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SYNTHESIS AND ANTIMICROBIAL ACTIVITIES OF 4-ARYL-3,4-DIHYDROCOUMARINS AND 4-ARYLCOUMARINS

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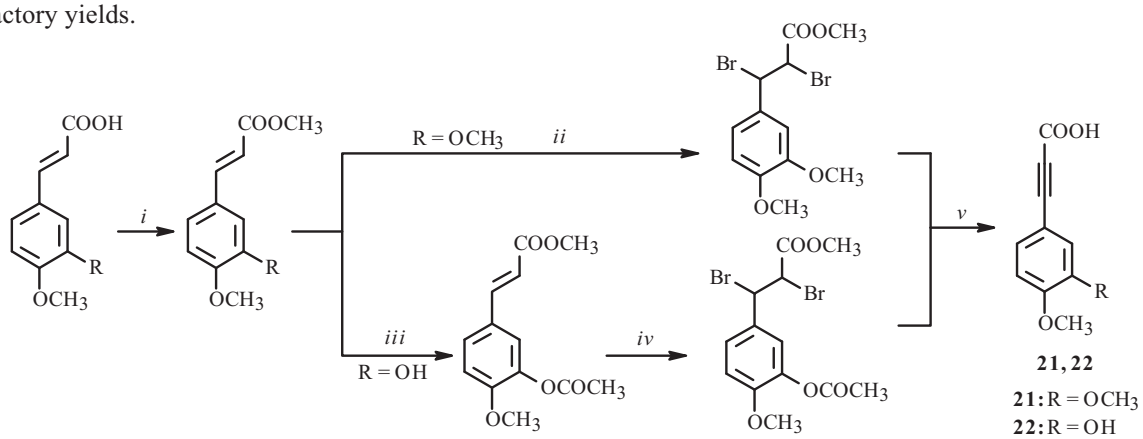
A new series of 4-aryl-3,4-dihydrocoumarins and 4-arylcoumarins were synthesized by the reaction of substituted cinnamic acids and 3-arylpropiolic acid with the corresponding phenols. These compounds were evaluated for antibacterial activity in vitro*. The synthesized compounds displayed different degrees of antimicrobial activity against* Staphylococcus aureus*,* Escherichia coli*, and* Bacillus dysenteriae*, and* Candida albicans *(a fungus). Compounds with catechol moieties and 7,8-substituted dihydroxyls in the A ring were the most active antimicrobial agents.*

Keywords: 4-aryl-3,4-dihydrocoumarin, 4-arylcoumarin, antimicrobial activity.

4-Arylcoumarin and 4-aryl-3,4-dihydrocoumarin constitute a specific branch of coumarin derivatives and are also regarded as a rare subgroup of flavonoids. In recent years, several 4-arylcoumarins and 4-aryl-3,4-dihydrocoumarins have been shown to possess antidiabetic [1], anti-inflammatory [2, 3], cytotoxic [4, 5], and antifungal [6] effects. Notably, antibacterial activity has also been reported for 4-arylcoumarins isolated from *Mesua ferrea* blossoms [7]. As part of our ongoing research into bioactive arylcoumarins, we report herein the synthesis and antimicrobial activity of five 4-arylcoumarins and twelve 4-aryl-3,4-dihydrocoumarins, some of which displayed a broad spectrum of activities.

The synthetic methods for 4-arylcoumarins have mainly involved Pechmann reaction, Perkin reaction, Suzuki or Stille reaction, Houben-Hoesch reaction, and Wittig reaction. Most of the reported methods suffered from one or more drawbacks, such as harsh reaction conditions, long reaction times, low yields, and inconvenient workup procedures [8–11]. We herein report a phenylpropiolic acid-based Ponndorf reaction with phloroglucinol to afford 4-arylcoumarins in mild conditions with satisfactory yields.



i. MeOH, $SOCl_2$, reflux, 4 h; *ii*. Br_2, CH_2Cl_2, 0°C, 20 min; *iii*. CH_3COOH, $SOCl_2$, reflux, 2 h; *iv*. Br_2, CH_2Cl_2, 0°C, 20 min; *v*. KOH, EtOH, reflux, 6 h.

Scheme 1

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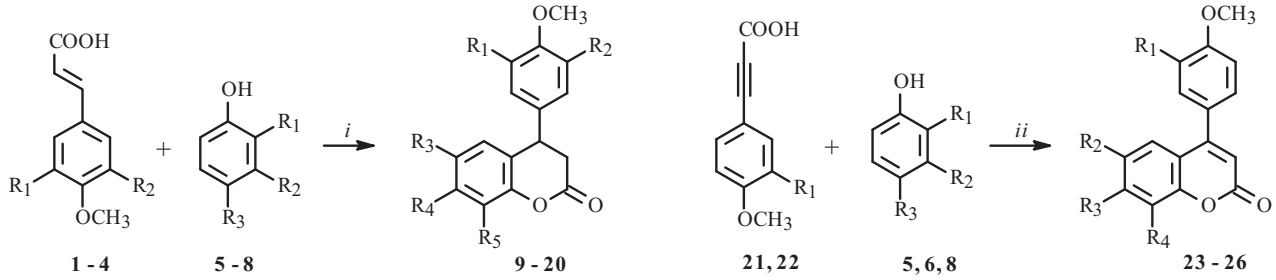
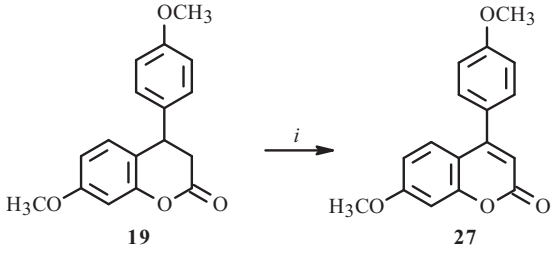


