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Synthesis, Identification, and Study of the Antimicrobial and Antioxidant Activities of Some New 2,5-Disubstituted-1,3,4-Oxadiazole Derivatives

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Abstract

This study includes the synthesis of 2,5-disubstituted-1,3,4-oxadiazole **3-12** on creatinine derivatives that have antioxidant and microbial properties. The reaction of creatinine with hydrazine hydrate in absolute ethanol was successfully achieved to afford hydrazide derivatives **1,2**. The desired 2,5-disubstituted-1,3,4-oxadiazoles **3-12** were obtained by the reaction of hydrazide derivatives with different aliphatic and aromatic acid derivatives in the presence of $POCl_3$. FT-IR and ^1H NMR spectroscopy were used to determine the structures of some compounds. The antioxidant activity of compounds **8**, **9**, **11**, and **12**, and the antimicrobial activity of compounds **1**, **5**, **11**, and **12** were tested *in vitro* and showed good results.

Keywords: Antimicrobial activity, Antioxidant activity, Hydrazide, 1,3,4-Oxadiazole.

تحضير، تحديد و دراسة الفعالية المضادة للميكروبات و الأكسدة لبعض المشتقات الجديدة من 2،5-ثنائي معوض1,3,4-اوكسادايازول

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الخلاصة

تضمنت هذه الدراسة تحضير 2,5-ثنائي معوض-1,3,4-اوكسادايازول من مشتقات الكرياتينين والتي لها خصائص مضادة للأكسدة و الميكروبات. تم مفاعلة مشتقات الكرياتينين مع الهيدرازين المائي في الايثانول المطلق بنجاح للحصول على مشتقات الهيدرازايد 1,2 . تم الحصول على 2,5-ثنائي معوض 1,3,4-اوكسادايازول 3-12 من تفاعل مشتقات الهيدرازايد مع مشتقات مختلفة من الحوامض الاليفاتية والاروماتية بوجود كلوريد الفوسفوريل. تم استخدام التحليل الطيفي (FTIR, ^1H-NMR) لتشخيص تراكيب المركبات الجديدة. تم اختبار الفعالية المضادة للأكسدة للمركبات 8،9،11 و 12 والمضادة للميكروبات للمركبات 1،5،11 و 12 في المختبر و اظهرت نتائج جيدة.

1. Introduction

Different strategies were reported to develop new heterocyclic compounds in order to produce therapeutic biological products. The compounds containing the hydrazide-hydrazone moiety have significant antibacterial, anticancer, and antioxidant properties [1,2]. Oxadiazole

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is a five-membered heterocyclic molecule that contains two nitrogen atoms and one oxygen atom [3]. It is one of the most commonly studied heterocyclic for its anticancer, antioxidant properties, and antimicrobial effects [4-7]. There are several different synthetic routes that can be used to synthesize 1,3,4-oxadiazole and its derivatives. Generally, 1,3,4-oxadiazole can be produced by the reaction of acid hydrazide or hydrazine with carboxylic acids/acid chlorides, as well as by the ring-closing of diacyl hydrazines with different cyclizing agents such as phosphorus oxychloride, thionyl chloride, phosphorus pentaoxide, polyphosphoric acid, and acetic anhydride [8-12]. In our work, 2,5-disubstituted-1,3,4-oxadiazole derivatives will be synthesized in two steps, and the prepared compounds will be characterized and studied for their biological activities

2. Experimental part

2.1. Materials and instruments

All the chemicals were purchased from BDH, Merck, and Fluka and used without further purification. The melting points were determined using a Gallen Kamp apparatus and are uncorrected. A Shimadzu FTIR-8400s Fourier transform infrared spectrophotometer was used to record the FT-IR spectra for the chemicals in a KBr disc. ^{1}H NMR spectral data for some of the synthesized compounds were measured using a Bruker spectrophotometer (400 MHz). TLC was performed on Merck silica gel 60 F_{254} and visualized by iodine vapor.

