SYNTHESIS OF SOME NEW SUBSTITUTED 1,2,4-TRIAZOLE AND 1,3,4-THIADIAZOLE AND STUDY OF THEIR ACTIVITIES ON SOME STRAINS OF BACTERIA

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Abstract

This research includes preparing of some substituted 1,2,4-triazoles and 1,3,4-thiadiazoles through the reaction of each of phthalimide and cis-1,2,3,6-tetrahydrophthalimide with ethyl bromo acetate in the presence of sodium hydroxide, and absolute ethanol to obtain the esters ethyl phthalimide acetate, then converted these esters to thiosemicarbazide and semicarbazide derivatives through their reaction with thiosemicarbazide and semicarbazide respectively. Furthermore, substituted triazole were prepared through the cyclization of thiosemicarbazide and semicarbazide in alkaline media such as sodium hydroxide solution, also thiadiazole through the treatment of esters, ethyl phthalimide acetate and ethyl cis-1,2,3,6-tetrahydrophthalimide acetate with thiosemicarbazide in the presence of a base. The prepared compounds were identified by spectral methods FTIR, ^{1}H-NMR, ^{13}C-NMR, and measurements some of its physical properties and some specific reactions, furthermore we were studied the effects of the preparing compounds on some strains of bacteria, *Staphylococus aureus,Escherichia Coli, Serrtia,* which exhibited antibacterial activity against *Escherichia Coli* specifically and have no effect on other strains.

Introduction

Imides are important in organic chemistry especially aromatic heterocyclic imides and have different chemical and biological activities N-aryl phthaimides have pronounced plant growth regulator[1]. Many cyclicimide and their derivatives have analgesic properties[2] and showed activity against some species of bacteria, some imides similar to phthalimide showed activity against yeast, fungus and must microorganisms[3,4]. In addition to that the compound cis-1,2,3,6-tetrahydro phthalimide (THPI) and its derivatives have activity against some plant fungus, and importance in industrial applications[5,6]. Furthermore, five memberd heterocyclic like 1,3,4-thiadiazoles and 1,2,4-trizole constitute a potential class of compounds which posses abroad spectrum of biological activity[7,8], including antimicrobial, sedative, anticonvulsant, and antinflamatory. Keeping the above facts, we made an attempt to condence phthalimide and cis-1,2,3,6-tetrahydro phthalimide (THPI) with five membered heterocyclic mentioned above to synthesis new derivatives that have biological activities.

Experimental

Melting point were determined on a Gallenkamp FB.600-olof. melting point apparatus.

FTIR spectra were recorded using solid KBr discs by tests scan Shimadzu FTIR 800 series. The ^{1}HNMR and ^{13}CNMR spectra were recorded on a make Bruker model DPX 300/300MIX NMR at the university of Jordan DMSO-d^{6} was used as solvent and TMS as internal reference. All chemicals were of reagent grade.

Preparation of cis-1,2,3,6-tetrahydro Phthalimide [7].

Standard literature procedure was followed for preparing the cis-1,2,3,6-tetrahydro phthalimide[9]. Physical properties of the product are listed in Table (1-2).

Preparation of ethyl N-(phthalimide) acetate or ethyl N-(cis-1,2,3,6-tetrahydrophthamilide) acetate [1,8].

Literature procedure was used with some modification[10]. Phthalimide or cis-1,2,3,6-tetrahydro phthalimide (0.1 mol) was dissolved in absolute ethanol (150 mL), and heated in a water bath until the solution become clear. The hot solution was poured

into a solution of sodium hydroxide (4.0 g ; 0.1 mol) in (20 mL) of ethanol, the mixture was coold the formed white precipitate was filtered. The products sodium phthalimide or sodium cis-1,2,3,6-tetrahydro phthalimide were transfered to 250 mL round bottom flask fitted with adropping funnel, then (150 mL). of absolute ethanol was added followed that (10.6 g ; 0.1 mol) sodium carbonate was added. The dropping funnel was loaded with (14.6 mL; 0.1 mol) ethyl bromo acetate. The addition was perfor-med dropwise with stirring for 1 hr. the stirring and refluxing continued for 4 hrs. The reaction mixture was filtered and evaporated to give white crystals, which was recrystallized from ethanol to give the ester [1,8]. Physical properties of the products are listed in Table (1-2).

