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Synthesis, spectroscopy and electrochemistry of new 4-(4-acetyl-5-substituted-4, 5-dihydro-1, 3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones as a novel class of potential antibacterial and antioxidant derivatives

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ABSTRACT

The synthesis of the new 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones derivatives **5** was accomplished by the use of 4-hydroxycoumarine as a starting material. The structures of the compounds were confirmed by analytical UV, IR, ^{1}H, ^{13}C-NMR, NOESY and HMBC NMR spectra to elucidate the different positions of protons and carbons. All the compounds exhibited one quasireversible redox process. The UV absorption spectra of the obtained compounds showed strong absorption bands between 264 and 291 nm assigned to π-π^* transitions of the oxadiazole group. All the newly synthesized compounds were screened for their antibacterial and antioxidant activities. Antimicrobial studies revealed that compounds **5a** and **5b** showed significant antibacterial activity against *Escherichia coli* and *Pseudomonas Aeruginosa* 27853. Furthermore these compounds showed antioxidant activities of different extents with respect to individual compounds as well as to the antioxidant methods. The compounds **5a-d** was found to be the most active antioxidant in the series then Trolox, which makes the investigated complexes promising a new class of antibacterial compounds.

1. Introduction

1,3,4-Oxodiazole derivatives are highly attractive compounds for the development of materials for organic electroluminescent (EL) devices since they possess high electron-accepting properties and display strong fluorescence with high quantum yield [1]. This behavior is exemplified by 2,5-diphenyl-1,3,4-oxodiazole and 2,5-di-2-naphthyl-1,3,4-oxodiazole, for which quantum yields of 0.80 and 0.85 in cyclohexane solution, respectively, were reported [2]. Thus, compounds involving 1, 3,4-oxadiazole rings have been used as electron transporting materials and emitters in organic EL devices [3–5]. Recently 1,3,4-oxodiazole derivatives have aroused considerable interest in the area of organic light-emitting diodes (OLEDs) [6–9]. Oxadiazole fragments have also been connected to classical chelating ligands (such as bipyridines) in luminescent complexes, to obtain multifunctional (emitting and charge transporting) molecular species [10–17]. Furthermore, it has been reported that substituted 1, 3,4-oxadiazole derivatives show a broad spectrum of biological activity including anticancer effects [18,19]. In recent years, the application of 1,3,4-oxodiazole consisting of five membered heterocyclic ring have been described [20–24].

On the other hand, coumarins and structurally related compounds have been shown to inhibit replication of HIV

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and thus exhibit a therapeutic potential [25]. A large number of structurally novel coumarin derivatives have been reported to show substantial cytotoxic and anti-HIV activity in vitro and in vivo [26,27]. A variety of synthetic coumarins have unique mechanisms of action referring to the different stages of HIV replication [28]. Thus, coumarins are important lead compounds for the development of antiviral and/or virucidal drugs against HIV [29–31].

Keeping in view of the properties of 1,3,4 oxodiazole derivatives and in prolongation of our research on biologically active molecules [32,33], we have carried out the present study to describe new convenient and general procedures to afford novel 2-[(coumarin-4-oxy)-methyl]-4-acetyl-5-substitued–1,3,4-oxodiazole **5** containing 4-hydroxycoumarine moieties and investigate both their electrochemistry and antimicrobial properties against *Staphylococcus aureus* (CIP 7625), *S. aureus*, *Escherichia coli ATCC 25922*, *Klebsiella pneumonia CIP 104727* and *Pseudomonas aeruginosa* 27853 (CIP 76110) using the agar disk diffusion assay. The antioxidant properties of these compounds have been studied using two different test methods, namely 2,2-diphenyl-1-picryl-hydrazyl and ABTS radicals, respectively. The differences of radical scavenging and antioxidant properties of 2-[(coumarin-4-oxy) methyl]-4-acetyl-5-substitued–1,3,4-oxodiazole were compared with similar doses of Trolox, a standard antioxidant commonly used in food and pharmaceutical industries.

2. Results and discussion

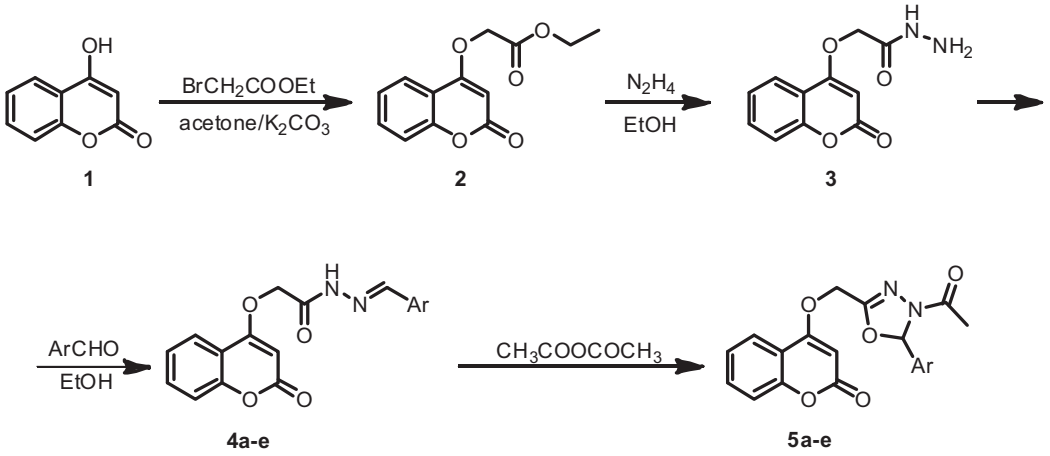
Compound **2** was prepared by reaction of 4-hydroxycoumarine with ethylbromoacetate in the presence of anhydrous potassium carbonate in dry acetone, followed by refluxing with hydrazine hydrate in absolute ethanol, generating a colorless crystalline product for which structure **3** was assigned.

