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Original article

Synthesis, structure, antimicrobial and antioxidant investigations of dicoumarol and related compounds

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Abstract

Different substituted 3,3′-arylidenebis-4-hydroxycoumarins (**1**–**7**) and tetrakis-4-hydroxycoumarin derivative **8** are the final products when 4-hydroxycoumarin and aromatic aldehydes containing different groups in *ortho*, *meta* or *para* positions condense in boiling ethanol or acetic acid. Upon heating 3,3′-arylidenebis-4-hydroxycoumarins, and tetrakis-4-hydroxycoumarin derivative in anhydride acetic acid, the epoxydicoumarins (**9**–**16**) were formed. From a study of nuclear magnetic resonance and infrared spectra, intramolecularly hydrogen-bonded structures are proposed for the dicoumarols (**1**–**8**). A possible relationship between such hydrogen-bonded structures and the antimicrobial and the antioxidant activities of compounds **1**–**8** is suggested.

Keywords: 4-Hydroxycoumarin derivatives; Aromatic aldehydes; Antioxidant activity; Antimicrobial activity

1. Introduction

Many coumarin derivatives are biologically active [1–3]. Much research has been focused on the inhibition of bacterial growth by naturally occurring coumarins (xanthoxin, herniarin, umbelliferone, and scopoletin) and on the antifungal activity of umbelliferone, scopoletin, and coumarin itself [4–7]. Some coumarin derivatives (novobiocin and analogues) have proven very active as antibiotics [8,9]. Among synthetic derivatives, several antibacterial 3-acyl [10–14] and 3-carbamoyl-4-hydroxycoumarins [13,15] have been described. The simplest one that proved moderately active, 3-acetyl-4-hydroxycoumarin, exists in its crystalline form as shown in a X-ray diffraction study, predominantly as tautomer A (Scheme 1: tautomers of 3-acyl-4-4-hydroxycoumarin) with an intermolecular H-bond [16,17]. There has been continuous interest in the synthesis of these materials.

Though many efforts have been paid to the synthesis of 3-substitued-4-hydroxycoumarin derivatives, there is shortage of studies on the synthesis of dicoumarols and related compounds. In studies on the antiproliferative actions of coumarin compounds, we discovered that dicoumarol (a coumarin anticoagulant, 3,3′-methylenebis[4-hydroxycoumarin]) inhibits the first cleavage of *Stronglylocentrotus purpuratus* (sea urchin) embryos in a concentration-dependant manner with 50% inhibition occurring at a concentration of 10 μM [18]. It was found that dicoumarol binds to bovine brain tubulin with a K_d of 22 μM and that 0.1 μM dicoumarol strongly stabilizes the growing and shortening dynamics at the plus ends of the microtubules in vitro [19]. Dicoumarol and taxol in combination gave results in a synergistic inhibition of cell division of sea urchin embryos.

These two compounds can be used in combination in order to reduce the high toxicity of taxol.

The third position in 4-hydroxycoumarin ring is highly activated, because of the influence of hydroxyl group with electron-donating properties and electron-withdrawing effects of carbonyl oxygen atom at the second place. There is a conjugation of p

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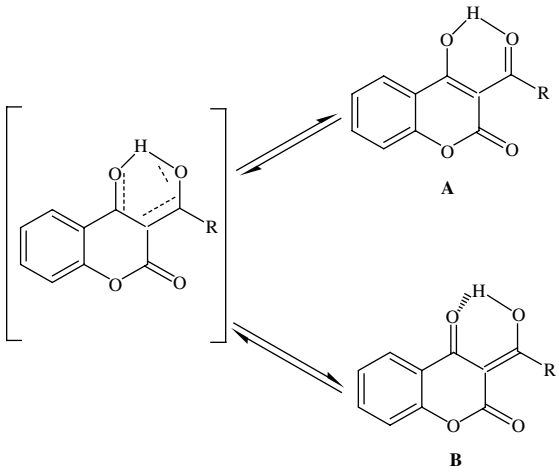


Scheme 1.

electrons from the double bond and lone p-electron pairs from oxygen atom. These factors make the third position in the coumarin ring very convenient for Michael addition to the compounds with activated double bond (Michael acceptors).

The parent compound 4-hydroxycoumarin (a, R = H) may contribute for building a new chemical library, used for treatment of diseases, which are estrogenic dependant and can be represented as one of the three tautomeric structures **a**, **b** or **c** (Scheme 2).

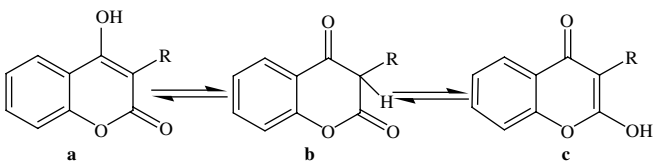
The possible places of electrophilic attack and hydrogen-bond formation were predicted. The different tautomeric forms of 4-hydroxycoumarin have been established [20]. The nature of these three forms was elucidated by using different spectral methods.

