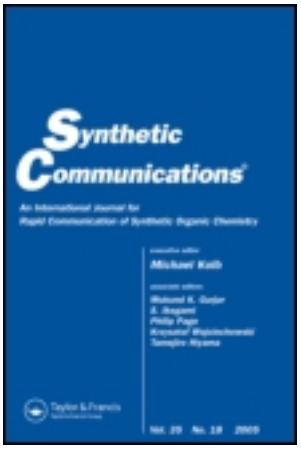

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Conventional and Microwave-Assisted Syntheses and Solid-State Structure of New Coumarins and Angelicins

Elżbieta Hejchman [a], Joanna Trykowska Konc [a], Dorota Maciejewska [a] & Hanna Kruszewska [b]
[a] Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland
[b] Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
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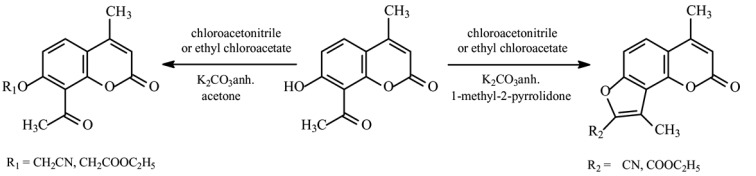
CONVENTIONAL AND MICROWAVE-ASSISTED SYNTHESES AND SOLID-STATE STRUCTURE OF NEW COUMARINS AND ANGELICINS

Elżbieta Hejchman,[1] Joanna Trykowska Konc,[1] Dorota Maciejewska,[1] and Hanna Kruszewska[2]

[1]*Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland*

[2]*Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland*

GRAPHICAL ABSTRACT



Abstract *New derivatives of coumarin and angelicin, 8-acetyl-7-cyanomethoxy-4-methyl-chromen-2-one (**1**), 8-acetyl-7-ethoxycarbonylmethoxy-4-methyl-chromen-2-one (**2**), 8-cyano-4,9-dimethyl-2H-furo[2,3-h]-1-chromen-2-one (**3**), and 8-ethoxycarbonyl-4,9-dimethyl-(2H-furo[2,3-h]-1-chromen-2-one) (**4**) were obtained by conventional synthesis and by efficient and high-yielding microwave-assisted synthesis. The solid structures were analyzed using ^{13}C cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy. Compounds **1** and **3** were evaluated for potential anticancer activity in an in vitro screening panel of 60 human tumor cell lines. Selected leukemia, non-small-cell lung, and renal cancer lines showed promising sensitivity to these compounds. Additionally, compound **4** was shown to inhibit growth of Gram-positive strains.*

Keywords Angelicin; coumarin; microwave-assisted synthesis

INTRODUCTION

Coumarin (*2H*-chromen-2-one) and its derivatives have been targets of our research because of their diverse biological activities.[1,2] Presently, we are interested in the synthesis of new, potentially bioactive angular furocoumarins, derivatives of angelicin [*2H*-furo(2,3-*h*)-1-chromen-2-one]. Antifungal activity of angelicin and its 8-substituted derivatives against *Candida albicans, Cryptococcus neoformans,*

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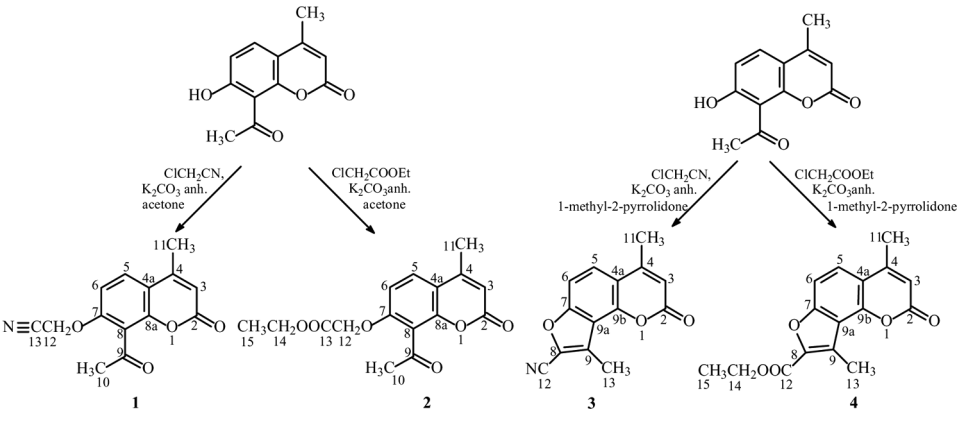
Address correspondence to Dorota Maciejewska, Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha Str., 02 097 Warsaw, Poland. E-mail: dmaciejewska@wum.edu.pl