2.2. Syntheses

*2.2.1. Synthesis of hydrazide derivatives **1** and **2***

Creatinine derivatives **1** and **2** were prepared according to the literature procedure [13]. A mixture of creatinine derivatives (0.01 mol) and hydrazine hydrate (0.03 mol, 99%) in absolute ethanol (20 mL) was refluxed for 6 hours. The solvent was then evaporated, and the residue solid material was washed with water before recrystallization from ethanol [14]. The physical properties of the prepared compounds are listed in Table 1.

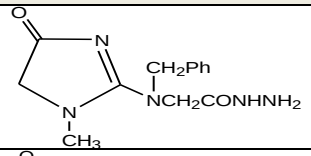
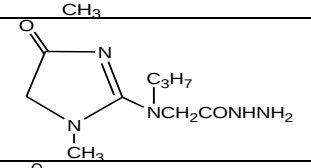
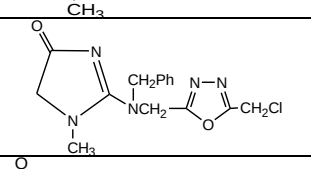
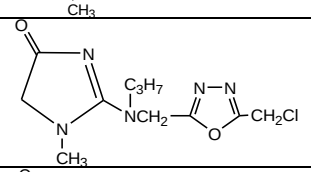
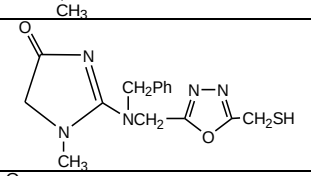
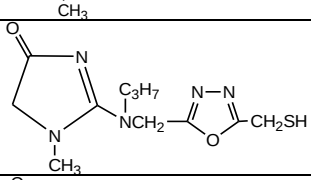
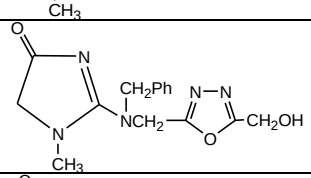
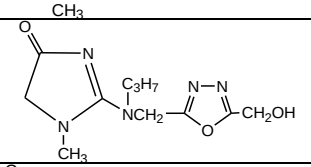
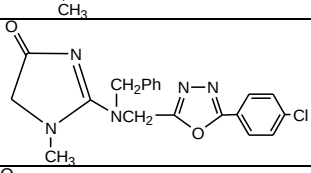
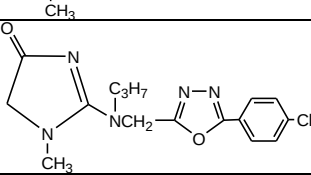
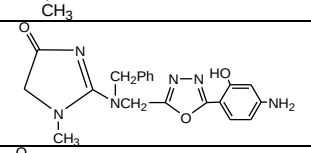
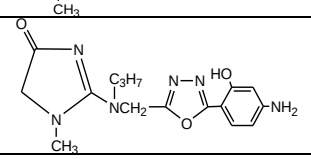
*2.2.2. Synthesis of 2-alkyl-5-substituted-1,3,4-oxadiazoles **3-8***

In a round-bottomed flask, a mixture of hydrazide derivatives **1** or **2** (0.001 mol), acetic acid derivatives (2-chloroacetic acid, 2-mercaptoacetic acid, or 2-hydroxyacetic acid) (0.001 mol), and phosphorus oxychloride (5 mL) was heated to reflux for 4 hours. The reaction mixture was then left to cool at room temperature before adding ice-cold water, filtration of the solid material, drying, and recrystallization from ethanol [15]. The physical properties of prepared compounds are given in Table 1.