In search for more active compounds, we prepared a series of dicoumarols **1**–**8** and epoxydicoumarins **9**–**16** and tested their activity on *Staphylococcus aureus* ATCC 6538, *Propionibacterium acnes* ATCC 11827, and *Staphylococcus epidermidis* ATCC 12228. Results shed light on the relative importance of antibacterial action of the 4-hydroxycoumarin nucleus. In addition the antioxidant activity of the obtained dicoumarols has been investigated. A possible relationship between such hydrogen-bonded structures and the antimicrobial and antioxidant activities of the obtained dicoumarols is suggested.

2. Results and discussion

2.1. Chemistry

Different substituted aromatic aldehydes were condensed with 4-hydroxycoumarin in ethanol or glacial acetic acid in a molar ratio 1:2. The products obtained were 3,3′-arylidene-bis-4-hydroxycoumarins and tetrakis-4-hydroxycoumarin derivatives. The characterization of these compounds was carried out by mass spectral, IR, ^{1}H NMR and ^{13}C NMR analyses.



Scheme 2.

The condensation process lasted for 5 h until the appearance of an insoluble product. The analytical results showed the products were 3,3′-phenylmethylenebis-(4-hydroxy-2*H*-1-benzopyran-2-one **1**) (without a substituent in the aromatic nucleus), 3,3′-(4-methoxyphenylmethylene)bis-(4-hydroxy-2*H*-1-benzopyran-2-one **6**) and 3,3′-(4-methylyphenylmethylene)bis-(4-hydroxy-2*H*-1-benzopyran-2-one **7**).

When 4-hydroxycoumarin was refluxed for 60 min in ethanol with both 3,4,5-trimethoxy benzaldehyde and 4-nitro benzaldehyde at molar ratio 2:1, two crystalline products appeared namely, respectively, 3,3′-(3,4,5-trimethoxy methylene)bis-(4-hydroxy-2*H*-1-benzopyran-2-one) and 3,3′-(4-nitrophenylmethylene)bis-(4-hydroxy-2*H*-1-benzopyran-2-one).

The condensation of 4-hydroxycoumarin with both 2,3-dihydroxy benzaldehyde and 2-hydroxy-5-nitro benzaldehyde in boiling glacial acetic acid for 6 h produced, respectively, 3,3′-(2,3-dihydroxyphenylmethylene)bis-(4-hydroxy-2*H*-1-benzopyran-2-one) and 3,3′-(2-hydroxy-5-nitro phenylmethylene)bis-(4-hydroxy-2*H*-1-benzopyran-2-one).

The brief heating of 4-hydroxyl coumarin with dialdehyde in boiling acetic acid led to an expected final product namely 3,3′,3″,3‴-(*p*-phenylenedimethylidine)tetrakis-(4-hydroxy-2*H*-1-benzopyran-2-one) (Scheme 3).

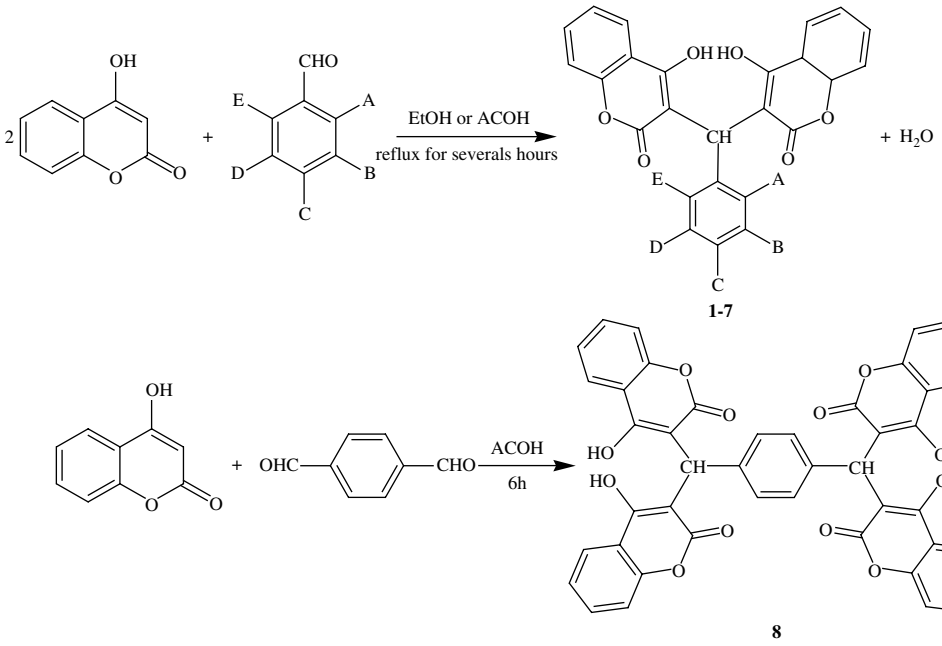
The mass spectral investigation proved that the molecular cation radical was rather unstable and decomposed releasing a water molecule.

