

Natural and Synthetic 2,2-Dimethylpyranocoumarins with Antibacterial Activity

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A new efficient synthetic approach to the natural coumarins 5-hydroxyseselin (**5**), 5-methoxyseselin (**3**), and (\pm) *cis*-grandmarin (**9**) is described as well as the synthesis of some new derivatives in the 5-methoxyseselin series (**10**–**15**). The natural coumarins 7-hydroxyalloxanthyletin (**6**), alloxanthoxyletin (**8**), and dipetalolactone (**7**) have also been obtained as secondary products. The type of fusion of the pyrano ring in all cases has been established by 2D NMR spectroscopy. The compounds have been studied for their in vitro antibacterial activity, which has been compared with that of some previously synthesized seselin derivatives. The most active compounds were **3**, **7**, **8**, **11**, and **14**. Some structure–activity relationships are discussed.

Coumarins are widely distributed natural products exhibiting a broad pharmacological profile.¹ A few years ago, we described the synthesis of some seselin (**1**) and xanthyletin (**2**) derivatives with interesting cytotoxic activities.² Some seselin derivatives, including derivatives of 5-methoxyseselin (**3**), were found to be potent anti-HIV agents.³ Besides the cytotoxic and antiviral activities, coumarins also possess antibacterial activities.⁴

As part of our research program on bioactive compounds with a pyranocoumarin nucleus, the synthesis of 5-methoxyseselin (**3**) as well as the synthesis of some natural and semisynthetic derivatives was envisaged. Synthesis of aromatic oxygenated pyranocoumarins is difficult, and thus, the development of new synthetic approaches is of interest. The antibacterial activity of the synthesized compounds is presented herein. Additionally, from comparison with the previously synthesized non-oxygenated 2,2-dimethylpyranocoumarins,² some structure–activity relationships are discussed.

Results and Discussion

Our synthetic approach toward the aforementioned pyranocoumarins was based on the successful use of 3-methyl-2-butenal in the synthesis of pyranoacridones⁵ and pyranoquinones.⁶ As starting material we used 5,7-dihydroxycoumarin (**4**), which was easily obtained in almost quantitative yield from phloroglucinol and ethyl propiolate.⁷ 5,7-Diacetoxycoumarin (**4a**) and 5,7-dimethoxycoumarin (**4b**) were also prepared, for structure–activity studies. Direct cyclization of **4** with 3-methyl-2-butenal in pyridine for 4 h led to a separable mixture of 5-hydroxyseselin (**5**) (26%) and 7-hydroxyalloxanthyletin (**6**) (8%). Dipetalolactone (**7**) was obtained as a byproduct (4%). No trace of the linear isomer 5-hydroxyxanthyletin could be detected. The yield of 5-hydroxyseselin (**5**) could be increased to 42% if the residual starting material (62%) was reacted again. As expected, the yield could not be increased using excess 3-methyl-2-butenal or by prolonged reaction

because these conditions led to higher yields of dipetalolactone (**7**). Subsequent methylation of 5-hydroxyseselin (**5**) and 7-hydroxyalloxanthyletin (**6**) with methyl iodide in acetone led to 5-methoxyseselin (**3**) and alloxanthoxyletin or 7-methoxyalloxanthyletin (**8**), respectively. Acetylation of **5** and **6** with acetic anhydride in pyridine led to the corresponding acetate esters **3a** and **8a**.

Compounds **3**, **5**, **6**, **7**, and **8** obtained in the above-described synthetic approach occur in nature. 5-Methoxyseselin (**3**) has been isolated from *Citrus grandis*⁸ and *Citrus paradisi* x *Citrus tangerine*,⁹ 5-hydroxyseselin (**5**) from *Citrus paradisi* x *Citrus tangerina*⁹ and from *Citrus natsudaidai*,¹⁰ alloxanthoxyletin (**8**) from *Zanthoxylum americanum*,¹¹ 7-hydroxyalloxanthyletin (**6**) from *Pilocarpus goudotianus*,¹² and dipetalolactone (**7**) from *Zanthoxylum dipetalum*¹³ and *Hortia arborea*.¹⁴

Interestingly, compounds **3**, **5**, **6**, and **7** were identified by ¹H NMR spectroscopy, and detailed 2D NMR evidence for the type of fusion of the pyrano ring was never provided. Alloxanthoxyletin (**8**) was recently synthesized, but ¹³C NMR data¹⁵ were incomplete.

