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Synthesis, Spectroscopy and Electrochemistry of New 3-(5-Aryl-4,5-Dihydro-1H-Pyrazol-3-yl)-4-Hydroxy-2H-Chromene-2-One 4, 5 as a Novel Class of Potential Antibacterial and Antioxidant Derivatives

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Abstract

3-((2E)-3(aryl)prop-2-enoyl)-2H-chromen-2-one **3** was synthesized from 4-hydroxy coumarin by refluxing 3-acetyl-4-hydroxy coumarin with aromatic aldehydes in chloroform in the presence of a catalytic amount of piperidine. **3** was converted to pyrazoles **4**, **5** by treatment with hydrazine and phenylhydrazine in toluene, respectively. The structures of the new compounds were confirmed by elemental analysis, IR, and multinuclear/multidimensional NMR spectroscopy (^{1}H, ^{13}C-NMR, NOESY, HMBC) which allowed us to assign the complete network of proton and carbon atoms. All the compounds exhibited one quasireversible redox process. All the newly synthesized compounds were screened for their antibacterial and antioxidant activities. Antimicrobial studies revealed that 3-(5-(2,5-dimethylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **5c** showed significant antibacterial activity against *Escherichia coli* and *Pseudomonas Aeruginosa* 27853. Furthermore, 3-(5-(aryl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones **4**, **5** showed antioxidant activities of different extents with respect to individual compounds as well as to the antioxidant methods. The 3-(5-(phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones **4a** was found to be the most active antioxidant in the series and more active than trolox which makes the investigated complexes a new promising class of antibacterial compounds.

Keywords: 4-Hydroxycoumarin, Pyrazole, Antibacterial Activity, Antioxydant Activity

1. Introduction

Chalcones are important precursors of flavonoids and isoflavonoids [1]. A large number of chalcones have been prepared by Claisen-Schmidt condensation of aldehydes with methyl ketones under basic conditions [2]. These compounds have shown *in vitro* antimalarial activity against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* [3]. Recently authors have reported the synthesis of chalcones under acidic conditions using perchloric acid and acetic acid [4]. The activity of a variety of chalcones as potent tyrosinase inhibitors and antioxidants has been also reported which are, thus, used as new depigmentation agents [5]. Nitrogen heterocycles containing chalcone moiety have been reported as active compounds against herpes simplex virus-1 (HSV-1) and human immunodeficiency virus 1 (HIV-1) [6,7]. This class of compounds also exhibits cytotoxic activity towards leukemia cell lines [8,9]. Various other chalcones exhibit insecticidal, antichinoviral, and antipicorniviral properties [10].

On the other hand, coumarins and structurally related compounds have been shown to inhibit replication of HIV and thus exhibit a therapeutic potential [11]. A large number of structurally novel coumarin derivatives have been reported to show substantial cytotoxic and anti-HIV activity both *in vitro* and *in vivo* [12,13]. A variety of synthetic coumarins have unique action mechanisms referring to the different stages of HIV replication [14]. Thus, coumarins are important lead compounds for the development of antiviral and/or virucidal drugs against HIV [15-17].

In view of the variety of pharmacological properties exhibited by chalcones, we were prompted to undertake

the synthesis of new compounds of this class and to study their conversion to other heterocycles which may show different or better physiological activities. We report herein the synthesis of new chalcone derivatives and their conversion to pyrazoles using nitrogen bases. In this regard it is worth stressing that also pyrazoles have been reported to show anti-inflammatory [18,19], cytotoxic [20], insecticidal [21], herbicidal [22], and fungicidal [23,24] activity.

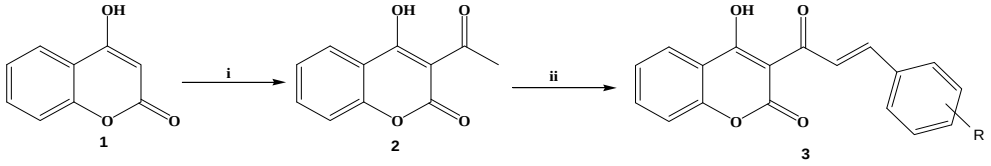
1.1. Chemistry

Due to the exceptional reactivity of the acetyl group in 3-acetylchromone as well as the versatile biological activities of coumarin derivatives, the chalcone **3** was synthesized from 4-hydroxycoumarin (1) under mild basic conditions.

