Synthesis and Antibacterial Activity of some Hdrazides, Substituted Thiosemicarbazide, 1,3,4-Oxadiazoles, Thiadiazoles and 1,2,4-Triazoles

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Abstract

In the present work five ethyl esters (1-5) were treated with hydrazine hydrate in ethanol to give the corresponding acid hydrazides (6-10). The hydrazides were converted to substituted thiosemicarbazides (11-14) by their reaction with phenyl isothiocyanate, while on heating at 150-200 °C substituted 1,2,4-triazole was obtained (15). The thiosemicarbazides were converted to substituted 1,2,4-triazoles (16-19), 1,3,4-oxadiazoles (20-22) and 1,3,4-thiadiazoles (23-26) by their reaction with sodium hydroxide, mercuric oxide in methanol and concentrated sulfuric acid respectively. The synthesized compounds (7, 11, 15, 18, 20, 22, 24 and 26) were tested against *Staph. Aureus, Bacillus subtilis, Proteus mirabilis, E. coli, K. pneumonia* and *Pseudomonas aeroginosa*, and the compounds (10, 16, 17, 19, 23 and 25) against *E. coli, Pseudomonas aeroginosa, Proteus mirabilis* and *Staph. aureus*. The structures of the synthesized compounds were established by physical and spectral methods.

الخلاصة

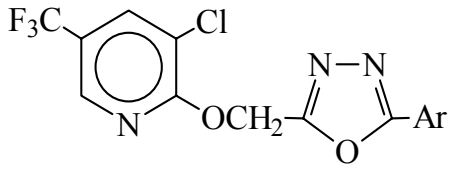
تم في هذا البحث تحويل خمسة من استرات الاثيل (1-5) الى الهيدرازيدات (6-10) المقابلة من خلال تفاعلها مع الهيدرازين المائي في الايثانول. حولت الهيدرازيدات الى ثايوسيميكاربازيدات معوضة (11-14) وذلك بتفاعلها مع ايزوثايوسيانات الفنيل، بينما اعطى تسخين الهيدرازيد الى درجة (150-200 °م) 4،2،1-ترايازول معوض (15). تم تحويل ثايوسيميكاربازيدات المعوضة الى 4،2،1-ترايازول معوض (16-19) و 4،3،1-اوكسادايازول معوض (20-22) و 4،3،1-ثايادايازول معوض (23-26) من خلال تفاعله مع محلول هيدروكسيد الصوديوم واوكسيد الزئبق في الميثانول وحامض الكبريتيك المركز على التوالي.

اختبرت فعالية المركبات (7، 11، 15، 18، 20، 22، 24، 26) تجاه البكتريا *Staph. Aureus* ، *Bacillus subtilis* ، *Proteus mirabilis* ، *E. coli* ، *K. pneumonia* ، والمركبات (10، 16، 17، 19، 23، 25) تجاه البكتريا *E. coli* ، *Pseudomonas aeroginosa* ، *Proteus mirabilis* ، *Staph. aureus* .

شخصت تراكيب المركبات المحضرة بالطرق الفيزياوية والطيفية.

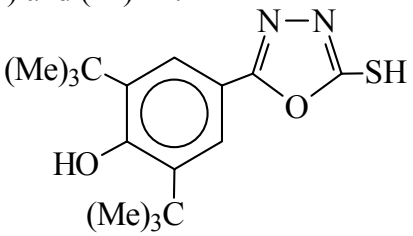
Introduction

The synthesis and the importance of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles were studied by many research workers, since, these compounds shows various biological, medical, industrial and agricultural activities, 1,3,4-oxadiazoles act against caterpillars of the type (Leucana separate walkan) as compound (1)[1].

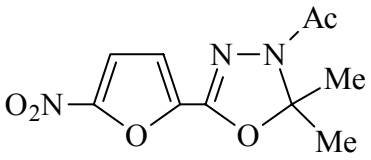


(I)

It was found that some substituted 1,3,4-oxadiazoles have anti-inflammatory properties as compound (II) and (III)[2,3].



(II)



(III)

In industry 2,5-bis (4-dimethyl aminophenyl) 1,3,4-oxadiazole was used as steel corrosion inhibitor in acidic media[4].

