Short Communication:

Synthesis, Antibacterial and Antioxidant Evaluation of 2-Substituted-4-arylidene-5(4*H*)-oxazolone Derivatives

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Abstract: *In this research, the synthesis of new substituted oxazolone derivatives is described via Erlenmeyer synthesis of* N*-acyl amino acid. Firstly, the azo derivative* ***1*** *was prepared by coupling the diazonium salt of 3-amino-4-methoxybenzoic acid with 4,5-dichloroimidazole in sodium hydroxide solution. Benzoyl chloride derivative* ***2****, the key intermediate of the synthesis, was synthesized by the acylation of azo-carboxylic acid derivative* ***1*** *with thionyl chloride. The resulting acyl chloride derivative reacted with glycine in a basic catalyst to form a hippuric acid derivative* ***3****. After that, oxazolone derivatives* ***4a–4f*** *were prepared via the reaction of the hippuric acid derivative with various aromatic aldehydes. All new compound structures were confirmed by spectral techniques, i.e., FTIR,* ^{1}*H-NMR,* ^{13}*C-NMR spectroscopy, and elemental analysis. The antimicrobial activity (*Staphylococcus aureus *and* Escherichia coli*) of all new compounds was screened* in vitro*. The results against* S. aureus *and* E. coli *showed that most of the tested compounds have an activity ranging from moderate to low. The antioxidant activity of derivative* ***4a*** *was also evaluated and showed good antioxidant activity.*

Keywords: *antibacterial; antioxidants; Erlenmeyer reaction; glycine; oxazolone*

■ INTRODUCTION

The Erlenmeyer azlactones, also known as 4-arylidene-2-substituted-5(4*H*)-oxazolones, were first introduced in 1983 by Friedrich Gustav Carl Emil Erlenmeyer [1]. This family of compounds is distinguished by its five-membered heterocyclic (γ-lactone) ring that contains nitrogen, oxygen atoms, and an exocyclic C=C [2]. Due to their broad range of biological and pharmacological qualities, they have attracted much more attention [3]. They serve as intermediates and suitable building blocks for the production of a variety of physiologic active compounds like amino acids and heterocyclic compounds [4-6]. In addition, these compounds are particularly effective as antioxidants [7], antitumors [8], antimicrobials [9], and antihypertensives [10] agents. The Erlenmeyer method is the most used method for creating oxazolones [11], which includes a condensation reaction of hippuric acid or its derivatives with aryl aldehydes in dehydrating agent as acetic anhydride and basic catalyst as anhydrous sodium acetate [12]. Among the numerous heterocyclic compounds containing nitrogen atoms, the imidazole ring, a typical component of natural products, has particular structural features and a variety of biological activities [13]. The unique structure of the imidazole ring was utilized to design, formulate, and develop imidazole-based therapeutic agents in medical fields, including anticonvulsant, antimicrobial, anticancer, anti-HIV, anti-hypertensive, antidepressant, anti-inflammatory, antileishmanial, pain-relieving, and anti-inflammatory [14-16]. Moreover, azo compounds have been used as hypotensive, anticancer, antifungal, antibacterial, anti-inflammatory, and antiviral therapeutic agents [17-20].

Due to various pharmaceutical activities to oxazolone, imidazole, and azo functions and since differing substituents is a common method in medicinal

chemistry for drug design and as a continuation of previous studies, this study aimed to synthesize and study antibacterial and *in vitro* antioxidant activity of the new oxazolone derivatives.

■ EXPERIMENTAL SECTION

Materials

In this study, the chemicals utilized included *p*-anisic acid and 4,5-dichloroimidazole were purchased from Fluorochem. Thionyl chloride and *p*-nitrobenzaldehyde were purchased from CDH while 3,4-methoxybenzaldehyde, *p*-tolualdehyde, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, DMSO, methanol and ethanol 99% were purchased from Sigma Aldrich. The vanillin, 4-bromobenzaldehyde, 4-hydroxybenzaldehyde (TCL), acetic anhydride 99% (Scharlau), glycine, hydrochloric acid, sodium nitrite, Mueller–Hinton agar (Himedia), sodium hydroxide (B.D.H), and anhydrous sodium acetate (Fluka) were also used in this work.