The resulting of carbohydrazide **3** was reacted with arylaldehydes to give rise the (E)2-(coumarin-4-oxyacetic)-N-benzylideneacetohydrazide **4**, which precipitated by mixing the carbohydrazide **3** and the corresponding ArCHO in hot ethanol.

The obtained compounds **4** were then refluxed with acetic anhydride to give the corresponding 4-(4-acetyl-5-substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** in good yields according to Scheme 1.

All the new 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5**, have been characterized by UV, IR, 1H, ^{13}C-NMR spectra as well as by NOESY and HMBC NMR experiments to elucidate their structures and assign completely the structural network of both protons and carbons. The obtained spectral data were in accordance with the proposed structures.

As example, the IR spectra of compound **5c** showed the characteristic absorption bands for 1612(C=C), 1472(N=N) and 1289(C–O–C). In addition, the detection of a strong C=N stretching band at 1555 cm^{-1} evidenced the formation of the 1,3,4-oxodiazol ring. The ^{1}H NMR spectra of **5c** displays a signal at δ 6.07 ppm that ascribable to the proton H-3 from the coumarine moiety. A characteristic singlet proton signal at δ 8.53 ppm was assigned to H-5' proton from the oxodiazole fragment. In addition, the aromatic protons (both coumarinic and oxodiazolinic) are observed between δ 7.08 and δ 7.91 ppm (see experimental), and the expected singlet for methylenic moiety is observed at δ 5.47 ppm. The methyl protons of the acyl group arose at δ



Compound		Ar
4a	5a	phenyl
4b	5b	4-fluorophenyl
4c	5c	4-methoxy-phenyl
4d	5d	4-nitro-phenyl
4e	5e	3,4,5-trimethoxy-phenyl

Scheme 1. Synthesis of 4-(4-acetyl-5- substituted -4, 5-dihydro-1, 3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones5.

2.39 ppm. The singlet at 3.84 ppm was assigned to the three protons of the methoxy group. Full assignment of the ^{1}H NMR spectra of **5c** was deduced from the NOESY spectrum. An observed NOE cross peak between both H-5' and methylic proton CH_3 placed these two units on the same side of the oxodiazole ring while H-3 and CH_2 are, therefore, on the other side. The ^{13}C{1H} NMR spectrum of **5c** in DMSO-d_6 showed two downfield signals at δ 166.7 ppm ($C_{2'}$) and δ 162.0 ppm (lactone C_2=O) as well as a signal to up field at δ 55.9 ppm (OCH_3). The signals at δ 26.0, δ 91.9 and δ 168.1 ppm were assigned to the $COCH_3$, the C_3 and $C_{5'}$, respectively.

The structure of **5c** was finally elucidated through the analysis of the ^{1}H, ^{13}C HMBC spectrum (Fig. 1), which indicates that the methylenic protons at δ 5.47 ppm correlate with $C_{2'}$ (δ 166.7 ppm) and C_4 (δ 164.8 ppm). The hydrogen atom H-5', at 8.53 ppm, correlate with Ca (δ 125.5 ppm). Moreover, the proton H-3 (δ 6.07 ppm) correlates with C_4 (δ 164.8 ppm). Furthermore, methylic protons CH_3CO (δ 2.39 ppm) and the CO carbon signal (δ 171.2 ppm) are also correlated.

Assignment of the protons of 2-[(coumarin-4-oxy) methyl]-4-acetyl-5-substitued–1,3,4-oxodiazole **5** was deduced from their NOESY spectra. An observed nOe cross peak between $H_{5'}$ and methylic proton CH_3 places these two units on the same side of the average oxadiazoline ring plane. H_3 and H_b are therefore on the other side of this plane.

A mechanistic rationalization for this reaction is provided in Scheme 2.

The formation of the 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** occurred via two steps: the carbohydrazide 3 initially formed rearranges to generate a negative oxygen which then attacks on the C=N bond, followed by attack of the nitrogen atom to the C=O bond of the acetic anhydride accompanied by the loss of acetic acid yielding the 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5**.

3. Absorption spectral characteristics of the compounds 5a–e

The UV spectra of the compounds **5** measured in methanol solutions are shown in Fig. 2. As shown in this figure compounds **5** display similar absorptions ranging from 200 to 350 nm that are attributed to π- π* transition of conjugate system.

