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Synthesis, Characterization, and Preliminary Evaluation of Antimicrobial activity of Imines derived from Vanillic Acid Conjugated to Heterocyclic 4*H*- 1,2,4-Triazole-3-Thiol [#]

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Abstract

Triazole is a significant heterocyclic moiety that occupies a distinctive area in heterocyclic chemistry because of its numerous biological actions. In terms of clinically useful drug options, 1,2,4-triazole has a wide range of activities, including analgesic, antiseptic, antimicrobial, antioxidant, anti-inflammatory, diuretic, anticancer, anticonvulsant, and antidiabetic agents. Vanillic acid is a phenolic substance that can be found in many foods and medicinal plants; it has antioxidant, antimicrobial, anti-inflammatory, anti-cancer, and anti-obesity properties. The purpose of this research is to prepare new imines derived from vanillic acid conjugated to heterocyclic 4*H*- 1,2,4-triazole-3-thiol (**5-8**) and evaluate their antimicrobial activity. A multistep synthesis was established for the preparation of new vanillic acid-triazole conjugates, this is done by synthesis of the intermediate 4-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-2-methoxyphenol (compound **4**), which then reacts with different heterocyclic aldehydes to give the corresponding 4-(4- (substituted amino)-5-mercapto-4*H*-1,2,4-triazol-3-yl)-2- methoxy phenol derivatives (**5-8**). These compounds were characterized spectroscopically by FT- IR and ^{1}H- NMR. These imine derivatives (**5-8**) were tested for their antimicrobial activity and compared with three standard references (amoxicillin, ciprofloxacin, and fluconazole). Overall, compounds **6** and **8** exhibited varying degrees of inhibitory effects on the growth of the examined bacterial species and fungus. The most active one is compound **6** having pyrrole ring imine derivative showed potent activity against *C. albicans* and moderate activity against all tested bacteria compared to other derivatives but no activity toward *P. aeruginosa* and *P. mirabilis*. Moreover, only compounds **5** and **7** having thiophene group showed moderate inhibitory activity toward *P. aeruginosa*, while compounds **6** and **8** had no effect.

Keywords: Vanillic acid, Heterocyclic aldehydes, 4*H*-1,2,4- Triazole-3-thiol, Imine derivatives, Antimicrobial Activity.

تحضيرو تشخيص وتقييم أولي للنشاط المضاد للميكروبات للمركبات الأيمينية الجديدة المشتقة من حامض الفانيليك المقترن مع حلقة (٤,٢,١- ترايزول -٣- ثايول) غيرالمتجانسة[#]

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الخلاصة

التريازول عبارة عن جزء حلقي غير متجانس كبير يشغل منطقة مميزة في الكيمياء الحلقية غير المتجانسة نظرًا لتأثيراته البيولوجية العديدة. فيما يتعلق بخيارات الأدوية المفيدة سريريًا ، فإن ١،٢،٤-تريازول لديها مجموعة واسعة من الأنشطة ، بما في ذلك المسكنات ، المطهرات ، مضادات الميكروبات ، مضادات الأكسدة ، المضادة للالتهابات ، مدرر للبول ، مضاد للسرطان، مضادات الاختلاج ، مضادات السكري. حامض الفانيليك مادة فينولية يمكن العثور عليها في العديد من الأطعمة والنباتات الطبية، ولها خصائص مضادة للأكسدة ومضادة للميكروبات ومضادة للالتهابات ومضادة للسرطان ومضادة للسمنة ، والغرض من هذا البحث هو إعداد قواعد شيف جديدة(٥-٨) مشتقة من حامض الفانيليك المتصل مع (4*H*- 1,2,4-triazole-3-thiol) حلقة غير متجانسة وتقييم نشاطهم المضاد للميكروبات. تم إنشاء المركبات بخطوات متعددة لتحضيرها (اتحادات حمض الفانيليك-تريازول الجديدة)، يتفاعل المركب (٤) (4-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-2-methoxyphenol) مع الألدهيدات الحلقية غير المتجانسة المختلفة للحصول على 4-(4- (substituted amino)-5-mercapto-4*H*-1,2,4- triazol-3-yl)-2- لمشتقات ميثوكسي الفينول (٥-٨). تم تشخيص هذه المركبات طيفيًا بواسطة FT-IR و H-NMR[١]. تم اختبار هذه المشتقات الايمينية (٥-٨) لنشاطها المضاد للميكروبات ومقارنتها بثلاثة مراجع قياسية (أموكسيسيلين ، سيبروفلوكساسين ، فلوكونازول). بشكل عام، أظهر المركب ٦ و ٨ درجات متفاوتة من التأثيرات المثبطة على نمو الأنواع البكتيرية والفطريات المفحوصة. أظهر المركب ٦ الحاوي على حلقة البيرول نشاطًا قويًا ضد داء المبيضات (*C. albicans*) ونشاطًا معتدلًا ضد جميع البكتيريا المختبرة مقارنة بالمشتقات الأخرى ولكن لم يكن هناك أي نشاط تجاه الزائفة الزنجارية (*P. aeruginosa*)والمُتَقَلِّبَةُ الرَّائِعَة (*P. mirabilis*). علاوة على ذلك ، أظهرت المركبات ٥ و ٧ فقط التي تحتوي على مجموعة ثيوفين نشاطًا مثبطًا معتدلًا تجاه *P. aeruginosa* ، بينما لم يكن للمركبين ٦ و ٨ أي تأثير.