Instrumentation

The digital melting point device from Stuart, UK was used to measure the melting points. The reactions were monitored on Merck silica gel plates TLC 60 F254 and the products were visualized by iodine vapor. On a Bruker 400 MHz device, ^{1}H and ^{13}C-NMR spectra were obtained, tetramethylsilane was employed as an internal standard and DMSO-d_6 as a solvent, values in parts per million are supplied for all NMR chemical shifts in University of Basrah, College of Education of Pure Sciences, Chemistry Department, Iraq. On the EuroFA elemental analyzer apparatus, elemental analysis (C.H.N) were counted. Infrared spectra were recorded and represented in wavenumber on (Shimadzu FTIR-8400S) spectrophotometer.

Procedure

Synthesis of azo derivative 3-((4,5-dichloro-1,3-diazole-2-yl)diazenyl)-4-anisic acid (1)

The synthesis was carried out in two steps, the first step includes diazonium salt formation by dissolving *p*-anisic acid (0.16 g, 1 mmol) in an acidic solution consisting of distilled water (20 mL) and concentrated HCl (4 mL) with cooling to 0 °C. The solution was treated with sodium nitrite (0.069 g, 1 mmol) in distilled water (5 mL) at the same temperature and continued stirring the above mixture for half an hour. A clear solution of diazonium salt was obtained, which was used directly in the second step by adding it slowly to 4,5-dichloroimidazole (0.13 g, 1 mmol) in absolute ethyl alcohol (15 mL) and (10 mL) of 10% NaOH with stirring for 2 h below 5 °C. The mixture was adjusted at pH 6 [21], and the orange precipitate was formed, then isolated by filtering, washed many times with distilled water, dried, and recrystallization from absolute ethanol. An orange powder with a yield 90%, and a melting point 231–233 °C, as shown in Scheme 1.

FTIR using KBr, ν (cm^{-1}): 3173 (NH imidazole), 3400–2500 (OH carboxylic acid), 3070 (C–H aromatic), 2993 (C–H aliphatic), 1604 (C=N imidazole), 1681 (C=O), 1575–1504 (C=C aromatic), 1450 (N=N), 864 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 12.84 (1H, br, OH), 11.74 (1H, s, NH), 7.40–8.14 (3H, m, aromatic), 4.06 (3H, s, OCH_3). ^{13}C-NMR spectrum (DMSO-d_6, 100 MHz, δ_C, ppm): 56.8 (C-17), 114.0 (C-15), 117.9 (C-13), 123.5 (C-12), 128.5 (C-2), 131.0 (C-131), 134.8 (C-14), 141.0 (C-11), 150.9 (C-5), 160.3 (C-10), 167.0 (C-18). $C_{11}H_8Cl_2N_4O_3$, Found, %: C 41.73; H 2.74; N 17.75. Calculated, %: C 41.93; H 2.56; N 17.78.

Synthesis of 3-((4,5-dichloro-1,3-diazole-2-yl)diazenyl)-4-methoxybenzoyl chloride (2)

To a carboxylic acid derivative **1** (0.3 g, 1 mmol), thionyl chloride (13.7 mol, 6 mL) was added slowly by a dropping funnel with stirring at room temperature, then refluxed at 70 °C for 7 h. After the completion of the reaction (followed up by TLC), the excess thionyl chloride was removed by reduced pressure and the product was used immediately in the next step [22]. A red powder with a yield of 91%, and a melting point of 195–197 °C, as shown in Scheme 1.

FTIR using KBr, ν (cm^{-1}): 3172 (N–H), 3085 (C–H aromatic), 2846 (C–H aliphatic), 1751(C=O acyl), 1604 (C=N imidazole), 1535–1512 (C=C aromatic), 1442 (N=N aromatic) and 825 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.83 (s, 1H, NH), 7.25–8.09 (m, 3H, aromatic), 3.91 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz,

DMSO-d_6, δ, ppm): 171.1 (C-17), 155.8 (C-10), 148.8 (C-5), 142.2 (C-11), 131.8 (C-14), 131.5 (C-3), 129.8 (C-12), 128.7 (C-13), 122.7 (C-2), 114.2 (C-15) and 57.0 (C-20). Found, %: C 39.20; H 2.22; N 16.13. $C_{11}H_7Cl_3N_4O_2$. Calculated, %: C 39.60; H 2.12; N 16.97.