The lowest energy absorption bands are from the π- π* transitions by virtue of their large molar extinction coefficients. Two absorption bands were observed for **5a** and **5e** respectively, while only one was observed for **5b**.

Comparing compounds **5b** and **5e**, electron-withdrawing group (F) result in a blue shift (6 nm) with contrast to an electron-donating group (3.4.5 MeO). Compounds **5** have almost same maximum absorption (200–350 nm) that means substituent R in para position does not influence the absorption.

4. Electrochemistry

Electrochemical studies of 4-(4-acetyl-5- substituted -4, 5-dihydro-1, 3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** are of interest due to the electron-deficient nature of the oxadiazole unit.

Hence, the electrochemical properties of compounds **5a–e**, were determined by cyclic voltammetry in CH_3CN (1×10^{-3} M) solutions, using 0.1 M tetrabutylammonium bromide ($C_4H_{12}BrN$) as the supporting electrolyte. Both platinum and gold were used as working electrodes, Ag/AgCl (0.1 M) as the reference electrode, and platinum as the counter electrode. Under these electrochemical conditions, **5** show a quasi-reversible behavior for the first reduction process. This can be deduced from the fact that the cathodic–anodic peak separations (Epc–Epa) are ca. 100 mV. The ratio of the peak current intensity for the cathodic and anodic processes is about 0.5–0.7. As expected, the reduction peak potential of the 2-[(couma-

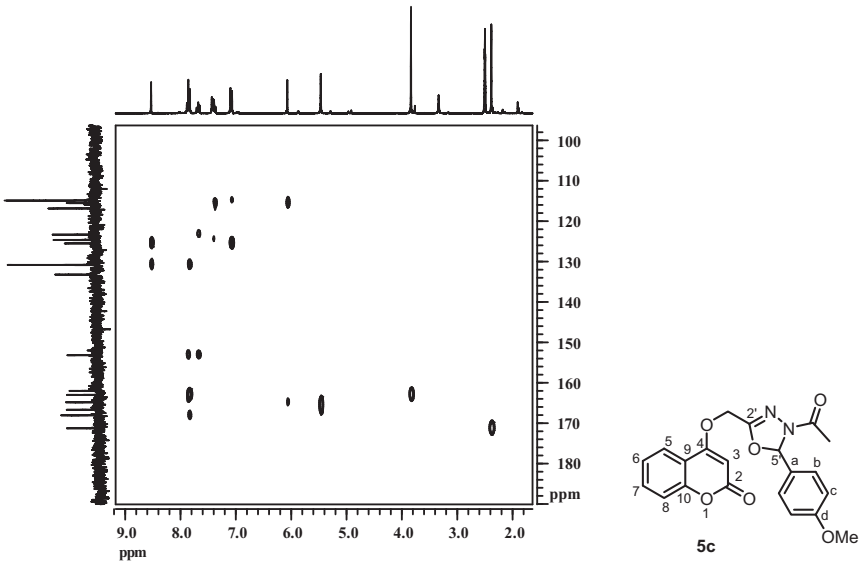
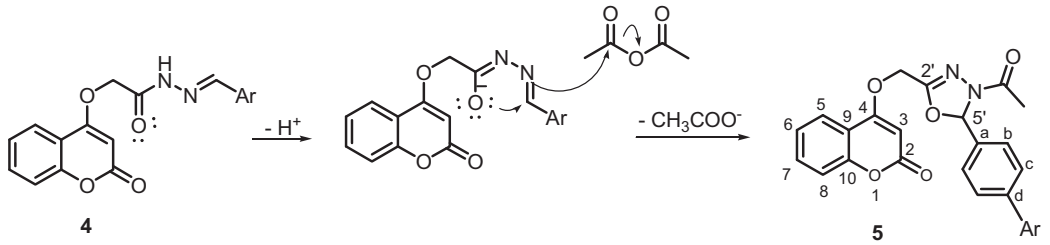


Fig. 1. ^{1}H ^{13}C HMBC spectrum of compound **5c**.



Compound	Ar
5a	phenyl
5b	4-fluorophenyl
5c	4-methoxy-phenyl
5d	4-nitro-phenyl
5e	3,4,5-trimethoxy-phenyl

Scheme 2. Proposed mechanism for the synthesis of **5a-e**.

rin-4-oxy) methyl]-4-acetyl-5-substitued–1,3,4-oxodiazole **5** are strongly influenced by the para substitutent on the phenylene ring. Compared to the unsubstituted compound **5**, the methoxy group, having a slight electron donor behavior, shifts the reduction peak potential of **5** to more negative values. The redox behaviors of all the 1,3,4-oxadiazolines are summarized in Fig. 3. All the compounds exhibited one quasireversible redox processes.

For example, compounds **5d** showed one quasireversible reduction at −0.8 and −1.15 V, respectively. We assume that the curve at lower reduction potential may be due to the more electron-deficient dications in the ring system, and the curve at higher reduction potential can be attributed to the redox behavior of the oxadiazole unit.