Saccharomyces cerevisiae, and *Aspergillus niger* was reported by Sardari et al.[3,4] Antiproliferative activity of 4-methylangelicin by inhibition of DNA synthesis on Ehrlich ascites tumor cells was shown.[5,6] The in vitro antiproliferative activities of some methyl derivatives of 5-methoxyangelicin on HL60 and A431 cell lines of human cancers were evaluated using 5-methoxypsoralen as the reference compound. 4,9-Dimethyl-5-methoxyangelicin and 3,9-dimethyl-5-methoxyangelicin have better activity than 5-methoxypsolaren. When the cytotoxicity data were coupled with the in vivo phototoxicity tests, 4,9-dimethyl-5-methoxyangelicin emerged as a promising candidate for use in clinical trials because of its high inhibitory effect on the growth of human cell lines and low skin phototoxicity.[7]

The most traditional and widely used method for the synthesis of angular furocoumarins is the introduction of the furan ring to the basic coumarin structure.[8] To prepare derivatives of angelicin with electron-attracting substituents in position 8 of the furan ring, base-catalyzed condensations of acetyl hydroxyheteroarenes with α-halocarbonyl compounds were applied. 4,9-Dimethylangelicin-8-carboxylic acid and its ethyl ester were prepared using 8-acetyl-7-hydroxy-4-methyl-chromen-2-one and diethyl bromomalonate.[9,10] Microwave-induced Claisen rearrangement was also used for the synthesis of 4,8,9,-substituted angelicins.[11] The microwave-assisted synthesis has many advantages over traditional methods, which are operational simplicity, good yields, short reaction times, and easy workup procedures. This type of synthesis was used in our previous studies on coumarins[1,2] as well as in the present study.

The ^{13}C cross polarization/magic angle spinning (CP/MAS) NMR spectroscopy in the solid state is used to the investigation of structures of those compounds for which we could not obtain the crystals suitable for x-ray diffraction (XRD) examinations. Its application to the studies of biologically active substances is of special interest.[12,13]

Here, we report a new synthetic route for two novel coumarins **1** and **2**, 8-cyanoangelicin **3**, and previously obtained 8-ethoxycarbonylangelicin **4** (see Scheme 1) by conventional and microwave-assisted syntheses. Additionally, we present the application of combined ^{13}C CP/MAS NMR spectroscopy and



Scheme 1. Reagents and conditions used for the preparation of **1**, **2**, **3**, and **4** with atom numbering.

molecular modeling approach to the structural studies of compounds **1**–**4**. The anticancer and antibacterial activities of compounds **1**, **3**, and **4** are also discussed.

RESULTS AND DISCUSSION

Chemistry

The *O*-alkylation reaction of 8-acetyl-4-methyl-7-hydroxycoumarin with the alkylating agents, chloroacetonitrile, and ethyl chloroacetate under reflux in acetone, using anhydrous potassium carbonate as a base, was performed, resulting in two novel *O*-substituted coumarins, **1** and **2**. Heating the 1-methyl-2-pyrrolidone solutions of 8-acetyl-4-methyl-7-hydroxycoumarin with the alkylating agents, chloroacetonitrile and ethyl chloroacetate, in the presence of anhydrous potassium carbonate at the temperature 70–75 °C yielded two products, the novel 8-cyanoangelicin **3** as well as 8-ethoxycarbonylangelicin **4**. Reflux was applied for 11.5 h and for 28.5 h to obtain the compounds **1** and **2**, respectively. Similarly, heating for 3 h and 6.5 h at 70–75 °C was required to obtain compounds **3** and **4**. Because of the prolonged heating used, the formation of the desired products is accompanied by the decomposition of the starting materials. To speed up these reactions and avoid the undesired side products, microwave irradiation was used.