HMBC and NOESY experiments were executed on **3** and **8**, because the carbon bearing the methoxy group could be unequivocally identified from the HMBC spectrum. For 5-methoxyseselin (**3**) C-5 was correlated with H-4 and not with H-4', while for alloxanthoxyletin (**8**) C-7 was correlated with H-4' and not with H-4. The above observations were confirmed by a NOESY spectrum, which was mainly used to exclude the linear isomer. Both **3** and **8** showed a correlation between the OMe protons and the aromatic proton, revealing that both isomers were angular.

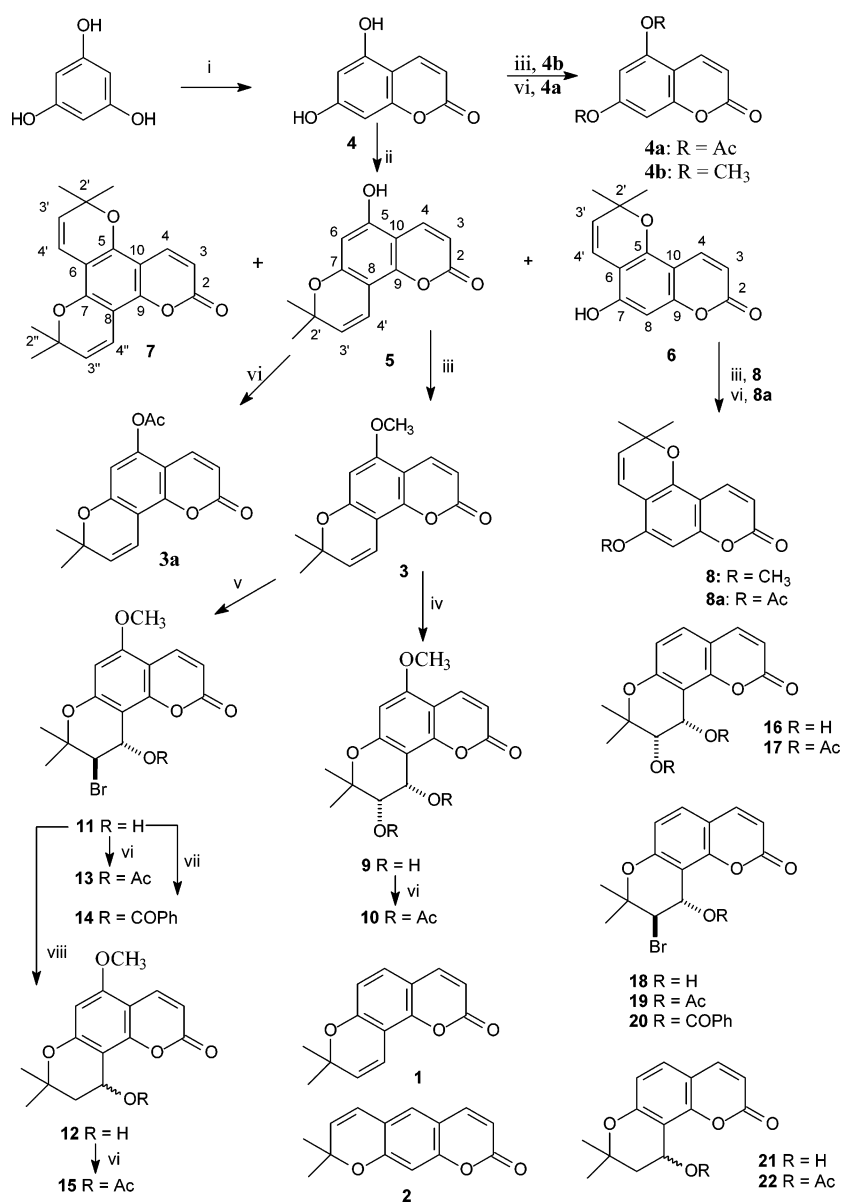
The overall yield from phloroglucinol to 5-methoxyseselin was 35% in only three steps, while previous synthetic approaches^{3,16,17} suffered from low yields over several reaction steps.³