5. Antibacterial and antioxidant studies

5.1. Free radical scavenging activity assay

The free radical scavenging activity of the new 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** was tested by utilizing DPPH scavenging [34].

DPPH is a free radical and accepts one electron or one-hydrogen radical to become a stable diamagnetic molecule [35]. The reduction capability of DPPH radical was determined by the decrease in absorbance induced by 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5**. Briefly, 1.5 ml ethanolic solution of the synthesized compounds (0.2 mM) was added to 1.5 ml (0.2 mM) solution of DPPH radical in ethanol (final concentration of DPPH and synthesized compounds was 0.1 mM). The mixture was shaken vigorously and allowed to stand for 30 min. After this, the absorbance at 534 nm was determined and the percentage of scavenging activity was calculated using the following formula:

$$\text{Scavenging activity} = \{[\text{Ab} + \text{As} - \text{Am}]/\text{Ab}\} \times 100\%$$

Ab: absorbance of 0.1 mM ethanolic solution of DPPH at 534 nm, As: absorbance of 0.1 mM ethanolic solution of test compound at 534 nm, Am: absorbance of ethanolic mixture of the drug and DPPH at 534 nm.

Trolox was used as reference compound. All tests and analyses were undertaken and averaged on three samples. The results are given in Scheme 3.

Among the compounds from the 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-

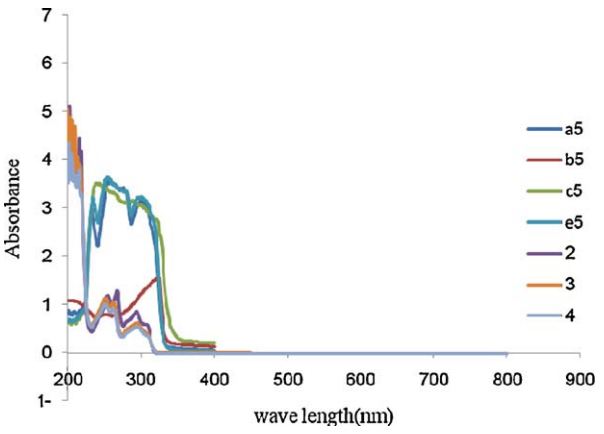


Fig. 2. The UV spectra of the compounds **5a–d** and **2–4** in methanol ($2\ 10^{-5}$ M).

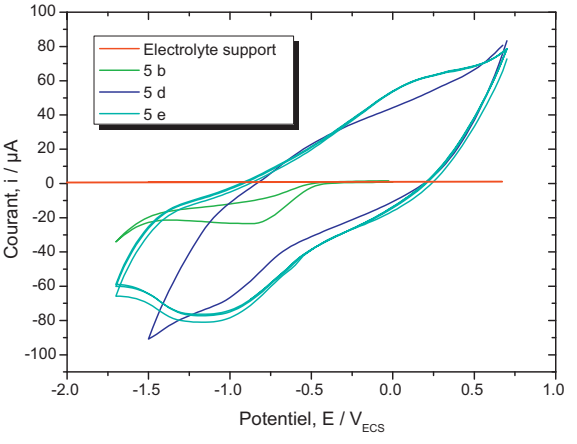
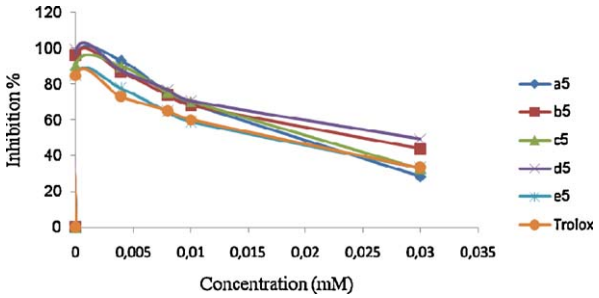
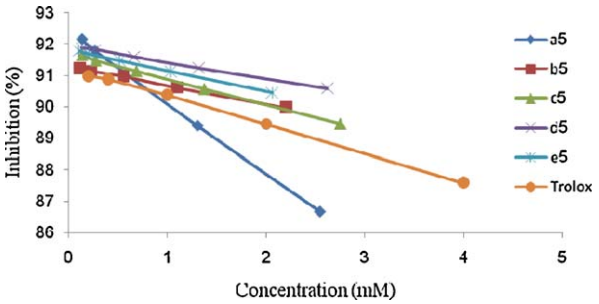


Fig. 3. Cyclic voltammograms of compounds **5d**, **5b** and **5e** (1×10^{-3} M) in CH_3CN, scanned at 100 mVs^{-1}.



Scheme 3. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of compounds **5**.



Scheme 4. Scavenging ability on ABTS radical of compounds **5**.

chromen-2-ones **5** series **5b** showed moderate antioxidant activity.

The activity exhibited by the compound **5e** was the highest. In addition the experimental data show that compound **5a** has a stronger effect of scavenging free radical than Trolox.

According to the experimental results, we can note that any increase in the concentration of the obtained 1,3,4 oxodiazole in the medium involves an attenuation of percentage of inhibition. Indeed, this last can reach 90% for a concentration about 1 μM for all the synthesized products. Thus, we can conclude that substituent's on the aryl group does not have an influence on the anti-oxidant activity.