We have found that the NMR spectrum of a solution of 3,3′-phenylmethylenebis-(4-hydroxy-2*H*-1-benzopyran-2-one) **1** in deuterochloroform shows three singlets at 6.1 bridge (CH: 1H), 11.34 (s, 1H, OH) and 11.57 (s, 1H, OH). The last singlets are independent of concentration and can be removed on the addition of traces of deuterium oxide. We assign these signals to the two strongly deshielded protons of the enolic hydroxyl group which are intramolecularly hydrogen bonded to carbonyl groups and we consider that this NMR evidence confirms the structural assignment for 3,3′-phenylmethylenebis-(4-hydroxy-2*H*-1-benzopyran-2-one **1**) mentioned above. The protons of the ring methylene groups give rise to a single resonance probably due to a rapid tautomeric exchange of their chemical shifts.

This indicates the presence of intramolecularly hydrogen-bonded protons and suggests the structure **A** for the obtained dicoumarols (Scheme 4).

It is also noticeable the reduction of the number of signals in comparison with those expected. All these points are in agreement with the existence of a symmetry plane perpendicular to the molecular plane (Scheme 5).

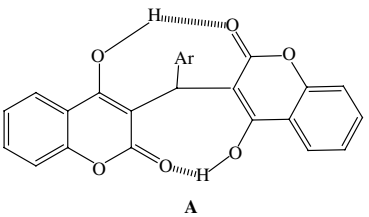
Examination of molecular models shows that in this case aryl substituent lies close to one of the hydroxyl protons accounting for the difference in their magnetic moments. This behaviour is also apparent for all dicoumarols (**1**–**8**) when both enolic protons can be distinguished in the NMR spectrum.



Compounds	A	B	C	D	E
1	H	H	H	H	H
2	H	OCH_3	OCH_3	OCH_3	H
3	H	H	NO_2	H	H
4	OH	OH	H	H	H
5	OH	H	H	NO_2	H
6	H	H	OMe	H	H
7	H	H	Me	H	H

Scheme 3.

In the IR spectrum of a solution (**a**, R = H) in chloroform or dioxane, the carbonyl stretching frequency occurs at 1660 cm^{-1}. The position of this band does not alter when the infrared spectrum is taken in the solid state (Nujol mull or potassium bromide pellet), while the carbonyl stretching frequencies of **a** (R = H or CH_3) vary from 1700 cm^{-1} (Nujol mull) to 1730 cm^{-1} (dioxane solution), as would be expected if intermolecular hydrogen bonding occurred. Moreover, the carbonyl stretching frequencies of the synthesized dicoumarols **1**–**3** occur, respectively, at 1725, 1710 and 1710 cm^{-1} (chloroform solution). On the other hand 2-methoxychromone and 2-methoxy-3-methylchromone both have carbonyl stretching frequencies at ca. 1630 cm^{-1} (chloroform solution). As this value is 30 cm^{-1} lower than that for **A**, a dichromone structure (**B**) for all the obtained dicoumarols (**1**–**8**) is proposed (Scheme 6).

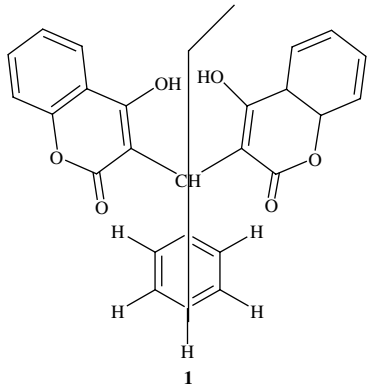


A

Scheme 4.

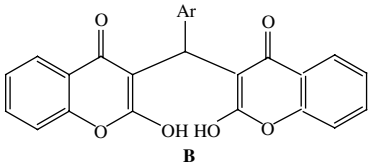
It is unlikely that any inter- or intramolecular hydrogen bonding in **A** would cause the carbonyl band to appear at even lower frequencies. We also believe that the spectroscopic evidence is inconsistent with a mixed coumarin-chromone structure (**C**) which can be also put forwarded to obtain dicoumarols (**1**–**8**) (Scheme 7).

On the other hand the heating of 3,3′-arylidenebis-4-hydroxycoumarins and tetrakis-4-hydroxycoumarin derivative in acetic acid anhydride was quickly transformed these compounds to the corresponding epoxycoumarins (Scheme 8).



1

Scheme 5.



B

Scheme 6.