5-Methoxyseselin (**3**) derivatives were also synthesized. (\pm)-*cis*-Grandmarin (**9**) was obtained by catalytic osmium oxidation of **3**, using 4-methyl-morpholine-*N*-oxide to regenerate the oxidizing agent (Scheme 1). Treatment of *cis* diol **9** with excess acetic anhydride in pyridine afforded the corresponding diester **10**. (\pm)-*trans*-3'-Bromo-4'-hydroxy-5-methoxy-3',4'-dihydroxyseselin (**11**) was obtained by treat-

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Scheme 1^a

^a Conditions: (i) ethyl propiolate, ZnCl₂, 100 °C; (ii) 3-methyl-2-butenal, Py, 115 °C; (iii) MeI, K₂CO₃, Me₂CO, 56 °C; (iv) OsO₄, *N*-methylmorpholine-*N*-oxide, *t*-BuOH, THF, H₂O, rt; (v) NBS, THF, H₂O, 0 °C; (vi) Ac₂O, Py, rt; (vii) (PhCO)₂O, Py, rt; (viii) Bu₃SnH, AIBN, toluene, 110 °C.

ment of **3** with *N*-bromosuccinimide in aqueous tetrahydrofuran solution. Bromohydrin **11** was smoothly debrominated with tributyltin hydride to afford **12**. Treatment of **11** with excess acetic anhydride or benzoic anhydride in pyridine gave the corresponding esters **13** and **14**, respectively. Similarly, alcohol **12** afforded the corresponding acetate **15**.

The antibacterial activity of compounds **3**–**15** and of the previously synthesized² non-oxygenated pyranocoumarins **1**, **2**, and **16**–**22** was evaluated *in vitro* using the diffusion technique of Bauer–Kirby (disk method) as previously described.¹⁸ The results are reported in Table 1. The most active pyranocoumarins were **3**, **7**, **8**, **11**, and **14**.

The compounds showed a broad diversity regarding growth-inhibitory activity (Table 1). Five compounds (5-methoxyseselin (**3**) and its brominated derivatives (**11**, **14**), alloxanthyletin (**8**), the acetylated derivatives **3a**, **8a**, and dipetalolactone (**7**)) were active against seven tested bacteria. A seselin derivative, 3-bromo-4-benzoyloxyseselin (**7**), showed moderate activity, while three other coumarins (**13**, **15**, **19**) expressed a specific activity only

against the two Gram-positive bacteria. Interestingly, seselin (**1**), xanthyletin (**2**), 5-hydroxyseselin (**5**), and 7-hydroxyalloxanthyletin (**6**) were found to be inactive.

Comparing the methoxy or acetoxy derivatives of seselin, alloxanthyletin, or simple coumarins with the respective non-oxygenated or hydroxy derivatives, it could be considered that the presence of additional oxygenated substituents in the ether or ester form generally enhances the antibacterial activity, while the presence of free hydroxyl reduces or abolishes the activity. This fact could be at least partially attributed to the reduced lipophilicity of the hydroxyl derivatives, which hinders the penetration through the bacterial cell wall.¹⁹ Further comparison showed that the brominated derivatives are generally more active than the unsubstituted compounds. Results for **13**, **15**, and **19** showed that the presence of an acetoxy group on the pyran ring enhances the selectivity against Gram-positive bacteria. Additionally, the activity found for dipyrano-coumarin (**20**), showed moderate activity, while three other coumarins (**13**, **15**, **19**) expressed a specific activity only

Table 1. Antibacterial Activity^a

compound	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>P. aeruginosa</i>	<i>E. cloacae</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>Proteus mirabilis</i>
3	+	+	++	+	+	++	++
3a	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+
4a	+	++	+	+	+	+	+
4b	+	++	++	+	+	++	+
7	++	+	++	+	+	++	+
8	+	+	++	+	+	++	+
8a	+	+	+	+	+	+	+
11	+	+	++	+	+	++	+
13	+	++	-	-	-	-	-
14	+	+	++	+	+	++	+
15	++	++	-	-	-	-	-
19	++	++	-	-	-	-	-
20	+	+	+	+	+	+	+
netilmicin	+++	+++	+++	+++	+++	+++	+++
amoxicillin	+++	+++	+++	+++	+++	+++	+++
clavulanic acid	+++	+++	+++	+++	+++	+++	+++

^a Zone of inhibition around each disk (in mm) <7 mm (-), 7–10 mm (+), 11–16 mm (++), >16 mm (+++). Compounds **1**, **2**, **5**, **6**, **9**, **10**, **12**, **16**, **17**, **18**, **21**, and **22** were tested and found to be inactive.

