



## Synthesis and Antioxidant Properties of Novel Galloyloxadiazole Hybrids

Nourah A. Al Zahrani<sup>1</sup>, Reda M. El-Shishtawy<sup>2,3</sup>

<sup>1</sup> Chemistry Department, Faculty of Science, University of Jeddah, Jeddah 21959, Saudi Arabia;  
nourh.a.z@hotmail.com (N.A.A.Z.)

<sup>2</sup> Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia  
<sup>3</sup> Dyeing, Printing and Textile Auxiliaries Department, Textile Research Division, National Research Centre,  
Dokki, Cairo 12611, Egypt

*In Loving Memory of Late Professor Doctor "Mohamed Refaat Hussein Mahran"*

### Abstract

Herein, the design, synthesis, and characterization of four novel galloyloxadiazole hybrids are described. Compounds were evaluated in vitro as antioxidant agents by DPPH assay in comparison with reference antioxidants (ascorbic acid and butylated hydroxytoluene (BHT)) (scavenging of free radical 2,2-diphenyl-1-picrylhydrazyl DPPH). It was found that, interestingly, all compounds showed very strong antioxidant activity, even more than the water-soluble ascorbic acid. Such strong activity is related to the gallic acid moiety, as it has three dissociable OH groups via hydrogen atom transfer. The overall results suggest that these compounds could, potentially, be further modified for the formation of more potent antioxidant, and more biological studies are under investigation. These studies should be conducted in the following study.

**Keywords:** oxadiazole; gallic acid; antioxidant; oxidative stress; hybrid molecules.

### 1. Introduction

A heteroaromatic ring system refers to a ring structure in organic chemistry that contains at least one heteroatom (an atom other than carbon) within the ring. Aromatic compounds are characterized by having conjugated  $\pi$  bonds, which provide stability to the molecule.

Heteroaromatic compounds play a significant role in organic chemistry and have diverse applications in various fields, including pharmaceuticals, materials science, and agrochemicals such as pyridine, which is a component of vitamin B3 (niacin), [1] furan, which is found in natural products, [2] thiophenes, which are used as building blocks in the synthesis of pharmaceuticals, dyes, and polymers, [3] and imidazoles, which are found in several drugs, including antifungal and antiviral agents [4-6].

Oxadiazoles have gained significant attention in organic synthesis and medicinal chemistry due to their versatile properties and wide range of applications. They exhibit interesting electronic and photophysical properties, making them useful in the development of organic electronic materials, such as light-emitting diodes (LEDs), organic field-effect transistors (OFETs), and organic solar cells. [7, 8]

In medicinal chemistry, oxadiazoles have shown potential as bioactive compounds. They have been investigated for their antimicrobial, anticancer, anti-inflammatory, and antioxidant activities. Oxadiazole derivatives have been

synthesized and evaluated as potential drug candidates for various diseases. [8, 9]

Furthermore, oxadiazoles have been used as building blocks in the synthesis of other heterocyclic compounds and as functional groups in the design of catalysts and ligands in organometallic chemistry. [10] Several methods have been reported in the literature for the synthesis of 1,3,4-oxadiazoles. The commonly used synthetic route for 1,3,4-oxadiazoles includes reactions of acid hydrazides (or hydrazine) with acid chlorides or carboxylic acids and direct cyclization of diacylhydrazines using a variety of dehydrating agents such as phosphorous oxychloride, thionyl chloride [11], phosphorous pentoxide, [12] triflic anhydride, [13] polyphosphoric acid, [14] or direct reaction of acid with (Nisocyanimino-) triphenylphosphorane. [15] 1,3,4-Oxadiazole pharmacophores have been subjected to extensive study in recent years due to their metabolic profile and ability to engage in hydrogen bonding with receptor sites. 1,3,4-Oxadiazole are very good bioisosteres of amides and esters, which can contribute significant pharmacokinetic properties due to the presence of an azole nucleus, which increases the lipophilicity that influences the ability of the drug to reach the target by transmembrane diffusion. This could benefit the drug's ability to reach the infection site through transmembrane diffusion. [16, 17]

Gallic acid is a phenolic compound found naturally in various plants, including fruits (such as grapes, strawberries, and blueberries), nuts, and tea leaves. It is

\*Corresponding author e-mail: [nalzahrani2@uj.edu.sa](mailto:nalzahrani2@uj.edu.sa); (Nourah A. Al Zahrani).