*2.2.3. Synthesis of 2-aryl-5-substituted-1,3,4-oxadiazoles **9-12***

A mixture of hydrazide derivatives **1** and **2** (0.001 mol), aromatic acid derivatives (4-chlorobenzoic acid or 4-aminosalicylic acid) (0.001 mol), and $POCl_3$ (3 mL) was dissolved in 1,4-dioxane (10 mL) before refluxing for 10-12 hours. A solution of sodium bicarbonate (10%) was used to neutralize the reaction mixture. The solid crude material was then filtered, dried, and recrystallized from ethanol [16]. Table 1 lists the physical properties of the title compounds **9-12**.

Table 1: Physical properties of compounds **1-15**

Comp. No.	Structure	Molecular formula	M.wt (g/mol)	Yield (%)	M.p (°C)	Color
1		$C_{13}H_{17}O_2N_5$	275.31	80	185-187	Pale Yellow
2		$C_9H_{17}N_5O_2$	227.26	85	212-213	White
3		$C_{15}H_{16}ClN_5O_2$	333.77	70	110-112	Yellow
4		$C_{11}H_{16}ClN_5O_2$	285.73	64	135-137	Yellow
5		$C_{15}H_{17}N_5O_2S$	331.39	79	107-109	Brown
6		$C_{11}H_{17}N_5O_2S$	283.35	83	125-127	Yellow
7		$C_{15}H_{17}N_5O_3$	315.33	63	179-181	Yellow
8		$C_{11}H_{17}N_5O_3$	267.13	88	79-81	Yellow
9		$C_{20}H_{18}N_5O_2Cl$	365.84	85	84-86	Orange
10		$C_{16}H_{18}N_5O_2Cl$	347.80	90	95-97	Off White
11		$C_{20}H_{20}N_6O_3$	392.41	89	180-182	Off White
12		$C_{16}H_{20}N_6O_3$	344.37	83	158-160	Off White

2.3. Antioxidant activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to test the samples' DPPH radical scavenging activities in **8**, **9**, **11**, and **12**. The DPPH solution in methanol was prepared daily at a concentration of 20 mg/L [17]. To a 1.5 mL of DPPH solution, 0.75 mL of sample or standard solution (25, 50, and 100 ppm) was added. The absorbance values were read at 517 nm against the blank after 30 minutes in the dark, and calculations were done using the formula below.

DPPH Radical Scavenging Activity (%) = $[(A_0-A_1) / (A_0)] \times 100$

A_0 is the absorbance control value; A_1 is the sample or standard absorbance value.