الكلمات المفتاحية: حامض الفانيليك، الألدهيدات الحلقية غير المتجانسة، ٤,٢,١- ترايزول -٣- ثايول،مشتقات الأيمين، النشاط المضاد للميكروبات.

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Introduction

Heterocyclic compounds are the most significant complicated toroidal branches of organic compounds whose atomic structures contain one (mostly five or six -membered rings) with at least one hetero-atom, the most prevalent heteroatoms are oxygen, nitrogen, and sulfur [(1)]. Heterocycles having Sulfur and nitrogen atoms are present in naturally occurring substances, commercially available medications, and substances with the potential to be active pharmaceutical constituents [(2)].

Imines are the nitrogen analogs of aldehydes and ketones, containing a C=N bond instead of a C=O bond. The electrophilic (C=O) carbon atoms found in aldehydes and ketones serve as substrates for nucleophilic round-through amine molecules. The termination product of the imination reaction results in a compound that contains either (CH=N) or (C=N), depending on the sort of carbonyl compound [(3)].

Schiff bases (imines) are investigated because of their simplicity and flexibility in the synthesis process. They also have a wide range of biological applications in the clinical and pharmaceutical fields. Mesbah *et al* [(4)] synthesized three different Schiff bases from the condensation of pyrrole-2-carboxaldehyde, thiophene-2-carboxaldehyde, and furan-2-carboxaldehyde (furfural) with 4,4' -diamino diphenyl sulfide the Schiff bases exhibited antibacterial activity against *E. coli*, while the strain *Pseudomonas aeruginosa* was not sensitive to any of these three compounds.

Imines derived from 1,2,4- triazole exhibited powerful biological effects. In particular, they are antibacterial1 [(5)], antifungal [(6)], antitubercular [(7)], antioxidant [(8)], analgesic [(9)], anti-inflammatory [(10)], and pesticide [(11)].

Vanillic acid or known as 4-hydroxy-3-methoxybenzoic acid is a vanillin oxidized form that is produced when vanillin is converted into ferulic acid and used as a flavoring agent, food additive, as well as a preservative in the food industry. [(12)]

The pharmacological activities of vanillic acid itself can be outlined; sedative activity [(13)], antidepressant effects [(14)], antinociceptive effects [(15)], antihypertensive [(13)], anticancer effects [(16)], antifungal activities [(17)], antioxidant activity [(18)] and anti-diabetic activity [(19)].

Many vanillic acid derivatives were produced and evaluated for different biological activities; vanillic Acid- oxadiazole Schiff bases showed interesting antibacterial activity [(20)], novel ester-hybrid derivatives of vanillic acid examined for their antibacterial activity, the methyl vanillate derivative has significant anti-bacterial activity against tested Gram-positive and Gram-negative bacteria [(21)]. Other series of amide derivatives of vanillic acid exhibit promising selective inhibitory effects against α–amylase, and α–glucosidase enzymes [(19)].