Compound **2** was prepared by reaction of 4-hydroxycoumarine with $POCl_3$ in chloroform in the presence of acetic acid, The resulting compound **2** was then reacted with arylaldehydes to give the (E) the coumarinic chalcones **3**, which precipitated out from the hot MeOH solution after mixing **2** with the corresponding ArCHO **Scheme 1.**

3-((2E)-3(aryl) prop-2-enoyl)-2H-chromen-2-one compounds **3a-3e** were identified from analysis of their spectroscopic data. The infrared (IR) spectrum of compound **3d** showed the coumarin carbonyl groups at 1768 cm^{-1}, in addition to a broad band for the C=C group at 1595 cm^{-1}. The ^1H NMR spectrum showed *trans* olefinic protons H_a and H_b as *ortho*-coupled doublets at 8.25 (J = 15.6 Hz) and 6.92 (J = 15.9 Hz), respectively. The remaining aromatic protons of the aromatic aldehydes and the four protons of the coumarin moiety appeared as a multiplet in the region δ 7.25 - 8.08.

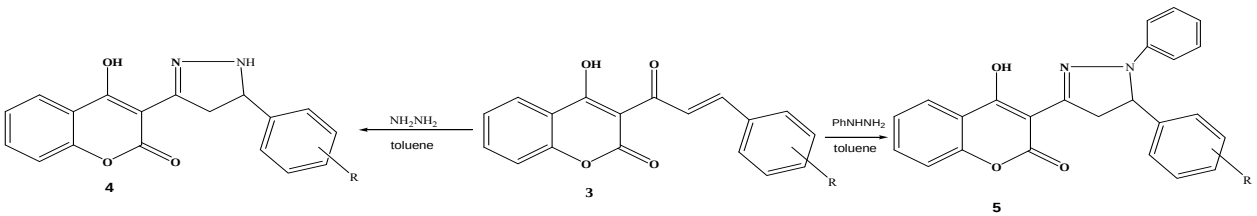
The $^{13}C\{^1H\}$ NMR spectrum of **3d** in DMSO-d_6 showed two downfield signals at ä 147.4ppm (C_4) and ä 162.4 ppm (lactone C = O) as well as an up field signal to at ä 55.4 ppm (OCH_3). The condensation of hydrazines with α,β-unsaturated carbonyl compounds usually gives pyrazolines [25] activities. Thus, when compound **3** was treated with nitrogen bases such as hydrazine and phenylhydrazine, the pyrazolines **4** and **5** were obtained, respectively. (**Scheme 2)**



i: $POCl_3$, ACOH, ii: $CHCl_3$, piperidine, Arylaldehyde

Compounds **3**	R
3a	H
3b	F
3c	2,5 CH_3
3d	OCH_3
3e	NO_2

Scheme 1. synthesis of chalcones 3.



Compounds **3**	R	Compounds **4** R'= H	Compounds **5** R'=ph	Yield (%)	
				4	5
3a	H	**4a**	5a	65	70
3b	F	**4b**	5b	75	72
3c	2,5 CH_3	**4c**	5c	80	78
3d	OCH_3	**4d**	5d	85	75
3e	NO_2	**4 e**	5e	80	75

Scheme 2. Synthesis of 3-(5-aryl-4,5-dihydro-1h-pyrazol-3-yl)-4-hydroxy-2h-chromene-2-ones 4, 5.

All the new 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones **4**, **5** were characterized by IR, ^{1}H, ^{13}C-NMR spectra as well as by NOESY and HMBC 2D-NMR experiments to elucidate their structures and assign completely the structural network of both protons and carbons. The spectral data were in accordance with the proposed structures (see experimental section).

The IR spectrum of **4d** showed broad band at 3207 cm^{-1} due to the presence of the NH group. A sharp and strong absorption band at 1668 cm^{-1} indicated a carbonyl group in the compound.

Since chromone carbonyl groups usually appear as sharp absorption bands in the region 1620 - 1650 cm^{-1} [26], the band at 1684 cm^{-1} was assigned to coumarin rather than the chromone carbonyl group.

In addition, the detection of a strong C=N stretching band at 1608 cm^{-1} evidenced the formation of the pyrazole ring. The ^{1}H NMR spectra of **4d** displays a signal at δ 4.12 ppm ascribable to the CH_2 protons of the pyrazole ring. A characteristic singlet proton signal at δ 4.81 ppm was assigned to CH proton from the pyrazole fragment. In addition, the aromatic protons (both coumarinic and aromatic) are observed between δ 6.82 and δ 8.1ppm (see experimental).

Full assignment of the ^{1}H NMR spectra of **4d** was deduced from the NOESY spectrum. An observed NOE cross peak between H-1' and aromatic protons confirms that these two units are located on the same side of the pyrazole ring.

