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Synthesis, Characterization, and Antibacterial Evaluation of New Vanillic Acid Derivatives

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Abstract

Hydrazide Schiff bases (hydrazones) and 2,5-disubstituted-1,3,4-oxadiazole derivatives exhibit diverse biological activities that include antibacterial, antifungal, antitubercular, antiviral, anticancer, anti-inflammatory, and analgesia; so that new derivatives, **compounds (5-8)** of vanillic acid based on 1,3,4-oxadiazole as scaffold unit were synthesized through multi-steps, and characterized by thin layer chromatography and spectroscopically by Fourier-transform Infrared (FTIR) and Proton nuclear magnetic resonance (^{1}HNMR). **Compounds (5-8)** were evaluated for their antibacterial activity by the disk diffusion method. **Compounds (5-8)** were showed moderate and comparable to the activities of amoxicillin and isoniazid against ***Escherichia coli*** (***E. coli***), but less than that of cefixime and nitrofurantoin which their activities were high. **Compound (6)** and **(7)** had shown moderate and comparable to the activities of amoxicillin and cefixime against ***Klebsiella pneumoniae*** (***K. pneumoniae***), but less than that of nitrofurantoin which its activity was high. **Compound (6)** and **(7)** had shown moderate and comparable to the activity of amoxicillin against ***Staphylococcus aureus*** (***S. aureus***), while the activities of cefixime and nitrofurantoin were high. **Compound (6)** was moderately active against ***Bacillus subtilis*** (***B. subtilis***), while the activities of amoxicillin, cefixime, and nitrofurantoin were high.

Keywords: Hydrazide, Schiff Base, Oxadiazole, Antibacterial.

تصنيع ، تشخيص ، وتقييم الفعالية المضادة للبكتريا لمشتقات جديدة لحامض الفانلين

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الخلاصة

قواعد شف و الحلقة الأروماتية الغير متجانسة ١،٣،٤-اوكسادايزول المعوضة في الموقعين ٢و٥ لهما العديد من الفعاليات الحيوية كأن تكون مضادات للبكتيريا والفطريات وبكتريا التدرن والفايروسات والخلايا السرطانية أو أن تكون مضادات للألتهابات أو تعمل كمسكنات للألم. لذلك تم تصنيع مشتقات جديدة لحامض الفانلين **المركبات (٥-٨)** بالأعتماد على الحلقة الأروماتية الغير متجانسة ١،٣،٤-اوكسادايزول كوحدة بناء رئيسية بعدة خطوات وهذه المركبات تم تشخيصها عن طريق الكروماتوغرافيا (استشراب الطبقة الرقيقة) و عن طريق مطياف الأشعة تحت الحمراء, الرنين النووي المغناطيسي للبروتون. **المركبات (٥-٨)** تم تقييم فعالياتها المضادة للبكتريا باستخدام طريقة الانتشار. **المركبات (٥-٨)** أظهرت فعالية متوسطة يمكن مقارنتها بفعالية الاموكسسلين والايزونيازايد ضد **البكتريا الاشريكية القولونية** ولكنها كانت أقل من فعالية السفيكزيم والنيتروفيورانتوين حيث كانت فعاليتهما عالية. **المركبان (٦)** و**(٧)** أظهرا فعالية متوسطة يمكن مقارنتها بفعالية الاموكسسلين والسفيكزيم ضد **البكتريا الكلبسيلة الرئوية** ولكنها كانت أقل من فعالية النيتروفيورانتوين حيث كانت له فعالية عالية. **المركبان (٦)** و**(٧)** أظهرا فعالية متوسطة يمكن مقارنتها بفعالية الاموكسسلين ضد **البكتيريا الكروية العنقودية الذهبية** بينما السفيكزيم والنيتروفيورانتوين كانت لهما فعالية عالية. **المركب (٦)** كانت له فعالية متوسطة ضد **البكتريا العصوية الرقيقة** بينما فعالية الاموكسسلين والسفيكزيم والنيتروفيورانتوين كانت فعالية عالية.

الكلمات المفتاحية: هيدرازايد, قواعد شف, اوكسادايزول, مضادات بكتيرية.

Introduction

Heterocyclic compounds are used in many biological fields, due to their different activities, and are considered as one of the principal classes of organic compounds, that are used in the development of several pharmaceutically essential compounds [(1,2)] .