1,3,4-Thiadiazoles acts as antibacterial[5,6], anticancer[7], anti-tuberculosis[8] agents. While 1,2,4-triazoles possess various activities, as antifungal especially *Candida albicans*[9], plant growth regulator[10], anticonvulsant activity[11,12] and antimicrobial[13].

The above mentioned activities and uses of 1,3,4-oxadiazoles, thiadiazoles and 1,2,4-triazoles gave these compounds a great importance and many research workers work on their synthesis, acid hydrazides were treated with carbon disulfide in potassium hydroxide[14,15] or in pyridine[16] to give 2-substituted-1,3,4-oxadiazole-5-thiol, oxidation of thiosemicarbazide with a different reagents as $Hg(OAc)_2$/EtOH and KI/I_2-NaOH gave 1,3,4-oxadiazole derivatives[17,18].

Whereas 1,3,4-thiadiazole, were synthesized from the reaction of substituted thiosemicarbazides with concentrated sulfuric[19] or phosphoric acid[20], 2-substituted-5-amino-1,3,4-thiadiazole was synthesized from the reaction of thiosemicarbazide with substituted benzoic acid in presence of phosphorous oxychloride[21].

Substituted 1,2,4-triazoles were synthesized from substituted thiosemicarbazides by their reaction with sodium hydroxide solution[22], while diacetyl hydrazine was treated with acetonitrile in presence of zinc chloride to give substituted 1,2,4-triazole[23].

In the present work the synthesis of new substituted 1,3,4-oxadiazoles, thiadiazoles, 1,2,4-triazoles and the antibacterial activity of some of the synthesized compounds were studied.

Experimental

All chemicals were purchased from Flucka and BDH Chemical Ltd. The melting points were measured on an Electrothermal 9300 Engineering LTD and were uncorrected. IR spectra were recorded on Infrared Spectrophotometer Model Tensor 27, Bruker Co., Germany, using KBr discs.

Ethyl N-substituted glycine (1-4)[24]

A mixture of amine (0.15 mole), ethyl bromoacetate (23 g, 0.15 mole) and sodium bicarbonate (12.5 g) in absolute ethanol (100 ml) was refluxed for three hours. The solvent then evaporated under reduced pressure and the residue extracted with methylene chloride, which dried and evaporated, to give the esters (1-4), Tables (1,2).

In the case of esters (3 and 4) (46 g, 0.3 mole) of ethyl bromoacetate and (25 g) sodium bicarbonate were used.

Hydroquinoxy diethyl acetate (5)[25]

A mixture of hydroquinone (15.51 g, 0.055 mole), anhydrous potassium carbonate (15.18 g, 0.11 mole), ethyl bromoacetate (16.8 g, 0.11 mole) in dry acetone (200 ml) was refluxed for (18 h). The solvent was evaporated under reduced pressure, cold water (100 ml) then added the precipitate, was formed filtered, washed with water, dried and recrystallized from ethanol, Tables (1,2).

Carboxylic acid hydrazides (6-10)[26]

A mixture of the ester (1,2,3,4 or 5) (0.04 mole) and hydrazine hydrate (0.2 mole) for the synthesis of (6, 7) and (0.4 mole) for the synthesis of compounds (8-10) in ethanol (80 ml) was refluxed for (3 h), the solvent was evaporated under reduced pressure to give the hydrazides (6-10), Tables (1,2).

4-Phenyl-1-acetyl thiosemicarbazide (11-14)[27]

Hydrazide (7) (0.002 mole) or hydrazide (8-10) (0.01 mole) was refluxed with phenyl isothiocyanate (0.002 mole) in absolute ethanol (40 ml) for (6 h), the precipitate was formed on cooling, filtered and recrystallized from ethanol, Tables (1,2).

3,5-Dialkyl amino-1,2,4-triazole (15)[28]

The hydrazide (7) (0.7 g, 0.004 mole) was heated between (150-200 °C) for (1 h), water (50 ml) was added and the mixture then refluxed for (15 min), the formed solid was collected and recrystallized from ethanol, Tables (1,2).

3-Substituted-4-phentyl-1,2,4-triazole-5-thiol (16-19)[29]

A mixture of thiosemicarbazides (11, 12, 13 or 14) (0.002 mole) and sodium hydroxide solution (25ml,4%) was refluxed for (3h), the resulting solution was acidified with 10% hydrochloric acid with cooling, the precipitate then filtered and recrystallized from ethanol-water (1:3), Tables (1,2).