The structure of **4d** was finally elucidated through the analysis of the ^{1}H, ^{13}C HMBC spectrum, which correlates the protons at δ 4.78 ppm with $C_{5'}$ (δ 55.05 ppm) and $C_{2'}$ (δ 153.3 ppm). The aromatic protons correlate with $C_{5'}$ (δ 55, 05 ppm). (**Table 1)**

Table 1. Correlations between HMBC and NOESY for compound 4d.

Proton	HMBC	NOESY
H-n	H-n-C-j	H-n-H-j
H-1'	2, 2', 5'	H_{arom}
H-5'	1',7'	
H-arom	5'	

A mechanistic rationalization for this reaction is straightforward and is provided in **Scheme 3**.

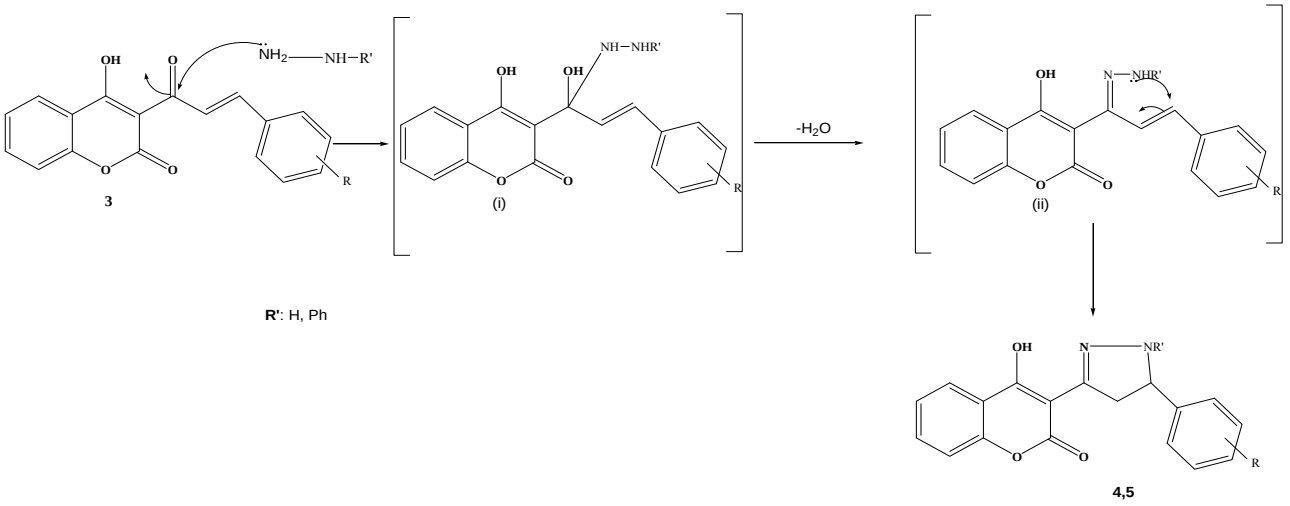
The first reaction step consists of a nucleophilic attack of the final hydrazine nitrogen atom to the carbonyl function followed by the elimination of water. The intermediate which forms may easily rearrange to afford the corresponding pyrazoles **4**, **5**.

1.2. Results and Discussion

1.2.1. Electrochemistry

Electrochemical studies of 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one, **5**, are of interest due to the electron-deficient nature of the pyrazole 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** shows 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.



Scheme 3. Proposed mechanism for the synthesis of 4, 5.

As expected, the reduction peak potential of the 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **5** are strongly influenced by the para substitutent on the phenylene ring. Compared to the unsubstituted compound **5**, the presence of electron donor groups such as the methoxy group shifts the reduction peak potential of **5** to more negative values. The redox behavior of all the new pyrazoles are summarized in **Figure 1** All the compounds exhibited one quasireversible redox processes.

For example, compounds **5d** showed one quasireversible reduction at –0.8 V 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 may be attributed to the redox behavior of the pyrazole unit.

1.2.2. Antibacterial and Antioxidant Studies

1.2.2.1. Free radical scavenging activity assay

The free radical scavenging activity of the new 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **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 [27]. The reduction capability of DPPH radical was determined by the decrease in absorbance induced by 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **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:

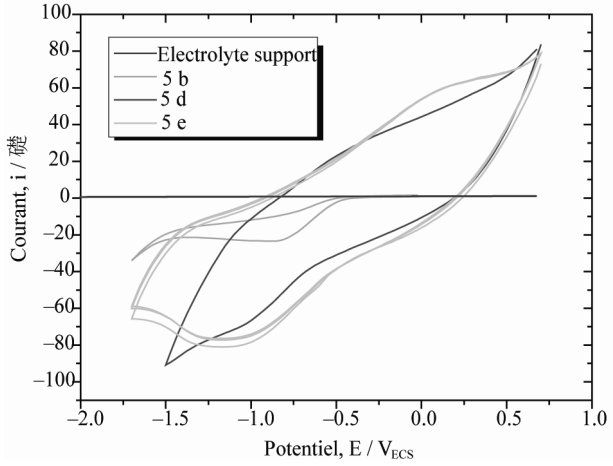


Figure 1 Cyclic voltammograms of compounds 5d, 5b and 5e (1×10^{-3} M) in CH_3CN, scan rate 100 mV·s^{-1}.