Oxadiazoles are important five-membered aromatic heterocyclic containing oxygen and two nitrogens in their structure. Because the oxadiazole ring is structurally rigid, various functional groups are easily introduced into the ring. Valuable biological activities are associated with oxadiazole derivatives [(3)], such as antitumor [(4)], anti-inflammatory [(5)], antimicrobial[(6)], antifungal[(7)], and anticonvulsant [(8)] . In synthetic medicinal chemistry, to improve the biological activity of new drugs with respect to the corresponding lead compounds, hybridization-*combination of different pharmacophores in one structure*-is one of the techniques being followed.[(9,10)] Hydrazide Schiff base derivatives (hydrazones) are good scaffolds for various pharmaceutical applications, and characterized by the presence of highly reactive azomethine group (–CO–NH–N=CH–).[(11)] Their biological activities include antibacterial[(12)], antifungal[(13)], antitubercular[(14)], antiviral[(15)], anticancer[(16)], anti-inflammatory[(17)], and analgesia.[(18)]

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Material and Methods

Chemicals supplied by hyper-chem (China) were used. Aluminum sheets pre-coated with Silica gel GF_{254} (type 60), exposed to UV-254 nm, were used for thin-layer chromatography (TLC) to monitor the completion of reactions and to test the purity of compounds. Two solvent systems **(S_1** and **S_2)** were used: **S_1**(toluene:ethylacetate:ethanol (**3:2:1)**) and **S_2**(ethylacetate:methanol:ammonia (**5:3.5:1.5**)). Melting points were incorrected and detected by using Stuart SMP3 melting point apparatus in open capillary tubes. All synthesized derivatives were characterized by spectroscopic analysis (Fourier-transform Infrared (FTIR) which was performed at Baghdad university-college of pharmacy) and (Proton nuclear magnetic resonance (^{1}HNMR) which was performed by Chemistry Analysis Center (CAC)) at Iraq/ Baghdad [19-22] .

Chemical synthesis

The target compounds were synthesized by multistep(s) reactions as shown in the scheme in Figure (1).

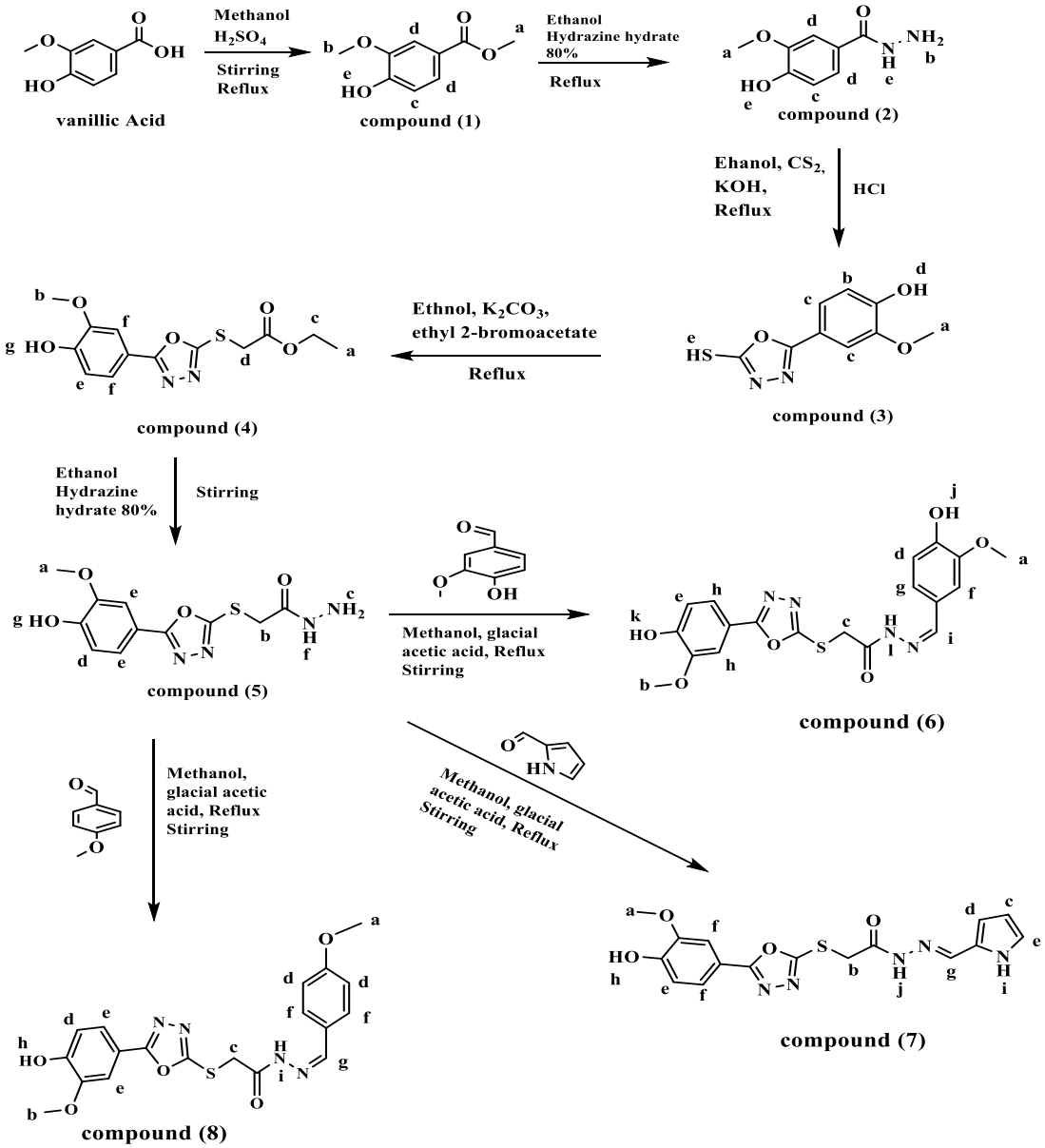


Figure 1. Multistep(s) scheme for synthesis of targeted compounds.