2-Phenyl amino-5-substituted-1,3,4-oxadiazole (20-22)[30]

To substituted thiosemicarbazide (11) (0.001 mole) or thiosemicarbazide (12 or 14) (0.0005 mole) in methanol (30 ml), mercuric oxide (0.24 g, 0.001 mole) was added the mixture then refluxed for (5 h), and filtered while the solution is hot the solvent was evaporated to give solid products which were dried and recrystallized from ethanol, Tables (1,2).

2-Phenyl amino-5-substituted-1,3,4-thiadiazole (23-26)[31]

Concentrated sulfuric acid (10 ml) was added to one of the substituted thiosemicarbazides (11-14), the mixture then heated on water bath at (90 °C) with stirring for (2 h), the mixture then poured on crushed ice, and neutralized with ammonium hydroxide solution with cooling, the precipitate formed, was filtered, washed with water and recrystallized from ethanol, Tables (1,2).

The biological activity

Some of the synthesized compounds (7, 11, 15, 18, 20, 22, 24, 26) were tested against the following bacteria *Staph. aureus*, *Bacillus subtilis*, *Proteus mirabilis*, *E. coli*, *K. pneumonae* and *Pseudomonas aereginosa* and the compounds (10, 16, 17, 19, 23, 25) against *E. coli*,

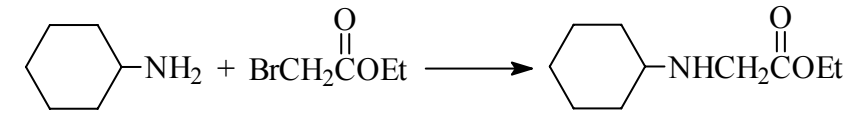
Pseudomonas aereginosa, *Proteus mirabilis* and *Staph. aureus*.

The procedure of Bauer[32] was used in sensitivity test as six colonies of the above mentioned bacteria which were transferred to the nutrient broth. The medium was incubated at 37 °C for 15-16 hours and then diluted with normal saline. (0.1 ml) of this medium was transferred to the nutrient agar and distributed on the surface of the Petri dishes, then left for about (30 min) at 37 °C in the incubator.

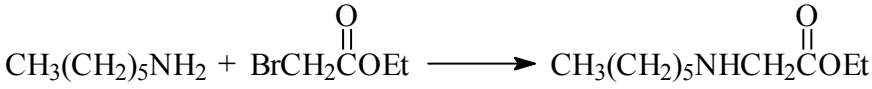
To determine the inhibitory effect, filter paper discs were saturated with different concentration of solution for tested compounds in DMSO and were distributed on the surface of the agar medium and then incubated on the surface of the agar medium for (15-16 h). The antibiotic ciprollaxin and chloramphenicol were used as a control.

Results and Discussion

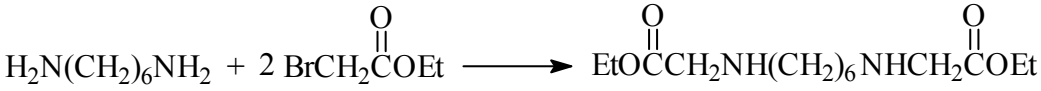
The synthesis of substituted 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles from hydrazide and substituted thiosemicarbazide (Scheme 1) and their antibacterial evaluation were reported. The esters (1-5) were synthesized from the reaction of amines (Cyclohexyl amine, Hexyl amine, 1,6-diaminohexane, p-phenylene diamine) and Hydroquinone with ethyl bromoacetate as follows:



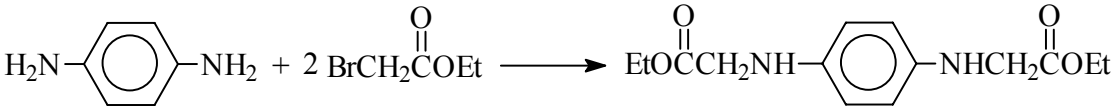
Cyclohexyl amine (1)