Synthesis of (3-((4,5-dichloro-1,3-diazole-2-yl)diazenyl)-4-methoxybenzoyl) glycine (3)

Aryl benzoyl chloride **2** (0.333 g, 1 mmol) in a minimum amount of acetone was added slowly over a 30 min period with portions to a mixture of glycine (0.075 g, 1 mmol) and sodium hydroxide (10 mL, 10%). The reaction mixture continued stirring vigorously for one day at room temperature, and the reaction progress was monitored through TLC (benzene: methanol 4:1, v/v). Crushed ice was added to the mixture and then acidified with HCl (concentrated) to pH 2–3. The formed precipitate was filtered and washed several times with cold distilled water, dried, and recrystallized from methanol [23]. The reddish-orange powder with a yield of 64% and the melting point of the compound (decompose at 196 °C), as shown in Scheme 1.

FTIR using KBr, ν (cm^{-1}): 2543–3548 (O–H acid), 3371 (N–H amide), 3178 (N–H), 3070 (C–H aromatic), 2985, 2846 (C–H aliphatic), 1712 (C=O acid), 1697 (C=O amide), 1604 (C=N imidazole), 1535–1504 (C=C aromatic), 1450 (N=N) and 825 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 12.95 (br s, 1H, OH acid), 11.96 (s, 1H, NH imidazole), 7.40–8.19 (m, 3H, aromatic), 7.84 (s, 1H, NH amide), 4.05 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 172.2 (C-22), 167.7 (C-16), 151.4 (C-10), 148.8 (C-5), 142.2 (C-11), 131.7 (C-14), 128.1 (C-13), 125.3 (C-3), 124.0 (C-12), 123.7 (C-2), 114.1 (C-15), 56.9 (C-21) and 46.3 (C-18). Found, %: C 41.61; H 2.83; N 18.16. $C_{13}H_{11}Cl_2N_5O_4$. Calculated, %: C 41.95; H 2.97; N 18.86.

Synthesis of 2-substituted-4-arylidene-5(4H)-oxazolones derivatives (4a-4f)

A mixture of *N*-acyl amino acid compound **3** (0.37 g, 1 mmol), various aromatic aldehydes (1 mmol), anhydrous sodium acetate (1 mmol), and acetic anhydride (20 mL, 10.5 mol) was shaken for 30 min at room temperature. Then, the mixture was refluxed at 80 °C. Absolute ethanol (5 mL) was added slowly with stirring after the completion of the reaction (TLC monitored), the pot of reaction was left standing overnight, and the solid product was filtered, washed with distilled water, dried, and recrystallized using ethanol [24], as shown in Scheme 1.

2-(3-((4,5-Dichloro-l,3-diazole-2-yl)diazenyl)-4-methoxyphenyl)-4-(4-hydroxybenzylidene)oxazol-5(4*H*)-one (4a). 4-Hydroxybenzaldehyde (0.12 g, 1 mmol), Dark brown powder with a yield (65%), and a melting point of 229–231 °C, R_f = 0.65 (benzene: methanol 4:1, v/v). FTIR using KBr, ν (cm^{-1}): 3394 (O–H), 3163 (N–H), 3132 (C–H aromatic), 3008 (C–H olefin), 2908, 2839 (C–H aliphatic), 1733 (C=O oxazolone), 1675 (C=N oxazolone), 1650 (C=N imidazole), 1604 (C=C alkene), 1558–1512 (C=C aromatic), 1458 (N=N aromatic), 1218 (C–O) and 840 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.32 (br s, 1H, NH), 8.80 (s, 1H, C–H olefin), 6.93–8.24 (m, 7H, aromatic), 7.82 (br s, 1H, OH), 3.99 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 168.9 (C-19), 166.1 (C-18), 160.1 (C-25), 155.4 (C-10), 148.8 (C-5), 144.2 (C-11), 137.5 (C-16), 134.7 (C-23,27), 132.3 (C-21), 130.1 (C-14), 128.5 (C-22), 126.7 (C-12), 126.4 (C-3), 124.1 (C-2), 121.7 (C-13), 116.3 (C-26,24), 111.7 (C-15), and 56.4 (C-30). Found, %: C 52.70; H 2.69; N 15.45. $C_{20}H_{13}Cl_2N_5O_4$. Calculated, %: C 52.42; H 2.86; N 15.28.