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known for its diverse biological activities and has been extensively studied for its potential health benefits, such as the fact that it exhibits strong antioxidant properties due to its ability to scavenge free radicals and inhibit oxidative stress. It can donate hydrogen atoms or electrons to neutralize reactive oxygen species (ROS) and protect cells and tissues from oxidative damage.[18, 19]

5-(Fur-2-yl)-2-(3,4,5-trihydroxyphenyl)-1,3,4-oxadiazole, which has an aryl substituent, is a good donor for electrons and hydrogen and can react with free radicals because its antioxidant activity is similar to that of butylated hydroxytoluene **BHT**. Furthermore, different derivatives of oxadiazole hybrids were synthesized to investigate the effect of substituents at position 5 of 1,3,4-oxadiazole on the performance of the compounds as antioxidants.[20, 21] In this context, for the first time in 2015, oxadiazole derivatives have been synthesized. These derivatives increased the inhibition of cancer cell proliferation. [22] Moreover, two-hybrid molecules, five-membered heterocycles with antitubercular activity derived from isoxazole-based chalcones composed of gallic acid moiety and dihydropyrazole, have been reported. These compounds were evaluated for their antioxidant, anticancer, and antimicrobial activities. [23] Recently, a series of substituted 3-(5-phenyl-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-ones have been synthesized by a green chemistry method using molecular iodine by a simple grinding technique. Coumarin-tethered compounds exhibit modest to good radical-scavenging susceptibilities.[24]

Antioxidant activity refers to the ability of a substance to neutralize or prevent the harmful effects of reactive molecules known as free radicals. Free radicals are generated in the body through various processes such as metabolism, exposure to environmental pollutants, and stress. They can cause damage to cells and tissues by oxidizing biomolecules like proteins, lipids, and DNA.[25, 26]

Several studies have suggested that oxidative stress caused by an imbalance between free radicals and antioxidants is associated with various chronic diseases, including cardiovascular diseases, neurodegenerative disorders, cancer, and aging-related conditions. Antioxidants help reduce oxidative stress and may provide protective effects against these diseases.[27, 28]

In the context of heteroaromatic compounds, many of them have been studied for their antioxidant activity. For example, certain heteroaromatic compounds such as flavonoids (which contain a heterocyclic ring with oxygen) and phenolic compounds have been found to exhibit strong antioxidant properties. These compounds are commonly found in fruits, vegetables, and other plant-based foods.[29-31]

Antioxidants work by donating an electron or hydrogen atom to free radicals, thereby stabilizing them and preventing them from causing oxidative damage. They can be endogenous (produced within the body) or exogenous (obtained from external sources, such as food or supplements).[32]

The mechanism of antioxidant activity can vary depending on the specific compound. Some heteroaromatic antioxidants directly scavenge free radicals, while others may enhance the activity of endogenous antioxidant enzymes or chelate metal ions involved in free radical generation.[25, 33]

Many studies proved that synergistic effects could be produced from a mixture of various phenolic antioxidants, which is higher than individual antioxidants.[34, 35] Moreover, the synergetic effect may result from both moieties of two antioxidants involved in one molecule through a covalent bond. [36] It is expected that the antioxidant efficiency produced by one molecule containing various moieties is higher than that of the sum of each moiety. [37, 38] Based on the previous studies, here we designed new oxadiazole compounds as antioxidant drugs in combination with gallic acid moiety to evaluate in vitro biological evaluation of synthesized compounds (scavenging of free radical 2,2-diphenyl-1-picrylhydrazyl DPPH).

