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Biscoumarin: new class of urease inhibitors; economical synthesis and activity

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Abstract—A variety of biscoumarins (**1**–**21**) with variable substituents at C-11 were synthesized with an improved method and evaluated as urease inhibitors. The synthesized compounds showed varying degree of urease inhibitory activity ranging from 15.06–91.35 μM. The size and electron donating or withdrawing effects of substituents influenced the activity, which lead to the urease inhibitors.

1. Introduction

Activity of urease (E.C 3.5.1.5) has been shown to be an important virulence determinant in the pathogenesis of many clinical conditions, which are detrimental for human and animal health as well as for agriculture. Urease is directly involved in the formation of infectious stones and contributes to the pathogenesis of urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, urinary catheter encrustation.[1,2] It is also known to be a major cause of pathologies induced by *Helicobacter pylori* (HP), which allows bacteria to survive at low pH of the stomach during colonization and therefore plays an important role in the pathogenesis of gastric and peptic ulcer, (which may lead to cancer).[2] In agriculture, high urease activity causes significant environmental and economic problems by releasing abnormally large amounts of ammonia into the atmosphere during urea fertilization. This further induces plant damage primarily by depriving them from their essential nutrient, and secondly ammonia toxicity and increase the pH of the soil.[1,2] Therefore, strategies based on urease inhibition are now considered as the first line of treatment for infections caused by urease producing bacteria.

Recently we explored different classes of compounds for their potential use in medicinal chemistry,[3] during our preliminary studies on biscoumarins,[4] it was observed that they have property to inhibit urease enzymes, in view of our initial results, we synthesized biscoumarins **1**–**21** and screened them for urease inhibition.

The biscoumarins are naturally occurring compounds[5–7] and also synthesized by different methods.[8–10] A variety of biological activities are associated with coumarin for example as anticoagulant, molluscicid, antianthelmintic, hypnotic and insecticidal.[11]

2. Results and discussion

2.1. Chemistry

Twenty-one biscoumarins **1**–**21** were synthesized by condensing different alkyl and aryl aldehydes with 4-hydroxycoumarin at room temperature in the presence of catalytic amount of piperidine in high yields. Previously, this condensation was achieved by heating the reaction mixture for 4 h and excess of aldehyde was required as well the reported yields were low.[8–10] Interestingly, we discovered that biscoumarin can be obtained at room temperature in high yield in contrast with reported in the literature. In a typical reaction, 2

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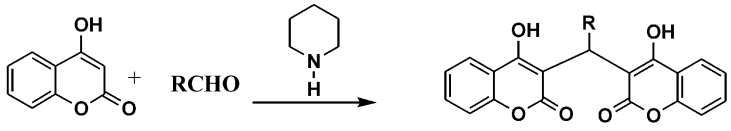
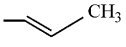
moles of 4-hydroxycomarin react with one mol of aldehyde in ethanol at room temperature in the presence of catalytic amount of piperidine to afford excellent yields Scheme 1. The structure of biscoumarins (**1**–**21**) were determined by modern spectroscopic techniques like UV, IR, NMR, mass and purity was confirmed by CHN analysis. The general structure of biscoumarin is shown in Figure 1 with its numbering used in this article.

As we described earlier some activity was found in biscoumarins against urease enzyme.[4]

The activity was carried out according to literature protocol[12] and thiourea was used as standard inhibitor having IC_{50} value 21 μM, the results are collected in Table 1.

Out of 21 biscoumarins tested for their urease inhibitory effects, five compounds **1**, **6**, **17**, **19** and **20** showed good urease enzyme inhibition at micromolar level. Compound 3,3′-methylenebis-4-hydroxycoumarin (**1**) showed exciting urease inhibitory activity with $IC_{50} = 15.01$ μM, where as standard inhibitor has



Comp. No.	R	Yield (%)
1	-H	92
2	$-CH_3$	95
3	$-(CH_2)_2CH_3$	97
4	$-(CH_2)_3CH_3$	90
5	$-(CH_2)_4CH_3$	92
6		92
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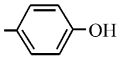
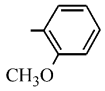
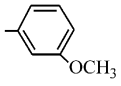
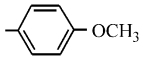
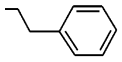
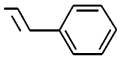
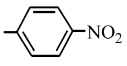
8		92
9		89
10		95
11		96
12		93
13		97
14		97
15		91
16		94
17		95
18		96
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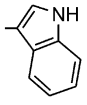
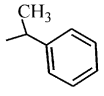
20		97
21		92

Scheme 1.