Scavenging activity = {[(Ab+ As) – Am]/Ab}×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 done on triplicate and averaged on three samples. The results are given in **Scheme 4**.

Among the compounds from the 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4** series, **4b** showed moderate antioxidant activity.

The activity exhibited by the compound **4e** was the highest. In addition the experimental data show that compound **4a** scavenges free radical better than Trolox.

According to the experimental results, we notice that increasing the concentration of pyrazole percentage of inhibition reaches 90% for a concentration about 1 µM for all the synthesized products. Thus, we can conclude that substituents on the aryl group do not influence significantly the anti-oxidant activity.

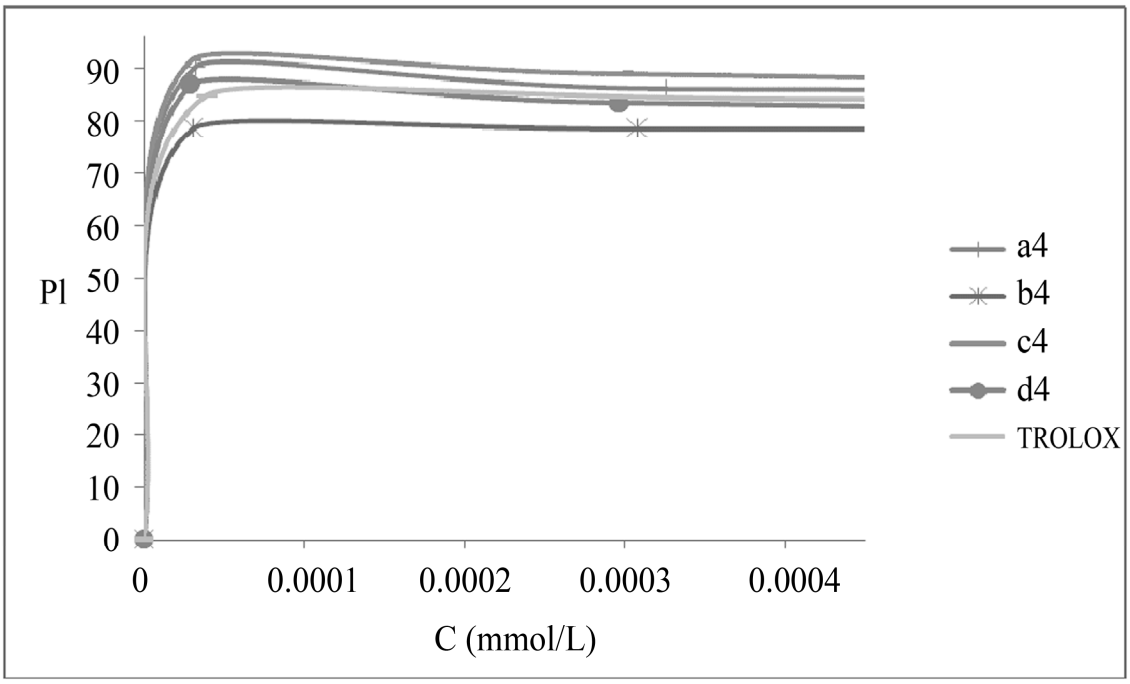
One parameter that has been introduced recently for the interpretation of the results from the DPPH method is the efficient concentration or EC_{50} value (otherwise called the IC_{50} value), which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) and corresponds to the endpoint of the titration. In all cases, any residual (yellow) color from the reduced form or any non specific absorbance from the sample should be considered in defining the ‘‘endpoint’’ of the titration, *i.e.,* the 50% point. Additionally, this IC_{50} parameter has also the drawback that the higher the antioxidant activity, the lower is the value of EC_{50}.

