JCRS

Journal of Chemical Reactivity and Synthesis 2023: 13(1) 1-19

Research Article

Synthesis of new coumarin based acetohydrazones, their correspounding oxadiazoles and oxadiazolines, and the investigation of their antibacterial activity

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ARTICLE INFO:

Received:
23 November 2022

Accepted:
6 December 2022

Available online:
10 January 2023

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ABSTRACT

Here we provide a report on the synthesis, structural recognition and tautomeric behavior of a new series of coumarin-based hydrazide-hydrazones (4-methylcoumarin-7-yl-oxymethyl arylaldehyde acetohydrazones) and their 1,3,4- oxadiazoline and oxadiazole derivatives. The final products were tested for antibacterial activity on both Gram-negative and Grampositive bacteria. Both types of hydrogen, the hydrazone N-H and methylene protons can take part in tautomeric isomerization equilibrium. The synthesis of 1,3,4-oxadiazoles and oxadiazoline bearing coumarin ring was conducted for inferring the isomerization-tautomerization of their hydrazone precursors According to the NMR investigation, We found that, the amide-iminol tautomerization is more probable than keto-enol one. It was further explained via converting the hydrazones to the corresponding 1,3,4-oxadiazoles and oxadiazolines based on the modified procedures. All the synthesized oxadiazoles and oxadiazolines have antibacterial effect on Gram-negative bacteria with respect to Gram-positive bacteria. The active ingredient of the synthesized derivative 8 has the best effect on both Gram-negative and Gram-positive bacteria.

Keywords: 4-Methylcoumarin; Oxadiazoles; Anti-bacterial agents; Heterocycles.

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1. Introduction

Aryl hydrazones and oxadiazoles are believed as two important categories of organic compounds. Oxadiazoles, depending on the position of nitrogen atoms, consist of four different isomers: 1,2,3-oxadiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole and 1,3,4-oxadiazole (Figure 1).

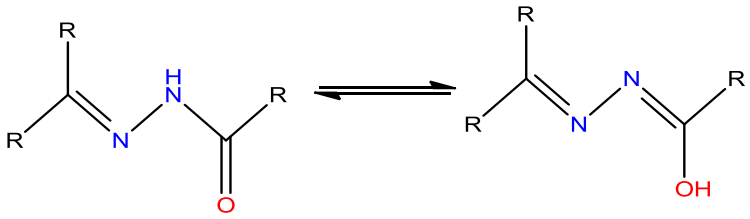
1,2,5-oxadiazole 1,2,3-oxadiazole 1,3,4-oxadiazole 1,2,4-oxadiazole

Fig. 1. Different oxadiazole isomers

Coumarin based hydrazone and oxadiazole derivatives have found a lot of interest because of their bioactivities [1-4]. Compounds containing 1,3,4-oxadiazole exhibit a wide range of biological activities such as anticancer, antiparasitic, antifungal, antibacterial, antidepressant, anti-tubercular and anti-inflammatory [5-8]. Complex 1,3,4-oxadiazole derivatives are supposed to be valuable compounds in this respect. These molecules are widely used in the production of many biopharmaceutical substances, showing a wide range of biological activities [9]. Oxadiazole heterocycles are therefore important structural motifs in the production of novel drugs [10-14].

Hydrazides and their derivatives could be transformed into various heterocyclic compounds either by cyclization [15-18] or cyclo-addition with numerous reagents. These acid hydrazides and their derivatives were recognized as beneficial synthons for the synthesis of various heterocyclic five, six or seven-membered rings including one or more heteroatoms with versatile applications and properties [19-24].

Acid hydrazones are regarded as important bidentate ligands, showing keto-enol (amido-iminol) tautomerism [25-27] They are usually presented in the keto form in solid state and preserved an equilibrium between keto and enol in solution state (Figure 2).

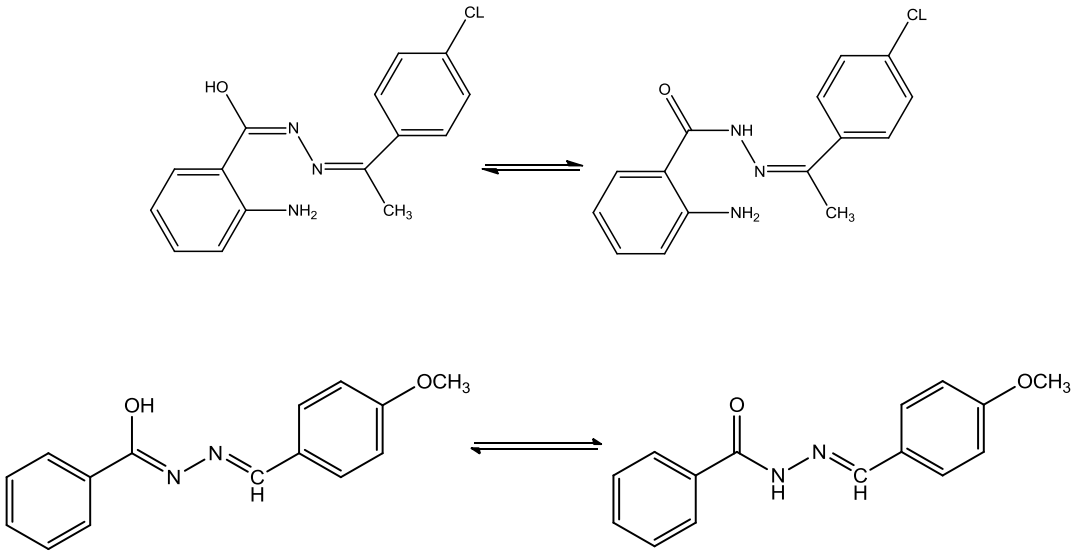


(keto form) (enol form)

Fig. 2. Keto-enol froms of hydrazones

An important aspect of the chemical structure of hydrazones is illustrated by the existence of prototropic tautomerism. Therefore, their structural categorization is an important feature in organic chemistry.

Some types of tautomerism are shown in the following examples (Figure 3).



Amide Iminol

Fig. 3. Amide-iminol tautomerism in the title compound

Due to their broad pharmaceutical utilization, hydrazides and hydrazones nowadays have many technical and commercial importance, among them are isocarboxazide, iproniazide, isoniazid, nifuroxazide, rifampisin [28].