1: $R_1 = R_2 = H$; **2:** $R_1 = H$, $R_2 = OH$; **3:** $R_1 = H$, $R_2 = OCH_3$; **4:** $R_1 = R_2 = OCH_3$; **5:** $R_1 = R_3 = H$, $R_2 = OH$
6: $R_1 = R_2 = OH$, $R_3 = H$; **7:** $R_1 = R_2 = H$, $R_3 = OH$; **8:** $R_1 = R_3 = H$, $R_2 = OCH_3$; **9:** $R_1 = R_3 = R_5 = H$, $R_2 = R_4 = OH$
10: $R_1 = R_3 = R_5 = H$, $R_2 = OCH_3$, $R_4 = OH$; **11:** $R_1 = R_3 = H$, $R_2 = R_4 = R_5 = OH$; **12:** $R_1 = R_3 = H$, $R_2 = OCH_3$, $R_4 = R_5 = OH$
13: $R_1 = R_2 = OCH_3$, $R_3 = R_5 = H$, $R_4 = OH$; **14:** $R_1 = R_2 = OCH_3$, $R_3 = H$, $R_4 = R_5 = OH$; **15:** $R_1 = R_2 = R_3 = H$, $R_4 = R_5 = OH$
16: $R_1 = R_4 = R_5 = H$, $R_2 = OCH_3$, $R_3 = OH$; **17:** $R_1 = R_2 = OCH_3$, $R_3 = OH$, $R_4 = R_5 = H$; **18:** $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OH$
19: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = OCH_3$; **20:** $R_1 = R_2 = R_4 = OCH_3$, $R_3 = R_5 = H$; **21:** $R_1 = OCH_3$; **22:** $R_1 = OH$
23: $R_1 = OCH_3$, $R_2 = R_4 = H$, $R_3 = OH$; **24:** $R_1 = OCH_3$, $R_2 = H$, $R_3 = R_4 = OH$; **25:** $R_1 = OCH_3$, $R_2 = R_4 = H$, $R_3 = OCH_3$
26: $R_1 = R_3 = R_4 = OH$, $R_2 = H$

i. $POCl_3$ and BF_3–Et_2O, 0°→room temperature, 4–18 h; *ii*. CF_3COOH, room temperature, 4–8 h.

Scheme 2



i. DDQ in dioxane, room temperature.

Scheme 3

The 3-arylpropiolic acid derivatives **21** and **22** were synthesized as previously described [12] in three or four steps with 37–44% yields (Scheme 1). Twelve 4-aryl-3,4-dihydrocoumarins **9–20** were synthesized by the condensation of substituted cinnamic acids **1–4** with the corresponding phenols **5–8** in the presence of BF_3–Et_2O and $POCl_3$, resulting in 52–73% yields (Scheme 2). Four 4-arylcoumarins **23–26** were synthesized by the condensation of substituted propargyl acids **21** and **22** with the corresponding phenols **5**, **6**, and **8** in the presence of CF_3COOH with 53–58% yields (Scheme 2). Compound **19** was dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxane at room temperature to yield compound **27** with 69% yield (Scheme 3). To date, we have attempted to transform the dihydrocoumarin by several methods; compound **19** was treated with SeO_2, Pd-C/PhOPh, MnO_2, or I_2/pyridine to introduce unsaturation into the lactone ring. However, compound **27** was not formed under these conditions. Dehydrogenation with DDQ finally yielded 4-arylcoumarin, and this proved to be a mild method for the synthesis of 4-arylcoumarins [5].