One parameter that has been introduced recently for the interpretation of the results from the DPPH method is the efficient concentration or EC_{50} value (otherwise called the IC_{50} value); this is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color), and it is corresponding to the endpoint of the titration. It should be noted that in all cases, any residual (yellow) color from the reduced form or any non specific absorbance from the sample has to be taken into account in defining the "endpoint" of the titration, or the 50% point, this IC_{50} parameter also has the drawback that the higher the antioxidant activity, the lower is the value of EC_{50}.

The EC_{50} values exhibited by 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** are summarized in Table 1.

It was found that oxodiazoles **5a–d** possess higher activity than Trolox with EC_{50} value of 0.00521, 0.005217, 0.00551and 0. 00506 mmol.l^{-1} respectively followed by **5e** with EC_{50} value of 0.00591. The low activity of the compound **5e** is attributed to the methoxy groups.

Table 1
The EC_{50} values exhibited by 4-(4-acetyl-5- substituted -4, 5-dihydro-1, 3, 4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones 5.

Compounds **5a–e**	EC_{50} (mmol.l^{-}1)
5a	0.00521
5b	0.005217
5c	0.00551
5d	0.00506
5e	0.00591
Trolox	0.005879

5.2. ABTS radical cation decolorization Assay

The potential of 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** to scavenge free radicals was also assessed by their ability to quench $ABTS^{+}$. Scheme 4 depicts the concentration-dependent decolourization of $ABTS^{+}$.

ABTS radical-scavenging activity of 4-(4-acetyl-5-substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** was determined according to Re et al. [30]. The $ABTS^{+\cdot}$. Cation radical was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. In a final volume of 1 ml, the reaction mixture comprised 950 μl of ABTS± solution and 50 μl of the 1,3,4 oxadiazoles **5** at various concentrations. The reaction mixture was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 ll of $ABTS^{+}$ solution and 50 μl of Trolox. As for the antiradical activity, ABTS scavenging ability was expressed as EC_{50} (<mu> g/ml). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging effect}\% = \{[A_0 + A_1 - A_0]\} \times 100\%$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

As shown for DPPH scavenging, these data indicate the higher capacity of 4-(4-acetyl-5- substituted -4,5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** to quench $ABTS^{+}$ as compared to the synthetic antioxidant Trolox.

The variation of the percentage of inhibition (PI) is almost constant starting from a value of the concentration equal to 1.34 mM. In addition, the synthesized products **5** have an antioxidant activity better than Trolox. Indeed, the antioxydant capacity seems to be attenuated when the concentration increases in the medium. This can be explained by the existence of the peroxides sites, which are susceptible for oxidizing when the concentration

Table 2
The EC_{50} values exhibited by 4-(4-acetyl-5- substituted -4, 5-dihydro-1, 3, 4-oxodiazol-2-yl) methoxy)-2H-chromen-2-ones 5.

Compounds **5a–e**	CI_{50} (mmol.l^{-1})
5a	0.073
5b	0.072
5c	0.07
5d	0.056
5e	0.06
Trolox	0.109

increases. We have just shown that the synthesized oxdiazole derivatives **5a–e** have a good antioxidant activity under weak concentration, but it proves to be necessary to determine the reaction time necessary to highlight the antioxidant effect to be able to use these derivatives in pharmay.

The EC_{50} values exhibited by 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl) methoxy)-2H-chromen-2-ones **5** are summarized in Table 2.

The 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** were shown to be efficient antioxidants. They showed higher free radical scavenging activity than Trolox scavenging activities.

These compounds have a remarkable capacity oxidizing which explains their susceptibility to fix free radicals DPPH and $ABTS^{+}$.

5.3. Antibacterial activity

The antibacterial activity of 4-(4-acetyl-5- substituted -4, 5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-ones **5** was assessed by the agar disk diffusion assay [36] against five human pathogenic bacteria: *Gram-positive* including *S. aureus* (CIP 7625), *S. aureus* and Gram-negative bacteria including *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853)(CIP 76110) and *K. pneumonia* CIP 104727. The bacterial strains were first grown on Muller Hinton medium at 37 °C for 24 h prior to seeding onto the nutrient agar. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs and compared with the known antibiotic *gentamycin*. Standard discs of *gentamycin* (10 UI) served as positive antibiotic controls according to CASFM 2005 guidelines. Discs with 10 μl of pure methanol were used as negative controls. The results are given in Table 3.

5.3.1. Methicillin-resistant clinical isolates

An examination of the data reveals that all the compounds showed antibacterial activity ranging from 25 to 100 μg ml^{-}1. The compounds **5a** and **5e** were highly active against all the five organisms employed. Compound **5c** was highly active against *E. coli*. From the screened results, it is observed that the presence of methoxy/NO_2 group at the phenyl ring increases the antibacterial activity. The activity is maximum in a compound with a methoxy group at 4th position.

Table 3
Antibacterial activity spectrum of compounds **5a–e**.