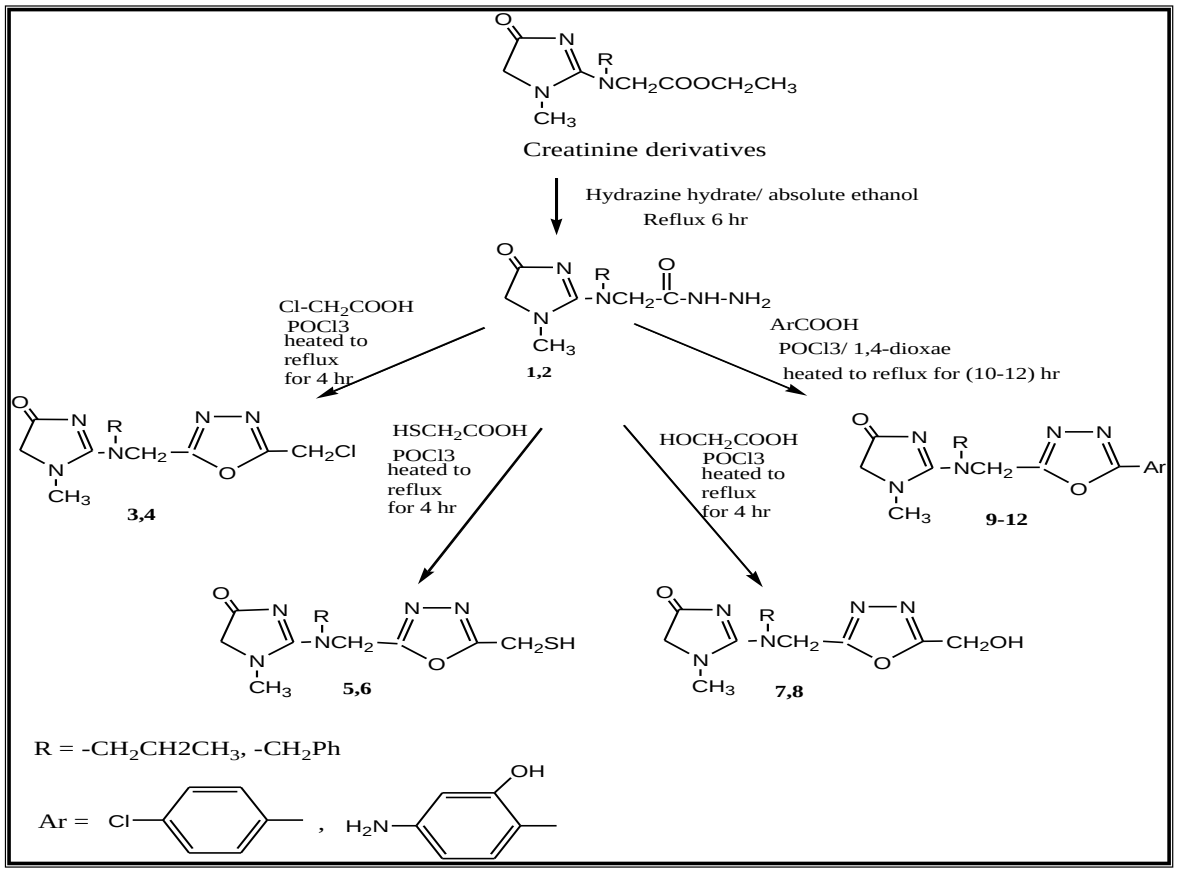
2.4. Antibacterial activity

The agar cup plate method was used to evaluate the biological activity of some of the produced compounds **1**, **5**, **11**, and **12** against *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria), as well as two against types of fungi (*Aspergillus flavus* and *C. albicans*). DMSO was used as a solvent, and the organisms were Borer cups were taken from the agar medium contained in a Petri plate that had previously been infected with the organism using sterilized cork. The Petri dishes were covered with the test compound solution (0.1 mL), and then incubated at 37 °C for 48 hours [18].

3. Result and discussion

3.1. Chemistry

Scheme 1 displays the synthetic route for the preparation of compounds **1-12**. Characteristic bands in the FT-IR spectra of the hydrazide derivatives were appeared between 1668 and 1690 cm^{-1} due to C=O amide) and absorptions in 3217-3284 cm^{-1} for N-H and NH_2 bands. The characteristic absorption bands are shown in Table 2.



Scheme 1: Synthesis of compounds **1-12**

Table 2: FT-IR spectral data (υ, cm-1) of hydrazide derivatives 1 and 2

Compound number	C=N and C-N	C-H aliphatic	C-H aromatic	C=C aromatic	C=O cyclic amide and C=O amide	NH	NH_2
1	1643, 1334	2983 2812	3050	1575 1502	1718, 1668	3217	3419 3375
2	1643, 1338	2941 2808	-	-	1720, 1690	3284	3415 3367

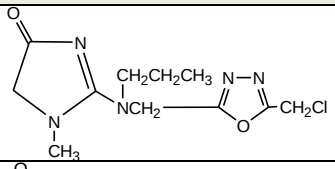
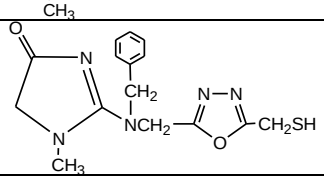
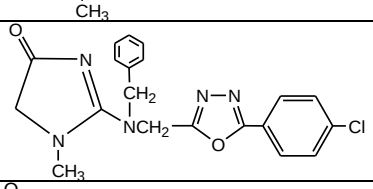
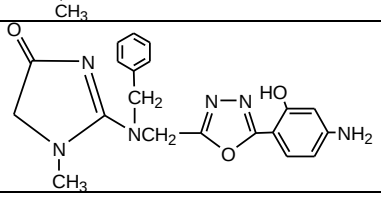
The FT-IR spectra of 2,5-disubstituted-1,3,4-oxadiazoles **3-12** showed new absorptions for the C-O-C and N-N bands, and the C=O amide absorptions disappeared. Table 3 shows the absorption bands of **3-12** [19].