The structures of all the synthesized compounds were supported by FT-IR, MS, ^{1}H NMR, and ^{13}C NMR spectral data. In the IR spectrum of 4-aryl-3,4-dihydrocoumarins, a prominent absorption band at 1714–1761cm^{-1} indicated the presence of a carbonyl group in the compound. The other absorption band at 3320–3513 cm^{-1} in the IR spectrum corresponded to a hydroxy group. The ^{1}H NMR spectrum of 4-aryl-3,4-dihydrocoumarins showed a multiplet at δ 2.96–3.03 due to the C-3 hydrogen and a quartet at 4.16–4.24 due to the C-4 hydrogen, and the aromatic hydrogens of rings A and B appeared as multiplets at 6.33–7.11. The ^{13}C NMR spectrum of 4-aryl-3,4-dihydrocoumarins showed the C-3 carbon at δ 36.8–37.9, the C-4 carbon at 39.2–41.0, the aromatic carbons at 104.0–160.0, and the carbonyl carbon at 166.9–168.5. In addition, the mass spectra of 4-aryl-3,4-dihydrocoumarins showed molecular ion peaks corresponding to their molecular formulas. The infrared spectra of 4-arylcoumarins showed an absorption band at 1695–1738 cm^{-1}, corresponding to a carbonyl group. The ^{1}H NMR spectra of 4-arylcoumarins showed a singlet at δ 6.03–6.20 due to H-3, and the aromatic hydrogens of rings A and B appeared as multiplets at 6.74–7.55. In the ^{13}C NMR spectrum, the C-3 carbon was detected at δ 110.6–111.8, the C-4 carbon around 112.7, and the carbonyl carbon at 161.2–163.8. The mass spectra of these compounds showed corresponding molecular ion peaks.

TABLE 1. *In vitro* Minimum Inhibitory Concentrations (MIC in μg/mL), Minimum Bactericidal Concentrations (MBC in μg/mL), and Minimum Fungicidal Concentrations (MFC in μg/mL) for the Synthesized Compounds

Compound	MIC, μg/mL				MBC (or MFC), μg/mL			
	S. aureus	*E. coli*	*B. dysenteriae*	*C. albicans*	*S. aureus*	*E. coli*	*B. dysenteriae*	*C. albicans*
9	625	469	234	469	1250	–	–	–
10	937.5	–	937.5	1250	–	–	–	–
11	234	58.5	156	117	–	625	625	–
12	469	39	469	469	1250	–	1250	1250
13	–	–	937.5	1250	–	–	–	–
14	937.5	58.5	117	234	–	312.5	–	–
15	312.5	78	117	312.5	625	625	625	–
16	156	117	117	469	625	1250	625	–
17	469	–	469	312.5	–	–	–	–
18	117	39	14.5	117	312.5	–	156	312.5
19	937.5	–	29	–	–	–	–	–
20	–	–	234	117	–	–	–	–
24	117	–	29.3	78	–	–	–	–
26	156	156	4.9	58.5	–	–	78	–
Ampicilin	<1.95	23	29	<1.95	<1.95	125	125	3.9
Gentamicin	<1.95	<1.95	<1.95	2.9	7.8	<1.95	31	125

The negative control, DMSO, showed no activity.

–: totally inactive (MIC > 1250 μg/mL); compounds **23**, **25**, and **27** were totally inactive.

All the synthesized compounds were evaluated for their antimicrobial activities against four microorganisms: *Staphylococcus aureus* (ATCC2592) (Gram-positive), *Escherichia coli* (ATCC25922) (Gram-negative), *Bacillus dysenteriae* (Bacillaceae), and *Candida albicans* (ATCC2002) (fungus). The antimicrobial activities of the seventeen 4-arylcoumarins derivatives, as indicated by the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) values, are listed in Table 1. These compounds exhibited a broad range of antimicrobial activities. Some of the 4-arylcoumarin derivatives exhibited activity against all four microorganisms tested, and a few of them were selectively active against one or two strains, while others displayed little or no activity against any of the microorganisms, even at the highest test concentration of 1250 μg/mL. In general, the activity against *Bacillus dysenteriae* was the highest of the four microorganisms tested. Compound **26** showed the highest activity against *Bacillus dysenteriae*, with an MIC value of 4.9 μg/mL (Table 1) and an MBC value of 78 μg/mL (Table 1). Compound **18** (MIC = 14.5 μg/mL, MBC = 156 μg/mL) and compound **24** (MIC = 29.3 μg/mL, MBC > 1250 μg/mL) (Table 1) also displayed strong activity against *Bacillus dysenteriae*. For most compounds, the MBC value was fourfold greater than the MIC value.

From these results, it is also possible to establish tentative relations between the structures of 4-arylcoumarin derivatives and their antimicrobial activities. The presence of a catechol moiety in the A ring increased the antimicrobial activity of the tested compounds. As a result, compounds **11**, **12**, **14**, **15**, **24**, and **26** displayed moderate to good activity. Derivatives containing 6-substituted hydroxy moieties showed higher activity than their corresponding 7-substituted hydroxy analogues. For example, compounds **16** and **17** with a 6-substituted hydroxyl group were more active than compounds **10** and **13** with a 7-substituted hydroxyl group. Simultaneously, 4′-methoxy substitution with a 3′-hydroxy group gave good antimicrobial activity; consequently, compounds **9**, **11**, and **26** all display antimicrobial activity. Finally, it is noteworthy that double bonds in the C ring were not a definite factor determining antimicrobial activity. While 4-arylcoumarins **24** and **26** were more active than 4-aryl-3,4-dihydrocoumarins with the same substituted group (**11** and **12**), 4-aryl-3,4-dihydrocoumarins **10** and **20** were more active than 4-arylcoumarins **23** and **27**.