4-(4-Bromobenzylidene)-2-(3-((4,5-dichloro-1,3-diazole-2-yl)diazenyl)-4-methoxyphenyl)oxazol-5(4*H*)-one (4b). 4-Bromobenzaldehyde (0.18 g, 1 mmol), Light brown powder with a yield 69%, and the melting point 210–212 °C, R_f = 0.3 (hexane: ethyl acetate 5:1, v/v). FTIR using KBr, ν (cm^{-1}): 3163 (N–H), 3101 (C–H aromatic), 3085 (C–H olefin), 2931, 2854 (C–H aliphatic), 1778 (C=O oxazolone), 1680 (C=N oxazolone), 1650 (C=N imidazole), 1612 (C=C alkene), 1558–1519 (C=C aromatic), 1458 (N=N aromatic), 1226 (C–O) and 887 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.82 (br s, 1H, NH), 8.92 (s, 1H, C–H olefin), 7.03–8.27 (m, 7H, aromatic), 3.89 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 167.3 (C-19), 161.9 (C-18), 155.4 (C-10), 149.1 (C-5), 138.3 (C-11), 135.4 (C-16), 134.4 (C-24,26),132.7 (C-22), 132.1 (C-23,27),

128.3 (C-12), 128.0 (C-3), 123.9 (C-25), 121.6 (C-2), 121.5 (C-13), 112.6 (C-15), and 57.2 (C-30). Found, %: C 46.21; H 2.55; N 13.63. $C_{20}H_{12}BrCl_2N_5O_3$. Calculated, %: C 46.09; H 2.32; N 13.43.

2-(3-((4,5-Dichloro-l,3-diazole-2-y1)diazenyl)-4-methoxyphenyl)-4-(3,4-dimethoxybenzylidene)oxazol-5(4*H*)-one (4c). 3,4-Dimethoxybenzaldehyde (0.16 g, 1 mmol), Caramel color powder with a yield 63%, and the melting point 137–139 °C, R_f = 0.6 (chloroform: methanol 4:1, v/v). FTIR using KBr, ν (cm^{-1}): 3178 (N–H), 3132 (C–H aromatic), 3078 (C–H olefin), 2962, 2839 (C–H aliphatic), 1727 (C=O oxazolone), 1668 (C=N oxazolone), 1634 (C=N imidazole), 1610 (C=C olefin), 1589–1512 (C=C aromatic), 1458 (N=N aromatic), 1272 (C–O) and 810 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.83 (br s, 1H, NH), 8.42 (s, 1H, C–H olefin), 7.02–8.28 (m, 6H, aromatic), 4.00 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 166.0 (C-19), 160.9 (C-18), 154.6 (C-10), 150.4 (C-25), 149.7 (C-26), 148.1 (C-5), 144.9 (C-11), 131.8 (C-16), 130.7 (C-24), 130 (C-14), 129.9 (C-22), 128.9 (C-23), 127.7 (C-3), 126.6 (C-12), 122.6 (C-2), 121.5 (C-13), 114.2 (C-27), 112.6 (C-24), 111.7 (C-15), 56.2 (C-29), 56.3 (C-32), and 56.9 (C-34). Found, %: C 52.86; H 3.09; N 13.52. $C_{22}H_{17}Cl_2N_5O_5$. Calculated, %: C 52.60; H 3.41; N 13.94.