Experimental Section

General Experimental Procedures. Spectra were recorded on the following apparatus. MS: Nermag R10-10C in desorption-chemical ionization, using NH₃ as reagent gas. NMR: Bruker AC200, ¹³C NMR (50 MHz); Bruker DRX400, ¹H NMR (400 MHz). Chemical shifts are given in δ values with TMS as an internal standard. Coupling constants (*J*) are given in Hz. The signals of ¹H and ¹³C spectra were unambiguously assigned using 2D NMR techniques: COSY, NOESY, HMQC, and HMBC. These 2D experiments were performed using standard Bruker microprograms. Column chromatography was conducted using Merck flash silica gel 60 (40–63 μ m), with an overpressure of 300 mbar.

5,7-Dihydroxycoumarin (4). A mixture of phloroglucinol (2.00 g, 15.8 mmol), ZnCl₂ (1.67 g, 12.3 mmol), and ethyl propiolate (1.9 mL) was stirred for 2 h at 100 °C. Then the mixture was cooled, and 5% hydrochloric acid (40 mL) was added. The precipitate was filtered off and washed with boiling H₂O to give **4** (2.53 g, 90%): ¹H NMR (DMSO, 400 MHz) δ 10.65 (1H, br s, OH), 10.40 (1H, br s, OH), 7.94 (1H, d, *J* = 9.7 Hz, H-4), 6.25 (1H, d, *J* = 2.2 Hz, H-8), 6.17 (1H, d, *J* = 2.2 Hz, H-6), 6.02 (1H, d, *J* = 9.7, H-3).

5,7-Diacetoxycoumarin (4a). To a solution of **4** (24 mg, 0.13 mmol) in dry pyridine (1.5 mL) was added Ac₂O (1 mL, 15 mmol). The reaction was stirred for 24 h at room temperature, and the reagents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel with cyclohexane–EtOAc (1:1) to give compound **4a** (28 mg, 92%). ¹H NMR as described in ref 20.

5,7-Dimethoxycoumarin (4b). To a solution of **4** (250 mg, 1.40 mmol) in dry acetone (15 mL) was added anhydrous K₂CO₃ (1.1 g) and MeI (3 mL). The reaction mixture was stirred for 4 h at 60 °C. Then, the mixture was filtered and the reagents were removed under reduced pressure. The solid residue was submitted to flash chromatography with CH₂Cl₂–MeOH (99.9:0.1) to give **4b** (260 mg, 90%). ¹H NMR as described in ref 21.

Reaction of 5,7-Dihydroxycoumarin (4) with 3-Methyl-2-butenal. To a solution of 5,7-dihydroxycoumarin (**4**) (1.000 g, 5.62 mmol) in dry pyridine (3.0 mL) was added 3-methyl-2-butenal (1.1 mL), and the reaction mixture was stirred for 4 h at 115 °C. Then, the reagents were removed under reduced pressure. The solid residue was submitted to flash chromatography with CH₂Cl₂–MeOH (99.9:0.1 to 94:6) to afford 5-hydroxyxseselin (**5**) (357 mg, 26%), 7-hydroxyalloxanthyletin (**6**) (110 mg, 8%), and dipetalolactone (**7**) (65 mg, 4%).

5-Hydroxyxseselin (5): mp 212 °C (EtOAc); ¹H NMR as described in ref 10; ¹³C NMR (CDCl₃, 50 MHz) δ 161.8 (C-2), 157.3 (C-7), 153.2 (C-5), 151.0 (C-9), 139.6 (C-4), 127.9 (C-3'), 115.4 (C-4'), 110.3 (C-3), 103.0 (C-8, 10), 99.3 (C-6), 78.1 (C-2'), 28.7 (2 \times CH₃); MS-DCI *m/z* 245 (M + H)⁺.

7-Hydroxyalloxanthyletin (6): mp 220 °C (EtOAc); ¹H NMR (CDCl₃, 400 MHz) δ 7.97 (1H, d, *J* = 9.8 Hz, H-4), 6.62 (1H, d, *J* = 9.8 Hz, H-4'), 6.53 (1H, s, H-8), 6.12 (1H, d, *J* = 9.8, H-3), 5.55 (1H, d, *J* = 9.8, H-3'), 1.44 (6H, s, 2 \times CH₃); ¹³C NMR (CDCl₃, 50 MHz) δ 162.6 (C-2), 155.9 (C-9), 151.1 (C-7), 150.6 (C-5), 139.5 (C-4), 127.5 (C-3'), 116.0 (C-4'), 110.1 (C-3), 106.4 (C-6), 103.5 (C-10), 95.5 (C-8), 77.9 (C-2'), 28.0 (2 \times CH₃); MS-DCI *m/z* 245 (M + H)⁺.