The reaction of 8-acetyl-4-methyl-7-hydroxycoumarin with chloroacetonitrile and ethyl chloroacetate under microwave irradiation afforded the products within a few minutes. However, the kind of products and their yields were dependent on the conditions applied. For example, the reflux in acetone resulted in the compounds **1** (43%) and **2** (20%); only traces of the cyclization products **3** and **4** were detected by thin-layer chromatography (TLC) in spite of the prolonged heating. Using 1-methyl-2-pyrrolidone as a solvent led exclusively to the preparation of the derivatives of angelicin **3** and **4**. The major achievement of this procedure is the considerable reduction of reaction times: from 3 h 45 min to 3 min for compound **3** and from 6.5 h to 12 min for compound **4**. The yields of the products obtained with microwave heating are greater or comparable with the yields of syntheses carried out in the conventional way. It made the microwave-assisted synthesis our method of choice in this case. The preparation of *O*-alkylated coumarins **1** and **2** *versus* furocoumarins **3** and **4** may be controlled by the choice of the reaction conditions. The formation of furocoumarins is favored through greater temperature, higher polarity, and basicity of 1-methyl-2-pyrrolidone as well as better solubility of potassium carbonate, resulting in greater concentration of the base in the reaction medium.

Solid-State Structural Analysis by ^{13}C CP/MAS NMR Spectra

The utilization of combined ^{13}C CP/MAS NMR and computational analysis to the structures of newly synthesized compounds **1**–**4** allowed us to propose their probable stable conformations in the solid state. Exemplary ^{13}C CP/MAS NMR spectra of **1** and **2** are presented in Fig. 1, and the most probable assignments of resonances in the spectra of **1, 2** and **3, 4** are given in Table 1 (the notation used in the discussion of the NMR results is given in Scheme 1). Since NMR spectra in the solid state show local conformational arrangement in opposition to NMR spectra in