## 2. Results and Discussion

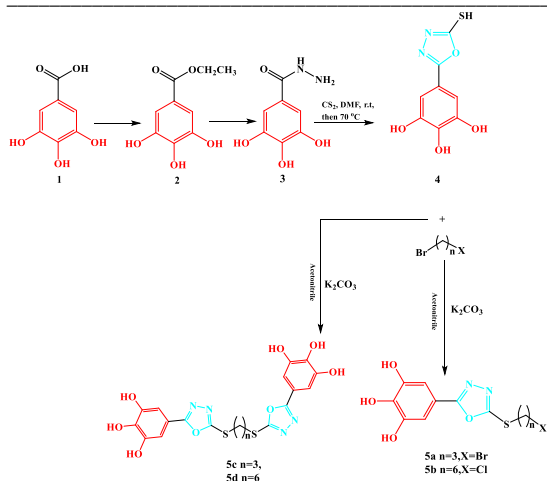
### 2.1. Chemistry

The synthetic route for the synthesis of hybrid molecules containing gallic acid and oxadiazole moieties is presented in (**Scheme 1**). Ethyl gallate **2** was prepared by esterifying the corresponding carboxylic acid **1** with ethanol and sulfuric acid. The reaction of ethyl gallate with hydrazine hydrate yielded the galloyl hydrazide **3**. For the preparation of new derivatives of galloyl-2-thioxo-1,3,4-oxadiazole, the key intermediate **3** was subjected to a reaction with carbon disulfide in the presence of dry DMF heated at 70°. The 1,3,4-oxadiazolyl **4** was subsequently S-alkylated with alkyl dihalide in the presence of K<sub>2</sub>CO<sub>3</sub> at room temperature for 4h with stirring to afford the 5-(5-((3-bromopropyl)thio)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol **5a** or with kept stirring overnight at room temperature to afford the corresponding dioxadiazole 5,5'-((propane-1,3-diylbis(sulfanediyl)) bis (1,3,4-oxadiazole-5,2-diyl))bis(benzene-1,2,3-triol) **5c**.

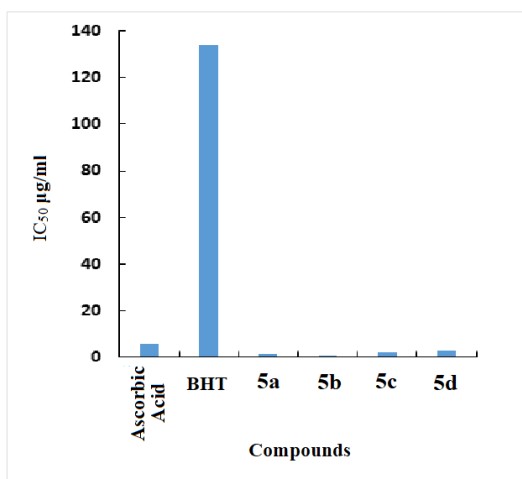
On the same way 5-(5-((6-chlorohexyl)thio)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol **5b** yielded by conventional heating at 50 °C for 1 hour in the presence of K<sub>2</sub>CO<sub>3</sub> and when the mixture was heated under reflux for overnight 5,5'-((hexane-1,6-diylbis(sulfanediyl))bis(1,3,4-oxadiazole-5,2-diyl))bis(benzene-1,2,3-triol) **5d** was obtained. The chemical structures of all compounds were confirmed by spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR, IR, and MS). The galloyl-2-thioxo-1,3,4-oxadiazole **4** showed -SH proton signal at 14.48 ppm in the <sup>1</sup>H-NMR spectrum, which disappeared upon S-alkylation to reveal the aliphatic CH vibration at 2799, 2752 cm<sup>-1</sup> in the IR of the product to confirm the chemical structure. <sup>1</sup>H-NMR spectrum of di oxadiazole compounds **5c** and **5d** showed increasing the number of aromatic protons at 6.86, 6.91 ppm and OH protons at 8.99, 9.49, 4.08 ppm compared with mono oxadiazole compounds.

### 2.2. Antioxidant activity

The antioxidant activity of oxadiazole-based gallic acid derivatives was assessed by DPPH assay and in comparison, with reference antioxidants (ascorbic acid and BHT). As shown in (**Fig. 2**) all compounds showed structural dependent antioxidant activity. Interestingly, all compounds showed very strong antioxidant activity even more than the water-soluble ascorbic acid. Such strong activity is related to the gallic acid moiety as it has three dissociable OH groups via hydrogen atom transfer .[18]