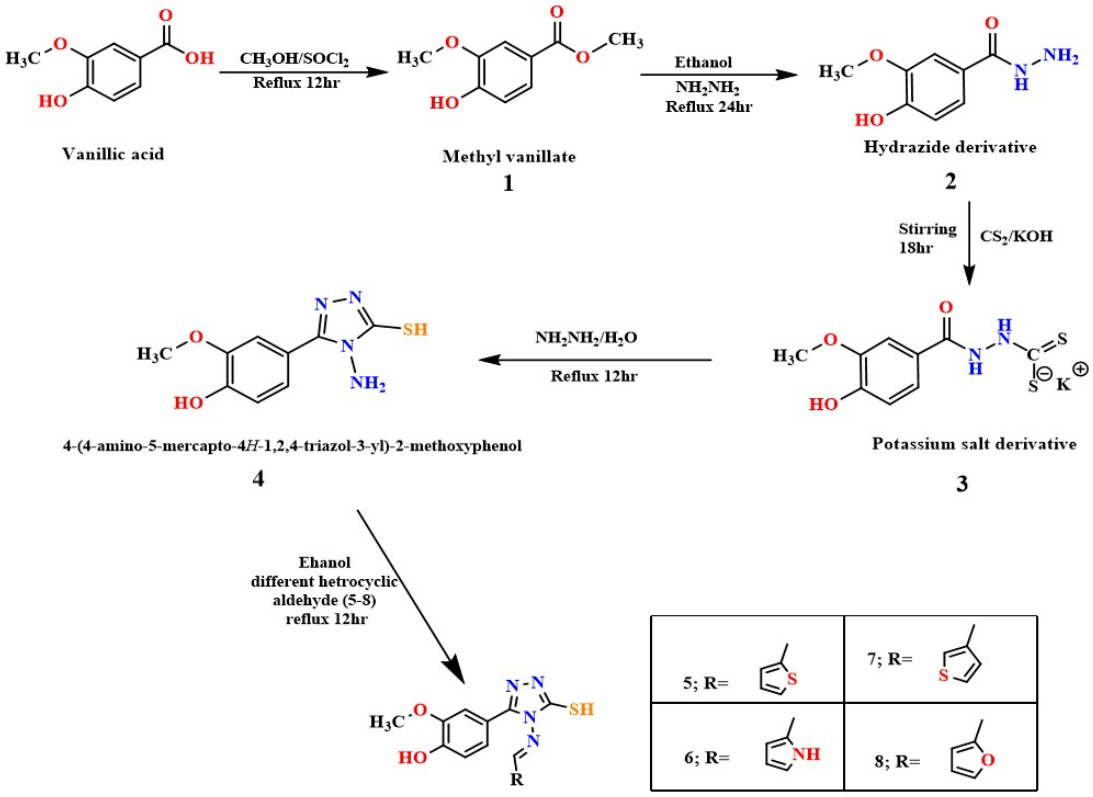
There is a great need for more effective antibacterial and antifungal medication today because of the high mortality rates connected to bacterial and fungal infections as well as the rising number of multidrug-resistant strains. Therefore, this study aimed to synthesize new derivatives of vanillic acid incorporating a 1,2,4-triazole-3- thiol ring and connecting to an imine moiety with expected antimicrobial activity.

Materials And Methods

All of the analytical-grade reagents and solvents were supplied by (Sigma- Aldrich Germany, Riedel–de Haën Germany, and Merck Germany). Incorrect melting points were obtained using the Stuart SMP3 melting point apparatus in open capillary tubes. The retention factor (R_f) values were estimated using two solvent systems: the first, **S1**: toluene: methanol (8:2), and the second, **S2**: chloroform: ethyl acetate (7:3). The infrared spectra were determined using a Fourier Transform Infrared (FT- IR) spectrophotometer, in Shimadzu, Japan, and the Proton Nuclear Magnetic Resonance (1H-NMR) spectrum was recorded using an NMR ultra shield spectrophotometer 500 MHz, BRUKER (Switzerland).

General synthetic procedure

Scheme (1) provides a summary of the steps for synthesizing the final compounds and their intermediates. Methyl vanillate (compound 1) was produced when the carboxylic group of vanillic acid was esterified in the presence of thionyl chloride ($SOCl_2$) in cold Methyl alcohol. Then, methyl vanillate was combined with hydrazine hydrate to produce a hydrazide derivative (compound 2), which was then involved in a reaction with carbon disulfide in presence of potassium hydroxide (KOH) to give potassium dithiocarbamate derivative (compound 3), which then underwent cyclization with hydrazine hydrate to produce 1,2,4-triazole-3-thiol heterocyclic ring derivative of vanillic acid (compound 4). Several new imine derivatives, as final compounds (5-8), were produced by the reaction of the primary amine group of the 1,2,4-triazole ring with various heterocyclic aldehydes.