Table 1. Tabular representation of urease inhibitory activity of compounds **1**–**21** against standard inhibitor

Compd	$IC_{50} \pm$ S.E.M[a]
1	15.01 ± 0.0070
2	61.30 ± 0.200
3	66.39 ± 0.0051
4	84.74 ± 0.0034
5	86.32 ± 0.0021
6	42.92 ± 0.0021
7	71.91 ± 0.0081
8	73.68 ± 0.0800
9	84.53 ± 0.037
10	72.93 ± 0.0300
11	73.85 ± 0.0037
12	81.81 ± 0.0068
13	59.78 ± 0.0049
14	55.30 ± 0.0051
15	67.28 ± 0.0031
16	59.06 ± 0.0300
17	35.03 ± 0.0021
18	65.01 ± 0.0061
19	46.09 ± 0.01
20	48.41 ± 0.0079
21	52.18 ± 0.09
4-Hydroxy Coumarin	Nil
Thiourea[b]	21 ± 0.011

[a] SEM is the standard error of the mean.
[b] The standard inhibitors of the enzyme thiourea.

$IC_{50} = 21.00$ μM. The compounds **6**, **17**, **19** and **20**, which were 2-propenyl, pyridyl, pyrolyl and indolyl analogues of compound **1** demonstrated interesting inhibitory activity in the range 35.05–48.41 μM. Rest of the compounds, exhibited varying degree of enzyme inhibitory activity ranges from 52.18–86.32 μM.

The inhibitory activities of compound **1** and **2** reflect that two hydroxyl groups present on two lactone rings of biscoumarin molecule may be only responsible for inhibitory activity and may coordinate with the nickel (active site) of enzyme. Any substituent present at C-11 may decrease the activity as indicated by all alkyl and aryl substituted biscoumarin. The least potent compound **5** was with an *n*-pentyl at C-11 correlates with the structures of the compounds **2**, **3** and **4**, respectively. These declines in activity rationalized, as the alkyl chain increasing the lowering in activity was occurred. This might be due to steric hindrance of alkyl groups, which may be responsible for less interaction with the nickel of the enzyme. In other words compound **1** having no substitution at C-11 may serve as a useful lead compound for urease inhibitors based on biscoumarin skeleton (Fig. 1).

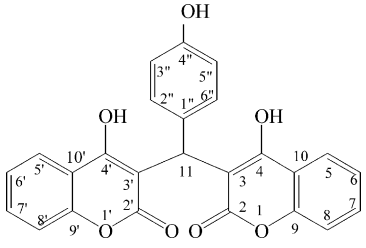


Figure 1.

On the basis of results it may be proposed that an open chain diol may act similar to biscoumarin for urease inhibition, but some other structural features may also contributing to activity. A further mechanism base study is required to see interaction of biscoumarins with enzyme.

3. Experimental

Melting Point: Melting points were determined on a Büchi 434 melting point apparatus and are uncorrected. NMR was performed on a Bruker AM 300 and 500 MHz, respectively. CHN analysis was performed on a Carlo Erba Strumentazion-Mod-1106 Italy. Ultraviolet spectra (UV) were recorded on Perkin–Elmer Lambda-5 UV/VIS spectrometer for DMSO. Infrared Spectra (IR) was recorded on JASCO IR-A-302 Spectrometer. Electron Impact Mass Spectra (EIMS) were recorded on a Finnigan MAT-311A Germany. Thin layer chromatography (TLC) was performed on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by, UV at 254 and 365 nm, followed by iodine vapors.

3.1. Urease assay and inhibition

Reaction mixtures comprising 25 μL of enzyme (Jack bean Urease) solution and 55 μL of buffers containing 100 mM urea were incubated with 5 μL of test compounds (1 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn.[12] Briefly, 45 μL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula $100-(OD_{testwell}/OD_{control})\times 100$. Thiourea was used as the standard inhibitor of urease.

3.2. General procedure for the synthesis of compounds (1–21)

To a mixture of aldehyde (1 mmol) and 4-hydroxycoumarin (2 mmol) dissolved in EtOH (10 mL), added a few drops of piperidine and stirred the mixture for 4 h at room temperature (TLC monitored completion), added water till precipitates occurred, cooled, filtered on suction filtration and washed with little ice-cold water followed by EtOH then drained to dryness to obtained pure product.