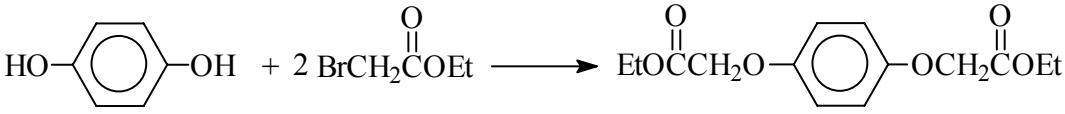
Hexyl amine (2)



1,6-diaminohexane (3)



p-phenylene diamine (4)



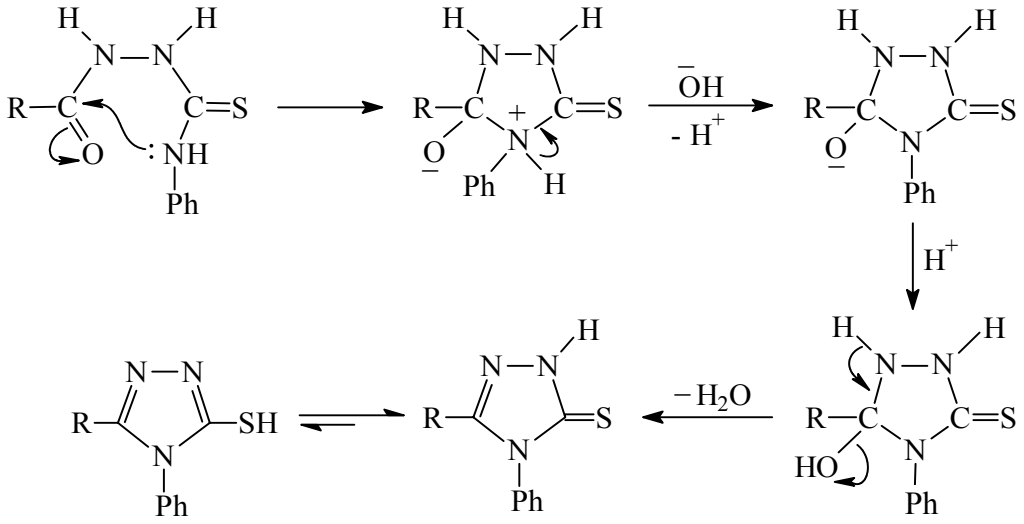
Hydroquinone (5)

The ethyl esters (1-5) were treated with hydrazine hydrate in ethanol to give acid hydrazides (6-10). The hydrazides (6) shows absorption ν cm^{-1} 3340 (N-H) and 1670 (C=O), while the ester (1) shows absorption for (C=O) ν cm^{-1} at 1745. The hydrazides (9,10) show absorption at 1555 and 1560 (C⋯C) respectively. The hydrazide then converted to substituted

thiosemicarbazides (11-14) by their reaction with phenyl isothiocyanate.

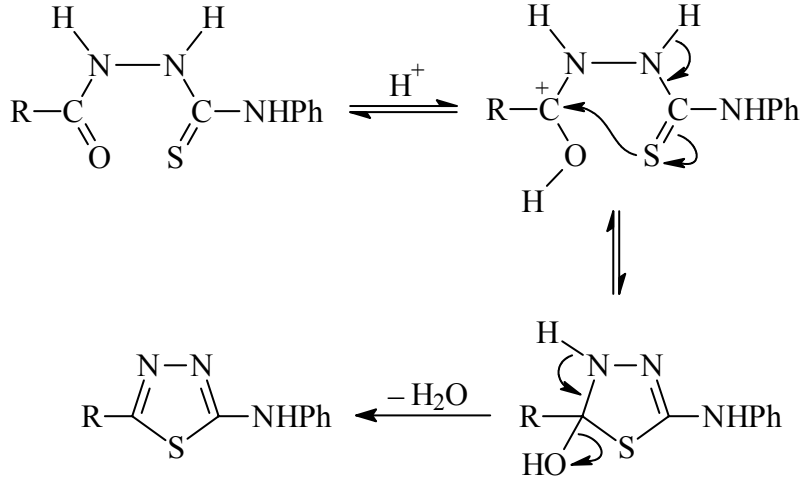
The thiosemicarbazide (11) show absorption at ν cm^{-1} 3060 (N-H), 1670 (C=O) and 1110 (C=S). The hydrazide (7) on heating at (150-200 °C) for one hour and refluxed after the addition of water for (15 min) gave substituted 1,2,4-triazole (15), which shows absorption ν cm^{-1} at 3375 (N-H) and 1650 (C=N).