Indicator organism	Inhibition zone (mm)	Compounds
Staphylococcus aureus (CIP 7625)	34	**5a**
	27	**5b**
	28	**5c**
	33	**5d**
	34	**5e**
	24–28	*Gentamycin*
*Staphylococcus aureus**	26	**5a**
	28	**5b**
	27	**5c**
	33	**5d**
	34	**5e**
	24	*Gentamycin*
Escherichia coli ATCC 25922	30	**5a**
	27	**5b**
	35	**5c**
	32	**5d**
	31	**5e**
	22–26	*Gentamycin*
Klebsiella pneumonia CIP 104727	25	**5a**
	27	**5b**
	31	**5c**
	30	**5d**
	28	**5e**
	21	*Gentamycin*
Pseudomonas aeruginosa 27853 (CIP 76110)	30	**5a**
	26	**5b**
	34	**5c**
	33	**5d**
	32	**5e**
	15–22	*Gentamycin*

The residual antibacterial activity in the compounds was tested by disc diffusion assay against the indicator strain in LB medium at 28 °C
ATCC: American Type Culture Collection, USA; CIP: collection de l'Institut Pasteur, Paris, France; LM: laboratoire de microbiologie, Centre national de Greffe de Moelle Osseuse, Tunis, Tunisia.

6. Conclusions

A new versatile synthetic route to 4-(4-acetyl-5-substituted -4, 5-dihydro-1, 3,4-oxodiazol-2-yl) methoxy)-2H-chromen-2-ones **5** by the treatment of 4-hydroxycoumarine with different reagents is described. The method is easy; rapid and produced the title compounds **5** in good yields: the structures were verified by, UV/IR| 1D| 2D NMR. All the newly synthesized compounds were screened for their antibacterial and antioxidant activities. Among the screened samples, compounds **5a** and **5b** showed excellent antibacterial activity against E. coli. Compounds with 4-phenyl, 4-methoxyphenyl, 4-fluorophenyl and 4-nitrophenyl substituents in the 1,3,4-oxodiazole ring exhibited highest antioxidant activity than Trolox and 1,3,4- oxodiazole bearing 3.4.5 trimethoxysubstituent in the phenyl ring exhibited enhanced antioxidant activity from the respective series. Absorption spectral characteristics of the compounds were investigated in methanol by UV absorption and they were correlated with substituent on benzene rings.

7. Experimental

7.1. General

All reactions were magnetically stirred. Commercially available reagents were used without further purification. All chemicals were supplied from Aldrich, Merck and Fluka Co. Melting points were determined by open capillary method and were uncorrected.

All reactions were monitored by thin layer chromatography (TLC). Compounds were visualized with UV light at 254 and 365 nm. Melting points were measured on a WRX-1S instrument. Infrared (IR) spectra were recorded with a Perkin-Elmer spectrum one B spectrometer. ^{1}H NMR spectra were recorded on a Varian-Unity spectrometer at 300 MHz using tetramethylsilane (TMS) as an internal standard. UV absorption spectra were recorded on a Lambda 20 UV spectrometer Perkin Elmer. Cyclic voltammetry (CV) was performed on a BAS 100BW electrochemical workstation. All CV measurements were carried out in tetrabutylammonium bromide ($C_4H_{12}BrN$) as a supporting electrolyte, purging with nitrogen prior to conduct the experiment. Platinum wire (MF-2013) was used as a working electrode, Ag/AgCl as a reference electrode, and another platinum wire (MF-1032) as a counter electrode.

7.2. Preparation of compounds 2–5

7.2.1. Preparation of ethyl (coumarin-4-oxy) acetate 2

4-hydroxycoumarin (16.2 g, 0.1 mol), anhydrous potassium carbonate (13.8 g, 0.1 mol), ethylbromoacetate (11.1 ml, 0.1 mol) are mixed in round bottomed flask. The latter is stirred at reflux in dry acetone for 10 h. The reaction mixture was filtered, while hot and the residue washed with boiling acetone. Yield (80%); mp 245 °C. IR spectrum, ν cm–1: 1732 (CO); 1525 (O–CO lactone). ^{1}H NMR spectrum (DMSO-d_6). δ ppm: 1.23(t,3H,CH_3); 4.23(q,2H,CH_2); 5.1(s,2H,OCH_2); 5.93 (s,1H,H_3); 7.36-7.82(m,4H, H_{arom}). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 14.2(CH_3); 61.4(CO–O–CH_2); 91.7(C_3), 65.7(OCH_2); 115.2–153.0(Carom); 161.7(C_2); 164.3(C_4); 167.3(CO).