Synthesis of Methyl 4-hydroxy-3-methoxybenzoate; compound (1)[23]:

Vanillic acid (7.0g, 42mmole) was dissolved in 75ml of absolute methanol (99.8%), the temperature of this solution was lowered to 0˚C by ethanol-water ice bath, 5ml of concentrated H_2SO_4 was added dropwise, and the mixture was stirred at room temperature for 48 hours, then refluxed for 7 hours. The reaction mixture was poured in beaker containing crashed ice (100ml), the formed precipitate was filtered, collected, and washed with 5% $NaHCO_3$ aqueous solution, filtered again, and dried by a warmed current of air, giving 7.0g of **compound (1)**.

Synthesis of 4-Hydroxy-3-methoxybenzohydrazide ; compound (2)[24]:

Compound (1), (5.0g, 27mmole) was dissolved in a minimum amount (10ml) of absolute ethanol (99.9%), (13.5g, 270mmole) of 80% hydrazine hydrate was added gradually. The mixture was refluxed for 3-4 hours (monitored by TLC). Then the reaction mixture was cooled and precipitate begin to appear, which was filtered and dried in oven (adjusted at 60˚C), giving 3.5g of **compound (2)**.

Synthesis of 4-(5-Mercapto-1,3,4-oxadiazol-2-yl)-2-methoxyphenol; compound (3)[25]:

To suspension of (1.25g, 6.86mmole) of compound **(2)** in 30ml of 50% aqueous ethanolic solution, (0.785g, 10.3mmole) of CS_2 was added with stirring for few minutes, followed by the addition of (0.7g, 10.3mmole) potassium hydroxide (KOH), and the mixture was refluxed for 12 hours (monitored by TLC, and notation the evolution of H_2S gas by strip soaked with lead acetate aqueous solution; which was turned black as indication of evolution of H_2S gas). The mixture poured in a beaker containing crashed ice (30 ml) and then concentrated HCl was added dropwise until pH became (2-3). The formed precipitate was filtered, dried, and purified by base-acid precipitation method (i.e.; the product was dissolved in water by the aid of equimilimole of sodium hydroxide or triethylamine, filtered, and the clear filtrate was treated with concentrated HCl which was added dropwise until the precipitate formed again), giving ٠.9g of **compound (3)**.

Synthesis of Ethyl 2-((5-(4-hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetate; compound (4)[26]:

Compound (3), (0.5g, 2.23mmole,) was dissolved in 2ml of absolute ethanol (99.9%), (0.155g, 1.115mmole) of anhydrous K_2CO_3 was added with stirring, after few minutes a white precipitate was formed, (0.37241g, 2.23mmole) of ethyl 2-bromoacetate was added dropwise. The reaction mixture refluxed for 2 hours (monitored by TLC) and left for stirring gently overnight. Then distilled water (D.W) was added gradually until precipitate was formed; then filtered, dried and crystallized from minimum amount (the amount that was just covered the powder) of boiled absolute ethanol giving 0.5g of pure product; **compound (4)**.

Synthesis of 2-((5-(4-Hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl) thio) acetohydrazide; compound (5)[27]:

Compound (4), (0.5g, 1.6167mmole) was dissolved in a 8ml of hot absolute ethanol (99.9%), (0.16167g, 3.233mmole) of 80% hydrazine hydrate was then added, after 2 hours of vigorous mixing at room temperature, precipitate was formed, and left to stir gently overnight. Then, washed with (10ml) absolute ethanol, filtered, dried, and used without further purification.

Synthesis of N'-(4-hydroxy-3-methoxybenzylidene)-2-((5-(4-hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl) thio)acetohydrazide; compound (6), *N'*-((1H-pyrrol-2-yl)methylene)-2-((5-(4-hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)acetohydrazide; compound (7), and 2-((5-(4-hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)thio)-N'-(4-methoxybenzylidene)acetohydrazide; compound (8)[28]:

An appropriate aldehydes; 4-hydroxy-3-methoxybenzaldehyde **(Vanillin)** (0.039g, 0.257mmole) for **compound (6)**, 1H-pyrrole-2-carbaldehyde (0.0257g, 0.257mmole) for **compound (7)**, and 4-methoxybenzaldehyde **(p-Anisaldehyde)** (0.035g, 0.257mmole) for **compound (8)**, were dissolved in 5ml of absolute methanol (99.8%) and stirred for 15-30 minutes in the presence of 2-3 drops of glacial acetic acid; then (0.075g, 0.25mmole) of **compound (5)** was added and the suspension that formed had been refluxed for 1-2 hours. The reaction mixture was left to stir overnight, then the formed precipitate was filtered, dried and crystallized from boiled methanol.