In this study, the antimicrobial potential of a number of 4-aryl-3,4-dihydrocoumarin and 4-arylcoumarin derivatives has been demonstrated. The presence of 7,8-substituted dihydroxyls in the A ring increased the antimicrobial activity of the tested compounds. Derivatives containing a 6-substituted hydroxy moiety demonstrated higher activity than the corresponding 7-substituted hydroxy analogues. In general, it has been demonstrated that the nature and position of substituent groups can change the antimicrobial activity of these compounds. Therefore, 4-arylcoumarins could be considered as candidates for further research to prove that they are potentially novel antimicrobial agents.

EXPERIMENTAL

Melting points were determined using a Thiele tube and were uncorrected. The FT-IR spectra were recorded using an Analect RFX-65A spectrometer with KBr pellets. The ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AM-400 spectrometer with TMS as the internal standard. Chemical shifts were reported on a δ scale (ppm) with $CDCl_3$ or CD_3COCD_3 as the solvents. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained with a Shimadzu QP-5050A spectrometer.

Synthesis of 3-Arylpropargyl Acids 21 and **22.** A mixture of cinnamic acid (10 mM) and $SOCl_2$ (1 mL) in MeOH (20 mL) was refluxed for 4 h, followed by removal of the solvent in vacuo. A mixture of the ester (10 mmol) and acetic acid (20 mL) in the presence of $SOCl_2$ (2 mL) was refluxed for 2 h, poured into ice water, and filtered to obtain the crude product. To a solution of the ester (10 mmol) in CH_2Cl_2 (20 mL), Br_2 (0.5 mL) was added dropwise at 0°C for 20 min. The solvent was removed under reduced pressure and the residue washed with water to obtain the crude product. A mixture of dibromide (10 mmol) and KOH (3 N) in EtOH (50 mL) was heated for 6 h, followed by removal of the solvent *in vacuo*. The residue was treated with 2 N HCl, washed with water, and then purified by chromatography (Scheme 1) [12].

General Procedure for the Synthesis of 4-Aryl-3,4-dihydrocoumarins 9–20 [13]. To a mixture of $POCl_3$ (10 mmol) and BF_3–Et_2O (20 mmol) at 0°C, a substituted cinnamic acid (5 mmol) was added and the reaction mixture was stirred for 15 min at 0°C. A substituted phenol (5 mmol) was added and the mixture stirred at room temperature for 4–18 h. The reaction mixture was poured onto ice water, extracted with ethyl acetate (2 × 150 mL), washed with water (150 mL), and dried. The solvent was evaporated under reduced pressure to obtain the crude product, which was purified by chromatography on silica gel or by crystallization from acetone and water. The physical properties of the synthesized compounds are listed below.

(±)-7-Hydroxy-4-(3-hydroxy-4-methoxyphenyl)-3,4-dihydrocoumarin (9). White solid, mp 165–168°C, yield 55.2%. IR (KBr, ν, cm^{-1}): 3513 (OH), 3334, 1733 (CO), 1627, 1597, 1513, 1450, 1300, 1273, 1223, 1151 and 815. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.98 (2H, m, H-3), 3.86 (3H, s, OCH_3), 4.16 (1H, t, J = 6.4, H-4), 6.54 and 6.59 (each H, dd, J = 2.4 and 8.4, H-6′ and H-6), 6.61 and 6.69 (each H, d, J = 2.4, H-2′ and H-8), 6.77, 6.83 (each H, d, J = 8.4, H-5′ and H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.9, 39.9, 56.2, 104.2, 112.3, 112.7, 114.9, 117.9, 119.1, 129.9, 135.6, 147.4, 147.6, 153.4, 158.5, 168.2. MS *m/z* (%): 286 $[M]^+$, 268 $[M - H_2O]^+$, 253 $[M - CH_3 - H_2O]^+$, 243, 227, 213, 176, 115.

(±)-7-Hydroxy-4-(3,4-dimethoxyphenyl)-3,4-dihydrocoumarin (10). Yellow solid, mp 144–146°C, yield 60.7%. IR (KBr, ν, cm^{-1}): 3429 (OH), 1761 (CO), 1626, 1597, 1516, 1462, 1419, 1335, 1271, 1244, 1159, 1103, 1024, 991, 847, and 812. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.98 (2H, m, H-3), 3.79 and 3.84 (each 3H, s, OCH_3), 4.20 (1H, t, J = 6.0, H-4), 6.56 (1H, dd, J = 2.4 and 8.4, H-6), 6.63–6.67 (3H, m, H-6′, H-5′, H-5), 6.80 and 6.82 (each H, d, J = 2.4, H-8 and H-2′). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.5, 39.6, 55.9, 104.2, 110.5, 111.6, 112.0, 117.7, 119.7, 129.1, 133.1, 148.3, 149.3, 152.2, 156.3, 168.5. MS *m/z* (%): 300 $[M]^+$, 282 $[M - H_2O]^+$, 267 $[M - CH_3 - H_2O]^+$, 257, 243, 227, 190 ,163, 129.