Scheme 1: Steps of the synthesis of final compounds and their intermediates

Synthesis of methyl 4-hydroxy-3-methoxybenzoate; methyl vanillate; Compound (1)

Vanillic acid (1.38 gm, 10 mmol) in methyl alcohol (50ml) was cooled to –20 °C, and then thionyl chloride ($SOCl_2$) (0.88 ml, 12.5 mmol) was added drop by drop. The obtained mixture was held for five hrs. at 40 °C and refluxed for another five hrs, then at room temperature for the remainder of the night. The Methyl alcohol was then evaporated to dryness and the residue of reaction redissolved in absolute ethyl alcohol and evaporated. This operation was repeated many times until all of the unreacted thionyl chloride ($SOCl_2$) was removed. The residual was recrystallized from ether/methanol to give a compound (**1**). Chemical formula; ($C_9H_{10}O_4$), appearance and color: powder of white color, yields 70%, and melting point: 60-62˚C. FT-IR (υ, cm^{-1}): 3468 (OH str.), 3017 (C-H, aromatic str.), 2978 & 2839 (C-H, asymm. and symm. str. of $-OCH_3$ group), 1686 (C=O str. of carbonyl ester), 1597 & 1528 (aromatic, C=C, str.), 1227 (C-O-CH_3, asymm. str.).

Synthesis of 4-Hydroxy-3-methoxybenzohydrazide ; Compound (2)

Compound (**1**) (4.99g, 27mmol) was dissolved in a small quantity of 12ml of 99.8% ethanol, and 80% of hydrazine hydrate (13.5g, 270mmol) was added drop by drop. The mixture was refluxed for 24 hrs, then monitored and checked for reaction by TLC. After cooling the reaction mixture, a precipitate began to form; it was filtered and dried in an oven set to 60˚C yielding 2g of compound (**2**). The precipitate of the vanillic acid hydrazide was recrystallized from 70% ethanol to get the crystals of the compound (**2**). Chemical formula; ($C_9H_{10}N_2O_3$), appearance and color: crystals off-white colored, yield 60%, melting point: 208-210˚C (the previously reported melting point was 210-211˚C) [22]. FT-IR (υ, cm^{-1}): 3306 (OH str. overlapped with N-H asymm. str. of primary amine), 3256 (N-H symm. str. of primary amine), 3209 (N-H symm. str. of sec. amide), 3051(Ar. C-H str.), 2939 & 2835 (C-H, asymm. and symm. str. of -CH_3 group), 1628 (C=O str. of amide), 1585 & 1504 (aromatic, C=C 1str.), 1273 (C-O-CH_3, asymm. str.).

Synthesis of potassium salt of 2-(4-hydroxy-3-methoxybenzoyl) hydrazine-1-carbodithioate, Compound (3) [23]

After mixing vanillic acid hydrazide (1.4 g, 10 mmol), potassium hydroxide (0.6 g, 15 mmol), and (2 ml, 25 mmol) carbon disulfide (CS_2) in 12 ml of absolute ethanol, followed by stirring for 18 hrs. and then isolating the formed product by diethyl ether.

Compound (**3**), the potassium salt, was produced and used in the next step without needing to be purified. Chemical formula: ($C_9H_9KN_2O_3S_2$), appearance and color: powder off-white. Yield 50%, m.p: 250˚C (decomp.). FT-IR (υ, cm^{-1}): 3333 (OH str. overlapped with (N-H) 1str. of NH-N<u>H</u>-CSS^- K^+), 3167(N-H str. of N<u>H</u>-NH-CSS^- K^+), 3059(Ar. C-H str.), 2973 & 2866 (C-H asymm. and symm. str.

of CH_3 group, 1651 (C=O) str. of amide, 1593 & 1508 (aromatic, C=C str.), 1277 (C=S str.).