Antibacterial essay

Well diffusion assay was carried out through using bacterial suspension of nearly (1.5×10^CFU/ml) obtained from McFarland turbidity standard (number 0.5). This was used to inoculate by swabbing the surface of Mueller Hinton Agar (MHA) plates. The excess liquid was dried by air under a sterile hood. In each agar plate of examined bacteria, four wells were made, and (80µl) of each concentration of the synthesized compound was poured to it. The plates were incubated at 37°C for 24 hours. The evaluation of antibacterial activity was based on the measurement of the diameter of the inhibition zone formed around the well. [29]

Results

Chemistry

Compound (1) ($C_9H_{10}O_4$): off-white powder, yield 91%, m.p: 60-62˚C (reported m.p: 64˚C)[30]. **The characteristic bands in FTIR spectrum in cm^{-1}**: (1686) C=O stretching vibration band, (1277) C-O stretching vibration band of aromatic ester.
^{1}HNMR (500MHz, DMSO-d_6) in ppm: 3.80, 3H, s, set (a) protons; 3.83, 3H, s, set (b) protons; 6.87-6.89, 1H, d, set (c) proton; 7.44-7.49, 2H, m, set (d) protons; 9.97, 1H, s, set (e) proton.

Compound (2) ($C_8H_{10}N_2O_3$): white powder, yield 70%, m.p: 208-210˚C (reported m.p: 210-211˚C)[31]. **The characteristic bands in FTIR spectrum in cm^{-1}**: (3310) phenolic OH stretching vibration band overlapped with NH_2 asymmetric stretching vibration band, (3256) NH amide stretching

vibration band, (3209) NH_2 symmetric stretching vibration band, (1628) stretching vibration band of carbonyl amide, (1601) NH bending vibration band of hydrazide amine, (1585) NH bending vibration band of hydrazide amide.
^{1}HNMR(400MHz, DMSO-d_6) in ppm: 3.82, 3H, s, set (a) protons; 4.42, 2H, s, set (b) protons; 6.81-6.83, 1H, d, set (c) proton; 7.33-7.44, 2H, m , set (d) protons; 9.56, 2H, s, set (e) protons.

Compound (3) ($C_9H_8N_2O_3S$): faint yellow powder, yield 59%, M.P: 186-189˚C. **The characteristic bands in FTIR spectrum in cm^{-1}**: (3452) phenolic OH stretching vibration band, (3155) NH broad (br) stretching vibration band, 2669 weak (w) SH stretching vibration band.
^{1}HNMR(400MHz, DMSO-d_6) in ppm: 3.84, 3H, s, set (a) protons; 6.92-6.94, 1H, d, set (b) protons; 7.30-7.37, 2H, m, set (c) protons; 10.04, 1H, s, set (d) proton; 14.58, 1H, s, set (e) proton.

Compound (4) ($C_{13}H_{14}N_2O_5S$): white crystalline powder, yield 65.5%, m.p: 118-120˚C. **The characteristic bands in FTIR spectrum in cm^{-1}**: (3400-2800) broad phenolic OH stretching vibration band, (1744) carbonyl stretching vibration band of aliphatic saturated ester, (1173) stretching vibration band of C-O of aliphatic saturated ester.
^{1}HNMR(500MHz, DMSO-d_6) in ppm: 1.18-1.21, 3H, t, set (a) protons; 3.86, 3H, s, set (b) protons; 4.14-4.18, 2H, q, set (c) protons; 4.26, 2H, s, set (d) protons; 6.95-6.96, 1H, d, set (e) proton; 7.41-7.43, 2H, m, set (f) protons; 9.97, 1H, s, set (g) proton.

Compound (5) ($C_{11}H_{12}N_4O_4S$): white powder, yield 65%, m.p: 196-199˚C. **The characteristic bands in FTIR spectrum in cm^{-1}**: (3506) stretching vibration band of phenolic OH, (3341) asymmetric NH_2 stretching vibration band, (3256) NH amide stretching vibration band, (3217) symmetric NH_2 stretching vibration band, (1682) C=O carbonyl amide stretching vibration band, (1655) NH_2 bending vibration band.
^{1}HNMR(500MHz, DMSO-d_6) in ppm: 3.86, 3H, s, set (a) protons; 4.00, 2H, s, set (b) protons; 4.36, 2H, s, set (c) protons; 6.93-6.95, 1H, d, set (d) proton; 7.41-7.44, 2H, m, set (e) protons; 9.41, 1H, s, set (f) proton; 9.93, 1H, s, set (g) proton.