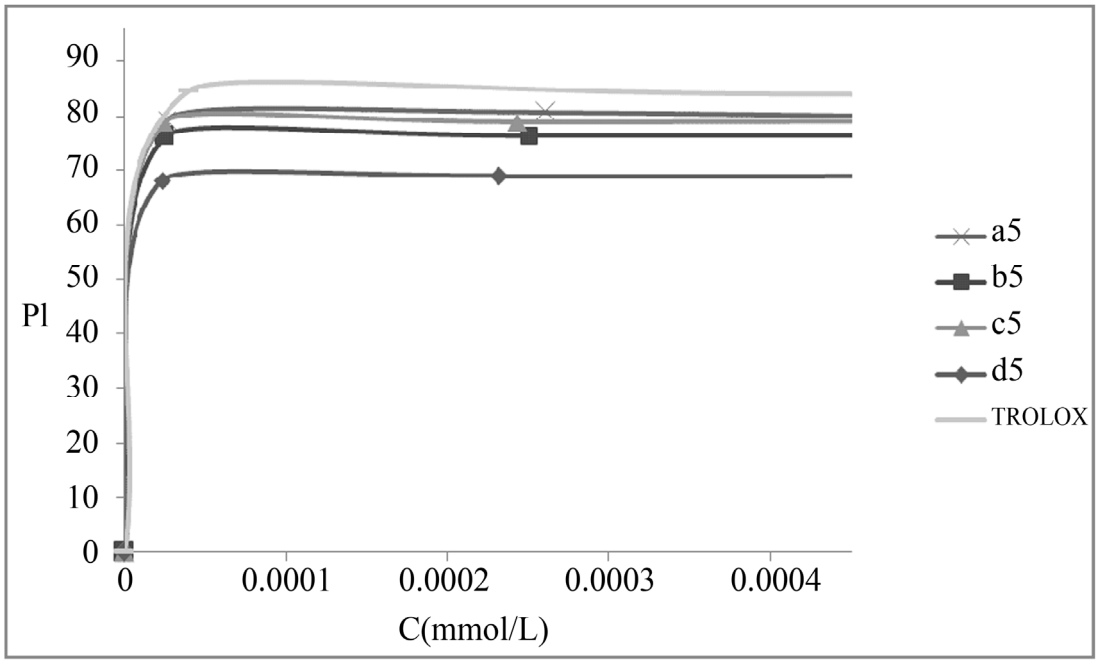
The EC_{50} values exhibited by 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **5** are summarized in the following **Table 2**.

From inspection of **Table 2**, it is evident that pyrazoles 4,5 are more active than trolox.

Table 2. The EC_{50} values exhibited by 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **5**

Compounds **4, 5**	CI_{50} (µmol.L^{-1}).
4a	4,8
4b	5
4c	3,8
4d	4,2
5a	3,8
5b	3,2
5c	2,7
5d	3
trolox	7,5





Scheme 4. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical of compounds 4, 5.

1.2.2.2 ABTS radical cation decolorization assay

The potential of 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4** to scavenge free radicals was also assessed by checking their ability to quench $ABTS^{+}$. **Scheme 5** depicts the concentration-dependent decolourization of $ABTS^{+}$.

ABTS radical-scavenging activity of 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **5** was determined according to Re et al. 30]. The $ABTS^{+}$. 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

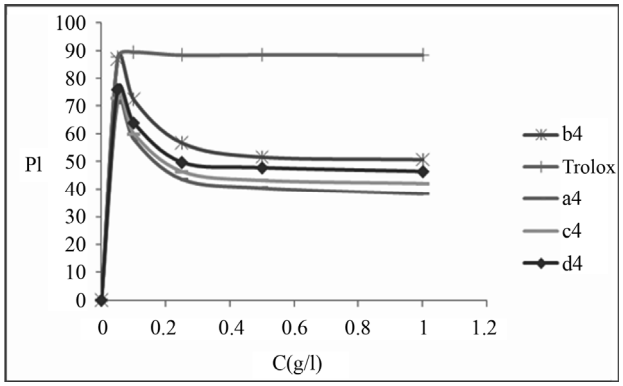
at 734 nm. In a final volume of 1 ml, the reaction mixture comprised 950 µl of ABTS ± solution and 50 µl of the pyrazoles **4**, **5** at various concentrations. The reaction mixture was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements 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 sca- venging ability was expressed as EC_{50} (<mu> g/ml). The inhibition percentage of ABTS radical was calculated using the following formula:

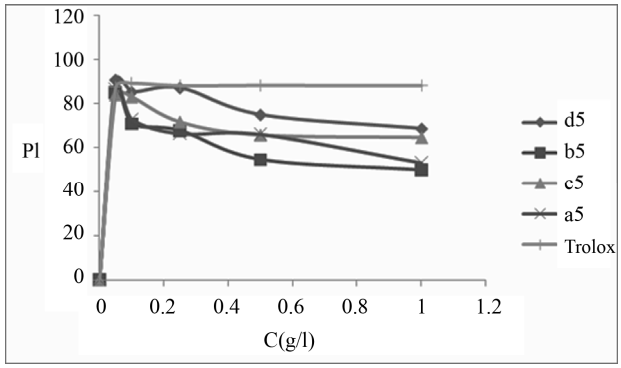
$$\text{ABTS scavenging effect \%} = \{[(A_0 - A_1)]/AO\} \times 100\%$$

where A_0 is the absorbance of the control at 30 min, and A1 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 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **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 increases. We have just shown that the synthesized pyrazoles derivatives **4**, **5** 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.