Dipetalolactone (7): ¹H NMR (CDCl₃, 400 MHz) δ 7.94 (1H, d, *J* = 9.8 Hz, H-4), 6.78 (1H, d, *J* = 10 Hz, H-4'), 6.62 (1H, d, *J* = 10 Hz, H-4''), 6.12 (1H, d, *J* = 9.8, H-3), 5.58 (1H, d, *J* = 10 Hz, H-3'), 5.54 (1H, d, *J* = 10, H-3''), 1.44 (12H, s, 4 \times CH₃); ¹³C NMR (CDCl₃, 50 MHz) δ 161.2 (C-2), 151.8 (C-7), 150.0 (C-5,9), 138.8 (C-4), 127.6 (C-3'*), 127.5 (C-3''*), 115.8 (C-4'*), 115.0 (C-4''*), 110.4 (C-3), 105.9 (C-8*), 103.1 (C-10), 102.1 (C-6*), 77.9 (C-2',2''), 28.0 (2 \times CH₃), 27.9 (2 \times CH₃); MS-DCI *m/z* 311 (M + H)⁺; *assignments can be interchanged.

5-Methoxyxseselin (3). Treatment of 5-hydroxyxseselin (**5**) (250 mg, 1.02 mmol) in conditions similar to those described for the preparation of **4b** afforded **3** (245 mg, 93%); mp 162 °C (diethyl ether); ¹H NMR as described in ref 3; ¹³C NMR (CDCl₃, 50 MHz) δ 161.1 (C-2), 157.3 (C-7), 156.4 (C-5), 150.9 (C-9), 138.9 (C-4), 127.4 (C-3'), 114.8 (C-4'), 110.2 (C-3), 103.5 (C-10), 102.4 (C-8), 95.2 (C-6), 77.8 (C-2'), 55.8 (OCH₃), 28.0 (2 \times CH₃); MS-DCI *m/z* 259 (M + H)⁺.

5-Acetoxyxseselin (3a). Treatment of **5** (25 mg, 0.10 mmol) in conditions similar to those described for the preparation of **4a** afforded **3a** (29 mg, 96%). ¹H NMR as described in ref 17.

Alloxanthyletin (8). Treatment of 7-hydroxyalloxanthyletin (**6**) (70 mg, 0.29 mmol) in conditions similar to those described for the preparation of **4b** afforded **8** (69 mg, 93%); mp 115 °C (EtOAc); ¹H NMR as described in ref 12; ¹³C NMR (CDCl₃, 50 MHz) δ 161.2 (C-2), 158.1 (C-7), 155.7 (C-9), 150.0 (C-5), 138.4 (C-4), 127.4 (C-3'), 115.9 (C-4'), 111.0 (C-3), 106.3 (C-6), 102.4 (C-10), 91.3 (C-8), 77.8 (C-2'), 55.8 (OCH₃), 27.7 (2 \times CH₃); MS-DCI *m/z* 259 (M + H)⁺.

7-Acetoxyalloxanthyletin (8a). Treatment of **6** (25 mg, 0.10 mmol) in conditions similar to those described for the preparation of **4a** afforded **8a** (29 mg, 96%). ¹H NMR as described in ref 12.

(±)-cis-3',4'-Dihydroxy-3',4'-dihydro-5-methoxyxseselin (9). To a solution of **3** (50 mg, 0.19 mmol) in 7 mL of *t*-BuOH–THF–H₂O (10:3:1 v/v/v) was added a solution of OsO₄ 2.5% (w/v) in *t*-BuOH (0.25 mL) and 50 mg (0.33 mmol) of 4-methylmorpholine-*N*-oxide. The reaction mixture was stirred for 48 h at room temperature. NaHSO₃ (sat.) was added, and the mixture was stirred for 1 h. The reaction mixture was extracted with CH₂Cl₂–H₂O, and the organic layer was collected. The solvent was removed under reduced pressure, and compound **9** was purified by flash chromatography on silica gel with cyclohexane–EtOAc (60:40 to 90:10) (32 mg, 58%); mp 230 °C (hexane–diethyl ether); ¹H NMR as described in

ref 10; ^{13}C NMR (CDCl_3 , 50 MHz) δ 161.9 (C-2), 157.7 (C-7), 157.2 (C-5), 156.0 (C-9), 140.0 (C-4), 110.5 (C-3), 104.3 (C-8-10), 96.0 (C-6), 79.5 (C-2'), 71.7 (C-3'), 61.4 (C-4'), 56.4 (OCH₃), 25.7 (CH₃), 21.9 (CH₃); MS-DCI m/z 293 (M + H)⁺.