In addition, synthesis of organic compounds containing the hydrazone group has been reported as they exhibit antibacterial activities. Hydrazones are the most important drugs against some microorganism cultures, Gram positive and negative bacteria and fungi. The acyl- and aroyl hydrazones are very important as chelating agents as well as versatile ligands in coordination chemistry [29].

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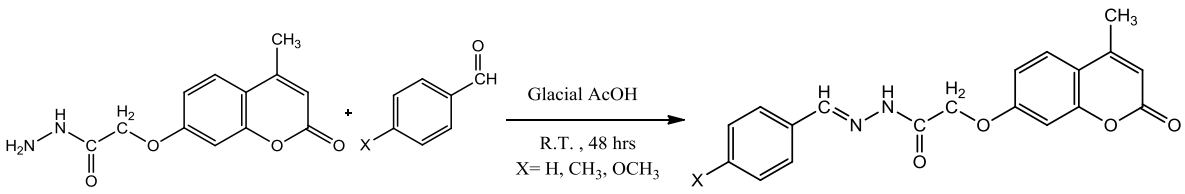
2. Experimental

2.1. Materials and method

Melting points were determined on Barnstead Electrothermal-9100 which were uncorrected. IR spectra were recorded on a Bruker IR spectrophotometer (Tensor model) as KBr pellets. The ^{1}H-NMR spectra were recorded on a Bruker Nuclear Magnetic Resonance (NMR) (300 MHz) using DMSO-d_6 or $CDCl_3$ as solvents and TMS as internal standard. Thin layer chromatography (TLC) was applied on silica gel plates using appropriate solvents as mobile phase. The ChemDrew-software was used for drawing and naming the synthesized compounds.

2.2 General procedure for the synthesis of 4-methylcoumarin-7-yl-oxymethyl-arylaldehyde acetohydrazone (1-3)



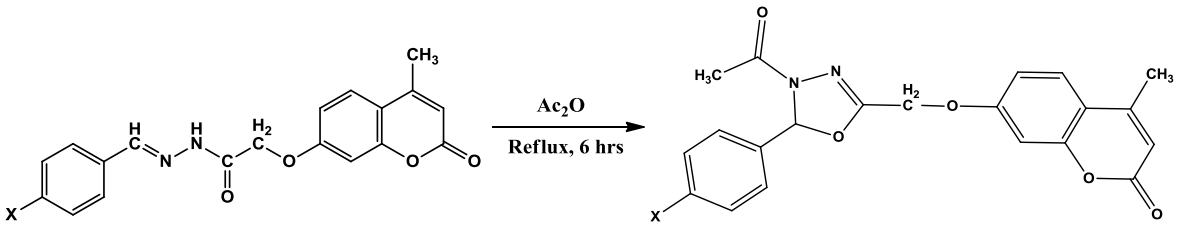
Scheme 1: synthesis of 4-methylcoumarin-7-yl-oxymethyl-arylaldehyde acetohydrazone

A (50 ml) round bottomed flask equipped with a magnet bar and magnetic stirrer was used as a reaction vessel and 15 ml glacial acetic acid was used as a solvent. 0.033 mole aryl aldehyde and (1 g) 4-methylcoumarin-7-yl-oxymethyl acetohydrazide were added to the reaction vessel respectively. The reaction mixture was stirred at room temperature for 48 hours. The progress of the reaction was monitored by thin layer chromatography (TLC) on silica-gel as the stationary phase, and ($CHCl_3$-MeOH 2:1) was used as the mobile phase. Completing of the reaction, the mixture was poured on (150 g) crushed ice. The crude product was precipitated and purified by recrystallization by acetone. The melting points of products were determined and the structures were characterized by IR and NMR spectroscopy. IR, $\bar{\upsilon}$ (Cm^{-1}) **(1)**: 3300, 3150, 3000 – 3082, 2850 – 2924 – 2971, 1686, 1710, 1415 – 1615, 1353 – 1391, 1057 – 1270, 1057 – 1270, 687 – 882; H-1 NMR, (300 MHz, DMSO), δ (ppm) **(1)**: 2.37 (S) 1H, 4.7 – 5.2 (S) 2H, amide tautomeric forms; 6.1 – 6.2 (S) 1H, 6.9 – 7.7 (S) 5H, 7.4 (S) 3H, 8 – 8.3 (S) 1H, imine tautomeric forms; 11.6 (S) 1H; IR, $\bar{\upsilon}$ (Cm^{-1}) **(2)**: 3311, 3000 – 3100, 3000 – 2850, 1690 – 1710, 1622 – 1400, 1390 – 1366, 1390 – 1366, 884 – 660; H-1 NMR, (300 MHz, DMSO), δ (ppm) **(2)**: 2.37 (S) 1H, 4.77 (S) 3H, 4.7 – 5.2 (S) 2H, amide tautomeric forms; 6.1 (S) 1H, 6.99 (m) 4H, 7.6 (S) 3H, 7.9 – 8.2 (S) 1H, imine tautomeric forms; 11.49 (S) 1H.

2.3 Synthesis of 4-methyl coumarin-7-yl-oxymethyl-p-methylbenzaldehyde acetohydrazone (3) as a typical procedure

A (50 ml) round bottomed flask with a magnet bar was used as a reaction vessel, the vessel equipped with a magnetic stirrer. (15 ml) glacial acetic acid as a solvent, (0.4 g) p-methyl benzaldehyde, and (1 g) 4-methylcoumarin-7-yl-oxymethyl acetohydrazide were added to the reaction vessel respectively. The reaction mixture was stirred at room temperature for (48 hours). The progress of the reaction was monitored by thin layer chromatography (TLC) on silica-gel as the stationary phase, and ($CHCl_3$- MeOH 2:1) was used as the mobile phase. Completing of the reaction, the mixture was poured on (150 gr) crushed ice. The crude product was formed as precipitate and purified by recrystallization by acetone (Mp: 264-266 ºC). IR, $\bar{\upsilon}$ (Cm^{-1}) **(3)**: 3300, 3150, 3000-3090, 2855-2969, 1684, 1710, 1616-1415, 1395.1, 1346.6, 1391-1353, 1269-1055, 882-687; H-1 NMR, (300 MHz, DMSO), δ (ppm) **(3)**: 2.31 (S) 3H, 2.37 (S) 3H, 4.7 - 5.2 (S) 2H, amide tautomeric forms; 6.19 (d) 1H, 6.9 - 7.7 (m) 7H, overlapped signals; 7.9 - 8.2 (S) 1H, imine tautomeric forms; 11.5 (S) 1H.