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

The EC_{50} values exhibited by 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **5** are summarized in the following **Table 3**.

The 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **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 $ABTS^+$.

2. Antibacterial Activity

The antibacterial activity of 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4** was assessed by the agar disk diffusion assay [28] against five human pathogenic bacteria: *Gram-positive* including *Staphylococcus aureus* (CIP 7625), *Staphylococcus aureus* and Gram-negative bacteria including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) (CIP 76110) and *Klebsiella 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 4** below.

Table 3. The EC_{50} values exhibited by 3-(5-aryl-4,5-dihydro-1H-pérazol-3-yl)-4-hydroxy-2H-chromène-2-one 4, 5.

Compounds **4, 5**	CI_{50} ($g \cdot l^{-1}$)
4a	0.72
4b	0.98
4c	0.78
4d	1.17
5a	0.938
5b	1
5c	0.773
5d	0.72
Trolox	0.549

Table 4. 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*
PseudomonasAeruginosa 27853 (*CIP* 76110)	30	*5a*
	26	*5b*
	34	*5c*
	33	*5d*
	32	*5e*
	15 - 22	*Gentamycin*

The residual antibacterial activity of 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

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 highest activity was found in compound **5b** bearing a methoxy group at 4-position.

3. Conclusions

A new versatile synthetic route to 3-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one **4**, **5** by the treatment of 4-hydroxycoumarine with different reagents is described. The method is easy, rapid and yielded the title compounds **4**, **5** in good yields. The structures of the novel compounds were verified by, IR 1D/2D NMR spectroscopy. 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 pyrazole ring exhibited higher antioxidant activity than trolox while the pyrazole bearing a *p*-methoxy substituent in the phenyl ring exhibited enhanced antioxidant activity.

4. Experimental Section

4.1. 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 and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Varian-Unity spectrometer at 300 MHz using tetramethylsilane (TMS) as an internal standard. Cyclic voltammetry (CV) was performed on a BAS 100 BW electrochemical workstation. All CV measurements were carried out using 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.

3-Acetyl-4-hydroxycoumarin

To a solution of 4-hydroxy-2H-chromen-2-one (3.0 g, 1.86mmol) in acetic acid (16 ml) phosphorus oxychloride (5.6 ml) was added. The mixture was heated at reflux for 30 min. After cooling to room temperature, the precipitate which separated out was collected by filtration and recrystallized from ethanol to give 3-acetyl-4-hydroxy-2Hchromen-2-one as white needles. Yield 2.7 g (90%); Mp = 135˚C. IR spectrum, ν cm^{-1}: 3185 (OH); 1705 (CO); 1700 (O-CO lactone). ^{1}H NMR spectrum ($CDCl_3$). δ: 2.72 (3H, s, CH_3); 7.98 (1H, s, H-5); 7.95 (1H, dd, ^{3}J 7.8, 8.35, ^{4}J 6.8, 1.2 Hz, H-8); 7.1 - 7.4 (2H, m, H-6 + H-7); 17.7 (1H, s, OH).$^{13}C\{^{1}H\}$ NMR spectrum ($CDCl_3$), δ: 29.9 (CH_3); 178.5 (CO);159.8 (C-4); 154.6 (C-2); 101.3 (C-3); 115.0 - 136.0 (C_{arom}).

General procedure for the preparation of the coumarinic chalcones 4a-i

3-acetyl-4-hydroxy-2Hchromen-2-one (0.031 mol) and the substituted aromatic aldehyde (0.03 mol) were dissolved in 30 mL of chloroform. A catalytic amount of piperidine (0.02 mol) was added and the reaction mixture was refluxed for 1.5 h. The chloroform was removed under vacuum and the residue was washed

3-((2E)-3(phenyl)prop-2-enoyl)-2H-chromen-2-one: 3a

Yield 85%; mp 265˚C. IR spectrum, ν cm^{-1}: 1490 (C=O), 1728 (O-C-O), 1529 (C=C). ^{1}H NMR spectrum ($CDCl_3$). δ ppm: 6.5 - 8.3 (m, 11H, H_{arom}, H_{eth}); 18.56 (s, 1H, OH). ^{13}CNMR spectrum ($CDCl_3$). δ ppm: 116.9 (C_3); 135.8 (C_2); 147.1 (C_4); 134.5 (C_{ethyl})