7.2.2. Preparation of coumarin-4-oxyacetic hydrazide 3

Ethyl (coumarin-4-oxy) acetate (9.72 g, 0.037 mol) obtained in previous reaction and hydrazine hydrate (1.1 ml, 0.022 mol) in absolute ethanol. The reaction mixture was then stirred for 7 h at room temperature. The reaction is controlled by TLC (hexane: ethyl acetate 2:1) when the quantities of reagents are depleted, the stirring was stopped. The residue from the reaction mixture was filtered off and washed with ethanol to give a white precipitate of coumarin-4-oxyacetic hydrazide **3**, which was crystallized to give colourless plates. Yield 80%; mp 265 °C. IR spectrum, ν cm^{-}1: 1720(CO). 1715(O–CO lactone). ^{1}H NMR spectrum (DMSO-d_6). δ ppm: 5.71(s,3H,H_3); 4.78 (s,2H,OCH_2); 4.42(s,2H,NH_2); 7.34–8.03(m,4H, Harom); 9.51(s,1H,NH). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 67.4(OCH_2); 91.6 (C_3); 115.3–133.2(Carom); 161.8(C_2); 164.8(C_4); 165.3(CO).

7.2.3. Preparation of (E)- N- arylidene coumarin-4-oxyacetic hydrazones 4

Coumarin-4-oxyacetic hydrazide **3** (2.34 g, 0.01 mol) and the substituted aromatic aldehyde (0.01 mol) were dissolved in a boiling methanol–water mixture (50:6 v/v) mL of methanol. The reaction mixture was refluxed for 5 h. Then concentrated and cooled. The solid product was filtered off, washed with the appropriate solvent.

7.2.4. (E)-N- benzylidene coumarin-4-oxyacetic hydrazone: 4a

Yield 75%. mp 244 °C. IR spectrum, ν cm^{-1}: 1692 (C=O); 1430 (C=N); 3118 (NH).

^{1}H NMR spectrum (DMSO-d_6). δ ppm: 4.98(s,1H,H_3); 5.47(s,2H,OCH_2); 11.78(s,1H,NH); 7.38-8.03(m,8H, H_{arom}); 7.89(s,1H, N=CH). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 66.4(OCH_2); 91.6 (C_3); 144.6 (N=C);115.6–134.2(C_{arom}); 161.9(C_2); 165.1(C_4); 167.5($C_{1'}$).

7.2.5. (E)- N- (4-fluorobenzylidene) coumarin-4-oxyacetic hydrazone: 4b

Yield 72%; mp 234 °C. IR spectrum, ν cm^{-1}: 1692 (C=O); 1430 (C=N); 3118 (NH). ^{1}H NMR spectrum (DMSO-d_6). δ ppm: 4.98(s,1H,H_3); 5.47(s,2H,OCH_2); 11.79(s,1H,NH); 7.26–8.02(m,8H, H_{arom}); 5.92(s,1H, N=CH). ^{19}FNMR (DMSO-d6) −110.55 ppm. ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 66.4(OCH_2); 91.6 (C_3); 115.6-133.3(C_{arom}); 147.5 (N=C); 161.9(C_2); 165.1(C_4); 167.6($C_{1'}$).

7.2.6. (E)- N- (4-methoxybenzylidene) coumarin-4-oxyacetic hydrazone: 4c

Yield 73%; mp 265 °C. IR spectrum, ν cm^{-1}: 1684 (C=O); 1686 (C=N); 3106 (NH).

^{1}H NMR spectrum (DMSO-d_6). δ ppm: 3.79 (s,3H, OCH_3); 4.95(s,1H,H_3); 5.44(s,2H,OCH_2); 5.88(s,1H, N=CH); 11.64(s,1H,NH); 6.98-8.24(m,8H, H_{arom}). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 55.7(OCH_3); 66.4(OCH_2); 91.7 (C_3); 114.6–133.3(C_{arom}); 144.5 (N=C); 162.5(C_2); 165.1(C_4); 167.3($C_{1'}$).

7.2.7. (E)- N- (4-nitrobenzylidene) coumarin-4-oxyacetic hydrazone: 4d

Yield75%; mp 248 °C. IR spectrum, ν cm^{-1}: 1710 (C=O); 1628 (C=N); 2962 (NH). ^{1}H NMR spectrum (DMSO-d_6). δ ppm: 5.99(s,1H,H_3); 5.48(s,2H,OCH_2); 8.14(s,1H, N=CH); 11.86(s,1H,NH); 7.38–8.27(m,8H, H_{arom}). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 66.4(OCH_2); 91.9 (C_3); 115.2-133.3(C_{arom}); 142.2 (N=C); 161.9(C_2); 165.0(C_4); 168.0($C_{1'}$).

7.2.8. (E)- N- (3,4,5- trimethoxybenzylidene) coumarin-4-oxyacetic hydrazone: 4e

Yield75%; mp 216 °C. IR spectrum, ν cm^{-1}: 1692 (C=O); 141 0 (C=N); 3118 (NH).

^{1}H NMR spectrum (DMSO-d_6). δ ppm: 3.69(s,3H, OCH_3); 3.82(s,6H, OCH_3); 5.50(s,2H,OCH_2); 5.87(s,1H,H_3); 7.02–7.94(m,6H, H_{arom}); 7.97(s,1H, N=CH); CH); 11.80(s,1H,NH). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 56.3(OCH3); 60.5(OCH_3); 66.5(OCH_2); 91.6 (C_3); 104.7–144.5(C_{arom}); 144.5 (N=C); 162.7(C_2); 165.2(C_4); 167.6($C_{1'}$).