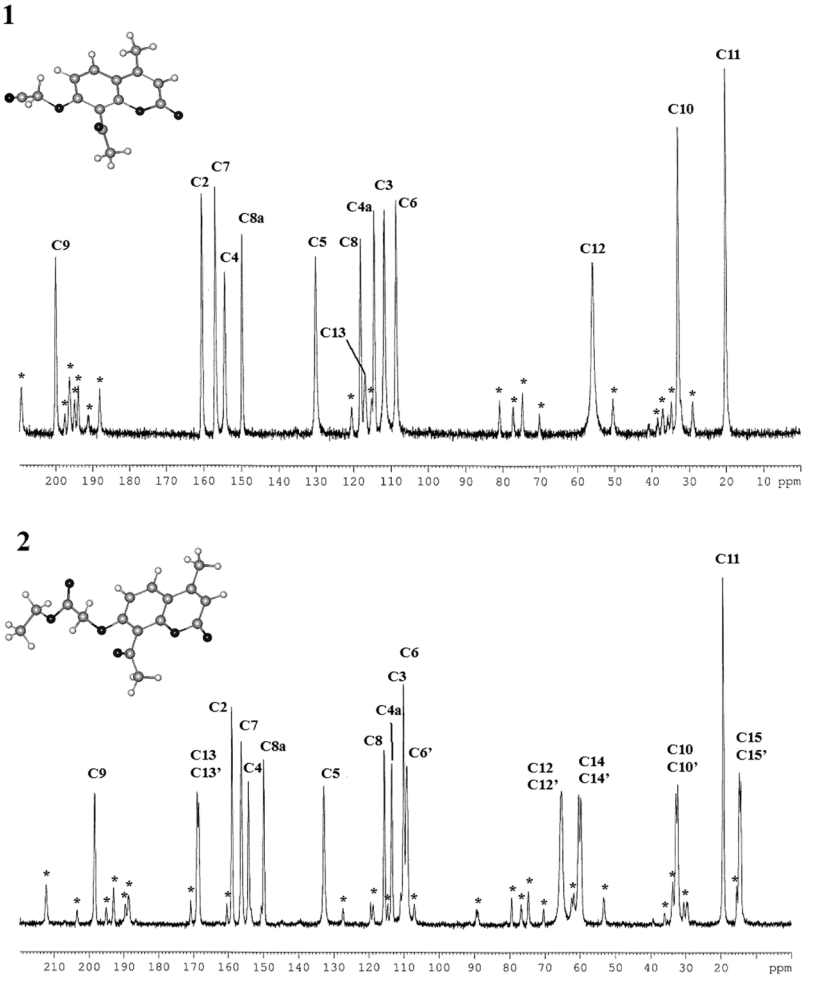


Figure 1. Exemplary ^{13}C CP/MAS NMR spectra of **1**, showing simple pattern of resonances, and of **2**, showing double pattern of resonances. Sidebands are marked with an asterisk.

solution, which detect average conformation of molecules, it was necessary to engage an additional tool for structural analysis: the computation of theoretical shielding constants for ^{13}C atoms. The density functional theory (DFT) method was used to examine the geometries and NMR resonances. For the structures corresponding to local energy minima, we have analyzed the correlation coefficients R^2 obtained for the linear correlations between the experimental chemical shifts δ (ppm) and the theoretical shielding constants σ (ppm). We have chosen as the most probable solid-state structures those for which the greatest values of R^2 (0.987–0.996) were obtained. Examination of the spectra of **1** and **3, 4** reveals the same number of peaks as the number of chemically distinct carbon sites in these molecules, but in the spectrum of **2** one can observe a significantly greater number of peaks. Those multiplicities and intensities of resonances are consistent with an interpretation

Table 1. Chemical shifts of ^{13}C NMR in solid state δ (ppm) for **1, 2** and **3, 4**

No.	**1**	**2**	**3**	**4**
C2	160.3	158.9	158.6	158.2
C3	111.6	110.0	112.6	113.6
C4	154.3	154.2	153.3	154.8
C4a	114.3	113.4	112.6	115.4
C5	129.9	132.7	123.5	124.8
C6	108.5	110.0;109.0	109.6	109.6
C7	156.8	156.2	156.3	156.2
C8	118.0	115.6	126.5	141.3
C8a	149.7	149.8	—	—
C9	199.9	198.3	130.3	124.8
C9a	32.9	32.7;32.2	114.1	116.8
C9b	—	—	147.7	150.1
C11	20.1	19.3	19.9	19.6
C12	55.8	65.4;65.3	112.6	160.0
C13	116.8	168.9;168.4	10.9	11.4
C14	—	60.3;59.7	—	63.4
C15	—	14.8;14.4	—	13.9

demanding the presence of a single molecule in the crystallographic asymmetric unit of compounds **1** and **3, 4** in contrast to compound **2**, for which two distinct molecules are expected. (Double resonances were previously observed for another *O*-alkyl-4-methylcoumarin,[2] and XRD measurement revealed two molecules in the crystallographic asymmetric unit.) The twofold multiplicity of resonances is particularly apparent in the regions of the spectrum of **2**, consisting of peaks due to the ester and acetyl groups. The small differences between two structures of **2** lie in the orientation of the ester side chain with respect to the fused bicyclic system. The energies of analyzed conformations are close to each other, and the torsion angles C8–C7–O–C12 should be very similar for both conformers. The resonances representing both structures in the spectrum of **2** are located very closely (the values are within the range of 1 ppm), and we could not deduce structures of both conformers. We decided to show only one conformation with the torsion angle C8–C7–O–C12 equal to $-165°$, corresponding to the *trans* ester bond for which the greatest R^2 value was obtained (see the hypothetical structure shown on the left-hand side of Fig. 1). To establish the correct orientations of cyanomethylene in **1** and acetyl substituents in **1** and **2**, we have additionally compared the signals of C6, C8, and C9 atoms in ^{13}C CP/MAS NMR spectra of **1** and **2** with those in solution. This comparison reveals a low-frequency shift of C6 resonances in the spectrum of **1** in solid state, which is in agreement with conformations in which the methylene groups $CH_2(12)$ are directed at C6 atom. An arrangement of methylene group in **1** resembles those proposed for $CH_2(12)$ in **2**. Such location of methylene groups force the acetyl group to deviate from the benzene ring plane. The acetyl group at C8 is principally perpendicular to the bicyclic plane in **1** and **2** (see the structures in Fig. 1). It is likely that this orientation is connected with the low-frequency shift of C8 resonance in solid state. Small high-frequency shift of C2 and C9 atoms in the spectra of **1** and **2** corresponds to the weak hydrogen bonding in which these groups are involved.