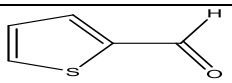
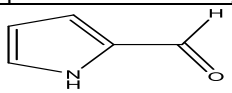
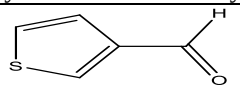
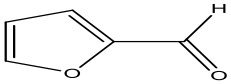
Synthesis of 4-(4-amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)-2-methoxyphenol, Compound (4)

In a suspension, hydrazine hydrate 80% (1.1 ml, 22.1 mmol), compound (**3**) (4 g, 10.98 mmol), and 20 ml of distilled water were refluxed for 12 hrs. A homogeneous solution was formed as a result of the evolution of hydrogen sulfide (H_2S) gas, which caused the reaction mixture to turn a greenish-brown color. After adding 100 ml of cold water and then acidifying it with a few drops of diluted 35% HCl solution, a pale yellow solid precipitated [24]. The solid product was filtered and washed with 50 ml of cold water, twice, then recrystallized from 70% ethanol to form a faint yellow powder. Chemical formula :($C_9H_{10}O_2N_4S$), color and appearance: faint yellowish powder, yield 60%, melting point: (190-192˚C). FT-IR (υ, cm^{-1}): 3310 (OH str. overlapped with N-H asymm. str. of primary amine), 3229 (N-H symm. str. of primary amine), 3109 (Ar. C-H str.), 2935 & 2822 (C-H asymm. and symm. str. of CH_3 group), 2534 (S-H str. of thiol tautomer), 1604 (C=N str.), 1593 & 1504 (aromatic, C=C str.), 1207 (C=S str.). ^{1}H-1NMR (δ ppm): 3.81 (s, 3H, OC<u>H</u>$_3$), 9.66 (s, 1H, O<u>H</u>), 6.88 to 7.57 (m, 3H, Ar-<u>H</u>), 5.79 (s, 2H, N-N<u>H</u>$_2$), 13.8 (s, 1H, S<u>H</u>).

Synthesis of Final compounds (5-8)

Compound (**4**) (1.1g, 3.29mmol) and (3.29 mmol) suitable heterocyclic aldehydes listed in Table (1), were mixed, separately, with 25 ml absolute ethanol and heated to reflux on a water bath for several hours depending on the type of aldehyde as given in Table (1), during the refluxing process 3 or 4 drops from glacial acetic acid were added. At the end of the reaction time, a rotary evaporator successfully evaporated the solvent, and the product was then generated by adding the residue to ice-cooled water. It underwent filtering, rinsing with cold water, and drying. The final product was purified and recrystallized from hot ethanol [25].

Table 1. Type of aldehydes, quantity, and time used to prepare the final compounds

Final compound number	Heterocyclic aldehyde's structure and name	Amount	Time required in the reaction
5	 Thiophene-2-carboxaldehyde	0.45ml	12 hrs reflux then left overnight stirring at room temp.
6	 Pyrrole-2-carboxaldehyde	0.4g	8 hrs reflux then left overnight stirring at room temp.
7	 Thiophene-3-carboxaldehyde	0.45ml	12 hrs reflux then left for 8 hrs. stirring at room temp.
8	 Furfural	0.50ml	15 hrs reflux then left for 8 hrs. stirring at room temp.

4-(5-mercapto-4-((thiophen-2-ylmethylene)amino) - 4H -1, 2,4-triazol-3-yl)-2-methoxyphenol, Compound (5)Chemical Formula: $C_{14}H_{12}N_4O_2S_2$, appearance, and color: powder of faint yellow color, yield 60%, m.p: (185-186˚C). FT-IR (υ, cm^{-1}): 3180 (OH str. of phenol), 3400-2700 (hydrogen bonded O-H broadband), 3090 (Ar. C-H str.), 2962 & 2831 (C-H str. of CH_3 group asymm. and symm.), 1589 (C=N str.), 1540 & 1512 (Ar. C=C str.). ^{1}H-NMR (δ ppm): 14.12 (s, 1H, S<u>H</u>), 9.89 (s, 1H, O<u>H</u>), 9.78 (s, 1H, N-N=C<u>H</u>), 7.97 to 7.29 (m, 6H, Ar-<u>H</u>), 3.82 (s, 3H, OC<u>H</u>$_3$).