7.2.9. Preparation of 2-[(coumarin-4-oxy) methyl]-4-acetyl-5-substitued – 1, 3,4-oxodiazoline **5**

The appropriate 2-[(coumarin-4-oxy) methyl]-4-acetyl-5-substitued–1,3,4-oxadiazolines **5** (0.001 mol) were refluxed with acetic anhydride (10 ml) for 8 h, unreacted acetic anhydride was removed and the residue was solidified by trituration with ether and crystallized from the proper solvent.

7.2.10. 4-(4-acetyl-5-phenyl-4,5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-one **5a**

Yield 65%. mp 218 °C. IR spectrum, ν cm^{-1}: 1624 (C=N); 1414 (N–N); 1284 (C–O–C).^{1}H NMR spectrum (DMSO-d_6). δ ppm: 1.89(s,3H, CH_3); 4.91(s,2H,OCH_2); 5.88(s,1H,H_3); 7.35–7.97(m,9H, H_{arom}); 7.99(s,1H,$H_{5'}$). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 20.8(CH_3); 65.8(OCH_2); 91.6 (C_3); 115.4 ($C_{5'}$);116.7–133.2(C_{arom}); 153.1(C_2); 161.8(C_4); 165.3(C=O); 168.8($C_{2'}$).

7.2.11. 4-(4-acetyl-5-(4-fluorophenyl)-4,5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-one **5b**

Yield 65%, mp 222 °C. IR spectrum, ν cm^{-1}: 1630 (C=N); 1404 (N–N); 1246 (C–O–C).^{1}H NMR spectrum (DMSO-d_6). δ ppm: 2.41(s,3H, CH_3); 5.48(s,2H,OCH_2); 6.0(s,1H,H_3); 7.34-7.99(m,8H, H_{arom}); 8.67(s,1H,$H_{5'}$). ^{19}F NMR (DMSO-d_6) −107.13 ppm.^{13}CNMR spectrum (DMSO-d_6). δ ppm: 26.0(CH_3); 69.3(OCH_2); 91.9 (C_3); 115.5 ($C_{5'}$);116.4-133.2(C_{arom}); 162.0(C_2); 164.8(C_4); 166.8(C=O); 171.2($C_{2'}$).

7.2.12. 4-(4-acetyl-5-(4-methoxyphenyl)-4,5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-one **5c**

Yield 67%; mp 258 °C. IR spectrum, ν cm^{-1}: 1624 (C=N); 1388 (N–N); 1246 (C–O–C).^{1}H NMR spectrum (DMSO-d_6). δ ppm: 2.38(s,3H, CH_3); 3.83(s,3H,OCH_3); 5.46(s,2H,OCH_2); 6.07(s,1H,H_3); 7.07-7.85(m,8H, H_{arom}); 8.53(s,1H,$H_{5'}$).^{13}CNMR spectrum (DMSO-d_6). δ ppm: 25.9(CH_3); 55.9(OCH_3); 69.3(OCH_2); 91.9 (C_3); 114.9 ($C_{5'}$);115.5–153.1(C_{arom}); 162.0(C_2); 164.8(C_4); 168.0(C=O); 171.2($C_{2'}$).

7.2.13. 4-(4-acetyl-5-(4-nitrophenyl)-4,5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-one **5d**

Yield 70%. mp 216 °C. IR spectrum, ν cm^{-1}: 1628 (C=N); 1410 (N–N); 1242 (C–O–C).^{1}H NMR spectrum (DMSO-d6). δ ppm: 2.18(s,3H, CH_3); 5.40(s,2H,OCH_2); 5.92(s,1H,H_3); 7.18-8.29(m,8H, H_{arom}); 7.80(s,1H,$H_{5'}$). ^{13}CNMR spectrum (DMSO-d_6). δ ppm: 20.8(CH_3); 66.4(OCH_2); 91.9 (C_3); 115.4 ($C_{5'}$);115.4–153.1(C_{arom}); 157.6(C_2); 161.8(C_4); 163.0(C=O); 164.8($C_{2'}$).

7.2.14. (4-acetyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1,3,4-oxodiazol-2-yl)methoxy)-2H-chromen-2-one **5e**

Yield 72%; mp 248 °C. IR spectrum, ν cm^{-1}: 1634 (C=N); 1408 (N–N); 1284 (C–O–C).^{1}H NMR spectrum (DMSO-d_6). δ ppm: 2.41(s,3H, CH_3); 3.75(s,3H,OCH_3); 3.78(s,6H,OCH_3); 4.99(s,2H,OCH_2); 5.88(s,1H,H_3); 7.21–7.67(m,6H, H_{arom}); 8.43(s,1H,$H_{5'}$).^{13}CNMR spectrum (DMSO-d_6). δ ppm: 20.8(CH_3); 56.3(OCH_3); 56.4(OCH_3); 60.6(OCH_2); 91.9 (C_3); 116.8 ($C_{5'}$);123.3–153.7(C_{arom}); 162.0(C_2); 164.8(C_4); 172.4(C=O); 192.2($C_{2'}$).

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