Table 2. Activity of compound **4** against Gram-positive bacterial strains

Strain	MIC (mg/mL)
Micrococcus luteus ATCC 9341	10
Micrococcus luteus ATCC 10240	10
Bacillus subtilis ATCC 6633	7,5
Bacillus cereus ATCC 11778	2,5
Staphylococcus epidermidis ATCC 12228	10
Staphylococcus aureus ATCC 6538	10
Staphylococcus aureus ATCC 6538P	10

Those were not observed for C2 resonances in the spectra of furocoumarins **3** and **4** and could be the evidence that C2=O groups in these compounds are probably not engaged in hydrogen bonding. The conformations of **3** and **4**, in which the orientations of all substituents are in plane of the tricyclic fused ring system, are proposed according to the values of the relevant shielding constants obtained for the low-energy structures and the greatest values of the correlation coefficient R^2.

Biological Activity Assays

Compounds **1** and **3** were accepted for evaluation by the Division of Cancer Treatment and Diagnosis National Cancer Institute (Bethesda, USA) in the full panel of 60 different cell lines, representing human leukemia, non-small-cell lung cancer, colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines. The details are available at the web site http://dtp.nci.nih.gov. The inhibition of growth was measured at a single dose of the tested compounds, at the concentration of 10^{-5} M. Compound **1** inhibited the growth of leukemia HL-60(TB) (growth 81.57%), K-562 (68.99), RPMI-8226 (80.00), non-small-cell lung cancer NCI-H522 (77.26), and prostate cancer PC-3 (87.23). Compound **3** appeared to be more active. It inhibited the growth of leukemia CCRF-CEM cell lines (growth 6.77%), and, to a lesser extent, non-small-cell lung cancer HOP-92 (81.97) and renal cancer A498 (75.10).

The angelicin derivative **4** was screened for activity on 14 microbial strains. Compound **4** inhibited growth of only Gram-positive strains (Table 2). It inhibited growth of *Bacillus subtilis* in the minimal concentration of 2.5 mg/mL (minimal inhibitory concentration, MIC). The lack of good water solubility of this compound may prevent it from having better antimicrobial activity.

EXPERIMENTAL

Instruments and Methods

Melting points were determined with a digital melting-point apparatus 9001 and are uncorrected. Microwave oven Plazmatronika (1000 W), equipped with a single-mode cavity suitable for the microscale synthesis and microwave-choked outlet connected to external condenser set to 30% power, was used (http://www.

plazmatronika.com.pl). ^{1}H NMR, ^{13}C NMR, HSQC, and heteronuclear multiple-bond correlation (HMBC) spectra in solution were recorded at 25 °C with Varian NMRS-300 or Varian 500 spectrometers. The calculated shielding constants were used as an aid in an assignment of resonances of ^{13}C atoms. The solid state ^{13}C CP/MAS NMR spectra were measured using a Bruker Avance DMX 400. The powdered samples were spun at 8 kHz. Contact time of 4 ms, repetition time of 16 s, and spectral width of 24 kHz were used for the total of 1,000 scans. The notation used in the detailed description of NMR resonances is given in Scheme 1. Starting geometries of compounds **1–4** were optimized at PM3 semi-empirical level of theory.[14] Final geometries were obtained using DFT method with B3LYP/6-31** hybrid functional, and the coupled perturbed Hartree–Fock–gauge including atomic orbitals (CPHF-GIAO) approach was employed for shielding constants calculations as implemented in Gaussian 03 package.[15] Infrared (IR) spectra were recorded on a Fourier transform (FT)–IR Perkin-Elmer instrument. High-resolution mass spectra (HRMS) were recorded on Quattro LCT (TOF).