3.2.1. 3,3′-Methylenebis-(4-hydroxycoumarin) (1). Yield: 0.475 g (95.0%); mp 273 °C; $R_f = 0.60$ (ethyl acetate); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (d, 2H, $J = 7.8$ Hz,

H-5/5′), 7.56 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-7/7′), 7.33 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.20 (td, 2H, $J=7.8$, $J=2.1$ Hz, H-6/6′), 3.95 (s, 2H); IR (KBr) ν_{max} 1567, 1598, 1648, 3064, 3648 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.6 (3.11); MS (*m*/*z*) 336 (M^+, 39), 215 (28), 174 (22), 162 (39), 121(100), 92 (71), 65 (37). Anal. calcd for $C_{19}H_{12}O_6$: C 67.86; H 3.60; Found: C 67.88; H 3.65.

3.2.2. 3,3′-Ethylidenebis-(4-hydroxycoumarin) (2). Yield: 0.465 g (93%); mp 175 °C; $R_f=0.64$ (ethyl acetate,); H^1 NMR (400 MHz, DMSO-d_6) δ 7.72 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.41 (td, 2H, $J=7.8$, $J=2.3$ Hz, H-7/7′), 7.33 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.25 (td, 2H, $J=7.8$, $J=2.5$ Hz, H-6/6′), 4.3 (s, 1H, H-11), 1.45 (d, 3H, $J=5.6$ Hz, –C$\underline{H}_3$); IR (KBr) ν_{max} 1562, 1587, 1654, 3058, 3642 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.6 (3.21) nm; MS (*m*/*z*) 350, (M^+, 23), 232 (24), 189 (54), 174 (15), 162 (18), 121 (100), 92 (37), 65 (29). Anal. calcd for $C_{20}H_{14}O_6$: C 68.57; H 4.03; Found: C 68.62; H 4.08.

3.2.3. 3,3′-*N*-Butylidenebis-(4-hydroxycoumarin) (3). Yield: 0.465 g (93%); mp 123 °C; $R_f=0.71$ (ethyl acetate); H^1 NMR (400 MHz, DMSO-d_6): δ 7.91 (d, 2H, $J=7.9$ Hz, H-5/5′), 7.56 (td, 2H, $J=7.9$ $J=1.8$ Hz, H-7/7′), 7.30 (d, 2H, $J=7.9$ Hz, H-8/8′), 7.24 (td, 2H, $J=7.9$ $J=1.7$ Hz, H-6/6′), 4.4 (t, 1H, $J=6.2$ Hz, H-11), 2.06 (m, 2H, –C$\underline{H}_2$–CH_2-CH_3), 1.24–1.15 (m, 2H, –CH_2–C$\underline{H}_2$–CH_3), 0.83 (3H, t, $J=6.4$ Hz, –CH_2–CH_2–C$\underline{H}_3$); IR (KBr) ν_{max} 1557, 1638, 1672, 3054, 3647 cm^{-1}; UV (DMSO) λ_{max} (log ε) 306.8 (3.18) nm; MS (*m*/*z*) 378 (M^+, 21), 335 (15), 241 (26), 217 (12), 187 (18), 162 (17), 121 (100), 92 (25), 65 (20). Anal. calcd for $C_{22}H_{18}O_6$: C 69.83; H 4.79; Found: C 69.89; H 4.75.

3.2.4. 3,3′-*N*-Pentylidenebis-(4-hydroxycoumarin) (4). Yield: 0.46 g (92%); mp 113 °C; $R_f=0.71$(ethyl acetate); H^1 NMR (300 MHz, DMSO-d_6): 7.89 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.66 (td, 2H, $J=7.8$, $J=2.2$ Hz, H-7/7′), 7.26 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.21 (td, 2H, $J=7.8$, $J=2.5$ Hz, H-6/6′), 4.16 (t, 1H, $J=6.5$ Hz, H-11), 1.67(m, 2H,–C$\underline{H}_2$–CH_2–CH_2-CH_3), 1.52 (m, 2H,–CH_2–C$\underline{H}_2$–CH_2–CH_3), 1.28 (m, 2H,–CH_2–CH_2–C$\underline{H}_2$–CH_3), 0.80 (t, 3H, $J=5.6$ Hz, –CH_2–CH_2–CH_2–C$\underline{H}_3$); IR (KBr) ν_{max} 1532, 1628, 1662, 2895, 3646 cm^{-1}; UV (DMSO) λ_{max} 307.5 (log ε) (3.22) nm; MS (*m*/*z*) 392, (M^+ 32), 336 (40), 274 (17), 231 (29), 174 (36), 162 (16), 121 (100), 92 (25), 65 (18). Anal. calcd for $C_{23}H_{20}O_6$: C 70.40; H 4.14; Found: C 70.48; H 5.17.