2.4 General procedure for the synthesis of of 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-4-acetyl-5-aryl-1, 3, 4-oxadiazolines (4,5)



X= H, CH_3, OCH_3

Scheme 2: synthesis of of 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-4-acetyl-5-aryl-1, 3, 4-oxadiazolines

A (50 ml) round bottomed flask with a magnet bar is used as a reaction vessel, the vessel equipped with a condenser and heater stirrer, (5 ml) dry acetic anhydride as a solvent and

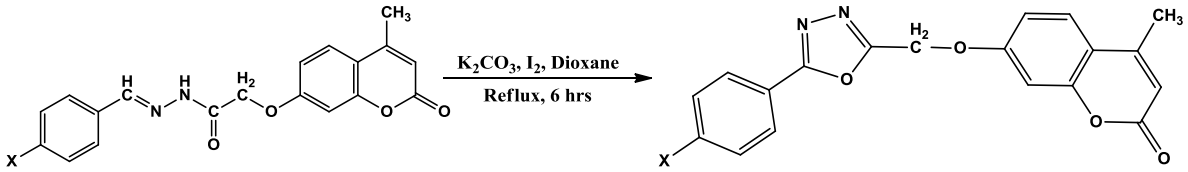
appropriate amounts of methylcoumarin-7-yl-oxymethyl arylaldehyde aceto hydrazone were added to the reaction vessel and refluxed for (6 hours). The progress of the reaction was monitored by thin layer chromatography .(TLC) on silica-gel as the stationary phase, and ($CHCl_3$- MeOH 2:1) was used as the mobile phase. After completion of the reaction, the mixture was poured on (100 g) crushed ice. The crude product was precipitated and purified by recrystallization from acetone. The melting points of products were determined and the structures were characterized by IR and NMR spectroscopy. IR, ῡ (Cm^{-1}) (**5**): 3080, 3075, 2925, 2850, 1730, 1711.12, 1620.4, 1511.8, 1429, 1387.72, 1300.5, 1260, 1191.8, 1149.6, 1074.5, 1016.91, 951.36, 848.9, 829.41, 630; H-1 NMR, (300 MHz, DMSO), δ (PPM) (5):2.36 (S) 3H, (Acetyl CH_3); 2.38 (S) 3H, (Coumarin attached CH_3); 3.85 (S) 3H, (OCH_3)؛ 5.29 (S), 4.81 (S) 2H, (CH_2 Tautomers); 6.19 (S) 1H, (No.3-H of Coumarin); 6.94 – 8.5 (m) 7H, (Aromatic); 8.50 (S) 1H, (No.5-H of Oxadiazole ring); 12.85 (S) broad, N-H (Tautomeric form of oxadiazole ring); C-13 NMR, (300 MHz, DMSO), δ (ppm) (5):18.1, 25.6, 55.5, 64.8, 68.3, 101.5, 103.3, 111.2, 112.2, 113.5, 114.5, 125.1, 126.3, 127.8, 130.4, 152, 153, 154, 160.7, 162.5, 167.7, 170.7; Mass (5): MW= 410, Found: 410.2 (M+); Elemental analysis: Caculated for $C_{22}H_{21}N_2O_6$: C, 64.70%; H, 4.90%; N, 6.86%. Found: C, 64.66%; H, 4.85%; N, 6.78%.

2.5 Synthesis of 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-4-acetyl-5-(4-methylphenyl)-1,3,4-oxadiazolines (4) as a typical procedure

A (50 ml) round bottomed flask with a magnet bar used as a reaction vessel, the vessel equipped with a condenser and heater stirrer. (5 ml) dry acetic anhydride as a solvent and (0.2 g) 4-methylcoumarin-7-yl-oxymethyl p-methylbenzaldehyde aceto hydrazone were added to the reaction vessel and refluxed for (6 hours). The progress of the reaction was monitored by thin layer chromatography (TLC) on silica-gel as the stationary phase, and ($CHCl_3$-MeOH 2:1) was used as the mobile phase. After completion of the reaction, the mixture was poured on (100 g) crushed ice.

The crude product was precipitated and purified by recrystallization using acetone (Mp:168-170 ºC). IR, ῡ (Cm^{-1}) **(4)**: 3079, 3065, 2924, 2854.7, 1724, 1712, 1615, 1560.64, 1509.79, 1390.17, 1281.31, 1190, 1153.83, 1073.06, 980.47, 951.08, 845.96, 810.28, 625.33, 585.36; H-1 NMR, (300 MHz, DMSO), δ (ppm) (4): 2.37 (m) 9H, (Overlaped signals of Acetyl, Tolyl and Coumarin attached methyl groups; 5.3 (S), 4.8 (S) 2H, (CH_2 Tautomers)؛ 6.2 (S) 1H, (No.3-H of Coumarin)؛ 6.9 – 7.17 (m) 7H, (Aromatic)؛ 8.56 (S) 1H, (No.5-H of Oxadiazole ring)؛ 12.8 (S) - broad, N-H (Tautomeric from of Oxadiazole ring); C-13 NMR, (300 MHz, DMSO), δ (ppm) (4): 18, 21, 25, 68.3, 91.3, 101.5, 111, 112, 113, 126.2, 127.8, 129.5, 133.25, 140.07, 142.51, 153.3, 156.8, 160.06, 161.04, 165.96, 167.81, 170.79; Mass (4): MW= 394, Found: 394.2 (M+); Elemental analysis: Caculated for $C_{22}H_{21}N_2O_5$: C, 67.34%; H, 5.35%; N, 7.14%. Found: C, 67.30%; H, 5.30%; N, 6.12%.