(±)-**cis-3',4'-Diacetoxy-3',4'-dihydro-5-methoxyseselin (10)**. Treatment of **9** (24 mg, 0.08 mmol) in conditions similar to those described for the preparation of **4a** afforded **10** (28 mg, 92%): UV (CHCl_3) λ_{max} (log ϵ) 344 (sh), 326 (4.15), 259 (3.95), 249 (3.92) nm; IR (CHCl_3) ν_{max} 1747, 1729 (sh), 1629, 1603, 1236 cm^{-1} ; ^1H NMR (CDCl_3 , 200 MHz) δ 7.92 (1H, d, J = 9.5 Hz, H-4), 6.44 (1H, d, J = 5.0 Hz, H-4'), 6.22 (1H, s, H-6), 6.13 (1H, d, J = 9.5 Hz, H-3), 5.25 (1H, d, J = 5.0 Hz, H-3'), 3.86 (3H, s, OCH₃), 2.20 (3H, s, COCH₃), 2.09 (6H, s, COCH₃), 1.42 (3H, s, 2 × CH₃); ^{13}C NMR (CDCl_3 , 50 MHz) δ 171.0 (COCH₃), 161.9 (C-2), 157.7 (C-5), 157.2 (C-7), 156.0 (C-9), 139.7 (C-4), 112.8 (C-3), 104.3 (C-10), 100.0 (C-8), 96.7 (C-6), 78.5 (C-2'), 71.5 (C-3'), 62.3 (C-4'), 57.3 (OCH₃), 26.4 (CH₃), 25.4 (CH₃), 21.8 (COCH₃); MS-DCI m/z 377 (M + H)⁺; *anal.* C 60.75%, H 5.39%, calcd for C₁₉H₂₀O₈, C 60.64%, H 5.36%.

(±)-**trans-3'-Bromo-4'-hydroxy-3',4'-dihydro-5-methoxyseselin (11)**. To a solution of **3** (140 mg, 0.54 mmol) in THF (5 mL) and H₂O (5 mL) was added *N*-bromosuccinimide (100 mg, 0.56 mmol). The reaction mixture was stirred for 1 h at 0 °C, and then the reaction mixture was extracted with NaCl (sat.)–Et₂O and the organic layer was collected. The solvent was removed under reduced pressure, and compound **11** was purified by crystallization with Et₂O (110 mg, 57%): UV (CHCl_3) λ_{max} (log ϵ) 331 (3.99), 261 (3.75), 251 (3.71) nm; IR (CHCl_3) ν_{max} 3350, 1630, 1605, 1225 cm^{-1} ; ^1H NMR (DMSO, 200 MHz) δ 8.02 (1H, d, J = 9.7 Hz, H-4), 6.28 (1H, s, H-6), 6.20 (1H, d, J = 9.7 Hz, H-3), 5.35 (1H, d, J = 5.1 Hz, H-4'), 4.25 (1H, s, br, OH), 4.29 (1H, d, J = 5.1, H-3'), 3.90 (3H, s, OCH₃), 1.65 (3H, s, CH₃), 1.55 (3H, s, CH₃); ^{13}C NMR (DMSO, 50 MHz) δ 161.6 (C-2), 157.3 (C-5), 156.9 (C-7), 155.2 (C-9), 139.9 (C-4), 110.9 (C-3), 104.9 (C-10), 104.4 (C-8), 96.0 (C-6), 79.2 (C-2'), 67.2 (C-4'), 58.2 (C-3'), 56.4 (OCH₃), 26.5 (CH₃), 25.3 (CH₃); MS-DCI m/z 357, 355 (M + H)⁺; *anal.* C 50.61%, H 4.19%, Br 22.45% calcd for C₁₅H₁₅BrO₅, C 50.72%, H 4.26%, Br 22.50%.