Compound (6) ($C_{19}H_{18}N_4O_6S$): white powder, yield 40%, m.p: 196-200˚C. **The characteristic bands in FTIR spectrum in cm^{-1}:** (3449) two Phenolic OH_s stretching vibration bands, (3182) NH secondary amide stretching vibration band, (1678) C=O amide carbonyl stretching vibration band, (1601) C=N imine stretching vibration, C=N stretching vibration of oxadiazole and C=C aromatic skeletal stretching vibration (overlapped) bands.
^{1}HNMR(500MHz, DMSO-d_6) in ppm: 3.81, 3.80, 3H, 2s , set (a) protons [syn/anti-syn]; 3.83, 3H, s, set (b) protons; 4.16, 4.61, 2H, 2s, set (c) protons [syn/anti-syn]; 6.80-6.83, 1H, m, set (d) proton; 6.90-6.93, 1H, m, set (e) proton; 7.07-7.09, 1H, m, set (f) proton; 7.26-7.27, 1H, m , set (g) proton; 7.39-7.42, 2H, m, set (h) protons; 7.91, 8.08, 1H, 2s, set (i) proton [syn/anti-syn]; 9.52, 1H, s, set (j) proton; 9.94, 1H, s, set (k) proton; 11.60, 11.63, 1H, 2s, set (l) proton [cis/trans].

Compound (7) ($C_{16}H_{15}N_5O_4S$): white powder, yield 53.4%, m.p: 226-228˚C. **The characteristic bands in FTIR spectrum in cm^{-1}**: (3302) phenolic OH and NH pyrrole stretching vibration (overlapped) bands, (3209) NH amide stretching vibration band, (1663) amide carbonyl stretching vibration band, (1620) NH pyrrole bending vibration and imine stretching vibration (overlapped) bands.
^{1}HNMR(500MHz, DMSO-d_6) in ppm: 3.83, 3H, s, set (a) protons; 4.17, 4.60, 2H, 2s, set (b) protons [syn/anti-syn]; 6.11-6.14, 1H, m, set (c) proton; 6.44-6.49, 1H, m, set (d) proton; 6.90-6.95, 2H, m, set (e) protons; 7.40-7.43, 2H, m, set (f) protons; 7.86, 8.04, 1H, 2s, set (g) proton [syn/anti-syn]; 9.94, 1H, s, set (h) proton; 11.40, 1H, s, set (i) proton; 11.45, 11.48, 1H, 2s, set (j) proton [cis/trans].

Compound (8) ($C_{19}H_{18}N_4O_5S$): White powder**,** yield 72%, m.p: 190-193˚C. **The characteristic bands in FTIR spectrum in cm^{-1}**: (3495) Phenolic OH stretching vibration band, (3182) NH secondary amide stretching vibration band, (1666) C=O amide carbonyl stretching vibration band, (1609) C=N imine stretching vibration, C=N stretching vibration of oxadiazole and C=C aromatic skeletal vibration (overlapped) bands.
^{1}HNMR(500MHz, DMSO-d_6) in ppm: 3.79, 3.80, 3H, 2s , set (a) protons [syn/anti-syn]; 3.83, 3H, s, set (b) protons; 4.18, 4.59, 2H, 2s, set (c) protons [syn/anti-syn]; 6.90-7.01, 3H, m, set (d) protons; 7.39-7.42, 2H, m, set (e) protons; 7.61-7.65, 2H, m, set (f) protons; 7.97, 8.14, 1H, 2s, set (g) proton [syn/anti-syn]; 9.94, 1H, s, set (h) proton; 11.64, 11.69, 1H, 2s, set (i) proton [cis/trans].

Anti-bacterial evaluation

The antibacterial activities of the synthesized compounds; **compounds (5-8)** were evaluated against six bacteria and compared with four standard antibiotics; amoxicillin, cefixime, nitrofurantoin, and isoniazid. Dimethyl sulfoxide (DMSO) was used as solvent and control. It's evident from the data displayed in the table **(1)**, **compound (5)** showed moderate antibacterial activity against ***Escherichia coli*** (***E. coli***)**. Compound (6)** showed moderate activity towards ***Staphylococcus aureus*** (***S. aureus***), ***Bacillus subtilis*** (***B. subtilis***)**,** ***Escherichia coli*** (***E. coli***)**,** and ***Klebsiella pneumoniae*** (***K. pneumoniae***). **Compound (7)** showed moderate activity towards ***Staphylococcus aureus*** (***S. aureus***), ***Escherichia coli*** (***E. coli***)**,** and ***Klebsiella pneumoniae*** (***K. pneumoniae***)**. Compound (8)** was slightly active against ***Staphylococcus aureus*** (***S. aureus***) and moderately active against ***Escherichia coli*** (***E. coli***).

No one of the synthesized compounds had shown activity against **Streptococcus *pyogenes*** (***S. pyogenes***) and ***Pseudomonas aeruginosa*** (***P. aeruginosa***).

Table 1.The antibacterial activities of synthesized compounds.