4-(4-(((1H-pyrrol-2-yl)methylene)amino)-5-mercapto -4H-1,2,4-triazol-3-yl)-2-methoxypheno l, Compound (6)

Chemical Formula: $C_{14}H_{13}N_5O_2S$, appearance, and color: powder off-white color, yield 60%, m.p:(230-231˚C). FT-IR (υ, cm^{-1}): 3352 (NH str. of pyrrol) 3117 (O-H str. of phenol), 2700-3300 (hydrogen bonded O-H broadband), 3032 (Ar. C-H str.), 2962 & 2835 (C-H str. of CH_3 group asymm. and symm.), 1601 (C=N str.), 1540 & 1516 (Ar. C=C str.). ^{1}H-NMR
(δ ppm): 13.96 (s, 1H, S<u>H</u>), 12.09 (s, 1H, N<u>H</u>), 9.69 (s, 1H, O<u>H</u>), 9.00 (s, 1H, N-N=C<u>H</u>), 7.48 to 6.85 (m, 6H, Ar-<u>H</u>), 3.82 (s, 3H, OC<u>H</u>$_3$).

4-(5-mercapto-4-((thiophen-3-ylmethylene)amino)- 4H- 1 , 2 ,4- triazol -3 -yl) -2-methoxyphenol, Compound (7)

Chemical Formula: $C_{14}H_{12}N_4O_2S_2$, appearance, and color: powder of faint brown color, yield 55%,

m.p:(210-211˚C). FT-IR (υ, cm^{-1}): 3468 (OH str. of phenol), 3124 (Ar. C-H str.), 2962 & 2835 (C-H str. of CH_3 asymmetric and symmetric), 1593 (C=N str.), 1558 & 1508 (C=C str.). ^{1}H-NMR (δ ppm): 14.10 (s, 1H, SH), 9.76 (s, 1H, OH), 9.59 (s, 1H, N-N=CH), 7.79 to 6.91 (m, 6H, Ar-H), 3.87 (s, 3H, OCH_3).

4-(4-((furan-2-ylmethylene)amino)-5-mercapto-4H- 1, 2, 4 – triazol – 3 – yl) -2-methoxyphenol, Compound (8)

Chemical Formula: $C_{14}H_{12}N_4O_3S$, appearance, and color: powder of brown color, yield 50%, m.p: (184-185˚C). FT-IR (υ, cm^{-1}): 3124 (OH str.), 3300-2700 (hydrogen bonded O-H broadband) 3021 (Ar. C-H str.), 2963 & 2835 (C-H str. of CH_3 group asymmetric and symmetric), 1612 (C=N str.), 1562 & 1512 (C=C str.). ^{1}H-NMR (δ ppm): 14.09 (s, 1H, SH), 9.73 (s, 1H, OH), 9.58 (s, 1H, N-N=CH), 8.09 to 6.97 (m, 6H, Ar-H),), 3.83 (s, 3H, OCH_3).

In vitro Antimicrobial screening

The newly synthesized compounds (vanillate-1,2,4-triazole-3-thiol conjugates) were evaluated for antimicrobial activity as primary screening in one concentration (200mg/ml) against Gram-positive (*Staphylococcus aureus*, *Bacillus. subtilis*) and Gram- negative (*Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis,* and *Klebsiella pneumonia*) bacteria and (*Candida albicans*) fungus by using well-diffusion technique [26]. The inhibition zone (IZ) was measured in mm. and compared with three different standards; Amoxicillin, Ciprofloxacin, and Fluconazole. All the examined compounds and standards were dissolved in dimethyl sulfoxide (DMSO) to give a concentration of 200mg/ml. The *in vitro* antimicrobial activity was evaluated by the Aljazeera Company (J.P.I) Medical Laboratory.

Results And Discussion

Chemistry

The four final derivatives of vanillic acid Schiff bases (**5-8**) as well as the four intermediates (**1-4**) were produced using the classical chemical processes, which are described in Scheme 1. Vanillic acid was first esterified using methyl alcohol in the presence of thionyl chloride to produce an intermediate termed acyl chloride, which then reacted with the initial alcohol (Methyl alcohol) to yield methyl ester of vanillic acid, compound (**1**) was distinguished by carbonyl moiety of aromatic ester at 1686 cm^{-1} in its FT– IR spectrum.

The mechanism of compound (**2**) synthesis essentially occurred under basic conditions (hydrazinolysis of ester) that took place under standard basic circumstances, with two hydrazine molecules acting as the rate-determining step as a proton was transferred between them. The next step involves steadily leaving one molecule of hydrazine with one alcohol molecule [27].

\For compound (**2**), two stretching vibration bands for the primary amine of the hydrazide molecule were observed in the FT-IR spectrum at 3306 cm^{-1} and 3256 cm^{-1}, respectively, a characteristic NH amide band at 3209 cm^{-1} stretching vibration, as well as a stretching vibration carbonyl amide band at 1628 cm^{-1}, and NH_2 bending vibration band at 1601 cm^{-1}.