Table 3: FT-IR spectral data (υ, cm^{-1}) of compounds **3-12**

Compound number	C=N C-N	N-N	C-O-C	C-H aliphatic	C=O cyclic amide	C-H aromatic	C=C aromatic	Other
3	1640 1334	1438	1141	2937 2881	1712	3002	1575 1540	649 (C-Cl)
4	1635 1336	1425	1145	-	1685	-	-	649 (C-Cl)
5	1641 1342	1415	1143	2906 2870	1710	3050	1585 1566	2324 (S-H) 651 (C-S)
6	1633 1334	1423	1130	2939 2883	1710	-	-	2332 (S-H) 649 (C-S)
7	1640 1336	1421	1132	2910 2800	1699	3004	1575 1530	3463 (O-H)
8	1635 1336	1423	1132	2939 2877	1720	-	-	3490 (O-H)
9	1640 1325	1421	1130	2939 2875	1679	3050	1573 1520	649 (C-Cl)
10	1645 1326	1423	1130	2937 2883	1677	3097	1573 1530	649 (C-Cl)
11	1640 1336	1425	1132	2935 2850	1685	3020	1575 1550	3452 (O-H) 3431 3409 (NH_2)
12	1640 1334	1415	1130	2937 2835	1699	3055	1573 1530	3460 (O-H) 3460 3396 (NH_2)

The ^{1}H NMR spectral data were measured for some of the synthesized compounds (**7**, **8**, **12**, and **14**), as shown in Table 4 [20].

Table 4: ^{1}H NMR spectral data (δ, ppm) of some prepared compounds (**7**, **8**, **12**, and **14**)

Compound number	Compound structure	^{1}H NMR spectral data (δ, ppm)
4		0.9 (3H, t), 1.8 (3H, s), 2.5 (2H, m), 3.0 (2H, t), 3.2 (2H, t), 4 (2H, s), 4.1 (2H, s)
5		1.3 (3H, s), 2.9 (2H, t), 3.2 (2H, s), 3.6 (2H, s), 3.8 (2H, s), 7.3-7.4 (5H, m), 10.3 (1H, s)
9		1.2 (3H, s), 3.4 (2H, t), 3.5 (2H, s), 4.1 (2H, s), 7.1-7.9 (9H, m)
11		1.2 (3H, s), 3.3 (2H, s), 3.3 (2H, t), 3.4 (2H, s), 3.7 (2H, s), 7.4-8.3 (8H, m), 9.7 (1H, s)

3.2-Antioxidant activity

DPPH scavenging activity was dose-dependently observed in some compounds **8**, **9**, **11**, and **12**. The evaluated compounds **8**, **9**, **11**, and **12** showed DPPH scavenging ranging from 0.027, 0.023, 0.028, and 0.057 to 0.125, 0.113, 0.18, and 0.3, respectively, in accordance with Table 5 and Figure 1. Compound **12** showed the greatest DPPH scavenging activity, whereas compounds **8**, **9**, and **11** showed only moderate DPPH recycling in comparison to vitamin C [22].