2.6 General procedure for the synthesis of 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-5-aryl-1,3,4-oxadiazole (6-8)



X= H, CH_3 ,OCH_3

Scheme 3:synthesis of 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-5-aryl-1,3,4-oxadiazole

A (50 ml) round bottomed flask with a magnet bar used as a reaction vessel, the vessel equipped with a condenser and magnetic heater stirrer, (7 ml) 1,4-dioxane used as a solvent and appropriate amount of 4-methylcoumarin-7-yl-oxymethyl arylaldehyde aceto hydrazone, (0.2 g

) potassium carbonate and (0.15 g) iodine were added to the reaction vessel respectively and refluxed for (6 hours). The progress of the reaction was monitored by thin layer chromatography

(TLC) on silica-gel as the stationary phase, and ($CHCl_3$- MeOH 2:1) was used as the mobile phase. After cooling the vessel, (20 ml) sodium thiosulfate (5%) was added to the reaction, mixture and extracted with (1:1) methanol-dichlromethane (3x15 ml). The crude extract was dried on anhydrous sodium sulfate and the solvent was evaporated and the solid product recrystallized from ethylacetate. The melting points of products were determined and the structures were characterized by IR and NMR spectroscopy.

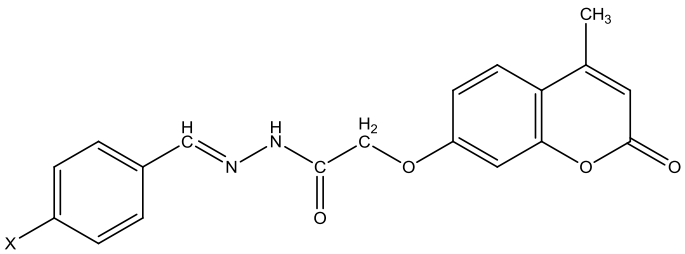
IR, ῡ (Cm^{-1}) **(7)**: 3088, 3061, 2954, 2923, 1714, 1688.25, 1612.76, 1561, 1500.7, 1415, 1390.32, 1272.28, 1196, 1156, 1138, 1068, 1019, 981, 834, 852, 733; H-1 NMR, (300 MHZ, DMSO), δ (ppm) **(7)**: 2.34 (S) 3H, (Tolyl CH_3); 2.38 (S) 3H, (Coumarin attached CH_3); 5.60 (S) 2H, CH_2, 6.22 (S) 1H, (No.3-H of Coumarin); 7.07 – 7.89 (m) 7H, (Aromatic); C-13 NMR, (300 MHz, DMSO), δ (ppm) **(7)**: 18.4, 21.10, 60.04, 101.97, 111.90, 115, 114, 120, 126.70, 126.96, 131, 139, 142, 154, 160, 161, 164, (some peaks are overlapped); Mass **(7)**: MW= 351, Found: 351.2 (M+), 352.2 (M+1); Elemental analysis: Caculated for $C_{20}H_{16}N_2O_4$: C, 68.96%; H, 4.59%; N, 8.04%. Found: C, 68.92%; H, 4.54%; N, 7.96%.; IR, ῡ (Cm^{-1}) **(8)**: 3080, 3065, 2954.24, 1713.06, 1611.03, 1551.2, 1483.74, 1449.90, 1389.04, 1275.17, 1255, 1196, 1155.99, 1138.45, 1068, 1020, 981.52, 832.39, 775.18, 712.91, 692.02, 632.49; H-1 NMR, (300 MHz, DMSO), δ (PPM) **(8)**: 2.3 (S) 3H, CH_3، 5.63 (S) 2H, CH_2، 6.24(S), 1H (No.3 H of coumarin) ; 7.09 – 8.02 (m) 8H Aromatic; C-13 NMR, (300 MHz, DMSO), δ (ppm) **(8)**: 18.17, 60, 66, 102, 111, 112, 114, 122, 126, 129, 132, 153, 154, 160, 162, 164, (some peaks are overlapped); Mass **(8)**: MW= 336, Found: 336.2 (M+), 337.2 (M+1); Elemental analysis: Caculated for $C_{19}H_{14}N_2O_4$: C, 68.26%; H, 4.19%; N, 8.38%. Found: C, 68.22%; H, 4.14%; N, 8.30%.

2.7 Synthesis of 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-5-(4-methoxyphenyl)-1,3,4-oxadiazole (6) as a typical procedure

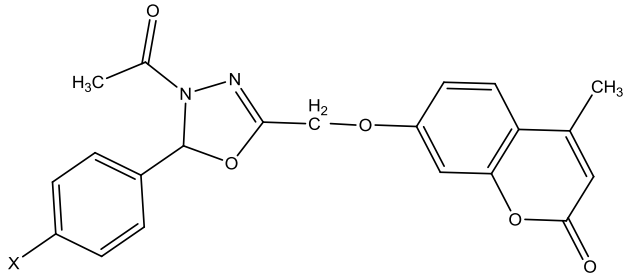
A (50 ml) round bottomed flask with a magnet bar used as a reaction vessel, the vessel equipped with a condenser and magnetic heater stirrer. (7 ml) 1,4-dioxane used as a solvent and (0.18 g) 4-methylcoumarin-7-yl-oxymethyl p-methoxy benzaldehyde aceto hydrazone, (0.2 g) potassium carbonate and (0.15 g) iodine were added to the reaction vessel respectively and refluxed for (6 hours). The progress of the reaction was monitored by thin layer chromatography (TLC) on silica-gel as the stationary phase, and ($CHCl_3$-MeOH 2:1) was used as the mobile phase. After cooling the vessel, (20 ml) sodium thiosulfate (5%) was added to the reaction, mixture and extracted with (1:1) methanol-dichlromethane (3x15 ml). The crude extract was dried on anhydrous sodium sulfate and the solvent was evaporated and the solid product recrystallized from ethylacetate (Mp:195-197 ºC). IR, ῡ (Cm^{-1}) **(6)**: 3086, 3061.2, 2953.9, 2922, 1724, 1615, 1502.9, 1426, 1390.29, 1308.1, 1263.48, 1206.9, 1146.89, 1071.9, 1014.4, 851.26, 834, 740.65, 707.9, 634.42; H-1 NMR, (300 MHZ, DMSO), δ (ppm) **(6)**: 2.39 (S) 3H, CH_3; 3.83 (S) 3H, OCH_3; 5.60 (S) 2H, CH_2; 6.24 (S) 1H, (No.3-H of Coumarin; 6.97-7.95 (m) 7H, (Aromatic); C-13 NMR, (300 MHz, DMSO), δ (ppm) **(6)**: 18.1, 60.04, 101.94, 112.4, 114, 115, 126, 128, 153, 154, 160, 161, 162, 164, (some peaks are overlapped); Mass **(6)**: MW= 367, Found: 367.1(M+); Elemental analysis: Calculated for $C_{20}H_{16}N_2O_5$: C, 65.93%; H, 4.39%; N, 7.69%. Found: C, 65.89%; H, 4.34%; N, 7.61%.