Conventional Syntheses

8-Acetyl-7-cyanomethoxy-4-methyl-chromen-2-one (1). 8-Acetyl-7-hydroxy-4-methylcoumarin (1.47 mmol, 0.32 g) and chloroacetonitrile (2.7 mmol, 0.17 mL) were dissolved in acetone (15 mL), and anhydrous K_2CO_3 (2.9 mmol, 0.4 g) was added to this solution. The mixture was refluxed and monitored by TLC on silica-gel plates (eluent $CHCl_3$–AcOEt 20:1). After completion of the reaction as indicated by TLC, the mixture was poured into the flask with water and ice (50 mL) and stirred for 30 min. The precipitate was filtered out and dried. Heating: 11 h and 30 min. $R_f = 0.10$. Yield 0.24 g (64%). The analytical sample was crystallized from ethanol. Mp 198.5–199 °C; IR (KBr) ν_{max}/cm^{-1}: 3082, 2961, 2935, 2259, 1735, 1701, 1591, 1566, 1446, 1493, 1368, 1293, 1102, 839; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.44 (d, $J = 1.0$ Hz, 3H, H-11), 2.63 (s, 3H, H-10), 4.88 (s, 2H, H-12), 6.25 (d, $J = 1.0$ Hz, 1H, H-3), 7.03 (d, $J = 9.0$ Hz, 1H, H-6), 7.66 (d, $J = 9.0$ Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz): 18.80 (C-11), 32.55 (C-10), 54.71 (C-12), 109.29 (C-6), 114.11 (C-3), 114.20 (C-13), 116.28 (C-4a), 121.12 (C-8), 126.67 (C-5), 150.97 (C-8a), 151.62 (C-4), 154.80 (C-7), 159.20 (C-2), 198.14 (C-9); TOF MS ES+ $[M + Na]^+ = 280.0539$ ($C_{14}H_{11}NO_4Na$).

8-Acetyl-7-ethoxycarbonylmethoxy-4-methyl-chromen-2-one (2). 8-Acetyl-7-hydroxy-4-methylcoumarin (1.5 mmol, 0.33 g) and ethyl chloroacetate (1.65 mmol, 0.176 mL) were dissolved in acetone (15 mL), and anhydrous K_2CO_3 (4.5 mmol, 0.625 g) was added to this solution. The mixture was refluxed and monitored by TLC on silica-gel plates (eluent $CHCl_3$–AcOEt 20:1). After completion of the reaction as indicated by TLC, the mixture was poured into the flask with water and ice (50 mL) and stirred for 30 min. The precipitate was filtered out and dried. Heating: 28 h and 30 min. $R_f = 0.18$. Yield 0.28 g (61%). The product was pure as indicated by TLC. Mp 105–105.5 °C; IR (KBr) ν_{max}/cm^{-1}: 3093, 2983, 2928, 1757, 1731, 1601, 1568, 1491, 1453, 1376, 1208, 1123, 843; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (t, $J = 7.2$ Hz, 3H, H-15), 2.40 (d, $J = 1.2$ Hz, 3H, H-11), 2.65 (s, 3H, H-10), 4.26 (q, $J = 7.2$ Hz, 2H, H-14), 4.75 (s, 2H, H-12), 6.17 (bq, $J = 1.2$ Hz, 1H, H-3), 6.76 (d,

$J = 9.0$ Hz, 1H, H-6), 7.55 (d, $J = 9.0$ Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.32 (C-15), 18.92 (C-11), 32.56 (C-10), 61.91 (C-14), 65.91 (C-12), 108.33 (C-6), 113.37 (C-3), 115.05 (C-4a), 120.43 (C-8), 126.41 (C-5), 150.94 (C-8a), 152.03 (C-4), 156.77 (C-7), 159.90 (C-2), 167.95 (C-13), 199.11 (C-9); TOF MS ES+ $[M + Na]^+ = 327.0827$ ($C_{16}H_{16}O_6Na$).