(±)-7,8-Dihydroxy-4-(3-hydroxy-4-methoxyphenyl)-3,4-dihydrocoumarin (11). White solid, mp 188–190°C, yield 69.5%. IR (KBr, ν, cm^{-1}): 3498 (OH), 3417, 1753 (CO), 1516, 1468, 1271, 1198, 1174 and 1126. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.99 (2H, m, H-3), 3.86 (3H, s, OCH_3), 4.19 (1H, t, J = 6.4, H-4), 6.43 and 6.65 (each H, d, J = 8.4, H-6′ and H-5′), 6.59 and 6.78 (each H, d, J = 8, H-6 and H-5), 6.69 (1H, s, H-2). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 37.9, 40.4, 56.2, 111.7, 112.7, 115.0, 118.6, 119.1, 119.1, 133.8, 135.5, 141.3, 146.3, 147.5, 147.6, 168.0. MS *m/z* (%): 302 $[M]^+$, 284 $[M - H_2O]^+$, 269 $[M - CH_3 - H_2O]^+$, 259, 243, 229, 194, 179.

(±)-4-(3,4-Dimethoxyphenyl)-7,8-dihydroxy-3,4-dihydrocoumarin (12). White solid, mp 174–176°C, yield 72.8%. IR (KBr, ν, cm^{-1}): 3423 (OH), 1755 (CO), 1516, 1468, 1275, 1238, 1192, 1169, and 1020. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.03 (2H, m, H-3), 3.81 and 3.85 (each 3H, s, OCH_3), 4.24 (1H, dd, J = 7.6 and 6.4, H-4), 6.41, 6.66, and 6.80 (4H, 4 × d, J = 8.4, H-6′, H-5′, H-6, and H-5), 6.64 (1H, s, H-2). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 37.4, 40.0, 55.9, 110.6, 111.3, 111.5, 118.1, 118.9, 119.7, 131.1, 132.7, 139.3, 144.2, 148.5, 149.4, 166.9. MS *m/z* (%): 316 $[M]^+$, 298 $[M - H_2O]^+$, 283 $[M - CH_3 - H_2O]^+$, 273, 259, 243, 190.

(±)-7-Hydroxy-4-(3,4,5-trimethoxyphenyl)-3,4-dihydrocoumarin (13). White solid, mp 176–178°C, yield 70.0%. IR (KBr, ν, cm^{-1}): 3381 (OH), 1747 (CO), 1626, 1597, 1510, 1456, 1358, 1230, 1271, 1240, 1122, 987, and 968. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.01 (2H, m, H-3), 3.78 and 3.82 (9H, 2s, 3H and 6H each, 3 × OCH_3), 4.18 (1H, t, J = 6.0, H-4), 6.33 (2H, s, H-2′ and H-6′), 6.56 (1H, dd, J = 2.4 and 8.4, H-6), 6.64 (1H, d, J = 2.4, H-8), 6.84 (1H, d, J = 8.4, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.4, 40.4, 56.1, 60.9, 104.3, 104.5, 112.0, 117.4, 129.1, 136.4, 137.2, 152.3, 153.6, 156.3, 167.9. MS *m/z* (%): 330 $[M]^+$, 315 $[M - CH_3]^+$, 297 $[M - CH_3 - H_2O]^+$, 287, 273, 257, 213, 128, 107.

(±)-7,8-Dihydroxy-4-(3,4,5-trimethoxyphenyl)-3,4-dihydrocoumarin (14). White solid, mp 221–223°C, yield 52.6%. IR (KBr, ν, cm^{-1}): 3471 (OH), 1751 (CO), 1597, 1512, 1466, 1311, 1246, 1122, and 1007. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.03 (2H, m, H-3), 3.78 (6H, s, 2 × OCH_3), 3.82 (3H, s, OCH_3), 4.22 (2H, dd, J = 6 and 7.6), 6.33 (2H, s, H-2′ and H-6′), 6.45 and 6.67 (each H, d, J = 8.8, H-5 and H-6). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 37.3, 40.7, 56.2, 60.9, 104.5, 111.3, 117.6, 119.0, 131.1, 136.0, 139.3, 144.3, 153.6, 166.7. MS *m/z* (%): 346 $[M]^+$, 328 $[M - H_2O]^+$, 313 $[M - CH_3 - H_2O]^+$, 273, 257, 243, 229, 181, 115.

(±)-7,8-Dihydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (15). White solid, mp 166–168°C, yield 62.2%. IR (KBr, ν, cm^{-1}): 3413 (OH), 1751 (CO), 1612, 1514, 1311, 1284, 1247, 1180, 1144, and 1007. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 3.01 (2H, m, H-3), 3.78 (3H, s, OCH_3), 4.24 (1H, t, J = 6.8, H-4), 6.39 (2H, 2 × d, J = 8.4, H-5 and H-6), 6.85 and 7.04 (4H, 2 × d, J = 8.8, H-2′, H-6′, H-3′, and H-5′). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 37.9, 40.2, 55.4, 111.7, 114.9, 114.9, 118.5, 119.1, 129.3, 133.8, 134.5, 141.2, 146.3, 159.6, 167.9. MS *m/z* (%): 286 $[M]^+$, 268 $[M - H_2O]^+$, 243, 229, 200, 115.