3. Results and discussion

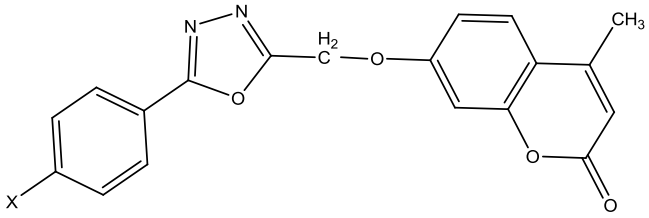
Chemical structures of 4-methylcoumarin-7-yl-oxymethyl-arylaldehyde acetohydrazone **(1-3)**, 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-4-acetyl-5-aryl-1, 3, 4-oxadiazolines **(4,5)** and 4-methyl-2-[(coumarin-7-yl-oxymethyl)]-5-aryl-1,3,4-oxadiazole **(6-8)** are shown below (figure 4).



(1) X= H **(2)** X= OCH_3 **(3)** X= CH_3



(4) X= CH_3 **(5)** X= OCH_3



(6) X= OCH_3 (7) X= CH_3 **(8)** X= H

Fig. 4. Chemical structures of products **(1-8)**

In this article the synthesis of 1,3,4-oxadiazoles and oxadiazoline bearing coumarin ring was pursued because of the isomerization-tautomerization of their hydrazone precursors. Conventional and modified synthesis of several coumarin-oxadiazole derivatives from starting compound, 4-methylcoumarinyl-7-oxyacetic acid hydrazide has been accomplished. Both types of hydrogen, the hydrazone N-H and methylene protons, can participate in tautomeric isomerization equilibrium (Figure 5 and 6).

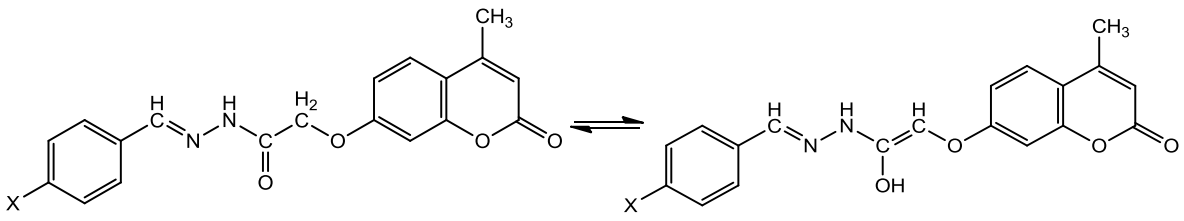


Fig. 5. The keto-enol tautomerization

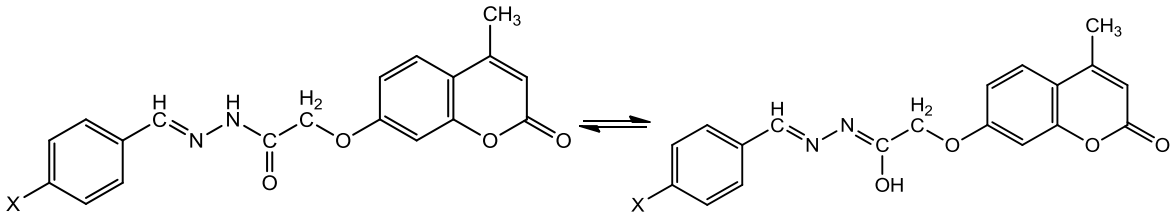
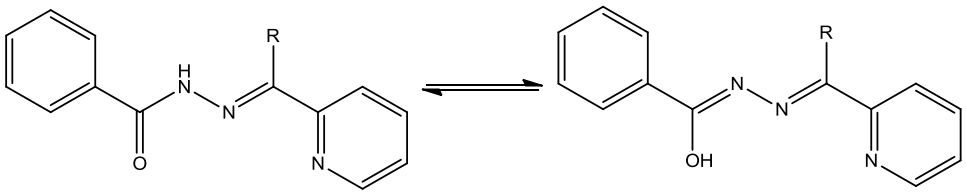


Fig. 6. The amide-iminol tautomerization

The conventional amide-iminol tautomerization is preferred in the absence of methylene protons (Figure 7).



amide form iminol from

Fig. 7. amide-iminol tautomerism of hydrazone molecules

Based on our best knowledge which well-established by H-NMR, the amide-iminol tautomerization occurred in the synthesized hydrazones **(1-3)**. The methylene protons signal of these hydrazones appears in two different chemical shifts, 4.7 and 5.2 ppm with overall integration of 2 protons. The signal at 5.2 ppm belongs to CH_2 protons of amide tautomer and the signal at 4.7 belong to CH_2 protons of iminol tautomer. Tries for separation of the tautomers was unsuccessful. The proportions of amide to iminol form shows the amide form well predominate to iminol form. According to integration of the H-NMR signals, the approximate concentration of amide form is 64% and the iminol form is 36% in the equilibrium mixture.

On the other hand we found that the hydrogens of methylene group in oxadiazoline **(4,5)** have taken part to imine-enamin tautoumerism by the adjacent nitrogen of the oxadiazoline ring (Figure 8). Whereas in the oxadiaxoles **(6-8)** they didn't show any tautoumerism with the adjacent nitrogen because of the aromatic stability of the oxadiazole ring.