(±)-**trans-3'-Bromo-4'-acetoxy-3',4'-dihydro-5-methoxyseselin (13)**. Treatment of **11** (35 mg, 0.10 mmol) in conditions essentially similar to those described for the preparation of **4a** afforded **13** (36 mg, 92%): UV (CHCl_3) λ_{max} (log ϵ) 344 (sh), 327 (4.11), 260 (3.90), 251 (3.82) nm; IR (CHCl_3) ν_{max} 1733, 1631, 1604, 1220 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 7.95 (1H, d, J = 9.7 Hz, H-4), 6.44 (1H, d, J = 4.2 Hz, H-4'), 6.28 (1H, s, H-6), 6.18 (1H, d, J = 9.7, H-3), 4.33 (1H, d, J = 4.2 Hz, H-3'), 3.90 (3H, s, OCH₃), 2.15 (3H, s, COCH₃), 1.59 (6H, s, 2 × CH₃); ^{13}C NMR (CDCl_3 , 50 MHz) δ 170.4 (COCH₃), 160.6 (C-2), 157.7 (C-5), 157.6 (C-7), 155.4 (C-9), 138.9 (C-4), 111.8 (C-3), 104.9 (C-10), 98.5 (C-8), 96.1 (C-6), 78.3 (C-2'), 67.4 (C-4'), 56.4 (OCH₃), 54.3 (C-3'), 26.4 (2 × CH₃), 21.3 (COCH₃); MS-DCI m/z 397, 395 (M + H)⁺; *anal.* C 51.41%, H 4.27%, Br 20.22% calcd for C₁₇H₁₇BrO₆, C 51.40%, H 4.31%, Br 20.12%.

(±)-**trans-3'-Bromo-4'-benzoyloxy-3',4'-dihydro-5-methoxyseselin (14)**. To a solution of **11** (28 mg, 0.08 mmol) in dry pyridine (1.5 mL) was added benzoic anhydride (62 mg, 0.29 mmol). The reaction mixture was stirred for 48 h at room temperature, and the reagents were removed under reduced pressure. The residue mixture was extracted with EtOAc–NaHCO₃ (sat.), and the organic layer was collected. The solvent was removed under reduced pressure, and the remaining residue was purified by flash chromatography on silica gel with cyclohexane–CH₂Cl₂ (1:1) to give compound **14** (23 mg, 63%): UV (CHCl_3) λ_{max} (log ϵ) 346 (sh), 329 (4.10), 297 (sh), 284 (sh), 258 (3.69), 242 (3.98) nm; IR (CHCl_3) ν_{max} 1736, 1629 (sh), 1608, 1604, 1220 cm^{-1} ; ^1H NMR (CDCl_3 , 200 MHz) δ 8.02 (2H, d, J = 7.5, H-2'',6''), 7.98 (1H, d, J = 9.5, H-4), 7.57 (1H, t, J = 7.5 Hz, H-4''), 7.43 (2H, t, J = 7.5 Hz, H-3'',5''), 6.70 (1H, d, J = 2.9, H-4'), 6.36 (1H, s, H-6), 6.16 (1H, d, J = 9.5, H-3), 4.53 (1H, d, J = 2.9, H-3'), 3.94 (3H, s, OCH₃), 1.67 (3H, s, CH₃), 1.66 (3H, s, CH₃); ^{13}C NMR (CDCl_3 , 50 MHz) δ 165.6 (COPh-9), 160.7 (C-2), 158.0 (C-5), 157.8 (C-7), 155.6 (C-9), 133.7 (C-4''), 130.3 (C-2'',6''), 129.8 (C-1'), 129.5 (C-3'',5''), 111.5 (C-3), 104.5 (C-10), 97.8 (C-8), 95.5 (C-6), 77.6 (C-2'), 67.3 (C-4'), 56.1 (OCH₃), 53.5 (C-3'), 28.4 (CH₃), 24.7 (CH₃); MS-DCI

m/z 461, 459 (M + H)⁺; *anal.* C 57.65%, H 4.21%, Br 17.55% calcd for C₂₂H₁₉BrO₆, C 57.53%, H 4.17%, Br 17.40%.