**Scheme 1.** Synthesis of new Galloyloxadiazole Hybrids **5a-5d**.



**Table 1** List of oxadiazoles compounds and some characterization data.

No.	Comp.Code.	Mol.Form	Mol.Wt.	Wight (g)	Yield (%)	Rf	m.p.
1.	2	C <sub>9</sub> H <sub>10</sub> O <sub>5</sub>	198.17	2	90	0.31	135
2.	3	C <sub>7</sub> H <sub>8</sub> N <sub>2</sub> O <sub>4</sub>	184.15	2.87	90	0	288-290
3.	4	C <sub>8</sub> H <sub>6</sub> N <sub>2</sub> O <sub>4</sub> S	226.21	0.17	77	0.54	168-170
4.	5a	C <sub>11</sub> H <sub>11</sub> BrN <sub>2</sub> O <sub>4</sub> S	347.18	0.1	80	0.22	150
5.	5b	C <sub>14</sub> H <sub>17</sub> BrN <sub>2</sub> O <sub>4</sub> S	389.26	0.1	80	0.19	149
6.	5c	C <sub>19</sub> H <sub>16</sub> N <sub>4</sub> O <sub>8</sub> S <sub>2</sub>	492.48	0.16	80	0.5	135
7.	5d	C <sub>22</sub> H <sub>22</sub> N <sub>4</sub> O <sub>8</sub> S <sub>2</sub>	534.56	1.91	80	0.47	164

### 3.3. Synthesis

#### 3.3.1. General procedure for the synthesis of ethyl 3,4,5-trihydroxybenzoate (2).

Gallic acid **1** (3.4 g, 20 mmol) and absolute ethanol (50 mL) were placed in a round bottom flask (RBF), the mixture was warmed slightly to dissolve GA then Conc. H<sub>2</sub>SO<sub>4</sub> (2.5 mL) was added as a catalyst.[39] The reaction mixture was refluxed for 1-2 h, then evaporated. The reaction was monitored by TLC elution with CHCl<sub>3</sub>/CH<sub>3</sub>OH, 90:10, v/v). After completion of the reaction, the reaction mixture was cooled. It was then neutralized with NH<sub>4</sub>OH (28%). The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuum to afford ethyl gallate. The crude product was then recrystallized from

**Fig. 2** The antioxidant activity of the synthesized compounds in comparison with ascorbic acid and BHT.

### 3. Experimental section

#### 3.1. General

All solvents were purchased from Sigma Aldrich and fisher and used directly without further purification. Thiosemicarbazide and trimethoxybenzaldehyde were purchased from sigma Aldrich. All other chemicals used herein were of analytical grade and were used without further purification. Chromatographic separations were carried out on silica gel (60–120 mesh).

#### 3.2. Apparatus

Structures of the synthesized ester was established by spectroscopic methods (IR, MS, and NMR) <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub>-d<sub>6</sub> or DMSO-d<sub>6</sub> on a Bruker Avance 600 and 850 MHz spectrometer, respectively. The reported chemical shifts were against TMS. The attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectrum was performed on a PerkinElmer spectrum 100 FT-IR spectrometer. Mass spectra were recorded on a positive ion mode on a Bruker Impact II, LC-MS/MS; Melting points were determined in open capillary tubes in Stuart Scientific melting point apparatus SMP3 and were uncorrected.

The physical data of oxadiazoles compounds are presented in (**Table 1**) and their structures was established by spectroscopic methods (IR, MS, and NMR).

boiling CHCl<sub>3</sub> as white powder in 90% yield.[40, 41] White solid in 90% Yield; mp: 135°C; <sup>1</sup>H NMR (850 MHz), DMSO-d<sub>6</sub> δ (ppm): 1.27 (t, 3H, CH<sub>3</sub>), 4.20 (q, 2H, OCH<sub>2</sub>), 6.95 (s, 2H, Ar-H), 9.21 (s, 3H, 3OH); <sup>13</sup>C NMR δ (ppm): 14.28, 60.03, 108.47, 119.58, 138.35, 145.56, 165.85; IR ν cm<sup>-1</sup>: 3448 (free O-H), 3286 (hydrogen bond O-H), 2976 (aliphatic C-H), 1704 (C=O), 1616, 1533 (aromatic C=C), 1311(C-O).

#### 3.3.2. General procedure for the synthesis of 3,4,5-trihydroxybenzoic acid hydrazide (3).

In a round bottom flask with stirring, 80% hydrazine hydrate (3.84 g, 5mmol) was added slowly to distilled water (1.2 g, 2.5 mmol). After completion of the addition,

