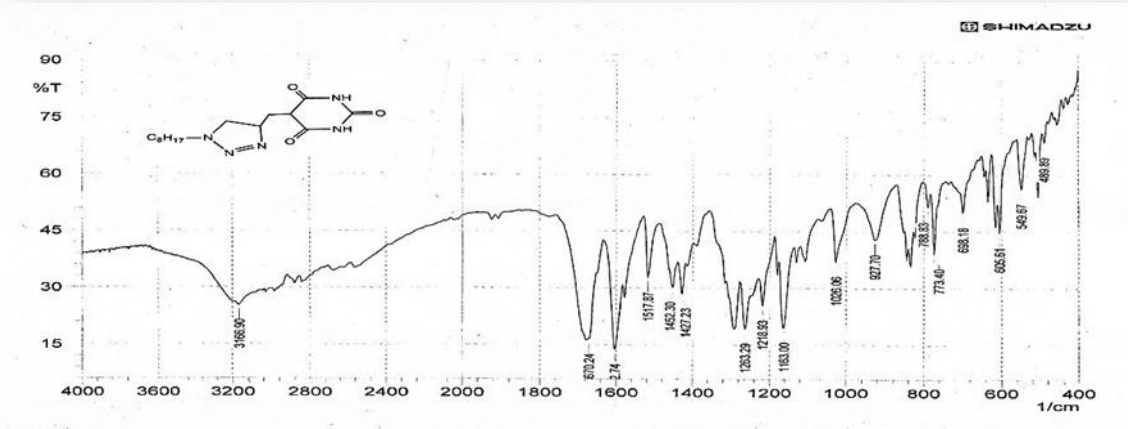


Fig.(3) FT-IR spectrum of compound (7b).

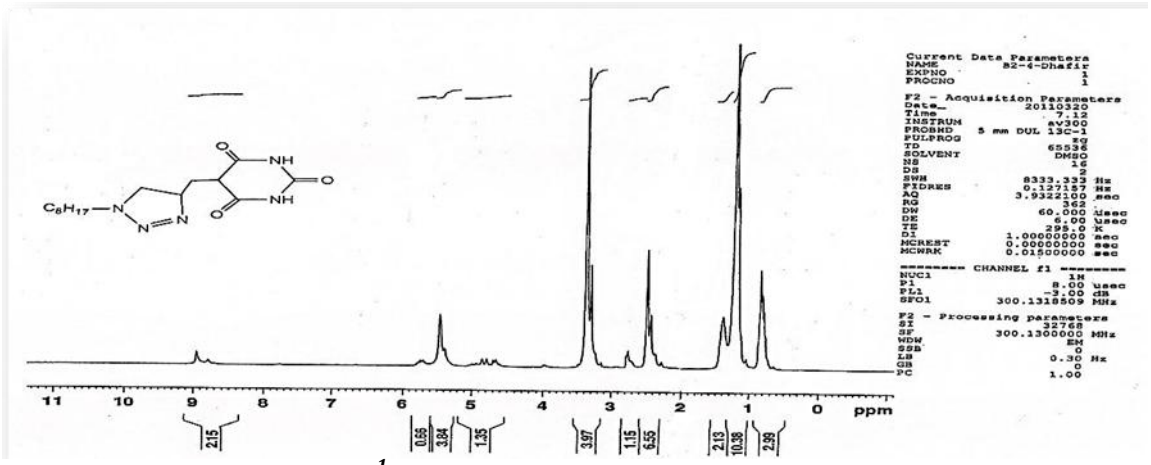


Fig.(4) ^{1}H NMR spectrum of compound (7b).