2-(3-((4,5-Dichloro-l,3-diazole-2-yl)diazenyl)-4-methoxyphenyl)-4-(4-hydroxy-3-methoxybenzylidene)oxazol-5(4*H*)-one (4d). Vanillin (0.15 g, 1 mmol), Umber color powder, Yield (71%), m.p = 151–153 °C, R_f = 0.5 (chloroform: methanol 4:1, v/v).FTIR using KBr, ν (cm^{-1}): 3271 (O–H), 3163 (N–H), 3132 (C–H aromatic), 3016 (C–H olefin), 2947, 2839 (C–H aliphatic), 1715 (C=O oxazolone), 1662 (C=N oxazolone), 1645 (C=N imidazole), 1635 (C=C olefin), 1589–1512 (C=C aromatic), 1458 (N=N aromatic), 1218 (C–O) and 825 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.83 (br s, 1H, NH), 9.27 (br s, 1H, OH), 8.25 (s, 1H, C–H olefin), 7.01–8.13 (m, 6H, aromatic), 3.98 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 166.9 (C-19), 160.2 (C-18), 155.1 (C-10), 153.6 (C-25), 149.5 (C-5), 148.6 (C-26), 144.5 (C-11), 136.1 (C-16), 135.9 (C-21), 131.8 (C-14), 129.9 (C-23), 129.09 (C-3), 126.5 (C-22), 123.9 (C-12), 123.2 (C-2), 119.8 (C-13), 114.2 (C-24), 113.0 (C-27), 111.1 (C-15), 56.0 (C-30), and 55.9 (C-33). Found, %: C 51.75; H 3.22; N 14.19. $C_{21}H_{15}Cl_2N_5O_5$. Calculated, %: C 51.65; H 3.09; N 14.34.

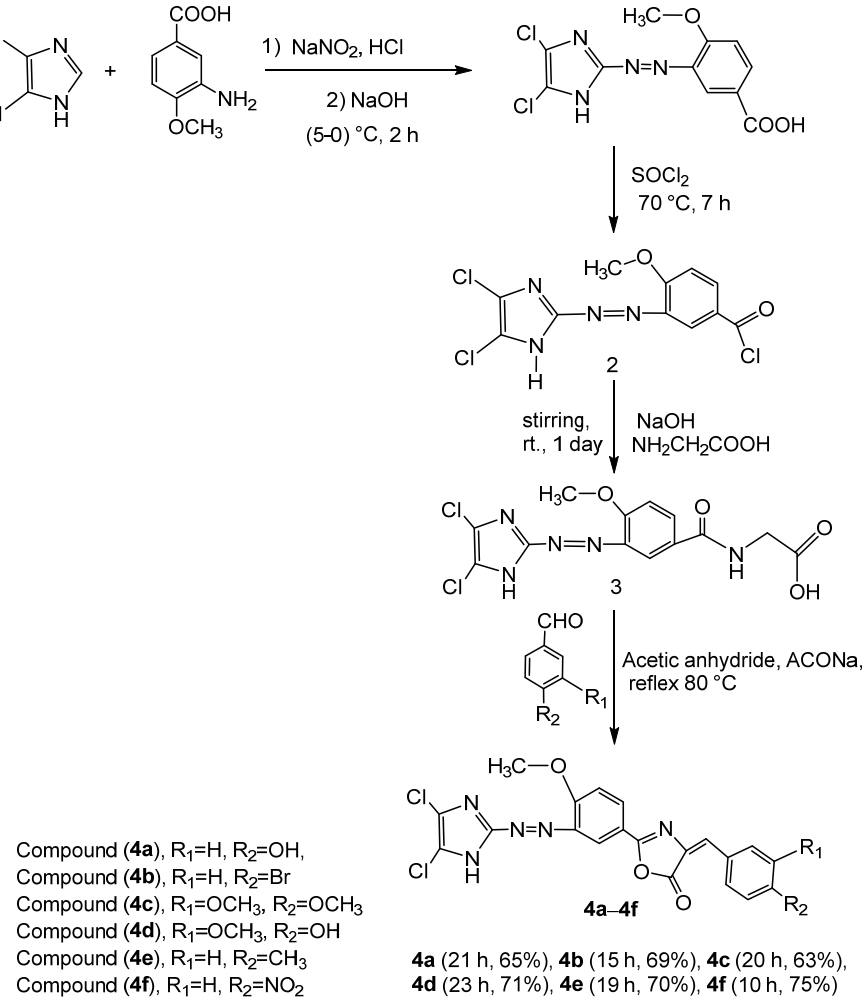
2-(3-((4,5-Dichloro-1,3-diazole-2-yl)diazenyl)-4-methoxyphenyl)-4-(4-methylbenzylidene)oxazol-5(4*H*)-one (4e). 4-Methylbenzaldehyde (0.12 g, 1 mmol), Brown powder with a yield 70%, and the melting point 215–217 °C, R_f = 0.35 (chloroform: methanol 4:1, v/v). FTIR using KBr, ν (cm^{-1}): 3209 (N–H), 3147 (C–H aromatic), 3093 (C–H olefin), 2923, 2854 (C–H aliphatic), 1740 (C=O oxazolone), 1685 (C=N oxazolone), 1660 (C=N imidazole), 1623 (C=C olefin), 1604–1512 (C=C aromatic), 1442 (N=N aromatic), 1265 (C–O) and 825 (C–Cl). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.83 (br s, 1H, NH), 8.63 (s, 1H, C–H olefin), 7.01–8.57 (m, 7H, aromatic), 3.99 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 166.7 (C-19), 159.3 (C-18), 155.0 (C-10), 145.6 (C-5), 144.6 (C-11), 138.1 (C-25), 136.9 (C-16), 136.0 (C-22), 135.1 (C-21), 133.2 (C-14), 131.8 (C-23,27), 129.8 (C-26,24), 129.6 (C-12), 126.3 (C-3), 123.3 (C-2), 121.3 (C-13), 114.2 (C-15), 56.0 (C-30), and 21.6 (C-28). Found, %: C 55.61; H 3.08; N 15.67. $C_{21}H_{15}Cl_2N_5O_3$. Calculated, %: C 55.29; H 3.31; N 15.35.