3.2.5. 3,3′-*N*-Hexylidenebis-(4-hydroxycoumarin) (5). Yield: 0.485 g (97%); mp 105 °C; $R_f=0.73$ (ethyl acetate); H^1 NMR (300 MHz, DMSO-d_6): 7.85 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.55 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-7/7′), 7.30 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.23 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-6/6′), 4.17 (t, 1H, $J=6.5$ Hz H-11), 1.66 (2H, m,-C$\underline{H}_2$–CH_2–CH_2–CH_2–CH_3), 1.30–1.52 (6H, m,-CH_2–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_2$–CH_3), 0.95 (t, 3H, $J=6.6$ Hz,–CH_2–CH_2–CH_2–CH_2–C$\underline{H}_3$); IR (KBr) ν_{max} 1513, 1630, 1665, 2940, 3690 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.4 (3.27) nm; MS (*m*/*z*) 406 (M^+, 22), 380 (22), 335 (15), 284 (23), 245 (20), 189 (10), 162 (15), 121 (100), 92 (21), 65 (17). Anal. calcd for $C_{24}H_{22}O_6$: C 70.92; H 5.46; Found: C 70.95; H 5.43.

3.2.6. 3,3′-(Crotonalidene)-bis-(4-hydroxycoumarin) (6). Yield: 0.475 g (95%); mp 169 °C; $R_f=0.67$ (ethyl acetate), H^1 NMR (300 MHz DMSO-d_6): δ 7.88 (d, 2H, $J=7.9$ Hz, H-5/5′), 7.68 (td, 2H, $J=7.9$, $J=1.5$ Hz, H-7/7′), 7.35 (d, 2H, $J=7.9$ Hz, H-8/8′), 7.28 (td, 2H, $J=7.9$, $J=1.8$ Hz, H-6/6′), 6.54 (d, 1H, $J=6.4$ Hz, H-11), 5.7 (dd, 1H, $J=14.4$, 6.4 Hz, –C$\underline{H}$=CH–CH_3), 5.4 (dd, 1H, $J=14.4$, 6.9 Hz, –CH=C$\underline{H}$–CH_3), 2.1 (d, 3H, $J=6.9$ Hz, –CH=CH–C$\underline{H}_3$); IR (KBr) $_{max}$ 1549, 1579, 1640, 3061, 3650.9 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.2 (3.69) nm; MS (*m*/*z*) 376, (M^+ 32), 232 (14), 215 (16), 187 (70), 162 (10), 121 (100), 92 (30), 65 (22). Anal. calcd for $C_{22}H_{16}O_6$: C 70.21; H 4.29; Found: C 70.26; H 4.23.

3.2.7. 3,3′-(3-Hydroxybenzylidene)-bis-(4-hydroxycoumarin) (7). Yield: 0.46 g (92%); mp 210.5 °C; $R_f=0.59$ (ethyl acetate21); H^1 NMR (300 MHz, DMSO-d_6): δ 7.92 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.62 (td, 2H, $J=7.8$, $J=2.1$ Hz, H-7/7′), 7.37 (1H, d, $J=8.4$ Hz, H-6″), 7.35 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.32 (s, 1H, H-2″), 7.28 (td, 2H, $J=7.8$, $J=1.7$ Hz, H-6/6′), 6.88 (td, 1H, $J=8.4$, $J=2.4$ Hz, H-5″), 6.71 (d, 1H, $J=8.4$ Hz, H-4″), 6.12 (s, 1H, H-11); IR (KBr) ν_{max} 1608, 1644, 1678, 2955, 3678 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.3 (2.99) nm; MS (*m*/*z*) 428, (M^+, 6), 307 (3), 265 (43), 221(2), 162 (56), 120 (100), 92 (61), 65 (20). Anal. calcd for $C_{25}H_{16}O_7$: C 70.09; H 3.76; Found: C 70.15; H 3.72.