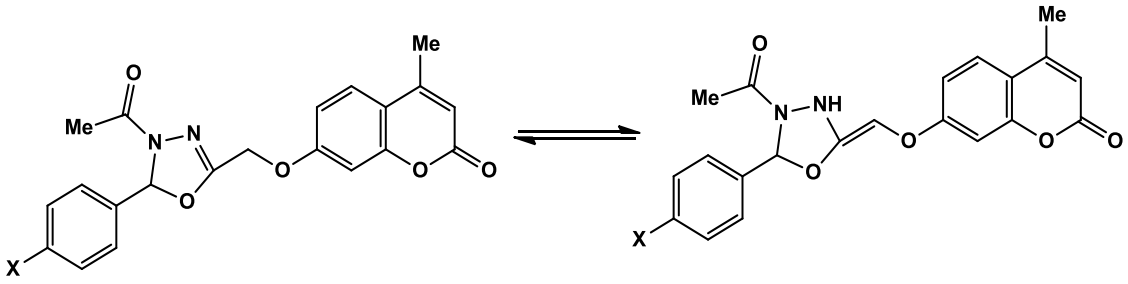


Fig. 8**.** imine-enamine tautomerism of oxadiazolines

Standard methods of Kirby-Bauer were used for assessment of antibacterial activities of each synthesized compound. Briefly, 0.2 mg/ml of each compound was test against Staphylococcus aureus (G+ bacteria) and Escherichia coli (G- bacteria) by well diffusion method. For MIC assessment, 100, 50, 25, 12.5, 6.25, 3.12, and 1.56 µg/ml of each compound were used as broth dilution method [Table 1]. The mean of inhibition zone of 25, 24, 20, 17,15, 12 and 10 mm, and 30. 25, 18, 17, and 15 were observed for S. aureus and E. coli respectively.

Table 1: Antibacterial effect of synthesized materials

By two methods of disk diffusion and welling on E.coli

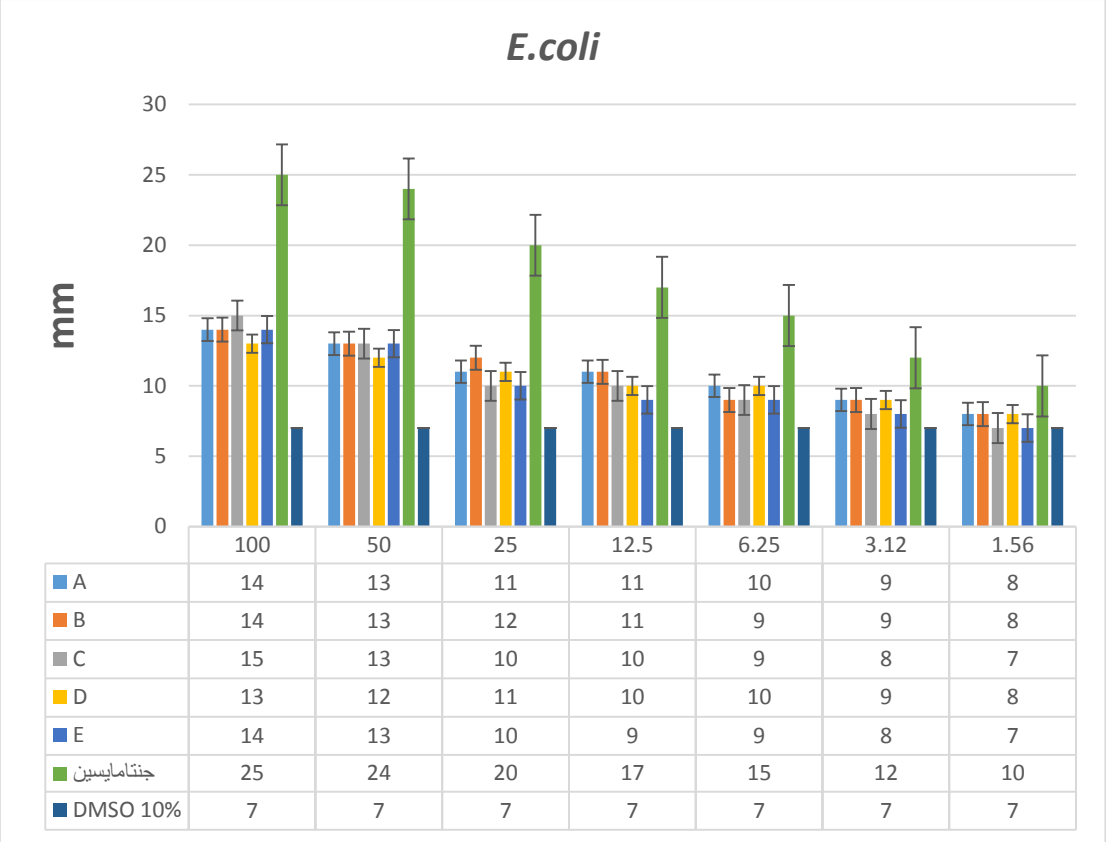
Product No.4: 4-methyl-2-[(coumarin-7-yl-oxymethyl-4acetyl-5-(4-methylphenyl)]-1,3,4-oxadiazolines

Product No.5: 4-methyl-2-[(coumarin-7-yl-oxymethyl-4acetyl-5-(4-methoxyphenyl)]-1,3,4-oxadiazolines

Product No.6: 4-methyl-2-[(coumarin-7-yl-oxymethyl-5-(4methoxyphenyl)]-1,3,4-oxadiazole

Product No.7: 4-methyl-2-[(coumarin-7-yl-oxymethyl-5-(4-methylphenyl)]-1,3,4-oxadiazole

Product No.8: 4-methyl-2-[(coumarin-7-yl-oxymethyl-5-(4-phenyl)]-1,3,4-oxadiazole



	100	50	25	12.5	6.25	3.12	1.56
A	14	13	11	11	10	9	8
B	14	13	12	11	9	9	8
C	15	13	10	10	9	8	7
D	13	12	11	10	10	9	8
E	14	13	10	9	9	8	7
جنتامایسین	25	24	20	17	15	12	10
DMSO 10%	7	7	7	7	7	7	7

Gentamycin and vancomycin were used as standard positive controls alongside of each compounds. The mean of three repeat of each experiment was mentioned as final result. The best antibacterial effect was observed for the eight derivative with a concentration of 100 µg/ml. Six, seven, and five derivatives at similar concentrations showed the same lack of growth. By reducing the concentration of the active substance to half, that is, 50 µg/ml, it shows a decrease in the halo of very low growth of 1 mm. It can be concluded that by reducing the amount of synthesized substance, the bactericidal power of the substance is still at the desired level [Table 2].