Differentiation of compounds **1**–**8** and **9**–**16** was made mainly by their ^{1}H, ^{13}C and IR spectra. In particular the main difference in the ^{1}H NMR spectra of compounds **6** and **13** was hydroxylic protons, which appeared as two singlets, respectively, at δ 11.34 and 11.57 ppm in compound **6**, whereas in compound **13** they were absent.

In the IR spectra, among the characteristic bands confirming the structure of epoxy derivatives, we should pick out the vibrations of the lactone group which appear at 1725 cm^{-1} and the absence of the vibrations of OH. The mass spectra of compound **13** the peak for most of the molecular ions has maximum intensity, which is typical for any bis-hetero aromatic compounds.

2.2. Antimicrobial activity

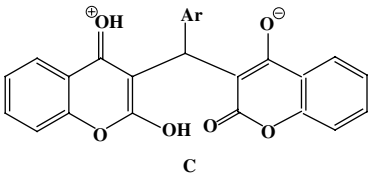
As shown in Table 1, most active against *Staphylococcus aureus* ATCC 6538 were dicoumarols **1**, **2**, **5**. Acute toxicities of compounds **3**, **4**, **6**–**8** were very low (all mice survived a single dose of 2 g/kg without any marked symptoms of intolerance).

The minimum inhibitory concentrations (MICs) of all compounds tested fell between 2.6 and 25.5 μg/mol (Table 1). The two most active compounds **3** and **4** were tested on *P. acnes* ATCC 11827 and *S. epidermidis* ATCC 12228 (Table 2), confirming that the activities of **3** and **4** were close to each other.

2.3. Antioxidant activity

2.3.1. Radical cation $ABTS^{+\bullet}$ scavenging activity

The standard method, described by Van den Berg [21], was adopted with minor modifications for determination of the TEAC values. This assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical ($ABTS^{+\bullet}$) in 5, 10, 15 and 20 min. The blue green ABTS radical form was produced through the reaction between ABTS and potassium persulfate in water. Briefly a concentrated $ABTS^{+\bullet}$ stock solution was diluted with phosphate-buffered saline (PBS), pH 7.4 to a final absorbance of 0.70 ± 0.02 with a wavelength of 734 nm and at a temperature of 37 °C. Solutions with different diluted concentrations of dicoumarols **1**–**8** were prepared in ethanol. Ten microliters of studied sample was added to 990 mL $ABTS^{+\bullet}$ solution and the absorbance at 734 nm was measured. Sample absorbance was compared to a blank where 10 mL of the solvent was added to 990 mL of the $ABTS^{+\bullet}$ solution. Absorbance was measured at 5, 10, 15 and 20 min after addition of the antioxidant (Figs. 1 and 2).



C

Scheme 7.

In biological systems, the formation of these intermolecular hydrogen bonds may hold both **A** and **13** in a suitable configuration for binding to an enzyme and hence may be an important factor in the antimicrobial and antioxidant activities of these compounds. It is of interest *S*-3-(α-acetonylbenzyl)-4-hydroxycoumarin (**13**) [22] and *S*-3-(α-ethylbenzyl)-4-hydroxycoumarin (**14**) [23] are more active as anticoagulants than their enantiomers [24] (Scheme 9).

Examination of molecular models of the two enantiomers of **13** shows that while both can form the intermolecular hydrogen bond, there is less steric interaction between the phenyl ring and the carbonyl group of the coumarin in the *S*- than the *R*-isomer. It is possible that compound **14** is oxidized in vivo to a derivative containing a carbonyl group at $C_{(3)}$ of the side chain, this derivative could then assume a suitable configuration for biological activity by virtue of formation of hydrogen-bonded structure similar to **13**. Moreover, we have found [25] that compounds which contain intermolecular hydrogen bonds e.g. **13** is uncoupler and inhibitor of mitochondrial oxidative phosphorylation, while compounds which can only form intermolecular hydrogen bonds, e.g. compound **a** is only uncoupler of oxidative phosphorylation. Here again, the formation of hydrogen bonds may be an important factor in assisting the synthesized dicoumarols to attain the correct configuration for antioxidant and antimicrobial activities.