3.2.8. 3,3′-(4-Hydroxybenzylidene)-bis-(4-hydroxycoumarin) (8). Yield: 0.475 g (95%); mp 193 °C; $R_f=0.73$ (ethyl acetate); H^1 NMR (300 MHz, DMSO-d_6): δ 7.91 (2H, d, $J=7.9$ Hz, H-5/5′), 7.59 (td, 2H, $J=7.9$, $J=2.0$ Hz, H-7/7′), 7.41 (d, 2H, $J=7.9$ Hz, H-8/8′), 7.30 (td, 2H, $J=7.9$, $J=2.4$ Hz, H-6/6′), 6.53 (d, 2H, $J=8.4$ Hz, H-3″/5″), 6.52 (d, 2H, $J=8.4$ Hz, H-2″/6″), 6.12 (s, 1H, H-11); IR (KBr) ν_{max} 1615, 1648, 1669, 2997, 3648, 3780 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.6 (3.40); MS (*m*/*z*) 428, (M^+, 10) 307 (10), 265 (43), 162 (56), 120 (100), 92 (61), 65 (20). Anal. calcd for $C_{25}H_{16}O_7$: C 70.09; H 3.76; Found: C 70.13; H 3.80.

3.2.9. 3,3′-(2-Chlorobenzylidene)-bis-(4-hydroxycoumarin) (9). Yield: 0.465 g (93%); mp 239 °C; $R_f=0.61$; (ethyl acetate); H^1 NMR (400 MHz, DMSO-d_6): δ 7.86 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.81 (d, 2H, $J=8.1$ Hz, H-6″), 7.55 (td, 2H, $J=7.8$, $J=2.5$ Hz, H-7/7′), 7.25 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.22 (td, 2H, $J=7.8$, $J=2.3$ Hz, H-6/6′), 7.14 (d, 1H, $J=8.1$ Hz, H-3″), 7.10 (td, 1H, $J=8.1$ $J=2.2$ Hz, H-5″), 7.07 (td, 1H, $J=8.1$ $J=2.5$ Hz, H-4″), 6.13 (s, 1H, H-11); IR (KBr) ν_{max} 1565, 1604, 1660, 2922, 3749 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.0 (3.179) nm; MS (*m*/*z*) 448 (M^+, 4), 446 (6),410 (5), 325 (2), 249 (100), 162 (14), 120 (21), 92 (21), 62 (10). Anal. calcd for $C_{25}H_{15}ClO_6$ C 67.20; H 3.38; Cl 7.93; Found: C 67.26; H 3.40; Cl 7.94.

3.2.10. 3,3′-(3-Chlorobenzylidene)-bis-(4-hydroxycoumarin) (10). Yield: 0.485 g (97%); mp 215 °C $R_f=0.68$ (ethyl acetate); ^1H NMR (400 MHz, DMSO-d_6): δ 7.87 (d, 2H, $J=7.9$ Hz, H-5/5′), 7.62 (s, 1H, H-2″), 7.58 (td, 2H, $J=8.7$, $J=1.9$ Hz, $J=7.9$ Hz, H-7/7′), 7.30 (d, 2H, $J=7.9$ Hz, H-8/8′), 7.29(d, 1H, $J=8.4$ Hz, H-4″), 7.27

(td, 2H, $J=7.9$, $J=1.6$ Hz, H-6/6′), 7.14 (td, 1H, $J=8.4$, $J=2.2$ Hz, H-5″), 6.17 (s, 1H, H-11); IR (KBr) ν_{max} 1565.7, 1604.2, 1660, 2922.3, 3749 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307 (3.18) nm; MS (*m*/*z*) 446 (M^+, 7), 446 (8), 410 (14), 325 (8), 249 (100), 162 (19), 120 (28), 92 (18), 62 (20). Anal. calcd for $C_{25}H_{15}O_6Cl$ C 67.20; H 3.38; Cl 7.93; Found: C 67.18; H 3.36; Cl 7.95.

3.2.11. 3,3′-(2-Bromobenzylidene)-bis-(4-hydroxycoumarin) (11). Yield: 0.465 g (93%); mp 203 °C; $R_f=0.62$ (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6): 7.80 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.78 (d, 2H, $J=8.3$ Hz, H-6″), 7.51 (td, 2H, $J=7.8$, $J=2.1$ Hz, H-7/7′), 7.31 (d, 1H, $J=8.3$ Hz, H-3″), 7.28 (td, 1H, $J=8.3$, $J=2.4$ Hz, H-5″), 7.25 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.21 (td, 2H, $J=7.8$, $J=1.5$ Hz, H-6/6′), 7.01 (d, 1H, $J=8.3$ Hz, H-3″), 5.98 (s, 1H, H-11); IR (KBr) ν_{max} 1530, 1616, 1650, 3010, 3672 cm^{-1}; UV (DMSO) λ_{max} (log ε) 308.1 (3.09) nm; MS (*m*/*z*) 493 (M^+, 4), 491 (5), 410 (11), 329 (2), 291 (12), 249 (100), 221 (17), 162 (53), 120 (64), 92 (66), 63 (56). Anal. calcd for $C_{25}H_{15}BrO_6$ C 61.12; H 3.08; Br 16.26; Found: C 61.15; H 3.09; Br 16.30.