Table 2: Antibacterial effect of synthesized materials

By two methods of disk diffusion and welling on S.aureus

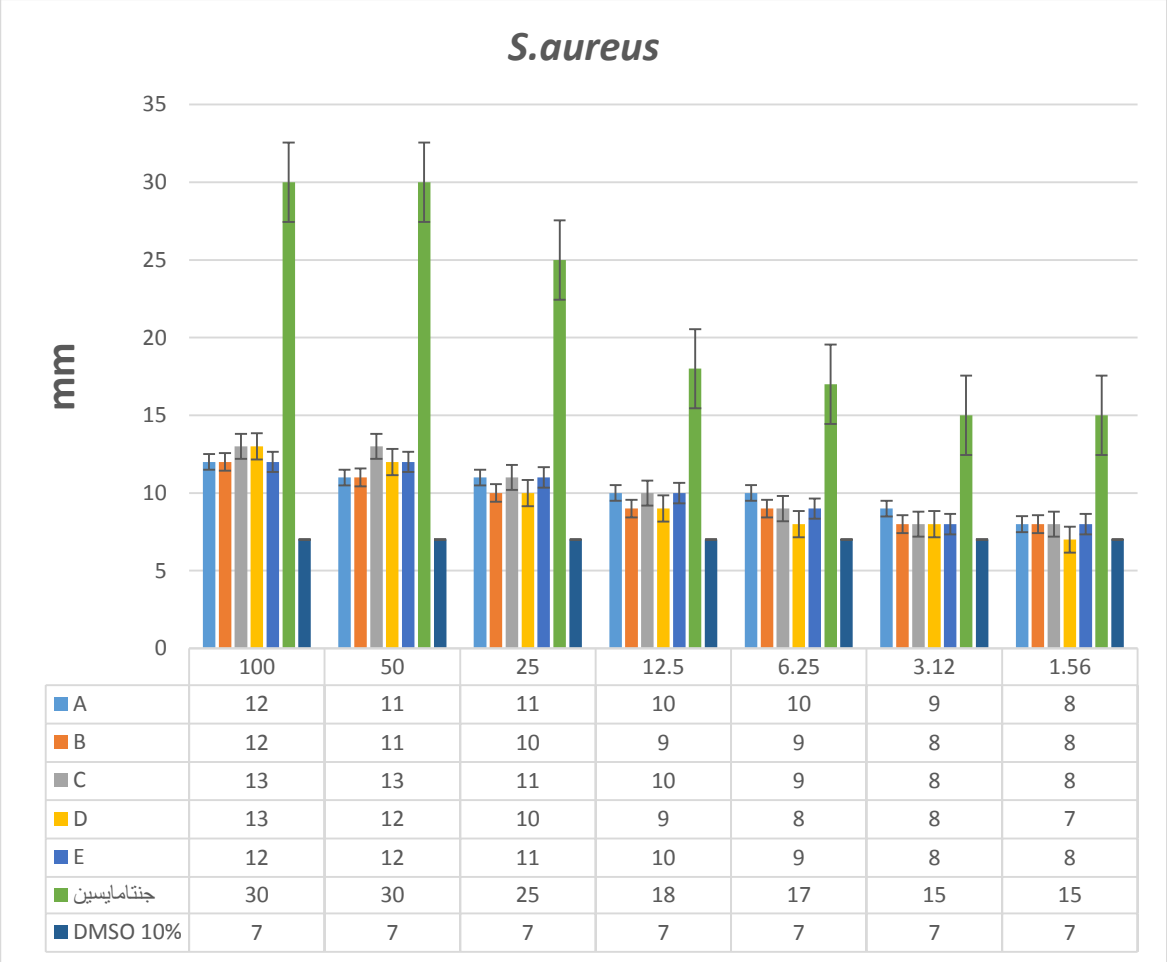
Product No.4: 4-methyl-2-[(coumarin-7-yl-oxymethyl-4-acetyl-5-(4-methylphenyl)]-1,3,4-oxadiazolines

Product No.5: 4-methyl-2-[(coumarin-7-yl-oxymethyl-4-acetyl-5-(4-methoxyphenyl)]-1,3,4-oxadiazolines

Product No.6: 4-methyl-2-[(coumarin-7-yl-oxymethyl-5-(4-methoxyphenyl)]-1,3,4-oxadiazole

Product No.7: 4-methyl-2-[(coumarin-7-yl-oxymethyl-5-(4-methylphenyl)]-1,3,4-oxadiazole

Product No.8: 4-methyl-2-[(coumarin-7-yl-oxymethyl-5-(4-phenyl)]-1,3,4-oxadiazole