5-(phthalimide or cis-1`,2`,3`,6`-tetrahydrophthalimide) aceto thiosemicarbazide [2,9][(11)].

A mixture of ethyl(N-phthalimide or N-cis1,2,3,6-tetrahydro phthalimide) acetate (0.01 mol) and thiosemicarbazide (0.91 g ; 0.01 mol) in ethanol (20 ml), was refluxed for 3hrs. The reaction mixture was filtered and poured on ice water. The precipitate was filtered and recrystallized from chlororform to give white crystals of the thiosemicarbazide derivative. Physical properties of the products are listed in Table (1-2).

5-(phthalimide or cis-1`,2`,3`,6`-tetrahydrophthalimide) aceto semicarbazide [3,10].

Literature procedures were followed in the preparation with minor modification[(11)]. A mixture of ethyl (N-phthalimide or cis-1,2,3,6-tetrahydro phthalimide) acetate (0.01 mol) semicarbazide hydrochloride (1.12 g ; 0.01 mol) and sodium acetate (0.82 g ; 0.01 mol) in ethanol (25 mL) was refluxed for 4hrs. The reaction mixture was filtered and poured on ice water, the precipitate was filtered and recrystallized from chloroform: petroleum ether to give white crystals of the semicarbazide derivative. Physical proper-ties of the products are listed in Table(1-2).

5-[(phthalimide or cis-1`,2`,3`,6`-tetrahydrophthalimide)N-methy]-1,2,4-triazol-3-thiol-or-ol.[5,12,4,11].

General Procedure[(12)]:-

In a round bottom flask (0.01 mol) of 5-(phthalimide or cis-1,2,3,6-tetrahydro phthalimide) aceto thiosemicarbazide or smicarbazide (from the previous step) was refluxed with 10% aqueous sodium hydroxide solution (25 mL) for (3-4) hrs. The reaction mixture was filtered, cooled, and neutralized by gradual addition of 10% hydrochloric acid, the formed white precipitate was filtered and recrystallized from suitable solvent to give white crystals. Physical properties of the dry products are listed in Tables (1-2) and (3-4).

2-Amino-5-[(phthalimide or cis-1`,2`,3`,6`-tetrahydrophthalimide)-N-methyl]-1,3,4-thiadiazole [6,13].

General Procedure[(13)]:-

A mixture of ethyl[N-phthalimide or cis-1,2,3,6-tetrahydrophthalimide] acetate (0.01 mol), thiosemicarbazide (0.91 g ; 0.01 mol) and (5 mL) phosphorus oxy chloride was refluxed for 8 hrs. The cold reaction mixture was poured on crushed ice and neutralized by adding sodium hydroxide solution. The resulting solid was filtered and recrystallized from chloroform to give a white crystals of amino thiadiazole derivarive [6,13]. Physical pro-perties of the products are listed in tables (1-2) and (3-4).