Comp. no.	Conc. µg/ml	Gram (+)ve			Gram (-)ve		
		S. aureus	***S. pyogenes***	***B. subtilis***	***E. coli***	***K. pneumoniae***	***P. aeruginosa***
		Zone of inhibition(ZI) in (mm)					
Comp.5	10^3	-	-	-	12	-	-
Comp.6	10^3	11	-	13	11	11	-
Comp.7	10^3	10	-	-	11	10.5	-
Comp.8	10^3	9.5	-	-	11	-	-
Amoxicillin	10^3	13	5	40	11	11.5	28
Cefixime	10^3	16	6	20	22	13	6
Nitrofurantoin	10^3	21	9.5	31	16	20	17
Isoniazid	10^3	8	-	-	12	-	-
DMSO	**Solvent control**	-	-	-	-	-	-

(-)= No activity, slightly active (ZI =5-10 mm), moderately active (ZI= 10-15 mm), highly active (ZI= more than 15 mm).[32,33]

Discussion

Chemistry

Compound (1) was esterification product which resulted from reaction between carboxylic acid and an alcohol in the presence of an acid as catalyst. Two stages were involved: **addition** of a *nucleophile* followed by **elimination** of a *leaving group*. Protonation and deprotonation steps also occur during the ester formation which could explain the role of acid in the reaction. Under basic conditions, carboxylate anion will be formed which does not react with an electron-rich nucleophile, so the esterification will be happened in the presence of an acid. Formation of ester is necessary for the success of **step 2** and **5** as explained later.[34].

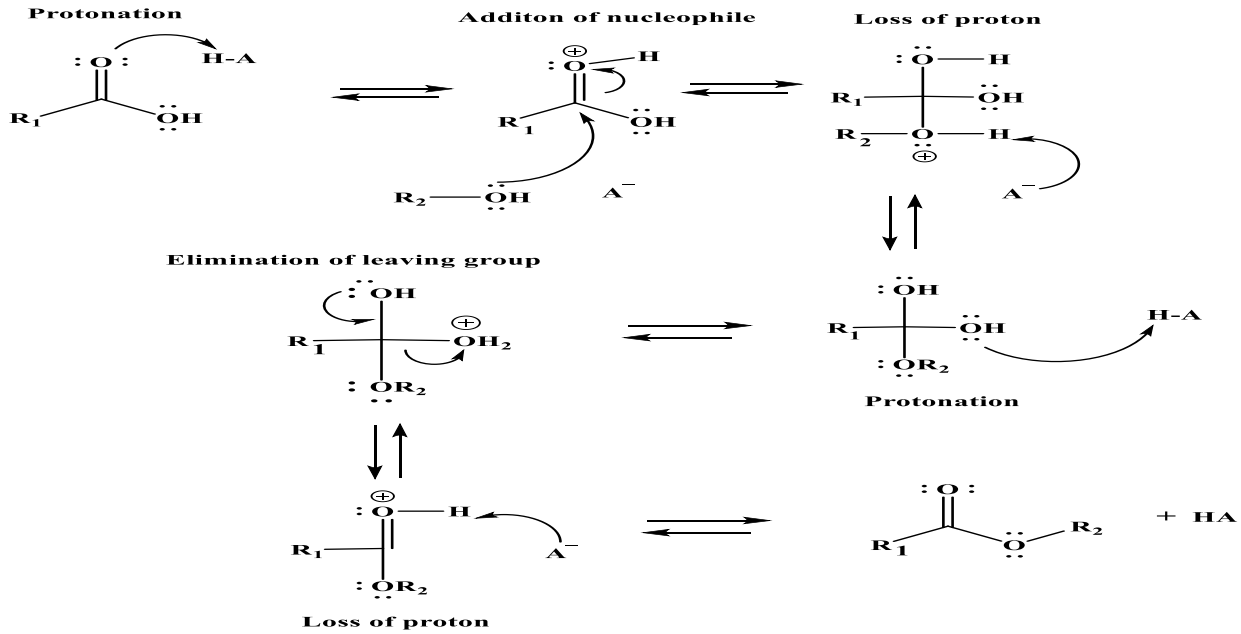


Figure 2. Steps of esterification.[34]

Compound (1) was characterized by carbonyl group of aromatic ester at 1686cm^{-1} in its **FTIR** spectrum and **^{1}HNMR** signals confirmed the presence of COOC$\underline{H}_3$ at 3.80 ppm.

Synthesis of **Compound (2) and (5)** is essentially a base catalyzed hydrolysis (hydrazinolysis of ester) which was run under

normal basic condition in which the rate-determining step involves two molecules of hydrazine in which a proton was being transferred between them. In the next step, one hydrazine molecule will be left slowly with one molecule of alcohol [35].