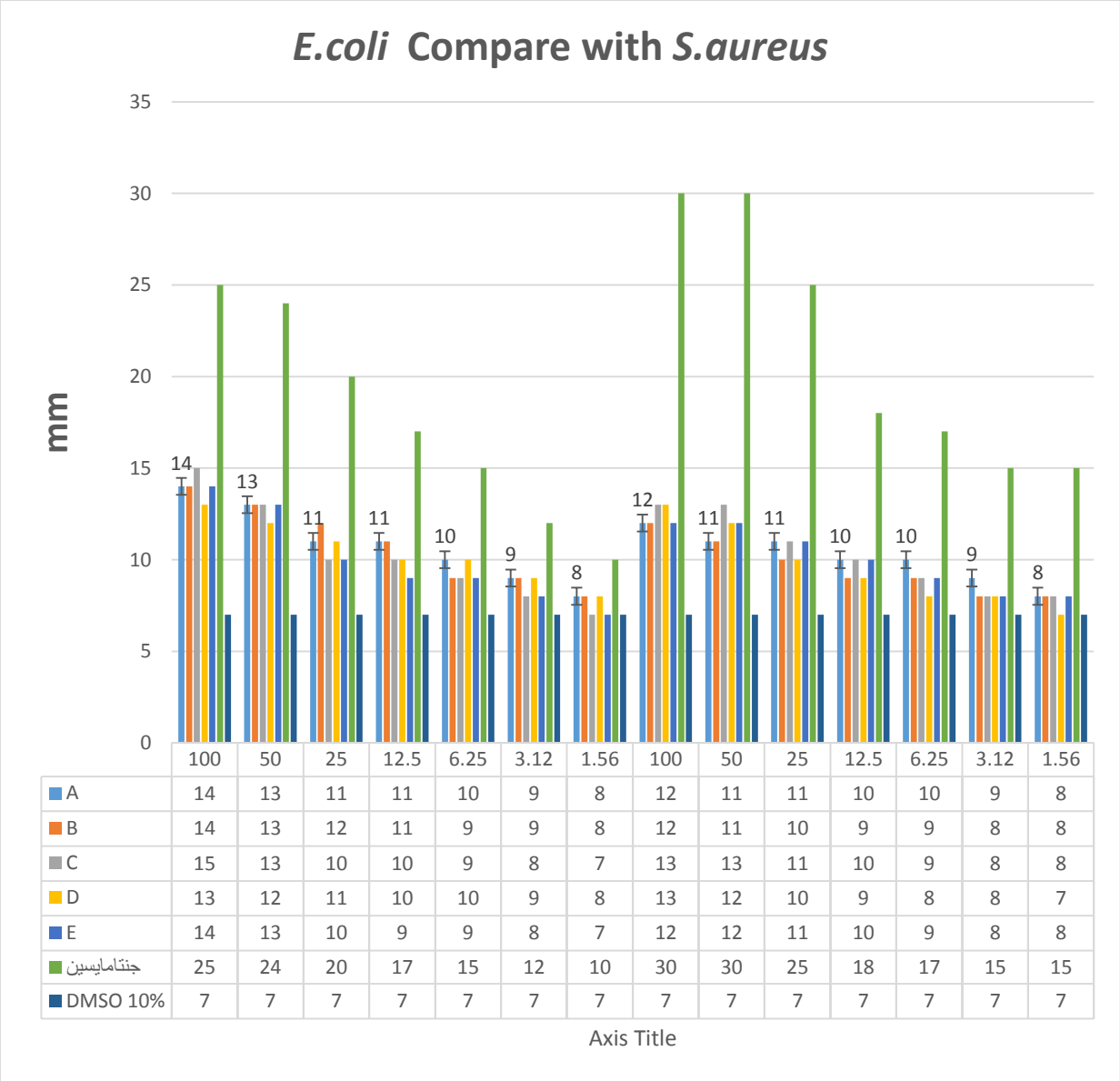


	100	50	25	12.5	6.25	3.12	1.56
A	12	11	11	10	10	9	8
B	12	11	10	9	9	8	8
C	13	13	11	10	9	8	8
D	13	12	10	9	8	8	7
E	12	12	11	10	9	8	8
جنتامایسین	30	30	25	18	17	15	15
DMSO 10%	7	7	7	7	7	7	7

Comparison of antibacterial properties of two bacteria In S. aureus, the largest growth inhibition zone was observed at a concentration of 100/µg/ml and for derivatives 8 and 4. As the concentration of the active ingredient decreased and reached the initial level of 50 µg/ml in S. aureus, we still saw the largest growth inhibition zone in eight derivative. However, at this concentration, the difference in derivative 4 with 1 mm still has the largest aura of lack of growth. Derivative 5, on the other hand, has a growth inhibition zone as the same of 4. Derivatives 6 and 7 are in the end of rank with a difference of 1 mm. For E. coli has the largest growth inhibition zone related to derivative 8 and with the same concentration of 100 µg/ml. At this concentration, derivatives 6, 7, and 5 have the

largest growth inhibition zone and are standing after 8, but by reducing the concentration of all derivatives, they show the same growth inhibition zone. Only the derivative 4 is in the end position with a difference of 1 mm [Table 3].

Table 3: Escherichia coli compare with Staphylococcus



	100	50	25	12.5	6.25	3.12	1.56	100	50	25	12.5	6.25	3.12	1.56
A	14	13	11	11	10	9	8	12	11	11	10	10	9	8
B	14	13	12	11	9	9	8	12	11	10	9	9	8	8
C	15	13	10	10	9	8	7	13	13	11	10	9	8	8
D	13	12	11	10	10	9	8	13	12	10	9	8	8	7
E	14	13	10	9	9	8	7	12	12	11	10	9	8	8
جنتامایسین	25	24	20	17	15	12	10	30	30	25	18	17	15	15
DMSO 10%	7	7	7	7	7	7	7	7	7	7	7	7	7	7

By looking at and comparing the growth inhibition halos created in the two bacteria, it can be concluded that the synthesized materials have antibacterial effect on Gramnegative bacteria with

respect to Gram-positive bacteria. Due to the fact that the cell membrane of Gram-negative bacteria has a complex and multi-layered structure. Their inner membrane, called the cytoplasmic membrane, is covered with a flat sheet of peptidoglycan and the outer membrane is attached to it and the bacteria Gram-positives usually do not have an outer membrane in their cell wall. Gram-positive bacteria have a relatively simple cell membrane consisting of 2–3 layers. Better treatment of the synthesized material on Gram-negative bacteria is an important fact. However, by comparing all the synthesized materials, it can be concluded that the active ingredient of the synthesized derivative 8 has the best effect on both Gram-negative and Gram-positive bacteria.

4. conclusion

In this study, different methods have been tried for the synthesis of novel, 4-methylcoumarin-7-yl-oxymethyl-arylaldehyde acetohydrazone **(1-3)**. By converting these acetohydrazones to the novel 4-methyl-2-(coumarin-7-oxymethyl)-4-acetyl-5-aryl-1,3, 4-oxadiazolines **(4,5)** and 4-methyl-2-(coumarin-7-oxy methyl)-5-aryl-1,3,4-oxadiazole **(6-8)** we found that the hydrogens of methylene group in oxadiazoline **(4,5)** have taken part to imine-enamin tautoumerism by the adjacent nitrogen of the oxadiazoline ring whereas in the oxadiaxoles **(6-8)** they didn't show any tautoumerism with the adjacent nitrogen because of the aromatic stability of the oxadiazole ring. All the synthesized oxadiazoles and oxadiazolines have antibacterial effect on Gram-negative bacteria with respect to Gram-positive bacteria. Due to the fact that the cell membrane of Gram-negative bacteria has a complex and multi-layered structure, their inner membrane, called the cytoplasmic membrane, is covered with a flat sheet of peptidoglycan and the outer membrane is attached to it and the bacteria Gram-positives usually do not have an outer membrane in their cell wall. Better treatment of the synthesized material on Gramnegative bacteria is an important fact. However, by comparing all the synthesized materials, it can be concluded that the active ingredient of the synthesized derivative (8) has the best effect on both Gram-negative and Gram-positive bacteria.

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