Table (1)
Physical properties of the prepared compound

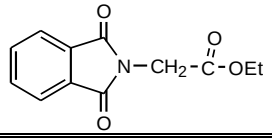
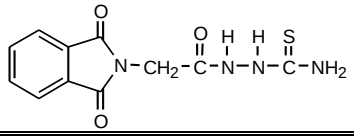
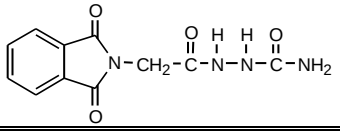
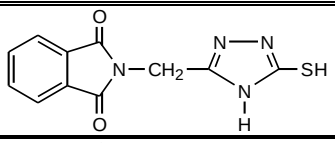
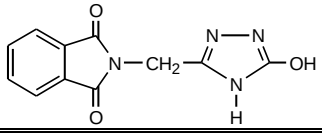
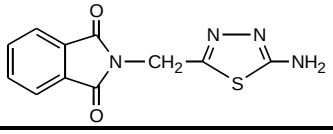
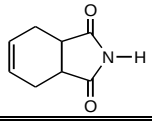
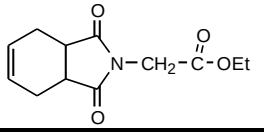
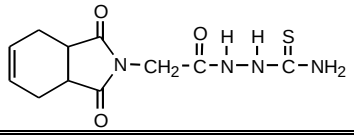
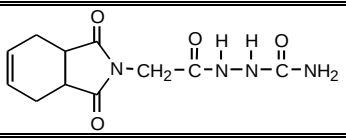
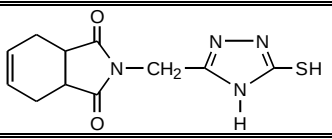
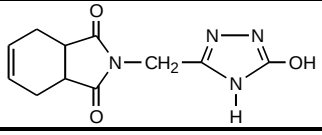
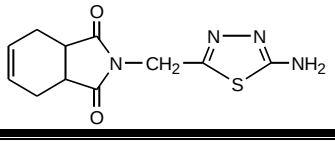
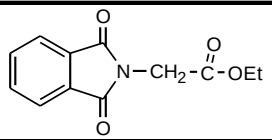
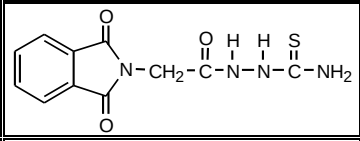
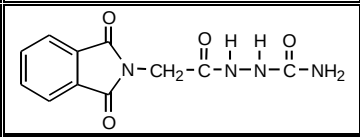
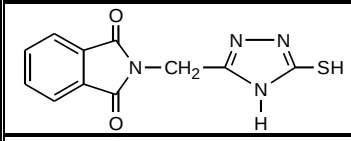
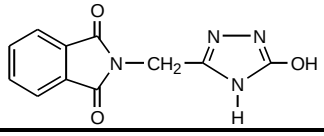
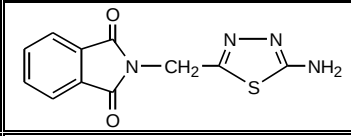
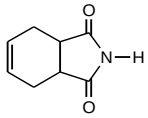
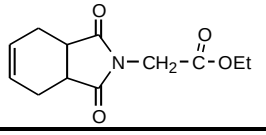
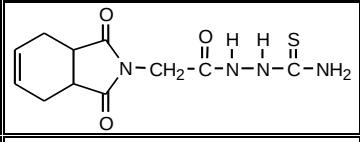
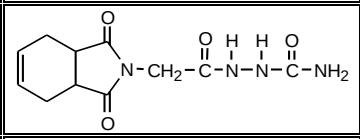
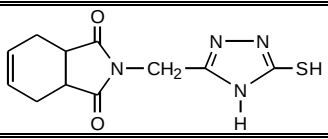
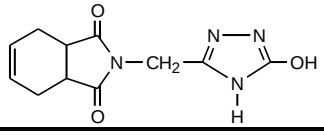
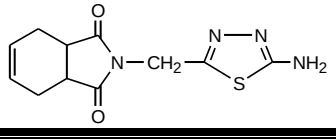
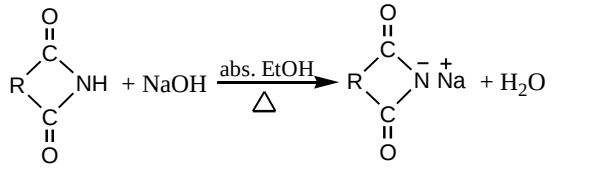
Comp. No.	Comp. Structure	m.p. °C	Yield%	Colour	Recryst. Solvents
1		97	88	White	Ethanol
2		105-106	75	White	Chloroform
3		116-118	65	White	Chloroform-petroleum ether
4		186	55	White	Chloroform
5		210	50	White	Ethanol
6		335dec.	45	Pink	Chloroform
7		135-137	85	White	Ethanol
8		82	84	White	Ethanol
9		78	72	White	Chloroform
10		90	68	White	Chloroform-petroleum ether
11		155	52	White	Ethanol
12		190	45	White	Ethanol-water
13		310dec.	40	yellow	Chloroform

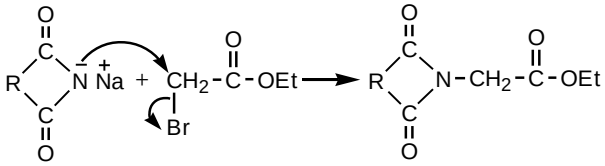
Table (2)
FTIR absorption spectra data (cm^{-1}) of the prepared compounds