3.2.12. 3,3′-(2-Methoxybenzylidene)-bis-(4-hydroxycoumarin) (12). Yield: 0.46 g (92%); mp 236 °C; $R_f=$ 0.46 (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.65 (d, 2H, $J=8.1$ Hz, H-6″), 7.46 (td, 2H, $J=7.8$, $J=2.3$ Hz, H-7/7′), 7.23 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.18 (td, 2H, $J=7.8$, $J=2.5$ Hz, H-6/6′), 6.44 (td, 1H, $J=8.1$, $J=2.3$ Hz, H-4″), 6.38 (td, 1H, $J=8.1$, $J=1.9$ Hz, H-5″), 6.35 (d, 1H, $J=8.1$ Hz, H-3″), 6.00 (1H, *s*, H-11), 2.9 (s, 3H, –OC$\underline{H}_3$); IR (KBr) ν_{max} 1556, 1610, 1645, 2945, 3746 cm^{-1}; UV (DMSO) λ_{max} (log ε) 308.2 (3.11) nm; MS (*m*/*z*) 442, (M^+, 34), 321 (2), 281 (2), 249 (100), 162 (30), 120 (45), 92 (25), 63 (17). Anal. calcd for $C_{26}H_{18}O_7$ C 70.58; H 4.10; Found: C 70.62; H 4.02.

3.2.13. 3,3′-(3-Methoxybenzylidene)-bis-(4-hydroxycoumarin) (13). Yield: 0.49 g (98%); mp 238 °C; $R_f=0.61$ (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (d, 2H, $J=7.9$ Hz, H-5/5′), 7.56 (td, 2H, $J=7.9$, $J=2.0$ Hz, H-7/7′), 7.39 (d, 1H, $J=8.6$ Hz, H-6″), 7.31 (d, 2H, $J=7.9$ Hz, H-8/8′), 7.29 (s, 1H, H-2″), 7.27 (td, 2H, $J=7.9$, $J=2.1$ Hz, H-6/6′), 6.98 (td, 1H, $J=8.6$, $J=2.3$ Hz, H-5″), 6.58 (td, 1H, $J=8.6$ $J=2.0$ Hz, H-4″), 6.11 (1H, *s*, H-11), 3.20 (3H, s, –OC$\underline{H}_3$); IR (KBr) ν_{max} 1564, 1609, 1670, 2934, 3649 cm^{-1}; UV (DMSO) λ_{max} (log ε) 308.6 (2.86) nm; MS (*m*/*z*) 442, (M^+, 40), 321 (17), 281 (52), 265 (53), 249 (100), 162 (83), 120 (99), 92 (86), 63 (67). Anal. calcd for $C_{26}H_{18}O_7$ C 70.58; H 4.10; Found: C 70.60; H 4.01.

3.2.14. 3,3′-(4-Methoxybenzylidene)-bis-(4-hydroxycoumarin) (14). Yield: 0.49 g (98%); mp 242 °C; $R_f=0.62$ (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.86 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.57 (td, 2H, $J=7.8$, $J=1.9$ Hz, H-7/7′), 7.32 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.27 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-6/6′), 7.01 (d, 2H, $J=8.3$ Hz, H-3″/5″), 6.70 (d, 2H, $J=8.3$ Hz, H-2″/6″), 5.89 (s, 1H, H-11), 3.41 (s, 3H, –OC$\underline{H}_3$); IR (KBr) ν_{max} 1565, 1611, 1665, 2937, 3675 cm^{-1}; UV (DMSO) λ_{max} (log ε) 288.6 (3.20) nm; MS (*m*/*z*) 442, (M^+, 76), 321 (12), 281 (21), 265 (18), 249 (100), 162 (64), 120 (99), 92 (97), 63 (61). Anal. calcd for $C_{26}H_{18}O_7$ C 70.58; H 4.10; Found: C 70.61; H 4.13.