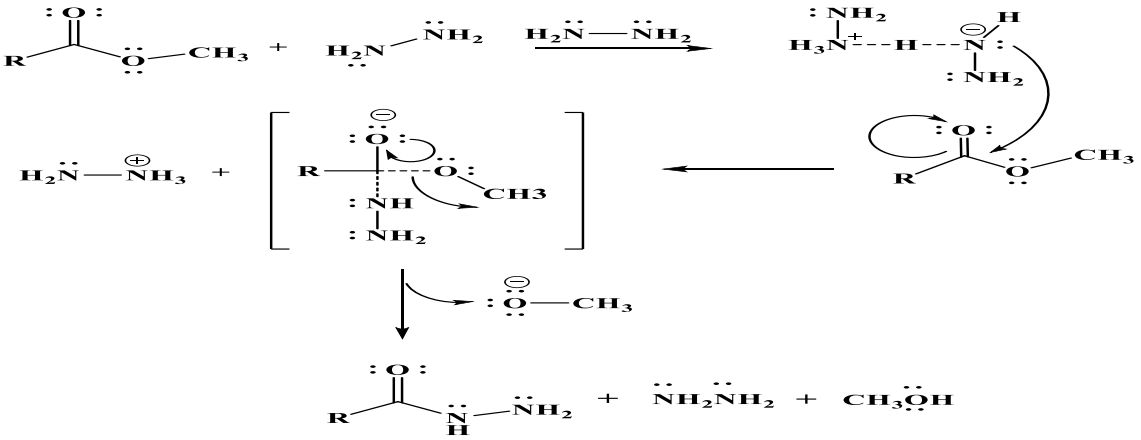


Figure 3. Hydrazinolysis of ester [35]**.**

In case of **compound (2)**; **FTIR** spectrum was characterized by two stretching vibration bands for the primary amine of hydrazide, at 3310 cm^{-1} and 3209 cm^{-1}, respectively, 3256 cm^{-1} NH amide stretching vibration band, 1628 cm^{-1}C=O stretching vibration band of amide, and 1601$cm^{-1}$$NH_2$ bending vibration band. **^{1}HNMR** signals confirmed the presence of CONH at 9.56 ppm, and NH_2 at 4.42 ppm.

For **compound (5)**; **FTIR** spectrum was characterized by asymmetric and symmetric stretching vibration bands of NH_2 at (3341 and 3217) cm^{-1}, respectively, 3256 cm^{-1} NH amide stretching vibration band, the amide carbonyl stretching vibration band at 1682 cm^{-1}, and NH_2 bending stretching vibration band at 1655 cm^{-1}. **^{1}HNMR** was revealed the presence of new signals at 9.41 ppm and 4.36 ppm related to CONH and $CONHNH_2$**,** respectively.

Synthesis of **compound (3)** had been carried out by refluxing an ethanolic suspension of **compound (2)** with CS_2, in the presence of (KOH) and the cyclization which involved formation of potassium salt of dithiocarbamate as an intermediate could be done through keto form of hydrazide carbonyl or enol form. Enol form is the preferred explanation because of the stability of enol form by intramolecular hydrogen bonding.[36]

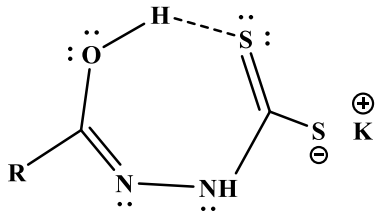
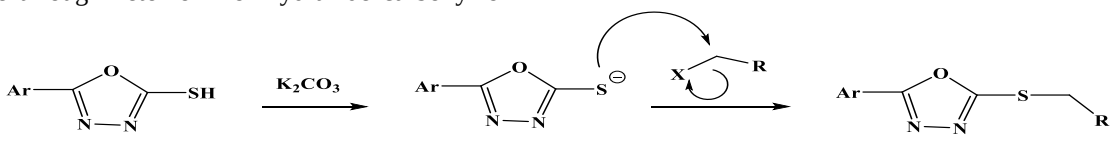


Figure 4. enol form of dithiocarbamate stabilized by intramolecular hydrogen bonding.[36]

FTIR spectrum of **compound (3)** was characterized by NH stretching vibration band at 3155cm^{-1}, weak SH stretching vibration band at 2669cm^{-1}, and in addition to the absence of amide band at 1628 cm^{-1}. **^{1}HNMR** was characterized by the appearance of new signal related to SH proton at 14.58 ppm.

Compound (4) was obtained by a nucleophilic substitution (SN^2) reaction between **compound (3)** and ethyl 2-bromoacetate in absolute ethanol and in the presence of anhydrous potassium carbonate as a catalyst.



X= Br
R= COO-CH_2CH_3

Figure 5. synthesis of compound (4) by nucleophilic substitution (SN^2) reaction [37]**.**

FTIR spectrum was characterized by 1744cm^{-1} stretching vibration band of saturated ester carbonyl. **^{1}HNMR** was characterized by the presence of new signals at 4.14-4.18(2H, q, $COCH_2CH_3$), 1.18-1.21(3H, t, $COCH_2CH_3$), and the absence of SH signal at 14.58 and instead of the appearance of new signal at 4.26 ppm which was related to –SCH_2.

Compounds (6-8) were Schiff base products (imines) which resulted from reaction between aldehydes with a primary amines in mildly acidic conditions and involves six steps; the first three steps produce an intermediate called a carbinolamine and the last three steps convert the carbinolamine into an imine [38].