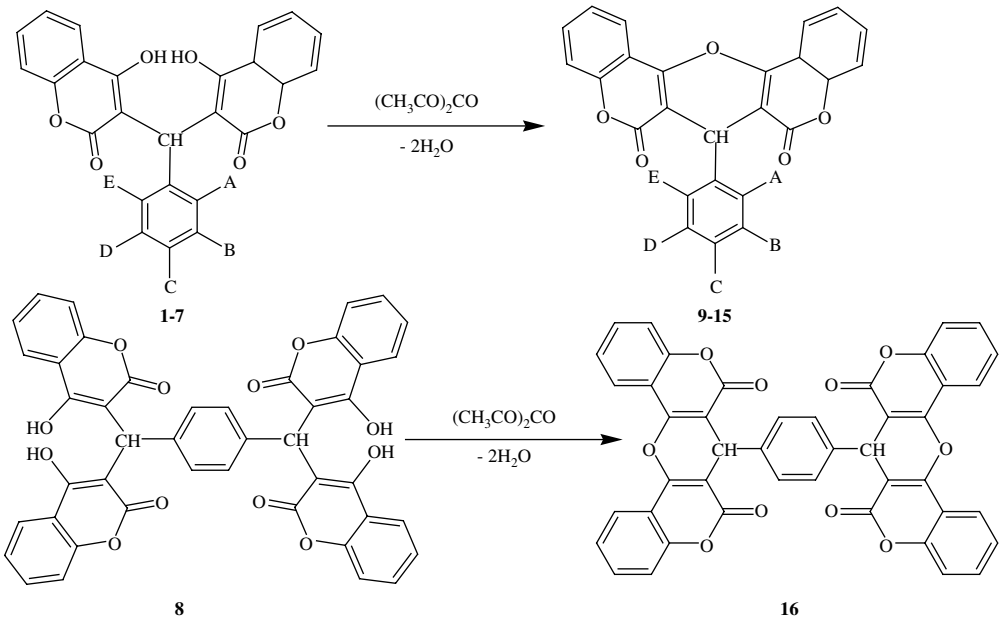
In conclusion we have found a simple and efficient route to the synthesis of 3,3′-arylidenebis-4-hydroxycoumarins and tetrakis-4-hydroxycoumarin derivative, which are not obtained easily or as the sole products by other method. They are characterized by spectral methods such as ^{1}H NMR, ^{13}C NMR, IR, mass spectrometries and also by TLC and melting point determination. These functionalized products are amenable to further transformations and we anticipate that they may have important applications in synthetic organic chemistry.

The present method carries the advantage that the reaction is not only performed under neutral conditions but also the starting materials and reagents can be mixed without any activation or modification.

We obtained in a good yield a number of dicoumarols **1**–**8** and epoxydicoumarins **9**–**16** that we found to be endowed with a marked antimicrobial and antioxidant activities.

The synthesized dicoumarols **1**–**8** by us could inhibit and exhibit antiradical activity by reacting with $ABTS^{+\bullet}$ and help to increase the overall antioxidant capacity of an organism.

In biological systems, the formation of these intermolecular hydrogen bonds may hold dicoumarols (**1**–**8**) in a suitable configuration for binding to an enzyme and hence may be an important factor in assisting the molecule to attain the correct configuration for antioxidant and antimicrobial activities.



Scheme 8.

3. Experimental

All reactions and manipulations were routinely performed. Reagents were obtained from Sigma–Aldrich–Fluka and used without purification. Mass spectra were obtained with a Hewlett–Packard 5880 spectrometer. In this case electron impact techniques were employed. Infrared spectra (λ in cm^{-1}) were recorded on an ATI Mattson Infinity series FT-IR spectrometer using potassium bromide pellets for solids and liquid films for oils. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer (300 and 75 MHz). The samples were dissolved in $CDCl_3$ (compounds **1–8**) and DMSO-d_6 (compounds **9–16**). The chemical shift values were referenced to tetramethylsilane as internal standard. Chemical shift values and IR data for all compounds are summarized in the experimental part and they are in agreement with the proposed structures. Melting points were determined on a Büchi No. 510 apparatus without corrections. Thin layer chromatography (TLC) was performed with silica gel plates HF254 (Merck) and the plates were viewed under UV-254 light. Silica gel (230–400 mesh) was used for chromatography separations. Spectrophotometer measurements were made on a Specord M-40 instrument.

3.1. General synthesis

4-Hydroxycoumarin and the respective aromatic aldehyde at a molar ratio 2:1 in ethanol or glacial acetic acid were mixed under stirring and heated at reflux until the appearance of an insoluble product. After cooling the product was filtered and recrystallized with the appropriate solvent. The following 3,3′-arylidenebis-(4-hydroxy-2*H*-1-benzopyran-2-ones) or tetrakis-4-hydroxycoumarin derivatives were synthesized according to this procedure.

*3.1.1. 4-Hydroxy-3-[(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-phenyl-methyl]-chromen-2-one (**1**)*

Yield: 70%; m.p. 114 °C. IR ν (cm^{-1}): 3078 (OH); 1673 (CO); 1562 (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 6.10 (s, 1H, H_{11}); 7.22–7.67 (m, 11H, H_{arom}); 8.04 (d, 2H, $H_{5,5'}$); 11.34 (s, 1H, OH); 11.57 (s, 1H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 36.12 (C_{11}); 116.59–135.15 (C_{arom}); 152.43 ($C_{9,9'}$); 164.47 ($C_{2,2'}$); 165.61 ($C_{4,4'}$). MS, *m/z* (%): 424 (M^+, 100).