(±)-6-Hydroxy-4-(3,4-dimethoxyphenyl)-3,4-dihydrocoumarin (16). White solid, mp 168–170°C, yield 62.3%. IR (KBr, ν, cm^{-1}): 3273 (OH), 1714 (CO), 1521, 1487, 1247, 1244, and 1186. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.0 (2H, m, H-3), 3.79 and 3.82 (each 3H, s, OCH_3), 4.17 (1H, dd, J = 6 and 8.4, H-4), 6.41 (1H, d, J = 2.8, H-2′), 6.63 (1H, d, J = 2.4, H-5), 6.68 (1H, dd, J = 2 and 8, H-7), 6.72 (1H, dd, J = 2.8 and 8.8, H-6′), 6.79 (1H, d, J = 8, H-8), 6.96 (1H, d, J = 8.8, H-5′). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 36.9, 40.4, 55.9, 110.6, 111.6, 114.5, 115.3, 118.0, 119.9, 127.4, 132.3, 145.4, 148.5, 149.4, 152.5, 168.2. MS *m/z* (%): 300 $[M]^+$, 282 $[M - H_2O]^+$, 267 $[M - CH_3 - H_2O]^+$, 257, 243, 227, 151, 115.

(±)-6-Hydroxy-4-(3,4,5-trimethoxyphenyl)-3,4-dihydrocoumarin (17). White solid, mp 174–176°C, yield 71.4%. IR (KBr, ν, cm^{-1}): 3421 (OH), 1757 (CO), 1504, 1454, 1232, 1195, and 1122. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.98 (2H, m, H-3), 3.77 and 3.80 (9H, 2s, 6H and 3H each, 3 × OCH_3), 4.17 (1H, t, J = 6.4, H-4), 6.33 (2H, s, H-2′ and H-6′), 6.55 (1H, dd, J = 2.4 and 8.6, H-6), 6.64 (1H, d, J = 2.4, H-8), 6.85 (1H, d, J = 8.6, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 36.8, 41.0, 56.1, 60.8, 104.6, 114.5, 115.5, 118.0, 126.9, 135.7, 137.2, 145.3, 152.6, 153.6, 168.1. MS *m/z* (%): 330 $[M]^+$, 312 $[M - H_2O]^+$, 297 $[M - H_2O - CH_3]^+$, 257, 213, 181, 128.

(±)-6-Hydroxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (18). White solid, mp 170–172°C, yield 52.2%. IR (KBr, ν, cm^{-1}): 3320 (OH), 1732 (CO), 1510, 1456, 1309, 1252, 1194, 1155, and 831. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.96 (2H, m, H-3), 3.75 (3H, s, OCH_3), 4.19 (1H, dd, J = 6.0 and 8.4, H-4), 6.38 (1H, d, J = 2.4, H-5), 6.71 (1H, dd, J = 3.2 and 8.8, H-7), 6.85 (2H, dd, J = 2 and 6.0, H-2′ and H-6′), 6.96 (1H, dd, J = 8.8, H-5), 7.05 (2H, dd, J = 2 and 6.0, H-3′ and H-5′). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.0, 39.9, 55.3, 76.7, 77.0, 77.2, 77.3, 114.5, 114.6, 114.7, 115.3, 118.0, 127.5, 128.7, 131.9, 145.5, 152.3, 159.0, 168.2. MS *m/z* (%): 270 $[M]^+$, 252 $[M - H_2O]^+$, 227 $[M - H_2O - CH_3]^+$, 213, 197, 128.

(±)-7-Methoxy-4-(4-methoxyphenyl)-3,4-dihydrocoumarin (19). White solid, mp 136–137°C, yield 52.1%. IR (KBr, ν, cm^{-1}): 2937, 1761 (CO), 1510, 1252, 1124, 1030, and 831. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 3.03 (2H, m, H-3), 3.76 and 3.81 (6H, 2s, 3H and 3H each, 2 × OCH_3), 4.37 (1H, t, J = 6.4, H-4), 6.66 (1H, d, J = 2.4, H-8), 6.69 (1H, dd, J = 2.4 and 8.4, H-6), 6.89 and 7.11 (each 2H, d, H-2′, H-3′, H-5′, H-6′), 6.95 (1H, d, J = 8.4, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.4, 39.2, 55.2, 55.4, 102.4, 110.6, 114.3, 117.9, 128.4, 128.7, 132.6, 152.3, 158.8, 159.8, 167.7. MS *m/z* (%): 284 $[M]^+$, 256 $[M - CO]^+$, 241 $[M - CO - CH_3]^+$, 227, 211, 128. The physical/spectral data of the compound matched well with those reported earlier [5, 14].