ethanolic solution of ethyl gallate **2** (4.76 g, 1 mmol) was added dropwise to the mixture at room temperature with stirring. After completion of the addition of ester the mixture was heated under reflux overnight. When TLC analysis indicated the end of the reaction, elution with  $\text{CHCl}_3/\text{CH}_3\text{OH}$ , 85:15, v/v), then the media was poured on ice, and the resulting precipitate was filtered, and washed several times with first cold water and ethanol and dried under vacuum to give the title compound galloyl hydrazide as pale white powder in 90% yield. White solid in 90% Yield; mp: 288-290°C;  $^1\text{H NMR}$  (850 MHz),  $\text{DMSO-d}_6$   $\delta$  (ppm): 4.32 (s, 2H,  $\text{NH}_2$ ), 6.79 (s, 2H, Ar-H), 8.96 (s, 3H, 3OH), 9.33 (s, 1H, NH);  $^{13}\text{C NMR}$   $\delta$  (ppm) : 106.92, 124.02, 136.60, 145.87, 166.88;  $\text{IR v cm}^{-1}$ : 3424 and 3390 (sharp peaks, free and intramolecular hydrogen bonded OH), 3297 (sharp peak, NH amide), 3204, 3144 (broad peaks, hydrogen bonded  $\text{NH}_2$  and OH), 1598 (C=O), 1502-1464 (aromatic C=C), 1341(C-O), 1203 (C-N), 1054 (N-N); **MS**: m/z calc. for  $\text{C}_7\text{H}_9\text{N}_2\text{O}_4$  185.1  $[\text{M}+2]^+$ , found 186.2.

### 3.3.3. General procedure for the synthesis of 5-(5-mercapto-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol (**4**).

Galloyl hydrazide **3** (0.18g, 1mmol) was dissolved in 15 ml dry dimethyl formamide (DMF) then, carbon disulfide (0.23 g, 3 mmol) was added to the mixture at room temperature with stirring for 30 min. Then, the reaction was heated at 70°C for 3 hrs. The reaction was monitored by TLC elution with  $\text{CHCl}_3/\text{CH}_3\text{OH}$ , 85:15, v/v). After completion of the reaction, the reaction mixture was cooled. The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuum to afford oxadiazol as pale brown powder in 77% yield. Yellow white solid in 77% Yield, mp: 168-170°C;  $^1\text{H NMR}$  (600 MHz),  $\text{DMSO-d}_6$   $\delta$  (ppm): 6.82 (s, 2H, Ar-H), 9.25 (s, 1H, OH), 9.52 (s, 2H, 2OH), 14.48 (s, 1H, SH);  $^{13}\text{C NMR}$   $\delta$  (ppm) :105.53, 112.45, 138.07, 146.94, 161.50, 177.37;  $\text{IR v cm}^{-1}$ : 3429, 3088 (hydrogen bonded O-H), 2922 (olefinic C-H), 2799, 2752 (S-H), 1651 (C=N), 1517 (aromatic C=C), 1301(C-O), 1179 (N-N); **MS**: m/z calc. for  $\text{C}_8\text{H}_7\text{N}_2\text{O}_4\text{S}$  227.0  $[\text{M}+1]^+$ , found 227.8.

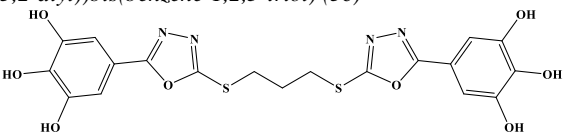
### 3.3.4. General procedure for the synthesis of 5-(5-((3-bromopropyl)thio)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol (**5a**).

5-(5-mercapto-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol **4** (0.23g, 1mmol) was dissolved in 15 ml dry acetonitrile then, 1,3-dibromopropane (1g, 5 mmol) was added to the mixture at room temperature with stirring. Then, anhydrous potassium carbonate (0.14g, 1 mmol) was added to the mixture and the reaction continued with stirring for 4 hrs at room temperature. The reaction was monitored by TLC elution with  $\text{CH}_3\text{OH}/\text{DCM}$ , 95:5, v/v). The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuum to afford alkylated oxadiazol as yellowish white powder in 80% yield. Yellow white solid in 80% Yield;  $^1\text{H NMR}$  (850 MHz),  $\text{DMSO-d}_6$   $\delta$  (ppm): 2.29 (quintet, 2H,  $J=6.8$  Hz,  $\text{CH}_2$ ), 3.36 (t, 2H,  $J=6.8$  Hz,  $\text{CH}_2$ ), 3.64 (t, 2H,  $J=5.95$  Hz,  $\text{CH}_2$ ), 6.91 (s, 2H, Ar-H);  $^{13}\text{C NMR}$   $\delta$  (ppm) : 30.99, 32.33, 33.24, 105.93, 113.19, 137.71, 146.90, 162.52, 166.09;  $\text{IR v cm}^{-1}$ : 3422(hydrogen bonded O-H), 1607(C=N), 1478 (aromatic C=C), 1187 (C-O), 1129 (N-N); **MS**: m/z calc. for  $\text{C}_{11}\text{H}_{12}\text{BrN}_2\text{O}_4\text{S}$  346.9  $[\text{M}+2]^+$ , found 347.0