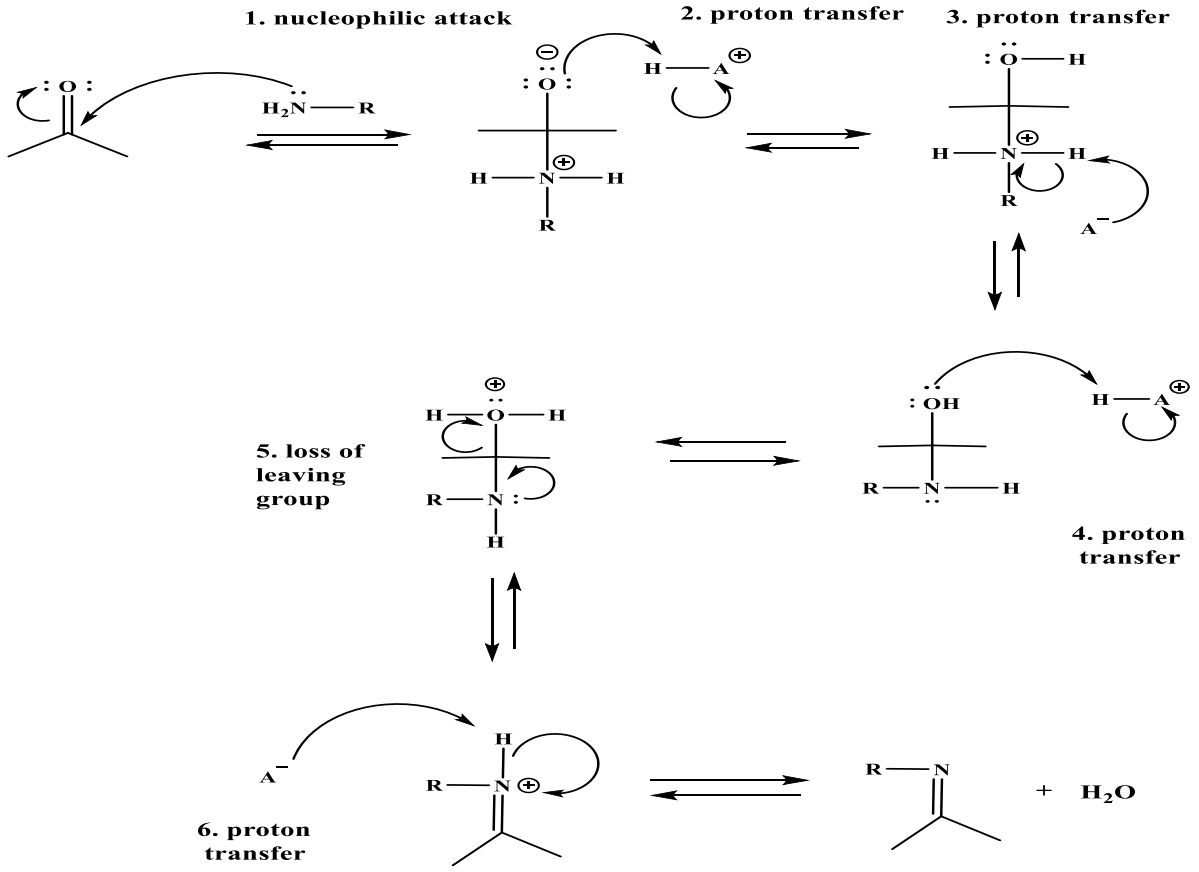


Figure 6. steps of Schiff base [38]**.**

FTIR spectra were characterized by absence of hydrazide NH_2 asymmetric and symmetric stretching vibration bands; while N=CH imine stretching vibration bands were overlapped with other bands in **FTIR** spectra. **^{1}HNMR** were characterized by the appearance of new signals related to CONHN which were due to ***cis*** and ***trans*** isomers showed ***2signals*** between [11.60,11.63 for **compound (6)**, 11.45,11.48 for **compound (7)**, 11.64,11.69 for **comp.8]**, and CONHN=CH which due to ***syn/anti-syn*** conformers showed ***2signals*** between [7.91, 8.08 for **compound (6)**, 7.86, 8.04 for **compound (7)**, 7.97, 8.14 for **compound (8)**].[39-42]

Antibacterial activities

Four antibacterial standards were used, amoxicillin to compare ***anti-gram (+)ve*** activities of the derivatives with it; cefixime to compare ***anti-gram(-)ve*** activities of the derivatives with it; nitrofurantoin because it is considered hydrazone and contains furan ring which is isoseter with oxadiazole ring, and isoniazid which is hydrazide compound resembles to **compound (5)**. Because **compound (6)** and **compound (7)** are more polar than **compound (8)**, they showed additional activities against ***K. pneumoniae*** [as polarity increased; there will be extended activity against ***gram(-)ve*** bacteria, while retained activities against ***gram(+)ve*** bacteria as in the case of penicillin G and aminopenicillins].[43] Because **Compound (5)** is hydrazide; it is expected to show limited activity against test bacteria and showed agreement with isoniazid.

Conclusion:

New oxadiazole derivatives (hydrazide and its Schiff bases), derived from vanillic acid were successfully synthesized by conventional methods. They were characterized and evaluated for their antibacterial activities. **Compound (6)** had shown the broadest spectrum against tested bacteria showed activities against four out of six bacteria. **Compound (7)** had shown moderate activities against ***S. aureus, E. coli,*** and ***K. pneumoniae***. **Compound (5)** was moderately and selectively active against ***E. coli***. **Compound (8)** was slightly active against ***S. aureus*** and moderately active against ***E. coli***.

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