*3.1.2. 4-Hydroxy-3-[(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-(3,4,5-trimethoxy-phenyl)-methyl]-chromen-2-one (**2**)*

Yield: (75%); m.p. 158 °C. IR ν (cm^{-1}): (OH); (CO); (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 3.73 (s, 9H, OCH_3); 4.14 (s, 1H, H_{11}); 5.80–7.27 (m, 10H, H_{arom}); 15.1 (d, 2H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 37.8 (C_{11}); 56.20 (OCH_3); 72.9–150.2 (C_{arom}); 164.5 ($C_{2,2'}$);

Table 1
Minimum inhibitory concentration (MIC) (mg/mL) on *Staphylococcus aureus* ATCC 6538

Compound	1	2	3	4	5	6	7	8
MIC (mg/mL)	2.6	5.2	24.2	25.5	5.9	23.5	23.2	23.4

Table 2
Antimicrobial activity (MIC) of compounds **3** and **4**

Organism	MIC (μg/mL)	
	3	4
Propionibacterium acnes ATCC 11827 (1.0×10^{-3} c.f.u/mL)	0.5	0.5
Staphylococcus epidermidis ATCC 12228 (1.6×10^{-3} c.f.u/mL)	1.3	1.9
Staphylococcus aureus ATCC 6538 (1.5×10^{-3} c.f.u/mL)	3.7	6.5

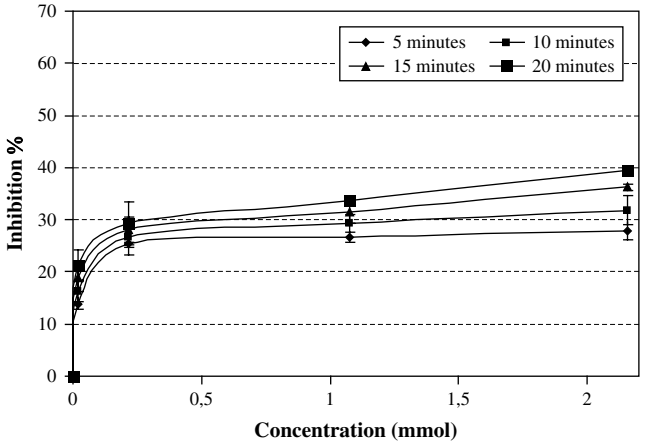


Fig. 1. Percentage inhibition of $ABTS^{+\bullet}$ in the presence of different concentrations of the dicoumarol (**4**) at 5, 10, 15 and 20 min.

101.4 ($C_{3,3'}$); 171.9 ($C_{4,4'}$). MS, *m/z* (%): 204 (M^+, 100); 189 (74); 161 (43); 502 (M^+, 100).

*3.1.3. 4-Hydroxy-3-[(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-(4-nitro-phenyl)-methyl]-chromen-2-one (**3**)*

Yield: (76%); m.p. 268 °C. IR ν (cm^{-1}): (OH); (CO); (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 4.11 (s, 1H, H_{11}); 5.80–7.27 (m, 10H, H_{arom}); 15.1 (d, 2H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 35.9 (C_{11}); 70.2–150.2 (C_{arom}); 100.3 ($C_{3,3'}$); 165.7 ($C_{2,2'}$); 157.9 ($C_{4,4'}$). MS, *m/z* (%): 204 (M^+, 100); 189 (74); 161 (43); 425 (M^+, 100).

*3.1.4. 3-[(2,3-Dihydroxy-phenyl)-(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-methyl]-4-hydroxy-chromen-2-one (**4**)*

Yield: (65%); m.p. 98 °C. IR ν (cm^{-1}): (OH); (CO); (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 4.2 (s, 1H, H_{11}); 5.80–7.02 (m, 10H, H_{arom}); 4.13 (s, 1H, OH); 5.00 (s, 1H, OH); 15.2 (d, 2H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 26.1 (C_{11}); 70.2–150.2 (C_{arom}); 165.7 ($C_{2,2'}$); 157.9 ($C_{4,4'}$); 100.4 ($C_{3,3'}$). MS, *m/z* (%): 204 (M^+, 100); 189 (74); 161 (43); 426 (M^+, 100).

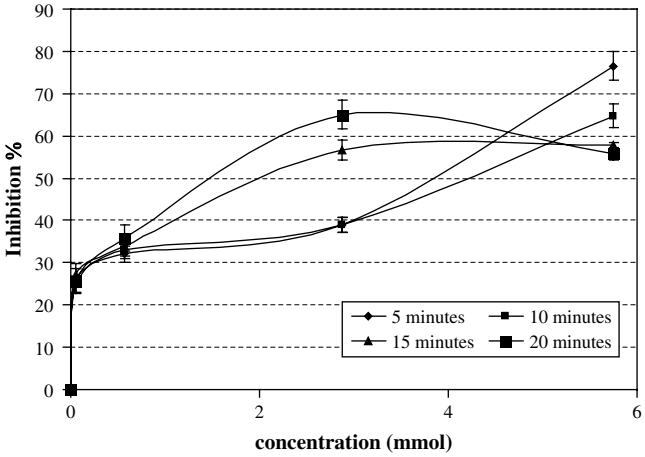
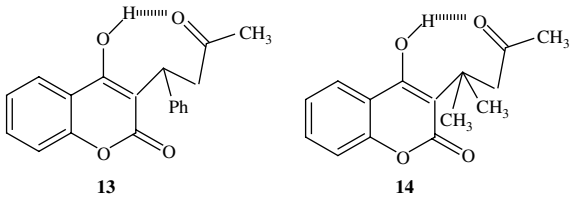