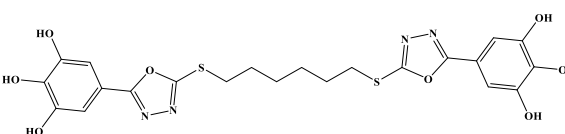
### 3.3.5. General procedure for the synthesis of 5-(5-((6-chlorohexyl)thio)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol (**5b**).

5-(5-mercapto-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol **4** (0.23g, 1mmol) was dissolved in 15 ml dry acetonitrile then, 1-bromo-6-chlorohexane (1g, 5 mmol) was added to the mixture at room temperature with stirring. Then, anhydrous potassium carbonate (0.14g, 1 mmol) was added to the mixture. Then, the reaction was heated at 50°C for 1 hour. The reaction was monitored by TLC elution with  $\text{CH}_3\text{OH}/\text{DCM}$ , 95:5, v/v). The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuum to afford alkylated oxadiazol as pale brown powder in 80% yield. Yellow white solid in 80% Yield;  $^1\text{H NMR}$  (600 MHz),  $\text{DMSO-d}_6$   $\delta$  (ppm): 1.42 (quintet, 4H,  $J=2.4$  Hz,  $2\text{CH}_2$ ), 1.72 (quintet, 2H,  $J=4.8$ Hz,  $\text{CH}_2$ ), 1.76 (quintet, 2H,  $J=4.8$  Hz,  $\text{CH}_2$ ), 3.26 (t, 2H,  $J=4.8$  Hz,  $\text{CH}_2$ ), 3.63 (t, 2H,  $J=4.8$  Hz,  $\text{CH}_2$ ), 6.89 (s, 2H, Ar-H), 8.99 (s, 1H, OH), 9.49 (s, 2H, 2OH);  $^{13}\text{C NMR}$   $\delta$  (ppm) : 26.14, 27.52, 29.30, 32.31, 32.40, 45.77, 105.87, 113.23, 137.66, 146.91, 162.97, 165.93;  $\text{IR v cm}^{-1}$ : 3433(hydrogen bonded O-H), 1112 (N-N); **MS**: m/z calc. for  $\text{C}_{14}\text{H}_{18}\text{ClN}_2\text{O}_4\text{S}$  345.1  $[\text{M}+1]^+$ , found 345.0.

### 3.3.6. General procedure for the synthesis of 5,5'-((propane-1,3-diylbis(sulfaneydiyl)) bis (1,3,4-oxadiazole-5,2-diyl))bis(benzene-1,2,3-triol) (**5c**)

  
5-(5-mercapto-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol **4** (0.96g, 4.2mmol) was dissolved in 15 ml dry acetonitrile then, 1,3-dibromopropane (0.2g, 1 mmol) was added to the mixture at room temperature with stirring. Then, anhydrous potassium carbonate (0.29g, 2.1 mmol) was added to the mixture and continued stirring overnight at room temperature. The reaction was monitored by TLC elution with  $\text{CH}_3\text{OH}/\text{DCM}$ , 90:10, v/v). The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was dried over  $\text{MgSO}_4$ , filtered, and concentrated in vacuum and product was purified by column chromatography (eluent: methanol: dichloromethane 90:10) on silica gel to afford alkylated oxadiazol as yellowish white powder in 80% yield. Yellow white solid in 80% Yield;  $^1\text{H NMR}$  (850 MHz),  $\text{DMSO-d}_6$   $\delta$  (ppm): 2.28 (quintet, 2H,  $J=6.8$ Hz,  $\text{CH}_2$ ), 3.41 (t, 4H,  $J=6.8$  Hz,  $2\text{CH}_2$ ), 6.91 (s, 4H, Ar-H), 8.99 (s, 2H, 2OH), 9.49 (s, 4H, 4OH);  $^{13}\text{C NMR}$   $\delta$  (ppm) : 29.42, 31.10, 105.92, 113.20, 137.69, 146.91, 162.73, 166.05;  $\text{IR v cm}^{-1}$ : 3175 (hydrogen bonded O-H), 1618 (C=N), 1527(aromatic C=C), 1323(C-O), 1172 (N-N); **MS**: m/z calc. for  $\text{C}_{19}\text{H}_{17}\text{N}_4\text{O}_8\text{S}_2$  493.0  $[\text{M}+1]^+$ , found 493.0.