2-(3-((4,5-Dichloro-1,3-diazole-2-yl)diazenyl)-4-methoxyphenyl)-4-(4-nitrobenzylidene)oxazol-5(4*H*)-one (4f). 4-Nitrobenzaldehyde (0.15 g, 1 mmol), Reddish brown powder with a yield 75%, and the melting point 240–242 °C, R_f = 0.55 (benzene: methanol 4:1, v/v). FTIR using KBr, ν (cm^{-1}): 3209 (N–H), 3109 (C–H aromatic), 3085 (C–H olefin), 2908, 2846 (C–H aliphatic), 1789 (C=O oxazolone), 1698 (C=N oxazolone), 1655 (C=N imidazole), 1633 (C=C olefin), 1527–1546 (C=C aromatic), 1458 (N=N aromatic), 1265 (C–O), 848 (C–Cl) and 1564, 1378 (NO_2). ^{1}H-NMR (400 MHz, DMSO-d_6), δ (ppm): 11.84 (br s, 1H, NH), 8.88 (s, 1H, C–H olefin), 7.20–8.42 (m, 7H, aromatic), 3.89 (s, 3H, OCH_3). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 167.4 (C-19), 160.9 (C-18), 155.8 (C-10), 148.2 (C-5), 147.6 (C-25), 144.9 (C-11), 136.8 (C-22), 131.8 (C-16), 131.4 (C-23,27), 131.1 (C-21), 130.8 (C-14),

124.7 (C-3), 124.5 (C-12), 124.2 (C-24,26), 123.4 (C-2), 122.3 (C-13), 114.2 (C-15), and 56.4 (C-29). Found, %: C 49.58; H 2.56; N 17.40. $C_{20}H_{12}Cl_2N_6O_5$. Calculated, %: C 49.30; H 2.48; N 17.25.

■ RESULTS AND DISCUSSION

The study design includes the synthesis of new oxazolone derivatives bearing imidazole moiety. The synthesis of different derivatives **1**–**3** and target compounds **4a**–**4f** is outlined in Scheme 1. At first, we prepared azo derivative **1** with a high yield of 90% through the general method that includes two steps, diazotization of primary amine and then coupling with aromatic compound. The produced azo derivative **1** was used to synthesize acid chloride derivative **2** under anhydrous conditions in a high yield of 91% with thionyl chloride. Then, compound **2** was used immediately in the Schotten Baumann reaction by reacting with glycine in 10% NaOH to afford hippuric acid derivative **3** through the nucleophilic displacement mechanism. Compound **3** was utilized to synthesize oxazolone derivatives **4a**–**4f** by Erlenmeyer condensation reaction with acetic anhydride in the presence of anhydrous sodium acetate and different aldehydes (4-hydroxybenzaldehyde, 4-bromobenzaldehyde, 3,4-dimethoxybenzaldehyde, vanillin, *p*-tolualdehyde, and 4-nitrobenzaldehyde). One of the observations that attracted our attention during the synthesis of oxazolone derivatives is that the reaction time is directly affected by the type of benzaldehyde used. It was observed that the substituted benzaldehyde with NO_2 and Br groups required 10 and 15 h to complete the reaction, respectively. Meanwhile, benzaldehydes



Scheme 1. Synthesis of oxazolone derivatives (**4a**–**4f**)

substituted with electron-donating groups such as OH and OCH_3, needed more time, 21 and 23 h, respectively. It is clear that the substituents in the aromatic ring play an essential role in determining the reaction time and rate. All synthesized compounds were characterized by spectral techniques like ^{1}H, ^{13}C-NMR, and FTIR in addition to C.H.N analyses.