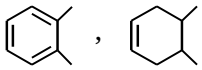
Comp. No.	Comp. Structure	νNH$_2$	νNH	νC-H aromatic	νC=O Imide	νC=C & νC=N	other bands
1				3030	1728		νC=O ester; νC-H alph. 1760 2943
2		3370 asym. 3263 sym.	3178	3020	1720 sholder 1740 amid		νC-H alph.; νC=S weak 2970 1130
3		3375 broad	3180	3065	1722 sholder 1745 amid		νC-H alph. 2975
4			3230	3085	1685	1558 1615	νC=S weak 1165
5			3175	3020	1692	1620	νC-H alph. 2950 , 2855
6		3310 asym. 3225 sym.	3030		1670	1620	νC-H alph. 2960
7			3223	νC-H Aromatic 3085	1707	νC=C Olefinic 1640	νC-H alph. 2950 , 2850
8				3055	1707	1635	νC=O ester; νC-H alph. 1747 2986
9		3310 asym.	3210	3095	1700s 1750 amide	1640	νC=S weak 1203
10		3376 broad	3205	3067	1700 1750 amide	1638	νC-H alph. 2985
11			3195	3055	1695	1635	νC=S 1150
12			3189	3060	1695	1638	νC=N 1625
13		3356 broad		3056	1703	1630	νC=N 1620

Results and Discussion

The synthesis of some new substituted 1,2,4-triazoles and 1,3,4-thiadiazoles were achieved from phthalimide and cis-1,2,3,6-tetrahydr-ophthalimide Scheme (I and II). The phthalimide and cis-1,2,3,6-trtrahydro phthalimide was treated with sodium hydroxide in absolute ethanol to give the salt, then added ethyl bromo acetate and sodium carbonate afforded compounds (1,8) respectively. Imide group ($>N-H$) is a neclophilic reagent which converted to $>N^{\ominus}$ after its reaction with a base, and achieved a neclophilic substitution of brome by SN_2 mechanism[14], according to the following equations:



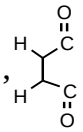


R = 

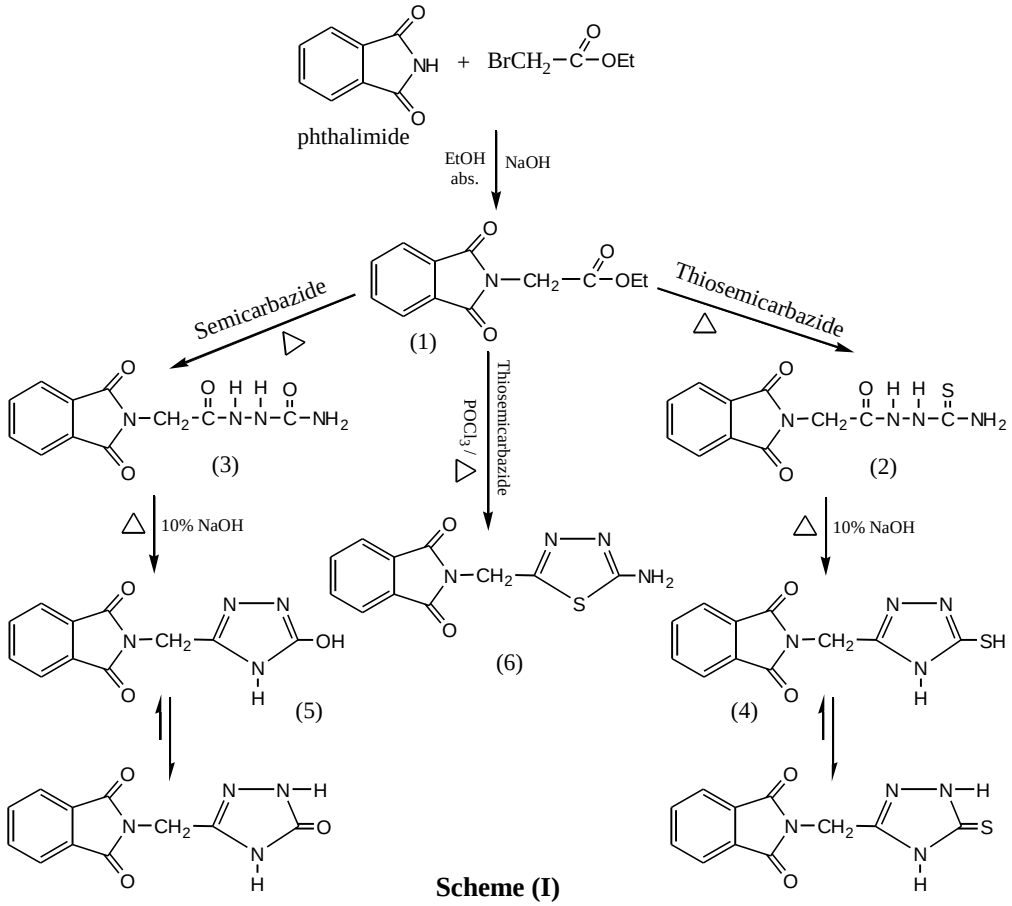
The structures of [1,8] were confirmed by physical properties which are listed in Table (1), FTIR spectra showing the absorption at ν 1760 cm^{-1} for (C=O) ester, 2934 for (C-H alph.), and disappearance the absorption of ($>N-H$) group. Other chemical test was carried out to characterize the prepared ester such as hydroxamic acid test[15] that confirmed the presence of ester group.