### 3.3.7 General procedure for the synthesis of 5, 5'- ((hexane-1,6-diylbis (sulfaneydiyl)) bis(1,3,4-oxadiazole-5,2-diyl)) bis (benzene-1,2,3-triol) (**5d**).

  
5-(5-mercapto-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol **4** (1.92g, 8.4 mmol) was dissolved in 15 ml dry acetonitrile then, 1-bromo-6-chlorohexane (0.2g, 1 mmol) was added to

the mixture at room temperature with stirring. Then, anhydrous potassium carbonate (0.44g, 3.2 mmol) was added to the mixture and continued stirring. The mixture was heated under reflux overnight. The reaction was monitored by TLC elution with CH<sub>3</sub>OH / DCM, 85:15, v/v). The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuum and product was purified by column chromatography (eluent: methanol: dichloromethane 90:10) on silica gel to afford alkylated oxadiazol as yellowish white powder in 80% yield. Yellow white solid in 80% Yield; <sup>1</sup>H NMR (850 MHz), DMSO-d<sub>6</sub> δ (ppm): 1.43 (quintet, 4H, J= 4.25 Hz, 2CH<sub>2</sub>), 1.74 (quintet, 4H, J= 6.8Hz, 2CH<sub>2</sub>), 3.24(t, 4H, J= 7.65 Hz, 2CH<sub>2</sub>), 4.08 (s, 6H, 6OH), 6.86 (s, 4H, Ar-H); <sup>13</sup>C NMR δ (ppm) : 27.67, 29.30, 32.38, 105.76, 112.44, 138.77, 147.19, 162.75, 166.13; IR ν cm<sup>-1</sup>: 3410 (hydrogen bonded O-H), 1528 (C=N), 1457 (aromatic C=C), 1130 (N-N); MS: m/z calcd. for C<sub>22</sub>H<sub>23</sub>N<sub>4</sub>O<sub>8</sub>S<sub>2</sub> 535.1 [M+1]<sup>+</sup>, found 535.

### 3.4. Antioxidant Activity:

The evaluation of antioxidant activity of the compounds was made in comparison with BHT as a soluble oil antioxidant and ascorbic acid as a water-soluble antioxidant using DPPH assay. The assay depends on the discoloration of the stable DPPH free radical from violet colour (lambda maximum 516 nm in methanol) to pale yellow colour. The colour change is dependent on the concentration of the antioxidant.[42] Briefly, 3 ml DPPH solution (0.1 mM, in methanol) was incubated for 30 min. in a dark room with different concentrations of the tested compounds and methanol was added to complete the volume to 10 ml. Since the tested compounds are soluble in DMSO, then stock solutions of 10 mg/10 ml (DMSO: methanol, 1:9 v/v) was made so that different volumes were mixed with DPPH as written above. For having the same condition of measurements, both ascorbic acids and BHT were dissolved in the same solvent mixture (DMSO: methanol, 1:9 v/v) to form two stock solution (10 mg/10 ml). After incubation, the absorbance was immediately measured at 516 nm against methanol spectrophotometrically. The IC<sub>50</sub> (µg/ml) is defined as the necessary concentration of an antioxidant to inhibit 50% of the radicals. The IC<sub>50</sub> was obtained from plotting the antioxidant activity versus the concentration using the following equation.

$$\% \text{ Antioxidant activity} = \left[ \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \right] \times 100\%$$

Data are average values of triplicate experiments.

### Conclusion:

A new series of novel Galloyloxadiazole hybrids were synthesized and fully characterized. The structures of the synthesized compounds were elucidated by spectroscopic techniques such as IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, Mass. The antioxidant activity of this class of compounds was evaluated based on DPPH radical scavenging to reveal comparable activities with ascorbic acid and BHT. All compounds showed very strong antioxidant activity even more than the water-soluble ascorbic acid. Based on antioxidant results, such strong activity is related to the gallic acid moiety as it has three dissociable OH groups via hydrogen atom transfer. The overall results suggest that these compounds could, potentially, be further modified for the formation of more potent antioxidant and more biological studies.

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