In an ethanolic solution of potassium hydroxide, the acid hydrazide compound (**2**) and carbon disulfide (CS_2) were combined to form the potassium salt derivative; compound (**3**). The reaction is a nucleophilic addition process in which the amine (NH_2) attacks the carbon of the carbon disulfide molecule. The product was the potassium salt of the more stable dithiocarbamic acid than the free acid. The FT-IR spectrum was distinguished by stretching vibration bands at 3333 cm^{-1} OH str. overlapped with (N-H) str. of NH-NH-CSS^-K^+, 3167cm^{-1} (N-H) str. of NH-NH-CSS^-K^+ and 1651cm^{-1} (C=O) str. of amide.

The 1,2,4-triazole-3-thiol derivative of vanillic acid was produced by cyclizing the potassium salt intermediate using hydrazine hydrate. Many intermediates were involved in this reaction, such as initially, the carbonyl group is attacked by the intermediate nucleophile hydrazine with loss of water molecules, intra-molecular cyclization by adjacent moiety nucleophile amine attacking the carbon of (CS_2) carbon disulfide by nucleophilic substitution reaction, and the formation of the potassium salt occurred with a loss of H_2S gas. Compound **4** is produced when concentrated hydrochloric acid (35%) is used to acidify potassium salt. Figure (1) shows thiol and thione in a stable equilibrium tautomer state. The free amine connected to the formed heterocyclic ring was reacted with various aldehydes to produce a variety of Schiff bases derivatives of vanillic acid. In this reaction, imines were produced as a result of the primary amine attacking the carbon of the carbonyl group in an acidic medium [28]. FT-IR spectrum was characterized by (N–H) asymmetric str. of primary amine overlapped with 3310 cm^{-1} OH str. and 3229 cm^{-1} (N–H) symmetric str. of primary amine and disappearance of 1651cm^{-1} carbonyl band of salt. ^{1}H-NMR was distinguished by the appearance of a distinct signal at 5.79 ppm (s, 2H, N-NH_2).

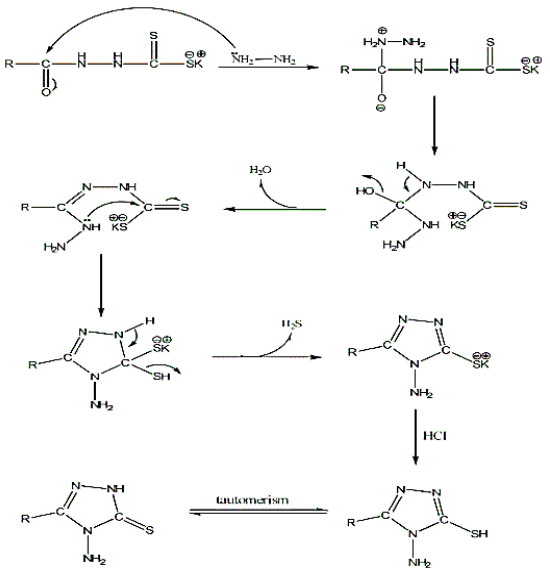


Figure 1. Mechanism of cyclization and formation of compound (4) [28]

Compounds (**5-8**) contain imine moiety that is a result of the reaction of heterocyclic aldehydes with primary amines under a weakly acidic solution which involves 6 steps: the first three steps produce an intermediate known as a carbinolamine and in the last steps the transformation of the carbinolamine into an imine as a final product. The absence of both vibration bands at 3310 cm^{-1} and 3229 cm^{-1} of NH_2 in FT-IR spectra indicates the formation of the imine group, as well as the new bands (1601-1604) cm^{-1}, indicating the (-C=N) formation in FT-IR spectra. ^{1}H-NMR was distinguished by the appearance of new signals at 9.75 to 9.16 (s, 1H, N-N=CH) for compounds (**5-8**), and the disappearance of the signal at the 5.79 ppm for the NH_2 group.

evaluation

According to the findings in Table 2; for the anti-bacterial assessment, all the prepared compounds (**5-8**) exhibited moderate to potent anti-bacterial activity towards *E. coli* at 200 mg/mL, and low active to inactive against *K. pneumonia, P. mirabilis, S. aureus,* and *B. subtills* at the same concentration compared to the standard drugs (amoxicillin and ciprofloxacin).