General Procedure for 3 and 4

8-Acetyl-7-hydroxy-4-methylcoumarin (1.5 mmol, 0.325 g) and the alkylating agent (chloroacetonitrile or ethyl chloroacetate, 1.65 mmol) were dissolved in 1-methyl-2-pyrrolidone (Fluka Chemika) (2 mL), and anhydrous K_2CO_3 (4.5 mmol, 0.625 g) was added to this solution. The mixture was heated in the oil bath and monitored by TLC on silica-gel plates (eluent $CHCl_3$–AcOEt 20:1). After completion of the reaction as indicated by TLC, the mixture was poured into the flask with water and ice (50 mL) and stirred for 30 min. The precipitate was filtered out and dried. The analytical samples were crystallized from ethanol.

8-Cyano-4,9-dimethyl-*2H*-furo[2,3-*h*]-1-chromen-2-one (3). Heating: 3 h and 45 min. Yield 0.123 g (34%). Mp 256–257 °C; IR (KBr) ν_{max}/cm^{-1}: 3090, 2963, 2828, 2225, 1748, 1612, 1481, 1366, 1232, 1074, 852; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.51 (d, $J = 1.5$ Hz, 3H, H-11), 2.74 (s, 3H, H-13), 6.32 (bq, $J = 1.5$ Hz, 1H, H-3), 7.42 (d, $J = 8.8$ Hz, 1H, H-6), 7.70 (d, $J = 8.8$ Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.64 (C-13), 19.48 (C-11), 108.68 (C-6), 111.02 (C-12), 113.65 (C-3), 115.45 (C-4a), 116.00 (C-9a), 124.64 (C-5), 125.99 (C-8), 129.89 (C-9), 149.59 (C-9b), 153.01 (C-4), 157.29 (C-7), 159.66 (C-2); TOF MS ES+ $[M + Na]^+ =$ 262.0480 ($C_{14}H_9NO_3Na$).

8-Ethoxycarbonyl-4,9-dimethyl-*2H*-furo[2,3-*h*]-1-chromen-2-one (4). Heating: 6.5 h. Yield 0.045 g (10%); mp 191.5–192 °C, lit. 192–192.5 °C[10]; IR (KBr) ν_{max}/cm^{-1}: 3084, 2986, 2866, 1729, 1609, 1571, 1442, 1471, 1365, 1156, 1078, 869; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.46 (t, $J = 7.0$ Hz, 3H, H-15), 2.50 (d, $J = 0.9$ Hz, 3H, H-11), 2.91 (s, 3 H, H-13), 4.48 (q, $J = 7.0$ Hz, 2H, H-14), 6.29 (d, $J = 0.9$ Hz, 1H, H-3), 7.46 (d, $J = 8.7$ Hz, 1H, H-6), 7.64 (d, $J = 8.7$ Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 11.36 (C-13), 14.59 (C-15), 19.72 (C-11), 61.68 (C-14), 109.12 (C-6), 113.27 (C-3), 115.07 (C-4a), 117.98 (C-9a), 123.96 (C-5), 126.02 (C-9), 142.00 (C-8), 150.51 (C-9b), 153.51 (C-4), 156.56 (C-2), 160.19 (C-7), 160.35 (C-12); TOF MS ES+ $[M + Na]^+ = 309.0739$ ($C_{16}H_{14}O_5Na$).

Microwave-Assisted Synthesis, General Procedure

8-Acetyl-7-hydroxy-4-methylcoumarin, chloroacetonitrile or ethyl chloroacetate, and K_2CO_3 were placed in a microwave flask (the amounts were the same as used in the traditional procedures), and 3 mL of acetone were added. The mixtures were refluxed in the monomode microwave oven (300 W) and monitored by TLC on silica-gel plates (eluent $CHCl_3$–AcOEt 20:1).