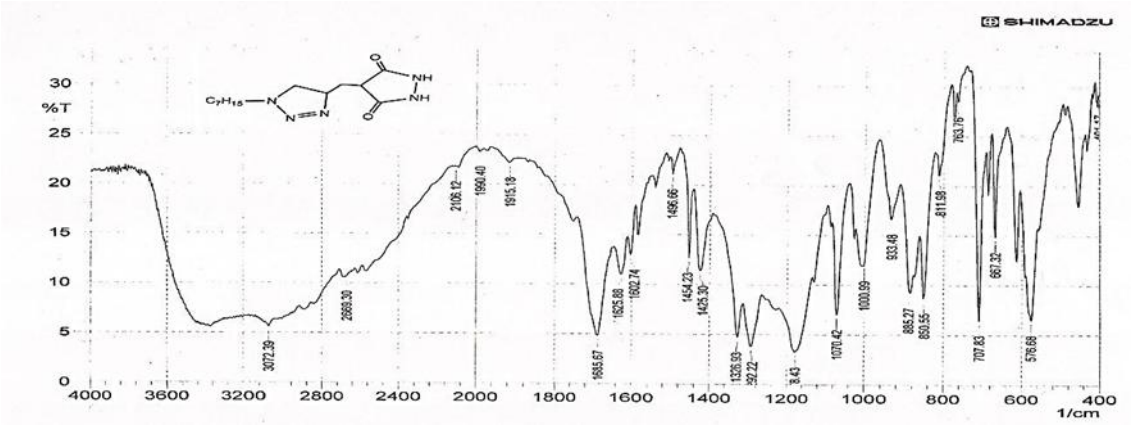


Fig.(5) FT-IR spectrum of compound (8a).

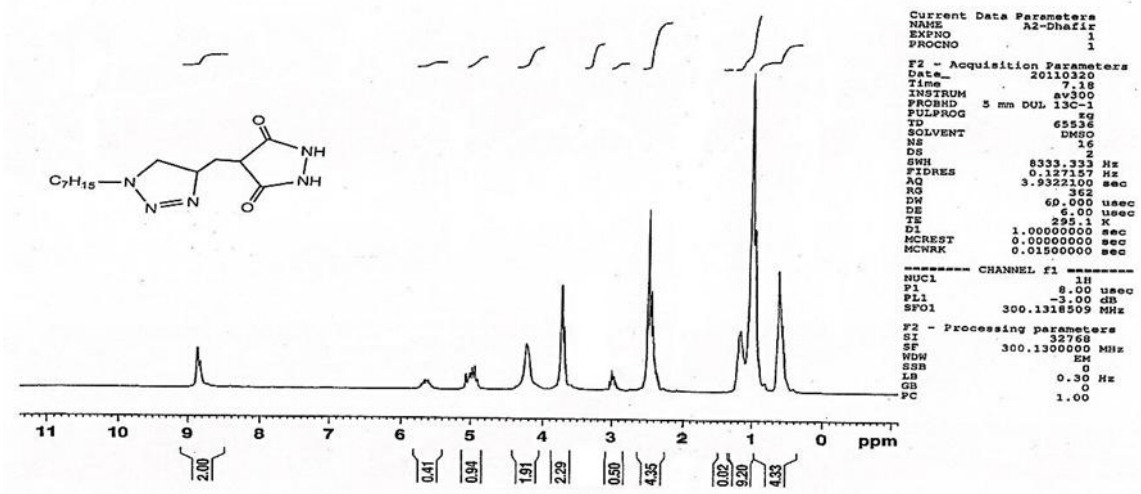


Fig.(6) ^{1}H NMR spectrum of compound (8a).

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الخلاصة

لقد تم تحويل الهبتانول والاوكتانول الى الازيدات الألكيلية المقابلة (2) بأستخدام حامض الهيدروبروميك 48% بخطوتين متتاليتين. تمت ألكلة ثنائي أثيل مالونيت بأستخدام يوديد الأليل فأعطى ثنائي أثيل 2–أليل مالونيت (4) وبمنتوج جيد. أن تفاعل المركب (4) مع اليوريا بوجود أيثوكسيد الصوديوم أدى ألى تكوين المركب 5–اليل حامض الباربتيوريك (5) وبمنتوج ممتاز بينما أعطى المركب 4–أليل بارازوليدين–5,3–دايون (6) من تفاعل المركب (4) مع الهايدرازين المائي.

ان تفاعل الأضافة الحلقية ثنائي الأقطاب للمركبين (5) و (6) مع الأزيدات الألكيلية (2) أدى ألى تكوين مركبات الترايازولين الجديدة (7) و (8). لقد تم تشخيص جميع المركبات المحضرة بأستخدام كروماتوغرافيا الطبقة الرقيقة، طيف الاشعة تحت الحمراء، الأضافة ألى طيف الرنين النووي المغناطيسي البروتوني للمركبين(7ب) و (8أ) فقط. لقد تم دراسة الفعالية البايولوجية للمركبات الجديدة المحضرة ضد البكتريا، فأظهرت معظم المركبات الجديدة قيد الدراسة فعالية حيوية ضد البكتريا.

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