The compounds [1,8] were converted to thiosemicarbazide or semicarbazide derivative [2,9,3,10] by the reaction with thiosemicarbazide or semicarbazide in ethanol. For the product compounds [2,9] FTIR spectral data showed absorption at ν (3370-3210)cm^{-1} asym. and sym. for ($-NH_2$) group which overlap with absorption (-NH) group, 1740 cm^{-1} for (C=O) amide group, (1700-1720) for (C=O) imide group 1130 for (C=S) weak peak while compounds [3,10] showed absorption at ν 3375 cm^{-1} broad for ($-NH_2$) group which overlap with absorption of (NH) group, 1750 cm^{-1} for (C=O) amid group, (1700-1722) cm^{-1} for (C=O) imide group.

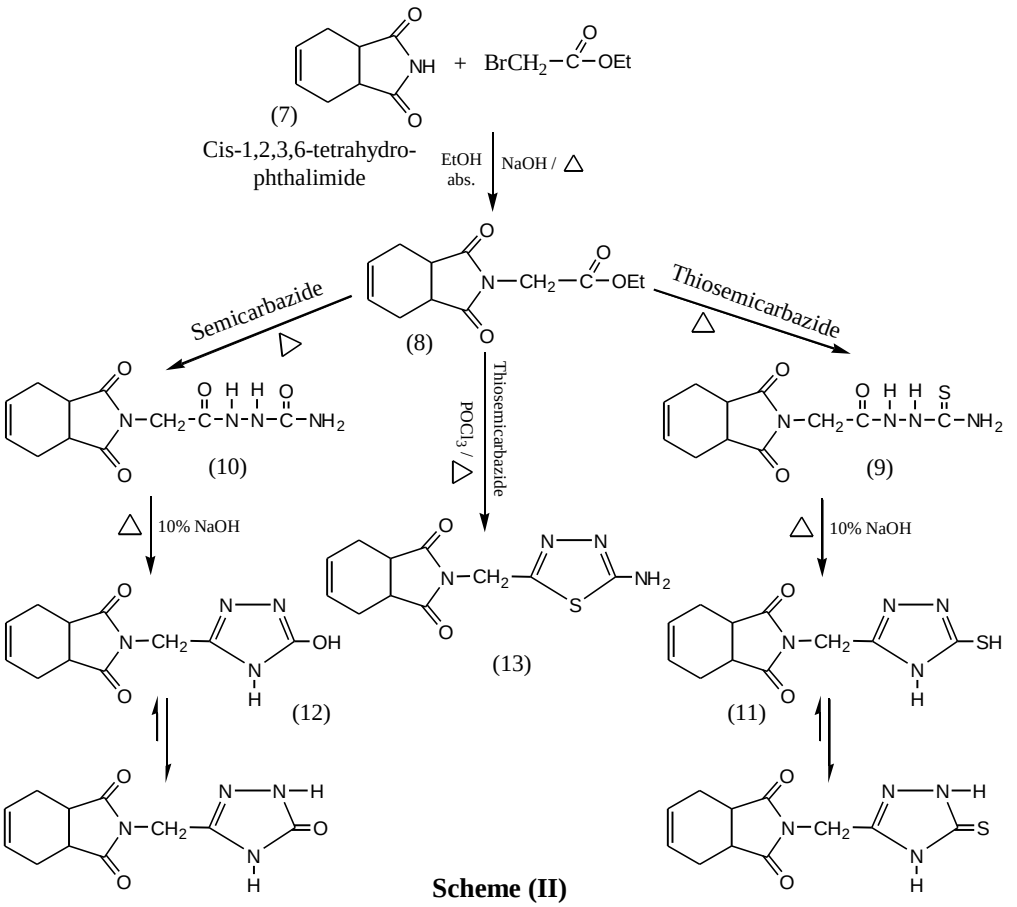
The thiosemicarbazide derivatives [2,9] were refluxed with 10% sodium hydroxide solution to give substituted 1,2,4-triazoles [4,11] which were showed absorption at ν (3230-3145) cm^{-1} for (NH), (1615-1635) for (C=N), (1150-1165) for (C=S), while the ^{1}HNMR spectra data of compound [4] δppm 4.40(s, 2H, $-CH_2-$); 7-7.6(m, 4H, Ar-H); 12.9(broad, 2H, NH) the absence of (-SH) proton in the ^{1}HNMR spectra of triazoles [4] may be due to the thio-thion structure formation, of (C=S) which shows ν at 1165 cm^{-1} [10]. The semicarbazide derivatives [3,10] were refluxed with 10% NaOH solution for 4hrs to give substituted triazole [5,12] which were showed absor-ption at ν (3189-3175) cm^{-1} for (NH), 1625 for (C=N), 1695 for (C=O), while