Thiosemicarbazides (11-14) were refluxed with 4% sodium hydroxide solution for three hours to give substituted 1,2,4-triazoles (16-19), compound (16) shows absorption ν cm^{-1} 3355 (N-H), 1645 (C=N) and 1170 (C=S). The reaction mechanism as follows:



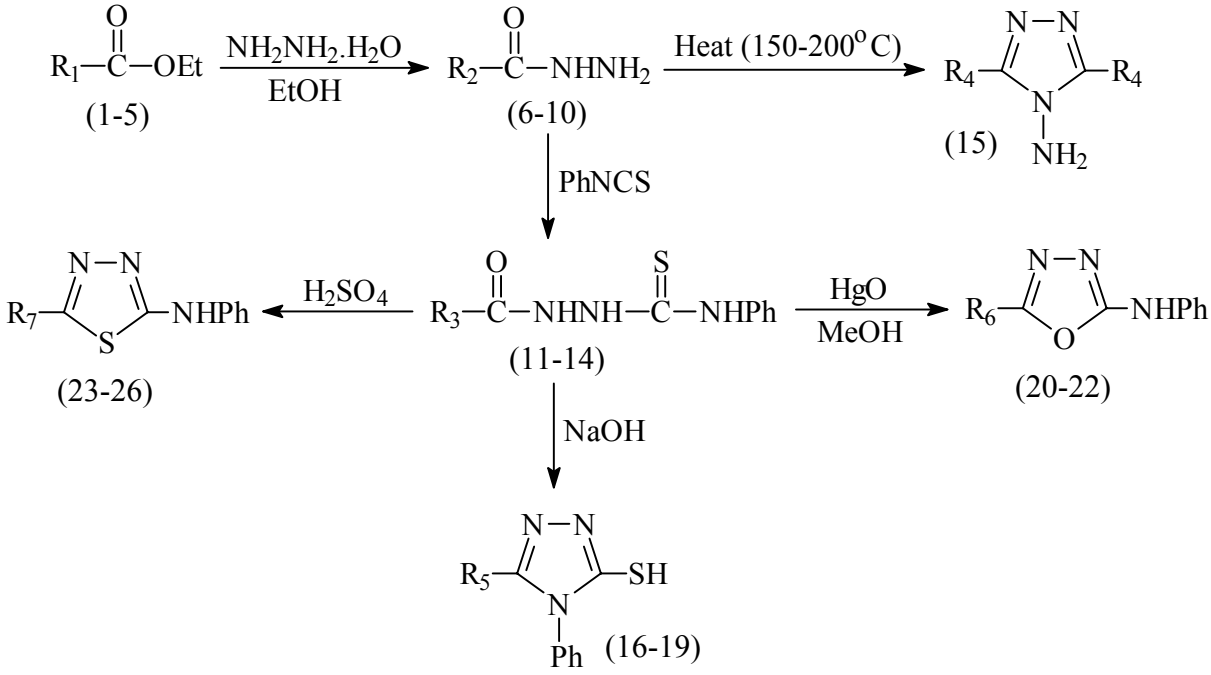
Substituted thiosemicarbazides (11, 12 and 14) were treated with mercuric oxide in methanol to give substituted 1,3,4-oxadiazole (20-22). Compound (20) shows absorption at 3370 (N-H), 1635 (C=N) and 1225 (C-O-C).