3.2.15. 3,3′-(Dihydrocinnamylidene)-bis-(4-hydroxycoumarin) (15). Yield: 0.465 g (93%); mp 190 °C; $R_f=0.58$ (ethyl acetate); H^1 NMR (400 MHz, DMSO-d_6); δ 7.88 (d, 2H, $J=8.0$ Hz, H-5/5′), 7.65 (td, 2H, $J=8.0$, $J=2.2$ Hz, H-7/7′), 7.33 (d, 2H, $J=8.0$ Hz, H-8/8′), 7.30 (td, 2H, $J=8.0$, $J=2.4$ Hz, H-6/6′), 6.5–6.8 (m, 5H, Ar-$\underline{H}$), 5.8 (t, 1H, $J=6.8$ Hz, H-11), 2.67 (m, 2H,-C$\underline{H}_2$-CH$_2$Ar), 1.89 (m, 3H, $J=6.5$ Hz, –CH$_2$-C$\underline{H}_2$-Ar); IR (KBr) ν_{max} 1563.9, 1615, 1658.3, 3026, 3650 cm^{-1}; UV (DMSO) λ_{max} (log ε) 309.2 (3.27) nm; MS (*m*/*z*) 440, (M^+, 4), 410 (17), 321 (12), 281 (21), 265 (18), 162 (64), 121 (100), 92 (97), 63 (61). Anal. calcd for $C_{27}H_{20}O_6$ C 73.63; H 4.58; Found: C 73.66; H 4.56.

3.2.16. 3,3′-(3-Cinnamylidene)-bis-(4-hydroxycoumarin) (16). Yield: 0.475 g (95%); mp 196 °C; $R_f=0.72$ (ethyl acetate); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.89 (d, 2H, $J=8.0$ Hz, H-5/5′), 7.51 (td, 2H, $J=8.0$, $J=1.7$ Hz, H-7/7′), 7.29 (d, 2H, $J=8.0$ Hz, H-8/8′), 7.25 (dd, 1H, $J=15.4$, 3.5 Hz, –C$\underline{H}$=CH-Ar), 7.20 (td, 2H, $J=8.0$, $J=2.5$ Hz, H-6/6′), 7.15–7.18 (5H, m, Ar-$\underline{H}$), 6.85 (d, 1H, $J=15.4$ Hz, –CH=C$\underline{H}$-Ar), 4.9 (d, 1H, $J=3.5$ Hz, H-11); IR (KBr) ν_{max} 1560, 1637, 1680, 3050, 3648 cm^{-1}; UV (DMSO) λ_{max} (log ε) 305.9 (3.16) nm; MS (*m*/*z*) 438, (M^+, 6), 277 (84), 202 (3), 187 (5), 162 (64), 121 (100), 92 (86), 63 (59). Anal. calcd for $C_{27}H_{18}O_6$ C 73.97; H 4.14; Found: C 73.92; H 4.10.

3.2.17. 3,3′-(2-Pyridyl-methylene-bis-(4-hydroxycoumarin) (17). Yield: 0.485 g (97%); mp 278 °C (decomposed); $R_f=0.63$ (ethyl acetate); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.61 (s, 1H, H-2″), 8.40 (d, 1H, $J=8.7$ Hz, H-4″), 7.80 (d, 2H, $J=8.0$ Hz, H-5/5′), 7.56 (td, 2H, $J=8.0$, $J=2.2$ Hz, H-7/7′), 7.35 (d, 2H, $J=8.0$ Hz, H-8/8′), 7.30 (td, 2H, $J=8.0$, $J=2.5$ Hz, H-6/6′), 6.93 (td, 1H, $J=8.7$, $J=2.3$ Hz, H-5″), 5.91 (1H, s, H-11); IR (KBr) $_{max}$ 1567, 1598, 1648, 3064, 3648 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.6 (3.35) nm; MS (*m*/*z*) 413, (M^+, 27), 252 (44), 162 (64), 120 (100), 92 (93), 63 (73). Anal. calcd for $C_{24}H_{15}NO_6$ C 69.73; H 3.66; N 3.39; Found: C 69.70; H 3.60; N 3.41.