the ^{1}HNMR spectra data of compound [5] δppm 4.26(s, 2H, $-CH_2-$); 7-7.6(m, 4H, Ar-H); 13.1(broad, 2H, NH). The absence of –OH protons in the NMR spectra of triazole [5] may be due to the keton-enol structure formation, of C=O which show ν 1695 cm^{-1} (C=O). ^{13}C-NMR spectra showed results were listed in Table (4). When esters [1,8] were refluxed with thiosemicarbazid and phosphorusoxy chlo-ride for 8hrs., to give thiadiazoles [6,13] showed absorption at ν cm^{-1} (3356-3225) broad for ($-NH_2$); 1620 for (C=N) and δppm 3.85(S, 2H, $-CH_2-$); 4.23(broad, 2H, $-NH_2$); 7-7.6(m, 4H, Ar-H) for compound [6] while compound [13] δppm

2.53(m,4H, 2 ) ; 3.43(q,2H , 

4.42(S, 2H, $-CH_2-$); 6.0(q, 2H, H-C=C-H); 4.0(broad, 2H, NH_2). ^{13}C-NMR spectra showed results were listed in Table (4).



Scheme (I)



Scheme (II)

Table (3)
^{1}H-NMR spectral data for selected compounds

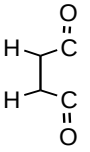
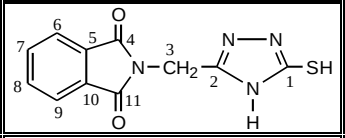
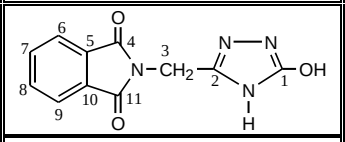
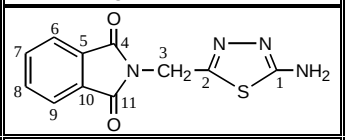
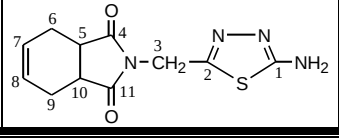
Comp. No.	^{1}H-NMR parameters (ppm) δ-H
4	4.40 (S,2H,-CH_2-) ; 7-7.6(m,4H,Ar-H) ; 12.9 (broad,2H,NH)
5	4.26 (S,2H,-CH_2-) ; 7-7.6 (m,4H,Ar-H) ; 13.1 (broad,2H,NH)
6	3.85 (S,2H,-CH_2-) ; 4.23 (broad,2H,-NH_2) ; 7-7.6 (m,4H,Ar-H)
13	2.53 (m,4H, 2 ) ; 3.43 (q,2H, ) ; 4.42 (S,2H,-CH_2-) ; 6.0 (q,2H, ) ; 4.0 (broad,2H,NH_2)