(±)-7-Methoxy-4-(3,4,5-trimethoxyphenyl)-3,4-dihydrocoumarin (20). White solid, mp 131–133°C, yield 57.2%. IR (KBr, ν, cm^{-1}): 3456, 1761 (CO), 1593, 1506, 1460, 1130. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.00 (2H, m, H-3), 3.78, 3.79, and 3.81 (12H, 3s, 6H, 3H, and 3H each, 4 × OCH_3), 4.19 (1H, t, J = 7.6, H-4), 6.33 (1H, s, H-2′ and H-6′), 6.64 (1H, dd, J = 2.4 and 8.4, H-6), 6.66 (1H, d, H-8), 6.89 (1H, d, J = 8.4, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 37.4, 40.5, 55.5, 56.1, 60.8, 102.5, 104.4, 110.8, 117.4, 128.9, 136.4, 137.3, 152.4, 153.6, 160.0, 167.6. MS *m/z* (%): 344 $[M]^+$, 329 $[M - CH_3]^+$, 311 $[M - CO - CH_3]^+$, 271, 220, 177, 121, 77. The physical/spectral data of the compound matched well with those reported earlier [5, 14].

Synthesis of 4-Arylcoumarins 23, 24, 25, 26. A solution of the appropriate phenol (11 mmol) and the appropriate arylpropargyl acid (10 mmol) in 10 mL CF_3COOH was stirred at room temperature for 4–8 h. The reaction mixture was poured onto ice water, extracted with ethyl acetate (2 × 150 mL), washed with water (150 mL), and dried. The solvent was removed under reduced pressure to obtain the crude product, which was then purified by chromatography on silica gel.

7-Hydroxy-4-(3,4-dimethoxyphenyl)coumarin (23). White solid, mp 233–234°C, yield 56.4%. IR (KBr, ν, cm^{-1}): 3194 (OH), 1695 (CO), 1624, 1599, 1518, 1263, and 1140. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 3.90 and 3.94 (6H, s, 3H and 3H, 2 × OCH_3), 6.20 (1H, s, H-3), 6.74 (1H, s, J = 2.4 and 8, H-6′), 6.91 (1H, s, J = 2.4, H-2′), 6.97 (1H, d, J = 8, H-5′), 6.98 (1H, d, J = 2, H-8), 7.02 (1H, dd, J = 2 and 8.8, H-6′), 7.45 (1H, d, J = 8.8, H-5′). ^{13}C NMR (100 MHz, CD_3COCD_3,

δ): 56.1, 56.2, 103.5, 111.0, 112.3, 112.5, 112.9, 113.6, 122.0, 128.9, 129.3, 150.3, 151.4, 156.5, 157.0, 161.3, 162.2. MS *m/z* (%): 298 $[M]^+$, 283 $[M - CH_3]^+$, 270, 255, 227, 199, 184, 113.

7,8-Dihydroxy-4-(3,4-dimethoxyphenyl)coumarin (24). White solid, mp 271–272°C, yield 58.0%. IR (KBr, ν, cm^{-1}): 2987, 1726 (CO), 1618, 1518, 1379, 1255, 1147, and 814. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 3.87 and 3.88 (each 3H, s, OCH_3), 6.07 (1H, s, H-3), 6.83 and 7.00 (2H, 2 × d, J = 8.8, H-5 and H-6), 7.06 (1H, dd, J = 2 and 8.4, H-5′), 7.10 (1H, d, J = 2, H-2′), 7.11 (1H, d, J = 8.4, H-6′). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 56.1, 56.2, 110.8, 112.5, 112.7, 113.0, 118.7, 122.1, 129.1, 133.3, 144.7, 149.9, 150.2, 151.4, 157.2, 161.2. MS *m/z* (%): 314 $[M]^+$, 286 $[M - CO]^+$, 271 $[M - CO - CH_3]^+$, 161, 133, 115, 103.

7-Methoxy-4-(3,4-dimethoxyphenyl)coumarin (25). White solid, mp 148–149°C, yield 53.8%. IR (KBr, ν, cm^{-1}): 3411 (CO), 1695 (CO), 1606, 1518, 1448, 1173, and 1140. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 3.88, 3.89, and 3.93 (9H, s, 3H, 3H, and 3H, 3 × OCH_3), 6.14 (1H, s, H-3), 6.90 and 7.08 (each H, dd, J = 8.8, 2.4 and J = 8.4, 2, H-6 and H-6′), 6.94 and 7.12 (each H, d, J = 2.4, H-8 and H-2′), 7.12 and 7.55 (2H, 2 × d, J = 8.4 and 8.8, H-5 and H-5′). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 56.1, 56.2, 56.3, 101.8, 111.8, 112.6, 112.9, 113.0, 113.2, 122.1, 128.7, 129.0, 150.4, 151.5, 156.3, 157.0, 161.1, 163.8. MS *m/z* (%): 312 $[M]^+$, 297 $[M - CH_3]^+$, 284 $[M - CO - CH_3]^+$, 269, 213, 183, 139.