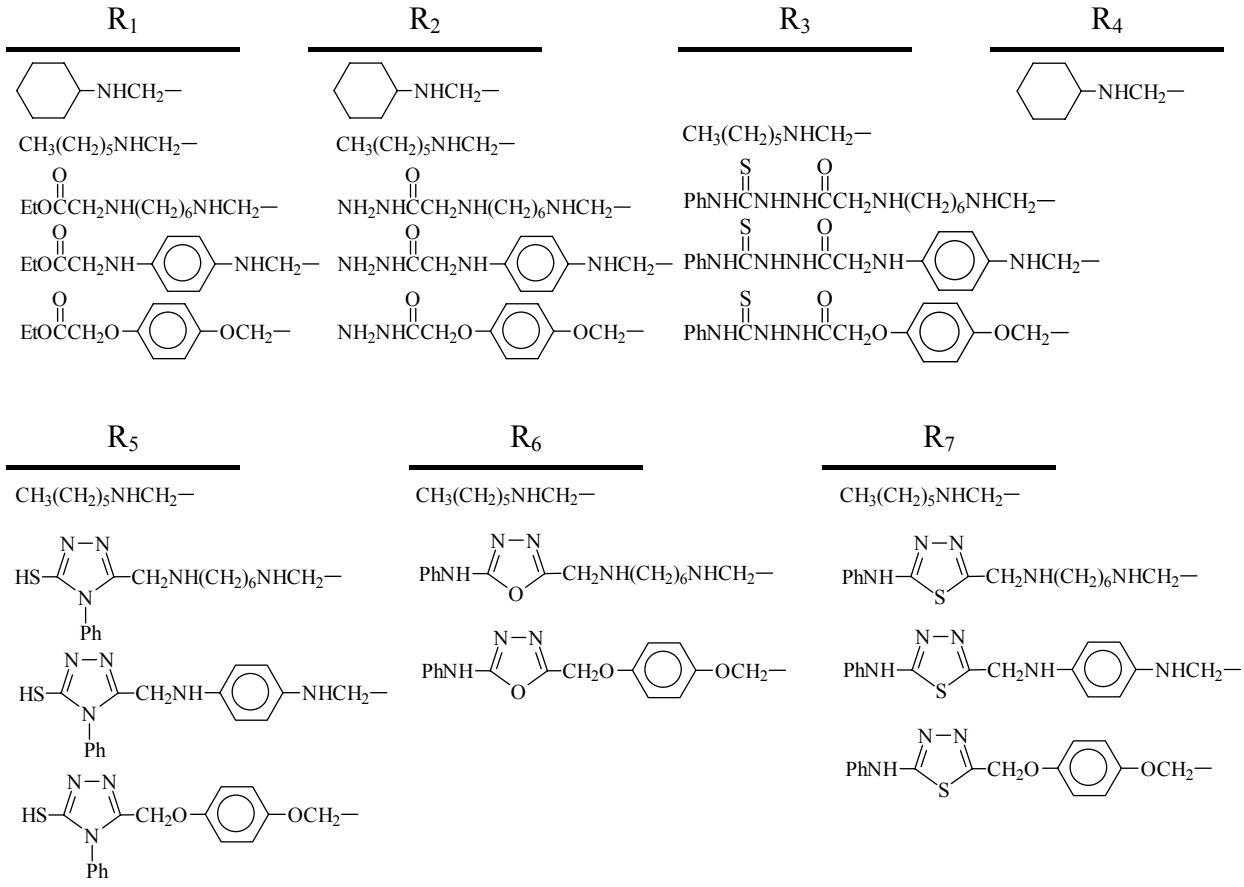
The substituted thiosemicarbazide (11-14) were heated on water bath with concentrated sulfuric acid for (2) hours to give substituted 1,3,4-thiadiazoles (23-26), the reaction proceed through the following mechanism.



1,3,4-Thiadiazole (23) shows absorption at ν cm^{-1} 3205 (N-H) and 1615 (C=N).

The physical and the IR data are listed in Tables (1,2).





Scheme (1)

Biological test

The antibacterial evaluation of some synthesized compounds (14 compounds) show that most of the tested compounds have a certain activity against the bacteria used in the test. The concentration of samples in DMSO were 10, 1, 0.1, 0.01 mg/disk, the highest activity at 10 mg/disk for some of the tested compounds were nearly in the same level as the antibiotic used.

The compounds 7, 11, 15, 18, 20, 22, 24 and 26 were inactive against *Proteus mirabilis*, while compounds 11, 15, 18, 20, 22 and 26 show no activity against *Bacillus subtilis* and compounds 7, 11, 15 and 26 were inactive against *E. coli*. The rest of the tested compounds shows varietal activities against the used bacteria in the test, according to the sample concentration and the kind of the bacteria. The antibacterial activity of the tested compounds are listed in Tables (3 and 4).