Attempts to obtain 8-acetyl-7-cyanomethoxy-4-methyl-chromen-2-one (**1**) or 8-cyano-4,9–dimethyl-*2H*-furo[2,3-*h*]-1-chromen-2-one (**3**) used 10 cycles. Heating

time for each cycle was 5 min. Yield of **1**, 43%; traces of **3** by TLC. Attempts to obtain 8-acetyl-7-ethoxycarbonylmethoxy-4-methyl-chromen-2-one (**2**) or 8-ethoxycarbonyl-4,9-dimethyl-*2H*-furo[2,3-*h*]-1-benzopyran-2-one (**4**) used 16 cycles. Heating time for each cycle 5 min. Yield of **2**, 20%; traces of **4** by TLC.

General Procedure for 3 and 4

8-Acetyl-7-hydroxy-4-methylcoumarin (7.5 mmol, 1.635 g) and the alkylating agent (chloroacetonitrile or ethyl chloroacetate) (8.25 mmol) were dissolved in 1-methyl-2-pyrrolidone (Fluka Chemika) (10 mL), and anhydrous K_2CO_3 (22.5 mmol, 3.105 g) was added to this solution. The mixture was heated in the monomode microwave oven (400 W).

8-Cyano-4,9-dimethyl-*2H*-furo[2,3-*h*]-1-chromen-2-one (3). One cycle: temperature 70–75 °C, time of heating 3 min. After completion of the reaction as indicated by TLC (silica-gel plates, eluent $CHCl_3$–AcOEt 20:1, $R_f = 0.59$), ice water (250 mL) was added to the reaction mixture and stirred magnetically for 30 min. The precipitate was filtered and dried to give an amorphous light brown solid (1.647 g, 92%).

8-Ethoxycarbonyl-4,9-dimethyl-*2H*-furo[2,3-*h*]-1-chromen-2-one (4). First trial: the mixture was heated in the monomode microwave oven (400 W) for four cycles: temperature 70–75 °C, time of heating 3 min (each cycle). After completion of the reaction as indicated by TLC (silica-gel plates, eluent $CHCl_3$–AcOEt 20:1, $R_f = 0.47$), ice water (50 mL) was added to the reaction mixture and hydrochloric acid (1:1) was added dropwise to neutralize the solution. The mixture was stirred magnetically for 30 min. The precipitate was filtered out and dried to give an amorphous brown solid. Yield 1.728 g (60%).

Second trial: the mixture was heated in the monomode microwave oven (400 W) for one cycle: temperature 80–85 °C, time of heating 3 min. After completion of the reaction as indicated by TLC (silica-gel plates, eluent $CHCl_3$–AcOEt 20:1, $R_f = 0.47$), the product was isolated from the reaction mixture as described in the first trial. Yield 1.155 g (54%).

Antibacterial Evaluation

The following microbial strains with various cell-wall structures were chosen: bacteria Gram-positive (*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 6538P, *Micrococcus luteus* ATCC 9341, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, and *Staphylococcus epidermidis* ATCC 12228); bacteria Gram-negative (*Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 27853, and *Pseudomonas aeruginosa* ATCC 15442), and fungi (yeast strains) (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Zygosaccharomyces rouxii* ATCC 28253). The cylinder-plate method was used in the preliminary antimicrobial activity tests.[16] One hundred μL of compound were placed into the cylinder in a suspension of 10% dimethylsulfoxide (DMSO) in a 0.08 M phosphate buffer with pH 7.0. The cylinders were put on a Muller-Hinton 2 or Sabouraud agar plate inoculated with

one of the tested strains. The plates with bacterial strains were incubated at 37 °C for 24 h, and plates with yeast strains were incubated at 30 °C for 48 h. MIC was obtained by mixing with 19 mL of a Mueller-Hinton 2 agar, cooled to 56 °C with 1 mL of appropriate dilution of the compound **4**. Then 2 μL of a particular cell suspension of optical density 0.5 unit on the McFarland scale were applied to the surface of the agar. The lowest concentration of compound **4** that totally inhibited growth of examined strain was evaluated as MIC value.[17]

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