7,8-Dihydroxy-4-(3-hydroxy-4-methoxyphenyl)coumarin (26). Yellow solid, mp 176–177°C, yield 53.7%. IR (KBr, ν, cm^{-1}): 3371 (OH), 1695 (CO), 1653, 1599, 1448, 1240, 1178, and 1130. ^{1}H NMR (400 MHz, CD_3COCD_3, δ, ppm, J/Hz): 3.91 (3H, s, OCH_3), 6.03 (1H, s, H-3), 6.83 (1H, d, J = 8.8, H-6), 6.95–6.99 (3H, m, H-5, H-2′, and H-6′), 7.10 (1H, d, J = 8, H-5′). ^{13}C NMR (100 MHz, CD_3COCD_3, δ): 56.2, 110.6, 112.4, 112.7, 113.0, 116.1, 118.7, 120.9, 129.3, 133.2, 144.6, 147.4, 149.6, 149.9, 157.2, 161.3. MS *m/z* (%): 300 $[M]^+$, 272 $[M - CO]^+$, 257 $[M - CO - CH_3]^+$, 229, 115.

Synthesis of 7-Methoxy-4-(4-methoxyphenyl)coumarin (27) [5]. To a solution of compound **19** (1 mmol) in 4.5 mL of dioxane, DDQ (1.5 mmol) was added. The mixture was stirred at room temperature until the starting material completely disappeared. After elimination of dioxane *in vacuo*, the crude product was purified by flash column chromatography on silica gel to yield compound **27**.

White solid, mp 155–157°C, yield 69%. IR (KBr, ν, cm^{-1}): 3440, 3064, 2993, 1738 (CO), 1612, 1510, 1375, 1250, 825. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.86 (6H, s, 2 × OCH_3), 6.18 (1H, s, H-3), 6.78 (1H, dd, J = 8.8 and 2.4, H-6), 6.87 (1H, d, J = 2.4, H-8), 7.01 and 7.37 (each 2H, d, J = 8.8, H-2′, H-3′, H-5′, H-6′), 7.43 (1H, d, J = 8.8, H-5). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 55.4, 55.8, 101.1, 111.4, 112.2, 112.7, 114.3, 127.8, 128.0, 129.9, 155.5, 156.0, 160.7, 161.4, 162.7. MS *m/z* (%): 282 $[M]^+$, 254 $[M - CO]^+$, 239 $[M - CO - CH_3]^+$, 211, 196, 168, 152, 140, 127.

Antibacterial Activity Assay. The synthesized compounds were tested for their antimicrobial activity against four microorganisms. The minimal inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), and minimum fungicidal concentrations (MFCs) of the tested compounds were determined by the dilution method. Each of the test compounds and standards were dissolved in DMSO at concentrations of 10 mg/mL. Further dilutions of the compounds and standards were made in test medium [15]. Standard bacterial and fungal strains were used in the antimicrobial assays. *Staphylococcus aureus* (ATCC2592) (Gram-positive), *Escherichia coli* (ATCC25922) (Gram-negative), *Bacillus dysenteriae* (Bacillaceae), and *Candida albicans* (ATCC2002) (fungus) were obtained from Kunming Medical College and were grown in Luria Bertani (LB) broth for 24 h at 37°C.

Microorganisms were first grown in LB broth to an OD_{600} of 0.8. Then, 10 μL was inoculated into 8 mL of fresh LB medium with 0.7% agar and poured over a 90 mm Petri dish containing 25 mL of 1.5% agar in LB medium. When the top agar hardened, 5 μL of filtered sample (50 mg/mL) was dropped onto the surface of the top agar and completely dried before incubation overnight at 37°C. If a clear zone formed on the surface of the top agar, growth of microorganism was inhibited [16].

Minimum inhibitory concentrations (MICs) were determined by the LB broth dilution method according to Tamokou et al. [17] with slight modifications. Samples in DMSO were diluted in LB to 0.049–25 mg/mL. Microorganisms were cultured for 12 h and then adjusted with LB to 10^6 colony-forming units (CFU) per mL. The 96-well cell culture plates were prepared by dispensing 180 μL of inoculum (10^6 CFU/mL for bacteria and 5×10^5 spores/mL for yeasts) and 20 μL test substances into each well. The plates were covered with their sterile sealer and incubated at 37°C for 24 h. The MIC was the lowest concentration of the test sample that prevented visible growth of microorganisms [17].

The minimum bactericidal concentrations (MBCs) or minimum fungicidal concentrations (MFCs) were determined by dropping 10 μL from each negative well and from the positive growth control onto LB agar and incubating them at 37°C for 24 h. The MBCs or MFCs were defined as the lowest concentration yielding negative subcultures or only one colony. For every experiment, sterility check (200 μL medium in one well), negative controls (adding 20 μL DMSO to medium), and positive controls (20 μL ampicilin or gentamicin at 400–0.78 μg/mL) were included. All the experiments were performed in triplicate.

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