3-((2E)-3(4-fluorophenyl)prop-2-enoyl)-2H-chromen-2-one: 3b

Yield 80%; mp 215˚C. IR spectrum, ν cm^{-1}: 1494 (C=O), 1716 (O-C-O), 1531 (C=C). ^{1}H NMR spectrum ($CDCl_3$, 400MHz). δ ppm: 6.51 - 8.21 (m, 10H, $H_{arom+éthyl}$); 18,4(s, 1H, OH). ^{13}CNMR spectrum ($CDCl_3$). δ ppm: 100.9 (C_3); 154.8 (C_2); 166 (C_4); 181.5 (CO); 136.2 ($C_{éthyl1}$); 131.5 ($C_{éthyl2}$); 116.5 - 131.51 (C_{arom}). NMR ^{19}F ($CDCl_3$, 400 MHz, t_{amb}) ppm: –107.8

***3-((2E)-3(2, 5-dimethylphenyl)prop-2-enoyl)-2H-chromen-2-one: 3c**

Yield 75%; mp 224˚C. IR spectrum, ν cm^{-1}: 1490 (C=O), 1720 (O-C-O), 1527 (C=C). ^{1}H NMR spectrum ($CDCl_3$). δ ppm: 2,41 (s, 1H, CH_3), 3,21 (s, 3H, CH_3), 6.82 – 8.21 (m, 9H, $H_{arom+éthy}$); 13,81 (s, 1H, OH). ^{13}CNMR spectrum ($CDCl_3$). δ ppm: 91.4 (C_3); 135.8 (C_2); 161.4 (C_4); 139.1 ($C_{éthyl}$), 134.1 ($C_{éthy2}$); 116.4 – 133.2 (C_{rom}), 176.2 (CO)

***3-((2E)-3(4-methoxyphenyl) prop-2-enoyl)-2H-chromen-2-one: 3d**

Yield 75%; mp 194˚C. IR spectrum, ν cm^{-1}: 1494 (C=O), 1708 (O-C-O), 1595 (C=C). ^{1}H NMR spectrum ($CDCl_3$). δ ppm: 3.84 (s, 3H, OCH_3); 6.92 (d, 1H, CH); 8.25 (d, 1H, CH); 7.25 - 8.08 (m, 8H, H_{arom}); 18.05 (s, 1H, OH). ^{13}CNMR spectrum ($CDCl_3$). δ ppm: 55.4 (OCH_3); 114.4 (C_3); 116.8 – 131.3 (C_{arom}); 135.6 (C_2); 147.48(C_4); 162.4 (CO).

***3-((2E)-3(4-nitrophenyl)prop-2-enoyl)-2H-chromen-2-one: 3e**

Yield 72%; mp 194˚C. IR spectrum, ν cm^{-1}: 1489 (C=O), 1712 (O-C-O), 1535 (C = C). ^{1}H NMR spectrum ($CDCl_3$). δ ppm: 7.1 - 8.6 (m,1OH, $H_{arom+ethyl}$); 18.4 (s1, H, OH). ^{13}CNMR spectrum ($CDCl_3$). δ ppm: 98.7 (C_3); 151.8 (C_2); 178.7 (C_4); 182.5 (CO); 151.86 (C_{ethyl1}); 122.5 (C_{ethyl2}).

3-(5-(phenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones: 4a

Yield 65%; mp 234˚C. IR spectrum, ν cm^{-1}: 1683 (C=O lactone), 1608 (C=N), 3230 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1.75(d, 2H, CH_2), 3.74 (t, 1H, CH), 6,82-7,27 (m, 9H, Harom). ^{13}CNMR spectrum (DMSO-d_6, 75 MHz, t_{amb}) δ ppm: 40, 6 (CH_2) 49,5 (CH), 91,1 (C_3); 158,6 (C_2); 166,4 (C_4); 155,7 (C = N), 121,5-127,1 (Carom). with methanol

3-(5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones: 4b

Yield 70%; mp 220˚C. IR spectrum, ν cm^{-1}: 1668 (C=O lactone), 1612 (C=N), 3192 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1,76(d, 2H, CH_2), 3.64 (t, 1H, CH), 6.79 – 7.35(m,8H, Harom). ^{13}CNMR spectrum (DMSO-d_6, 75 MHz, t_{amb}) δ ppm: 40.2 (CH_2) 48.7 (CH), 91.1 (C_3); 158.5 (C_2); 166.7 (C_4); 155.2 (C=N), 120.8 - 128.3 (Carom). NMR ^{19}F (DMSO-d_6, 300 MHz, t_{amb}) ppm: –139.26