Fig. 2. Percentage inhibition of $ABTS^{+\bullet}$ in the presence of different concentrations of dicoumarol (**5**) at 5, 10, 15 and 20 min.



13 **14**

Scheme 9.

*3.1.5. 4-Hydroxy-3-[(2-hydroxy-6-nitro-phenyl)-(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-methyl]-chromen-2-one (**5**)*

Yield: (75%); m.p. 248 °C. IR ν (cm^{-1}): (OH); (CO); (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 4.14 (s, 1H, H_{11}); 5.80–7.27 (m, 10H, H_{arom}); 15.01 (d, 2H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 17.1 (C_{11}); 70.2–134.8 (C_{arom}); 165.48 ($C_{2,2'}$); 157.9 ($C_{4,4'}$); 1002.8 ($C_{3,3'}$). MS, *m/z* (%): 204 (M^+, 100); 189 (74); 161 (43); 473 (M^+, 100).

*3.1.6. 4-Hydroxy-3-[(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-(4-methoxy-phenyl)-methyl]-chromen-2-one (**6**)*

Yield: 78%; m.p. 234 °C. IR ν (cm^{-1}): 3071 (OH); 1668 (CO); 1564 (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 3.75 (s, 3H, OCH_3); 6.00 (s, 1H, H_{11}); 7.99 (d, 2H, $H_{5,5'}$); 6.79–7.60 (m, 10H, H_{arom}). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 36.72 (C_{11}); 55.20 (OCH_3); 113.95–132.73 (C_{arom}); 152.27 ($C_{9,9'}$); 164.48 ($C_{2,2'}$); 165.56 ($C_{4,4'}$). MS, *m/z* (%): 204 (M^+, 100); 189 (74); 161 (43); 442 (M^+, 100).

*3.1.7. 4-Hydroxy-3-[(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-p-tolyl-methyl]-chromen-2-one (**7**)*

Yield: 75%; m.p. 125 °C. IR ν (cm^{-1}): 3071 (OH); 1669 (CO); 1564 (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 2.35 (s, 3H, CH_3); 6.08 (s, 1H, H_{11}); 7.10–7.67 (m, 10H, H_{arom}); 8.05 (d, 2H, $H_{5,5'}$); 11.53 (s, 2H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 20.90 (CH_3); 35.80 (C_{11}); 116.55–136.41 (C_{arom}); 152.43 ($C_{9,9'}$); 164.47 ($C_{2,2'}$); 165.61 ($C_{4,4'}$). MS, *m/z* (%): 204 (M^+, 100); 189 (74); 161 (43); 426 (M^+, 100).

*3.1.8. 4-Hydroxy-3-[(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-4-(bis(4-hydroxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)-methyl)-phenyl-methyl]-chromen-2-one (**8**)*

Yield: 70%; m.p. 224 °C. IR ν (cm^{-1}): (OH); (CO); (C=C). ^1H NMR (300 MHz, $CDCl_3$) (λ, ppm): 4.14 (d, 2H, H_{11}); 7.02–7.27 (m, H, H_{arom}); 15.1 (d, 2H, OH). ^{13}C NMR (75.47 MHz, $CDCl_3$) (δ, ppm): 36.4 (C_{11}); 117.5–150.2 (C_{arom}); 162.48 ($C_{2,2'}$); 100.2 ($C_{3,3'}$); 162.9 ($C_{4,4'}$). MS, *m/z* (%): 742 (M^+, 100).

*3.2. General synthesis of epoxydicoumarins (**9–16**)*

3,3′-Arylidenebis-4-hydroxycoumarins **1–7** and tetrakis-4-hydroxycoumarin derivatives **8** were dissolved by heating in

anhydride acetic. The reaction mixture was boiled for 2 h, kept in the refrigerator for 24 h, the precipitate was filtered off and recrystallized from the appropriate solvent.

*3.2.1. 7-Phenyl-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**9**)*

Yield: 65%; m.p. 195 °C. IR ν (cm^{-1}): 1675 (CO); 1572 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 4.9 (s, 1H, H_{11}); 7.02–7.27 (m, 12H, H_{arom}). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 41.2 (C_{11}); 121.5–150.1 (C_{arom}); 161.9 (CO). MS, *m/z* (%): 394 (M^+, 100).