(±)-**4'-Hydroxy-3',4'-dihydro-5-methoxyseselin (12)**. Compound **11** (78 mg, 0.22 mmol) was dissolved in anhydrous toluene (10 mL), and the solution was refluxed for 15 min under argon. Then AIBN (azo-bis-2,2'-(methyl-2-propionitrile)) (10 mg) was added, and after 5 min, a solution of tributyltin hydride (0.5 mL in 4 mL of toluene) was added over a period of 40 min. The reaction mixture was refluxed for 1 h. The solvent was evaporated and the residue was purified by flash chromatography on silica gel with cyclohexane–EtOAc (80:20) to give compound **12** (35 mg, 59%): UV (CHCl_3) λ_{max} (log ϵ) 330 (3.90), 261 (3.66), 251 (3.60) nm; IR (CHCl_3) ν_{max} 3350, 1630, 1604, 1220 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 8.02 (1H, d, J = 9.8 Hz, H-4), 6.24 (1H, s, H-6), 6.18 (1H, d, J = 9.8 Hz, H-3), 5.20 (1H, m, H-4'), 3.89 (3H, s, OCH₃), 3.27 (1H, d, J = 3.0 Hz, OH), 2.12 (1H, d, J = 4.9, H-3'), 1.51 (3H, s, CH₃), 1.43 (1H, s, CH₃); ^{13}C NMR (CDCl_3 , 50 MHz) δ 161.4 (C-2), 157.8 (C-7), 156.7 (C-5), 139.4 (C-4), 109.9 (C-3), 104.2 (C-8), 104.1 (C-10), 95.7 (C-6), 77.0 (C-2'), 59.0 (C-4'), 55.9 (OCH₃), 40.2 (C-3'), 28.0 (CH₃), 26.5 (CH₃); MS-DCI m/z : 277 (M + H)⁺; *anal.* C 65.33%, H 5.76%, calcd for C₁₅H₁₆O₅, C 65.21%, H 5.84%.

(±)-**4'-Acetoxy-3',4'-dihydro-5-methoxyseselin (15)**. Treatment of compound **12** (23 mg, 0.08 mmol) in conditions essentially similar to those described for the preparation of **4a** afforded **15** (24 mg, 92%): UV (CHCl_3) λ_{max} (log ϵ) 345 (sh), 329 (4.02), 261 (3.77), 251 (3.74) nm; IR (CHCl_3) ν_{max} 1732, 1630, 1604, 1220 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 7.98 (1H, d, J = 9.7 Hz, H-4), 6.24 (1H, s, H-6), 6.23 (1H, t, J = 4.9, H-4'), 6.17 (1H, d, J = 9.7, H-3), 3.90 (3H, s, OCH₃), 2.12 (2H, d, J = 4.9, H-3'), 2.12 (3H, s, OCOCH₃), 1.46 (1H, s, CH₃); ^{13}C NMR (CDCl_3 , 50 MHz) δ 170.8 (COCH₃), 161.3 (C-2), 158.0 (C-7), 157.6 (C-5), 155.3 (C-9), 138.6 (C-4), 110.8 (C-3), 104.1 (C-10), 100.6 (C-8), 95.5 (C-6), 76.6 (C-2'), 61.3 (C-4'), 55.9 (OCH₃), 38.4 (C-3'), 28.7 (CH₃), 25.8 (CH₃), 21.1 (COCH₃); MS-DCI m/z 319 (M + H)⁺; *anal.* C 64.09%, H 5.66%, calcd for C₁₇H₁₈O₆, C 64.14%, H 5.70%.

Antibacterial Activity. Antibacterial activities of compounds were determined using the diffusion technique of Bauer–Kirby (disk method) by measuring the zone of inhibition against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228), and five Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883), and *Proteus mirabilis*. Netilmicin, amoxicillin, and clavulanic acid were used as control antibiotics. The results were reported as the diameter of the zone of inhibition around each disk (in mm), and evaluation of inhibition corresponds to <7 mm (–), 7–10 mm (+), 11–16 mm (++), >16 mm (+++). The compounds were dissolved in DMSO. For each experiment control disks with solvent were used as negative control. All paper disks had a diameter of 6 mm and were deposited on the surface of the seeded trypticase Muller-Hinton agar. Petri dishes were previously inoculated with the organisms to give a final cell concentration of 10⁷ cell/mL. Volumes of 10 μL of the above solutions were required to wet the test paper disks. The incubation conditions used were 24 h at 37 °C. The experiments were repeated three times, and the results were expressed as average values. All strains were standard reference strains (American Type Culture Collection).

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