The FTIR spectrum results showed that compound **1** has a medium band at 1450 cm^{-1} due to the azo group. The stretching vibration of the carbonyl of acid showed a strong band at 1661 cm^{-1}. The ^{1}H-NMR spectrum of compound **1** shows a broad peak at 12.84 ppm due to acid proton and also a single peak at 11.74 ppm attributed to (NH imidazole) while ^{13}C-NMR spectra show signals at 167.0 for the carbon of carbonyl acid.

The FTIR analysis of compound **2** shows a shifting of the carbonyl band of acid to higher frequencies at 1751 cm^{-1}, indicating the formation of acid chloride in addition to the disappearance of a broad band of acid hydroxyl. The ^{1}H-NMR spectra of compound **2** show the disappearance of hydroxyl protons of acid while ^{13}C-NMR gives an important signal at 171.1 ppm belonging to the carbon of carbonyl group.

The FTIR analysis of compound **3** shows characterized new bands. The broad band at 2543–3548 cm^{-1} belongs to OH acid (Glycine). Also, sharp bands attributed to carbonyl groups at 1697 and 1712 cm^{-1}. In addition to a new stretching vibration of NH amide bond appearance at 3371 cm^{-1}.

The ^{1}H-NMR of compound **3** shows many peaks, at 12.95 ppm belonging to (OH acid). Another new single peak belongs to the NH amide proton at 7.89 ppm in addition to methylene (CH_2) protons at 4.05 ppm. On the other hand, ^{13}C-NMR gives new signals at 172 ppm belonging to the carbonyl of acid. A characteristic signal at 167.7 ppm was attributed to the carbonyl of amide. The methylene group also gave a new signal at 46.3 ppm.

Compounds **4a**–**4f** that synthesized by the reaction between compound **3** and various aromatic aldehydes. These compounds show many new bands at different regions in FTIR spectra; the new bands in the range 3008–3093 cm^{-1} belong to the olefin C–H bond in oxazolone derivatives. Other new bands in the range 1662–1698 cm^{-1} were attributed to (C=N Oxazolone), the stretching vibration at 1715–1789 cm^{-1} belong to (C=O oxazolone), new C=C olefin bands also appear in the range 1604–1635 cm^{-1} while absorption bands of C–O of oxazolone ring appear in the fingerprint region in the range 1218–1265 cm^{-1}.

^{1}H-NMR of compounds **4a**–**4f** shows the appearance of new peaks in the range 8.90–8.25 ppm belonging to =C-H olefin proton. On the other hand, ^{13}C-NMR spectra of compounds **4a**–**4f** were given special packages; the bands at the range 159–166 ppm were attributed to the carbon of C=N oxazolone (C-18), the bands in the range 160–168 ppm were attributed to the carbon of C=O oxazolone (C-19). Elemental analysis was also performed for the prepared compounds, and it was found that the theoretical or calculated value is close to the practical value, and thus this supports the validity of the synthesized chemical compositions.

The compounds were screened for their growth inhibition activity *in vitro* against two types of bacterial strains, Gram-positive and -negative, and derivative **4a** was also tested for its antioxidant activity.

Antibacterial Effectiveness

The well plate method was utilized to evaluate the newly synthesized derivatives *in vitro* against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) microorganisms [25-26] where was taken 100 μg/mL concentration from each compound in dimethyl sulfoxide (DMSO) as a solvent, after incubation for 24 h, the inhibitory zone diameter has been calculated. Most of the evaluated compounds that underwent antibacterial testing exhibited moderate activity against the growth of the tested microbial strains.