*3.2.2. 7-(3,4,5-Trimethoxy-phenyl)-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**10**)*

Yield: 70%; m.p. 160 °C. IR ν (cm^{-1}): 1667 (CO); 1570 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 3.73 (s, 9H, OCH_3); 4.14 (s, 1H, H_{11}); 5.80–7.27 (m, 12H, H_{arom}). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 32.8 (C_{11}); 55.20 (OCH_3); 121.5–148.3 (C_{arom}); 164.3 (CO). MS, *m/z* (%): 484 (M^+, 100).

*3.2.3. 7-(4-Nitro-phenyl)-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**11**)*

Yield: 67%; m.p. 175 °C. IR ν (cm^{-1}): 1670 (CO); 1572 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 4.2 (s, 1H, H_{11}); 7.02–8.07 (m, 12H, H_{arom}). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 40.3 (C_{11}); 121.1–150.2 (C_{arom}). MS, *m/z* (%): 394 (M^+, 100).

*3.2.4. 7-(2,3-Dihydroxy-phenyl)-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**12**)*

Yield: 70%; m.p. 155 °C. IR ν (cm^{-1}): 1672 (CO); 1570 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 3.94 (s, 1H, H_{11}); 6.37–7.27 (m, 12H, H_{arom}); 4.13 (s, 1H, OH); 5.00 (s, 1H, OH). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 31.4 (C_{11}); 121.5–150.2 (C_{arom}); 162.1 (CO). MS, *m/z* (%): 443 (M^+, 100).

*3.2.5. 7-(2-Hydroxy-6-nitro-phenyl)-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**13**)*

Yield: 75%; m.p. 185 °C. IR ν (cm^{-1}): 1673 (CO); 1570 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 4.84 (s, 1H, H_{11}); 7.02–7.63 (m, 12H, H_{arom}); 5.3 (s, 1H, OH). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 22.5 (C_{11}); 121.5–150.2 (C_{arom}). MS, *m/z* (%): 455 (M^+, 100).

*3.2.6. 7-(4-Methoxy-phenyl)-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**14**)*

Yield: 78%; m.p. 185 °C. IR ν (cm^{-1}): 1675 (CO); 1565 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 3.73 (s, 9H, OCH_3); 3.94 (s, 1H, H_{11}); 7.02–7.27 (m, 12H, H_{arom}). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 32.6 (C_{11}); 56.2 (OCH_3); 121.5–151.2 (C_{arom}); 161.1 (CO). MS, *m/z* (%): 424 (M^+, 100).

*3.2.7. 7-p-Tolyl-7,14b-dihydro-4aH-pyrano[3,2-c;5,6-c′]dichromene-6,8-dione (**15**)*

Yield: 70%; m.p. 170 °C. IR ν (cm^{-1}): 1672 (CO); 1570 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 2.35 (s, 3H, CH_3); 3.9 (s, 1H, H_{11}); 6.94–7.27 (m, 12H, H_{arom}). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 24.3 (CH_3); 41.2 (C_{11}); 121.5–150.2 (C_{arom}); 162.1 (CO). MS, *m/z* (%): 408 (M^+, 100).

*3.2.8. 3,3′,3″,3‴-(p-Phenylenedimethylidine)tetrakis-(4-hydroxy-2H-1-benzopyran-2-one) (**16**)*

Yield: 65%; m.p. 190 °C. IR ν (cm^{-1}): 1675 (CO); 1572 (C=C). ^1H NMR (300 MHz, DMSO-d_6) (λ, ppm): 4.14 (d, 2H, H_{11}); 7.02–7.27 (m, H, H_{arom}). ^{13}C NMR (75.47 MHz, DMSO-d_6) (δ, ppm): 36.4 (C_{11}); 117.5–150.2 (C_{arom}); 162.48 ($C_{2,2'}$); 100.2 ($C_{3,3'}$); 162.9 ($C_{4,4'}$). MS, *m/z* (%): 724 (M^+, 100).

*3.3. Reaction of **3**,**4** with 2-diphenyl-1-picrylhydrazyl*

The reaction was studied in ethanol containing dicoumarols (0.1 mM) and DPPH (50 μm).

The mixture was stirred, thermostatted at 25 °C, and monitored by the change of optical density at 517 nm.

3.4. Antimicrobial activity

Compounds to be tested were dissolved in acetone (10 mg/mL) and diluted with worm culture medium. Muller–Hinton broth containing 1% Tween 20 was used for tests with *S. aureus* and *S. epidermidis*, incubations were carried out aerobically at 36 ± 1 °C. Duplicate blanks were incubated alongside culture aliquots containing scalar dilution of each dicoumarol derivative. After 1–4 days of incubation, bacterial growth was estimated by turbidimetry or by plating on agar medium.

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