Table 5: Antioxidant activity of compounds **11, 12, 14**, and **15**

Comp. ~~Conc.~~	25 ppm	50 ppm	100 ppm
8	0.027	0.053	0.125
9	0.023	0.054	0.113
11	0.028	0.067	0.18
12	0.057	0.145	0.3
Vitamin C	0.45	0.67	0.8

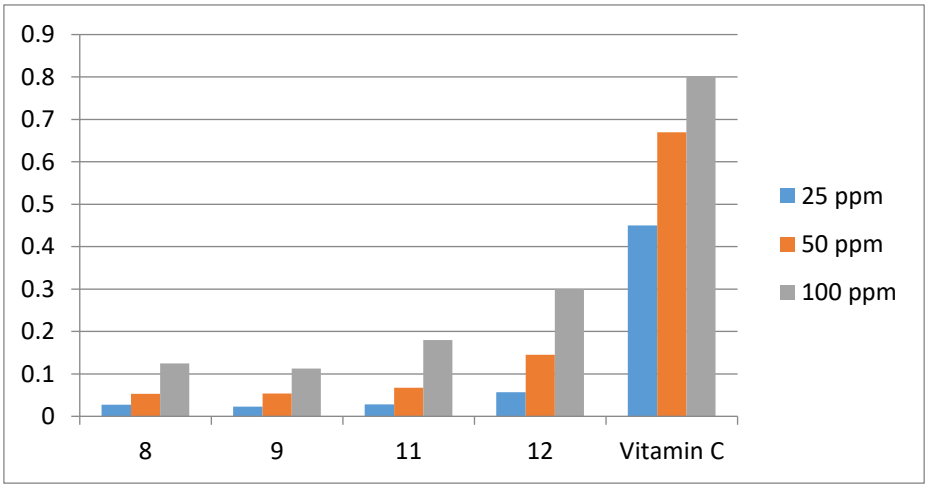


Figure 1: Antioxidant activity of compounds **8**, **9**, **11**, and **12**

3.3. Antibacterial activity

The synthesized compounds **1, 5, 11**, and **12** showed different biological activities against two types of bacteria: *staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) bacteria, using amoxicillin as a standard, as well as two kinds of fungi (*Aspergillus flavus* and *C. albicans)* in comparison to fluconazole as a standard. The results of the experiment showed that compounds **1**, **5**, **11**, and **12** are active against *staphylococcus aureus*. While compounds **1** and **5** showed the highest activity against *Escherichia coli* except for compound **11**, which was inactive, and compound **12**, which was moderately active. On the other hand, compounds **1** and **5** showed the highest activity toward *C. albicans*, while compounds **11** and **12** were inactive [23]. Additionally, compound **1** was highly active against *Asp.nigger* whereas compounds **5, 12**, and **12** were inactive, as shown in Table 6.

Table 6: Antimicrobial activity of compounds **2**, **4**, **8**, **14**, and **15**

Compound Number	*Staphylococcus*	*Escherichia coli*	*C.albicans*	*Aspergillus flavus*
	Conc. (0.02 g/ml) Inhibition zone diameter (mm)	Conc. (0.02 g/ml) Inhibition zone diameter (mm)	Conc. (0.02 g/ml) Inhibition zone diameter (mm)	Conc. (0.02 g/ml) Inhibition zone diameter (mm)
1	15	30	25	20
5	25	32	30	-
11	11	-	-	-
12	11	11	-	-
Amoxicillin	32	-	-	-
Fluconazole	-	-	25	27

4. Conclusion

New 2,5-disubstituted-1,3,4-oxadiazoles bearing a creatinine moiety were synthesized in yields of 63-90%. These compounds (**3**-**12**) were identified using FT-IR and ^{1}H NMR spectroscopy. Some of the produced compounds were examined for biological activity, such as antioxidants (compounds **8**, **9**, **11**, and **12**), anti-fungi, and anti-bacteria (compounds **1**, **5**, **11**, and **12**). The antioxidant effect of compound **12** was the highest compared with vitamin C, while compounds **8**, **9**, and **11** were lower. On the other hand, compounds **1** and **5** were found to have strong antifungal and antibacterial activity when compared with standard drugs.

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