Table (1): Physical data of compounds (1-26)

Comp. No.	Yield (%)	m.p. (°C)
1	65	239-242
2	61	144-146
3	70	Oily
4	80	90-92
5	40	92-95
6	90	78-80
7	88	Oily
8	83	Oily
9	55	232-234
10	91	244-246
11	90	184-186
12	85	180-183
13	68	158-160
14	71	170-172
15	60	Oily
16	60	213-216
17	56	215-218
18	63	254-256
19	66	266-268
20	91	230-233
21	82	246-248
22	67	234-236
23	78	245-248
24	82	128-130
25	55	223-225
26	62	237-240 d.

Table (2): I.R spectral data of compounds (1-26)

Comp. No.	I.R ν cm^{-1}				
	N-H	C=O	C=S	C=N	Others
1	3310	1745	-	-	-
2	3220	1775	-	-	-
3	3270	1720	-	-	-
4	3005	1760	-	-	-
5	3210	1735	-	-	-
6	3340	1670	-	-	-
7	3365	1680	-	-	-
8	3255	1670	-	-	-
9	3200	1685	-	-	1555 (C⋯C)
10	3300	1685	-	-	1560 (C⋯C)
11	3060	1670	1110	-	-
12	3330	1685	1105	-	-
13	3405	1735	1260	-	-
14	3155	1685	1255	-	-
15	3375	-	-	1650	-
16	3355	-	1170	1645	-
17	3255	-	1235	1620	-
18	3260	-	1220	1640	-
19	3135	-	1110	1610	-
20	3370	-	-	1635	1225 (C-O-C)
21	3150	-	-	1610	1260 (C-O-C)
22	3320	-	-	1640	1245 (C-O-C)
23	3205	-	-	1615	-
24	3290	-	-	1610	-
25	3300	-	-	1600	-
26	3100	-	-	1650	-

Table (3): The antibacterial activity of compounds (7, 11, 15, 18, 20, 22, 24, 26)

Comp. No.	Conc. (mg/disk)	Testing organism					
		Staph. aureus	*Bacillus subtilis*	*Proteus mirabilis*	*E. coli*	*K. pneumonia*	*Pseudomonas aeroginosa*
7	10	-	15	-	-	-	-
	1	-	13	-	-	-	-
	0.1	-	9	-	-	-	-
	0.01	-	-	-	-	-	-
11	10	18	-	-	-	9	16
	1	15	-	-	-	-	13
	0.1	13	-	-	-	-	10
	0.01	10	-	-	-	-	9
15	10	-	-	-	-	15	-
	1	-	-	-	-	13	-
	0.1	-	-	-	-	12	-
	0.01	-	-	-	-	10	-
18	10	9	-	-	15	17	17
	1	-	-	-	8	15	15
	0.1	-	-	-	-	11	13
	0.01	-	-	-	-	9	9
20	10	-	-	-	14	-	14
	1	-	-	-	-	-	12
	0.1	-	-	-	-	-	9
	0.01	-	-	-	-	-	-
22	10	-	15	-	13	-	20
	1	-	12	-	9	-	15
	0.1	-	9	-	-	-	13
	0.01	-	-	-	-	-	10
24	10	10	-	-	18	-	15
	1	-	-	-	13	-	12
	0.1	-	-	-	-	-	10
	0.01	-	-	-	-	-	-
26	10	15	-	-	13	13	13
	1	10	-	-	9	9	10
	0.1	9	-	-	-	-	-
	0.01	-	-	-	-	-	-

Table (4): The antibacterial activity of compounds (10, 16, 17, 19, 23, 25)

Comp. No.	Conc. (mg/disk)	Testing organism			
		E. coli	*Pseudomonas aeroginosa*	*Proteus mirabilis*	*Staph. aureus*
10	10	15	10	14	12
	1	13	7	13	10
	0.1	9	-	7	7
	0.01	7	-	-	-
16	10	16	16	13	13
	1	13	10	10	9
	0.1	10	8	7	8
	0.01	8	-	-	-
17	10	9	12	16	14
	1	8	10	9	12
	0.1	-	-	-	10
	0.01	-	-	-	-
19	10	18	15	15	18
	1	10	11	13	9
	0.1	9	8	8	7
	0.01	-	-	7	-
23	10	9	16	13	10
	1	7	12	11	9
	0.1	-	10	8	-
	0.01	-	7	-	-
25	10	16	18	9	9
	1	13	12	7	8
	0.1	12	10	4	-
	0.01	8	7	-	-
Tetracycline	Control	18	15	16	16
Gentamicine		15	14	13	17

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