The findings indicated that, among synthetic compounds, **4a** showed no activity against *S. aureus* (inhibition zone less than 5 mm), while **3**, **4b**, **4c**, **4d**, **4e**, and **4f** showed low activity against *S. aureus* bacteria. On the other hand, *E. coli* response to the produced chemicals was better, ranging between moderate and low activities, whereas compounds **3**, **4a**, **4d**, and **4f** showed

Table 1. Antimicrobial activity zone of synthesized compounds

Compounds	Inhibition zone diameter (mm)	
	E. coli	*S. aureus*
2	10	5
3	11	7
4a	12	3
4b	10	5
4c	9	10
4d	12	7
4e	6	8
4f	13	6
S1(Amoxicillin drug)	15	14
S2(Ciprofloxacin drug)	40	25
DMSO	-	-

moderate activities, which have a higher inhibition zone than the rest of the synthesized compounds. The inhibition zone diameters were classified as very strong (≥ 20 mm), strong (15–20 mm), moderate (10–15 mm), weak or low (< 10 mm) [27-28].

The results of the structure-activity relationship of the synthesized compounds oxazolone **4a**–**4f** have shown that the derivatives **4a**, **4d**, and **4f** that contain strong electron donating groups such as OH and OCH_3 in para and meta positions and strong electron withdrawal groups as NO_2 in the para position, respectively, showed higher inhibitory activity than the rest of the other derivatives. The presence of halogen group (**4b**) and methyl group (**4e**) in the *p*-position of the phenyl ring does not have a significant influence on the activity. It should be noted that the effect of hydroxy, methoxy, and nitro groups is higher on *E. coli* than *S. aureus*. Table 1 indicates antibacterial efficiency as compared to the reference drugs.

Antioxidant Activity

The antioxidant activity of the prepared oxazolone derivative containing the hydroxyl group was evaluated using the rapid method (DPPH radical scavenging) in which the DPPH radical is characterized by its strong absorption and at a wavelength of 517 nm [29-31], which changes color in the presence of an oxidizing substance from violet to yellow. Methanol-DMSO mixture was used as the sample solvent.

One of the target compounds, which is the oxazolone derivative **4a**, was chosen to test its ability to scavenge free radicals, as we expected that the oxazolone derivative **4a** could give higher antioxidant activity than the other synthesized compounds because it possesses a hydroxyl group at the phenyl ring that can donate a hydrogen atom to the free radical DPPH and converts it to a stable molecule. The experimental results of compound **4a** supported our previous expectations, as they demonstrated that compound **4a** has a significant free radical scavenging rate of 77% and is close to vitamin C. The antioxidant capacity of a synthetic derivative **4a** was examined utilizing several concentrations from 200 to 12.5 µg/mL. The percentage inhibition of DPPH scavenging activity showed that 200 µg/mL, the highest dose of the tested compound **4a** had the highest antioxidant activity (77.16%) with IC_{50} of 23.40 µg/mL compared to ascorbic acid (80.36%) with IC_{50} of 21.04 µg/mL, which was used as a standard. Data are depicted as mean ± SD. Table 2 illustrates the results of antioxidant efficiency.

■ CONCLUSION

In summary, this study has synthesized new imidazole-based oxazolone derivatives from the condensation of hippuric acid derivative with aromatic aldehydes with different substituents and evaluated their antibacterial and antioxidant activities. The results above indicate that the *p*-nitro substituted group on the benzene ring of oxazolone derivative highly affects the antibacterial activity against *E. coli*, the same effect shown in a hydroxy group at the same position and a methoxy group at the meta position. Furthermore, the oxazolone

Table 2. Antioxidant activity of synthesized compound **4a**

Compound	Inhibition of DPPH (%)					IC_{50}, µg/mL
	200 µg/mL	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	
4a	77.16 ± 2.41	71.37 ± 6.21	65.93 ± 2.41	51.47 ± 3.07	43.48 ± 3.57	23.40
Ascorbic acid (standard)	80.36 ± 2.87	69.48 ± 3.71	54.48 ± 2.41	40.43 ± 7.08	17.63 ± 7.20	21.04

derivative **4a** that evaluated its antioxidant activity exhibited good and close action to the activity of the standard drug used for the purpose of comparison in this study in the DPPH test. However, more research focusing on the mechanisms of action of oxazolones on bacterial response is needed.

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