3-(5-(2,5-dimethylphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones: 4c

Yield 80%; mp 252˚C. IR spectrum, ν cm^{-1}: 1683 (C=O lactone), 1606 (C=N), 3230 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1.68 (d, 2H, CH_2), 3,68 (t, 1H, CH), 2,34 (s, 6H, $2CH_3$), 6.85 - 7.32(m, Harom). ^{13}CNMR spectrum (DMSO-d_6,75 MHz, t_{amb}) δ ppm: 18.6 (CH_3), 24.5 (CH_3), 40.2 (CH_2), 42.6 (CH), 91.7 (C_3); 158.4 (C_2); 167.6 (C_4); 155.7 (C=N), 121.7 - 132.8 (Carom).

3-(5-(4-methoxyphényl)-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-ones: 4d

Yield 85%; mp 210˚C. IR spectrum, ν cm^{-1}: 1668 (C=O lactone), 1608 (C=N), 3207 (NH). ^{1}H NMR spectrum (DMSO-d_6,300 MHz,t_{amb}) δ ppm: 3.84 (s, 3H, OCH_3), 6.82 - 8.1 (m, 8H, H_{arom}), 4.12 (d, 2H, CH_2), 4,81 (t, 1H, CH). ^{13}CNMR spectrum (DMSO-d_6, 75 MHz, t_{amb}) δ ppm: 58.2 (OCH_3), 55.0 (CH), 43.7 (CH_2), 91.4 (C_3); 158.7 (C_2); 161.6 (C_4); 153.3 (C=N), 113.8 – 133.4 (Carom).

3-(5-(phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one:5a

Yield 70%; mp 230˚C. IR spectrum, ν cm^{-1}: 1668 (C=O lactone), 1608 (C=N), 3110 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1.65 (d, 2H, CH_2), 3.75 (t, 1H, CH), 6.42 - 7.35 (m Harom). ^{13}CNMR spectrum (DMSO-d_6, 75 MHz, t_{amb}) δ ppm: 36.4 (CH_2) 54.8 (CH), 92.3 (C_3); 159.7 (C_2); 167.8 (C_4); 158.1 (C=N), 113.5 - 133.1 (Carom).

3-(5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one: 5b
Yield 72%; mp 226˚C. IR spectrum, ν cm^{-1}: 1668 1683 (C=O lactone), 1606 (C=N), 3230 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1.62 (d, 2H, CH_2), 3.65 (t, 1H, CH), 6.35 – 7.45 (m Harom). ^{13}CNMR spectrum (DMSO-d_6,75 MHz, t_{amb}) δ ppm: 35.3 (CH_2) 52.7 (CH), 92.1 (C_3); 159.2 (C_2); 167.8 (C_4); 152.6 (C=N), 120.5 - 134.1 (Carom). NMR ^{19}F (DMSO-d_6, 300 MHz, t_{amb}) ppm: –129,42

3-(5-(2,5-dimethylphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one: 5c
Yield 78%; mp 265˚C. IR spectrum, ν cm^{-1}: 1681 (C=O lactone), 1608 (C=N), 3220 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1,63 (d, 2H, CH_2), 3.68 (t, 1H, CH), 6.35 – 7.68 (m Harom). ^{13}CNMR spectrum (DMSO-d_6, 75 MHz, t_{amb}) δ ppm: 35.3 (CH_2) 52.8 (CH), 92.1 (C_3); 158.8 (C_2); 168.4 (C_4); 159.6 (C=N), 126.5 - 142.1 (Carom).

3-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-4-hydroxy-2H-chromene-2-one: 5d
Yield 75%; mp 216˚C. IR spectrum, ν cm^{-1}: 1681 (C=O lactone), 1608 (C=N), 3234 (NH). ^{1}H NMR spectrum (DMSO-d_6, 300 MHz, t_{amb}) δ ppm: 1.62 (d, 2H, CH_2), 3.68 (t, 1H, CH), 3.78 (s, 3H, CH_3), 6.47 – 7.42 (m Harom). ^{13}CNMR spectrum (DMSO-d_6, 75 MHz, t_{amb}) δ ppm: 32.4 (CH_2) 51.4 (CH), 55.8 (OCH_3), 93.3 (C_3); 156.4 (C_2); 167.3 (C_4); 152.5 (C=N), 113.4 – 132.6 (Carom).

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NOESY: Nuclear Overhauser effect spectroscopy
HMBC: Heteronuclear Multiple Bond Correlation experiment