Table (4)
^{13}C-NMR spectral data for selected compounds

Comp. No.	Structure	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
4		135.29	133.36	40.49	169.15	131.23	128.90	123.88	123.88	128.90	131.23	169.15
5		148.92	133.36	40.49	169.15	131.23	128.90	123.88	123.88	128.90	131.23	169.15
6		138.23	133.36	40.49	169.15	131.23	128.90	123.88	123.88	128.90	131.23	169.15
13		139.23	132.31	40.44	170.20	130.2	123.31	127.90	127.91	123.31	130.21	170.21

Antimicrobial Activity Test

The test was performed according to the disk diffusion method[16]. The prepared compounds were tested against one strain of Gram +ve *staphyloccous*, and two strains of Gram –ve bacteria (*Escherichia Coli)*, and (*Serratia)*. Whatman No.1 filter paper disk of 5mm diameter were sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregnated with different compounds (600 μg/disk). Agar plates were surface inoculated uniformly with 100 μl from the broth culture of the tested microorganisms. The impregnated disks were placed on the medium suitably spaced apart and the plates incubated at 5°C for 1hr. to permit good diffution and then transferred to an incubator at 37°C for 24hrs. The inhibition zones caused by the various compounds on the microorganisms were examined. The results are listed in Table (5). The results refered that the prepared compounds are active against *Escherichio Coli*, but were found inactive against *Staphylococcus aureus*, and *Serratia*.

Table (5)
Results of antimicrobial activity of the tested prepared compounds

Comp. No.	E. Coli	*Serratia*	*S.aureus*
2	+++	-	-
3	++	-	-
4	+	-	-
5	+++	-	-
6	+++	-	-
9	++	-	-
10	+	-	-
11	+	-	-
12	+++	-	-
13	+	-	-

Key to symbols

Highly active=+++ (inhibition zone 15-20)
Moderately active=++(inhibition zone 1014)
Slightly active = + (inhibition zone 6-9mm)
Inactive = - (inhibition zone < 6mm)

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الخلاصة

تم في هذا البحث تحضير عدد من معوضات 4،2،1–ترايزول و4،3،1–ثايادايازول، وذلك عن طريق تفاعل كل من فثالئميد وسيز– 6،3،2،1–تتراهيدروفثالئميد اسيتت مع اثيل بروموا سيتت وهيدروكسيد الصوديوم في الايثانول المطلق ليعطي الاسترات، اثيل فثالئميد اسيتت واثيل (سيز– 6،3،2،1–تتراهيدروفثالئميد) اسيتت ثم تحويل هذه الاسترات الى مشتقات ثايوسيميكارباز ايد والسيميكارباز ايد، وذلك بتفاعلها مع ثايوسيميكارباز ايد والسيميكارباز ايد على التوالي.

حضرت معوضات الترايزول عن طريق حولقت مشتقات ثايوسيميكارباز ايد والسيميكارباز ايد في الوسط القاعدي من محلول هيدروكسيد الصوديوم المائي، كما تم تحضير معوضات ثايادايزول عن طريق معاملة الاسترات، [اثيل فثالئميد اسيتت واثيل (سيز– 6،3،2،1– تتراهيدروفثالئميد) اسيتت] مع الثايوسيميكارباز ايد بوجود $POCl_3$ مع القاعدة.

شخصت المركبات المحضرة بالطرق الطيفية [^{13}C-NMR, ^{1}H-NMR, FTIR] وتعيين بعض خواصها الفيزيائية واجراء بعض الكشوفات النوعية كما تم دراسة تاثير هذه المركبات على بعض انواع البكتريا *Escherichia Coli*، *Serrtia*، *Staphyloccus aureus*، واظهرت فعالية بصورة خاصة ضد *E. Coli*.