3.2.18. 3,3′-(4-Nitrobenzylidene)-bis-(4-hydroxycoumarin) (18). Yield: 0.465 g (93%); mp 219 °C; $R_f=0.59$ (ethyl acetate); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (d, 2H, $J=8.7$ Hz, H-2″/6″), 7.86 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.78 (d, 2H, $J=8.7$ Hz, H-3″/5″), 7.57 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-7/7′), 7.32 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.27 (td, 2H, $J=7.8$, $J=2.4$ Hz, H-6/6′), 5.89 (s, 1H, H-11); IR (KBr) ν_{max} 1562, 1637, 1664, 3010, 3614 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.1 (2.70) nm; MS (*m*/*z*) 457, (M^+, 32), 336 (7), 296 (10), 250 (16), 162 (42), 120 (85), 92 (100), 63 (30). Anal. calcd for $C_{25}H_{15}NO_8$ C 65.65; H 3.31; N 3.06; Found: C 65.71; H 3.29; N 3.10.

3.2.19. 3,3′-(2-Pyrolidene)-bis-(4-hydroxycoumarin) (19). Yield: 0.475 g (95%); mp 160 °C; $R_f=0.42$ (ethyl acetate); H^1 NMR (300 MHz, DMSO-d_6): δ 7.91 (d, 2H, $J=7.9$ Hz, H-5/5′), 7.55 (td, 2H, $J=7.9$, $J=1.7$ Hz, H-7/7′),

7.52 (d, 1H, $J=3.5$ Hz, H-3″), 7.33 (2H, d, $J=7.9$ Hz, H-8/8′), 7.30 (td, 2H, $J=7.9$, $J=1.7$ Hz, H-6/6′), 6.96 (dd, 1H, $J=3.5$ Hz, $J=2.4$ Hz, H-4″), 6.80 (d, 1H, $J=2.4$ Hz, H-5″), 6.03 (1H, s, H-11); IR (KBr) ν_{max} 1575, 1596, 1640, 3061, 3650 cm^{-1}; UV (DMSO) λ_{max} (log ε) 306.9 (3.07) nm; MS (*m*/*z*) 401, (M^+, 5), 357 (16) 335 (26), 240 (23), 162 (54), 120 (100), 63 (37). Caculated: Anal. calcd for $C_{23}H_{15}NO_6$: C 68.83; H 3.77; N 3.49; Found: C 68.89; H 3.75; N 3.43.

3.2.20. 3,3′-(3-Indolidene)-bis-(4-hydroxycoumarin) (20). Yield: 0.462 g (96%); mp 133 °C; $R_f=0.68$ (ethyl acetate); ^1H NMR (300MHz, DMSO-d_6): δ 7.79 (d, 2H, $J=7.8$ Hz, H-5/5′), 7.59 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-7/7′), 7.28 (d, 2H, $J=7.8$ Hz, H-8/8′), 7.20 (td, 2H, $J=7.8$, $J=2.0$ Hz, H-6/6′), 7.10–7.18 (m, 1H, H-4″-7″), 6.03 (s, 1H, H-11); IR (KBr) ν_{max} 1567, 1598, 1648, 3064, 3648 cm^{-1}; UV (DMSO) λ_{max} (log ε) 307.1 (3.17) nm; MS (*m*/*z*) 451, (M^+, 8), 367 (23) 331 (43), 291 (40), 289 (100), 162 (53), 120 (100), 92 (75), 63 (46). Anal. calcd for $C_{27}H_{17}NO_6$ C 71.84; H 3.80; N 3.10; Found: C 71.88; H 3.74; N 3.15.

3.2.21. 3,3′-(12-pheylpropylidene)-bis-(4-hydroxycoumarin) (21). Yield: 0.46 g (92%); mp 198 °C; $R_f=0.58$ (ethyl acetate); H^1 NMR (DMSO-d_6, 400 *MHz*); δ 7.8 (2H, d, $J=7.4$ Hz, H-5/5′), 7.6 (2H, td, $J=7.1$, $J=1.5$ Hz, H-7/7′), 7.33 (2H, d, $J=7.8$ Hz, H-8/8′), 7.30 (2H, td, $J=7.4$, $J=1.5$ Hz, H-6/6′), 6.5 (5H s, Ar.), 5.6 (1H, *m*,-CH-), 3.4 (3H, d, $J=7.8$, $-CH_3$); IR (KBr) ν_{max} 1563, 1615, 1658, 3026, 3650 cm^{-1}; UV (DMSO) λ_{max} (log ε) 306.2 (3.27) nm; MS (*m*/*z*) 440, (M^+, 6), 336 (30) 279 (5), 255 (5), 241 (10), 187 (12), 162 (35), 121 (100), 91 (99), 63 (46). Anal. calcd for C 73.63; H 4.58; Found: C 73.66; H 4.56.

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