Furthermore, compound **5** exhibited more activity against *E. coli* when compared to amoxicillin as a reference drug, while, compound **6** containing pyrrole ring at the same concentration showed potent activity *K. pneumonia* and was more reactive than amoxicillin with moderate activity against all other tested bacteria, and no activity against *P. aeruginosa* and *P. mirabilis*. The other targeting compounds were not effective against Gram-negative bacteria (*P. mirabilis*); while only compound 8 showed moderate activity. Another interesting point is that both compounds **5** and **7** with the S group showed moderate inhibitory activity toward *P. aeruginosa*, while compounds **6** and **8** have no effect. These differences in the effectiveness of different derivatives towards various microbes depend either on the impermeability of the cells of the pathogens or differences in the ribosomes of pathogen cells.

Regarding compounds **5** and **7** having the S group, the NH group in compound 6, and the O group in compound **8**, for the rest of these compounds have the same skeleton, it can be concluded that the NH group will potentiate the antibacterial activity of the vanillate-triazole imine derivatives and the O will be in the second place.

The antifungal activity against *C. albicans*; for tested compounds exhibited no activity, except compound **5** with slight activity and compound **6** exhibited potent activity compared to fluconazole (standard drug). Finally, the results obtained in Table (2) proved that these derivatives showed good antibacterial than antifungal effects

Table 2: Antimicrobial activities of the target compounds (5-8), in concentration (200 mg/ml)

Compound	(IZ) Inhibition zone in mm						
	Bacterial strains						Fungus
	Gram- positive		Gram- negative				
	S.aureus	*B.subtills*	*E.coli*	*K.pneumonia*	*P.mirabilis*	*P.aeruginosa*	*C.albicans*
5	-	-	18	-	-	12	5
6	16	12	14	12	-	-	18
7	-	-	12	-	-	14	-
8	-	-	10	8	10	-	-
Amoxicillin*	30	-	15	10	-	35	-
Ciprofloxacin*	52	28	30	18	45	40	-
Fluconazole**	-	-	-	-	-	-	30
DMSO***	-	-	-	-	-	-	-

***Standards for bacterial strains, **Standard for fungus, ***Control (–) means no activity, (IZ equal to 5–10 mm) slightly active, (IZ equal to 10-15 mm) moderately active, or highly active when (IZ is more than 15 mm) [29].**

Conclusion

Four derivatives of 4-(4-(substituted amino)-5-mercapto-4*H*-1,2,4- triazol-3-yl)-2-methoxy phenol were synthesized in good yields and characterized by FT–IR and ^{1}HNMR spectroscopies. 1,2,4- Triazoles are observed to have potential antibacterial activity and conjugation of the 4*H*-1,2,4-triazole-3- thiol ring with another natural antibacterial compound such as vanillic acid might provide new and more effective antibacterial candidates.

The conjugation of the heterocyclic ring (4*H*-1,2,4-triazole-3-thiol) to vanillic acid molecule will improve the anti-microbial activity of vanillic acid, this depends on the type of heterocyclic aldehyde molecule that forms an imine base with the mentioned heterocyclic ring. The incorporation of the pyrrole-2-carboxaldehyde into compound (**4**) will enhance the antimicrobial activity of the vanillate-triazole imine (derivative **6**) compared to other derivatives. Also, It can be concluded that (compound **6**) having NH and (compound **8**) with O group will potentiate the antibacterial activity of the vanillate-triazole imine derivatives. However, only compounds **5** and **7** with the S group showed moderate inhibitory activity toward *P. aeruginosa*, while compounds **6** and **8** had no effect.

In order to develop new 4*H*-1,2,4-triazole-3-thiol derivatives with improved potency and preserved safety profiles, further study in this area is made possible by the structure-activity relationship (SAR) evaluation.

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Conflicts of Interest

The authors have declared there is no conflict of interest.

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Ethics Statements

Authors declare that this study was *in-vitro* and synthesized, screening and evaluation of compounds need no ethical approval from an ethics committee.

Author Contribution

Noor, the first author, participated in the synthesis of the final compounds, the analysis of IR and ^{1}H-NMR data, the discussion of the antibacterial activity, the drafting of the text, and the critical revision of the manuscript. May, the second author, contributed to the design of the study, discussed the final results, and approved the final version of the manuscript.

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