

Synthesis and Evaluation of Coumarin α -Methylene- γ -butyrolactones: A New Class of Platelet Aggregation Inhibitors

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In a search for new inhibitors of platelet aggregation, certain coumarin-bearing α -methylene- γ -butyrolactones were synthesized and evaluated for inhibitory activity against thrombin (Thr)-, arachidonic acid (AA)-, collagen (Col)-, and platelet-activating factor (PAF)-induced aggregation in washed rabbit platelets. These compounds were efficiently synthesized from commercially available 7-hydroxycoumarin or its derivatives. Among them, 7-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-2*H*-1-benzopyran-2-one (**3d**) showed the most potent inhibition of AA- and PAF- induced aggregation, with IC₅₀ values of 3.65 and 16.36 μ M respectively.

Key words antiplatelet aggregation; coumarin; α -methylene- γ -butyrolactone

Natural products bearing an α -methylene- γ -butyrolactone functionality exhibit wide-ranging biological activities, which include antitumor, bacteriocidal, fungicidal and anthelmintic actions.^{1–3)} It was soon determined that the structural requirement for the biological activities is a O=C–C=CH₂ moiety, which acts as an alkylating agent in a Michael-type reaction with biological cellular nucleophiles or sulfhydryl-containing enzymes.⁴⁾ Because of their broad range of biological activities and their interesting structural features, α -methylene- γ -butyrolactones present an important scientific focus, and this is reflected in an increasing number of investigations and syntheses of these heterocycles.^{5–8)} Helenalin⁹⁾ and eremanthin¹⁰⁾ are representative compounds that show bacteriocidal, fungicidal, and anthelmintic properties. Heliangin and vernolepin cause plant growth inhibition,^{11,12)} and purine and pyrimidine α -methylene- γ -butyrolactones^{13–15)} were found to possess antitumor activity.

Coumarin derivatives such as bishydroxycoumarin and warfarin are important anticoagulants. Other clinically useful antiplatelet drugs are aspirin, eicosapentanoic acid (EPA), dipyridamole, and ticlopidine. However, their utilization is limited by potency and side effects. As part of our new drug discovery projects, we have synthesized several 4-hydroxycoumarin derivatives with various functional groups, such as 2-hydroxy-3-isopropylamino-propyl, 2,3-epoxypropyl, 2,3-dihydroxypropyl and α -methylene- γ -butyrolactones substituted at C₄-oxygen in the hope of discovering new coumarin anticoagulants. The α -methylene- γ -butyrolactone moiety proved to be the best for improvement of the antiplatelet activity of the coumarin skeleton.¹⁶⁾ The present report describes the preparation of 7-hydroxycoumarin α -methylene- γ -butyrolactones from commercially available coumarins *via* an efficient route. The products described herein are suitable for large-scale production and exhibit potent antiplatelet activity. Their structure–activity relationships are discussed.

Chemistry

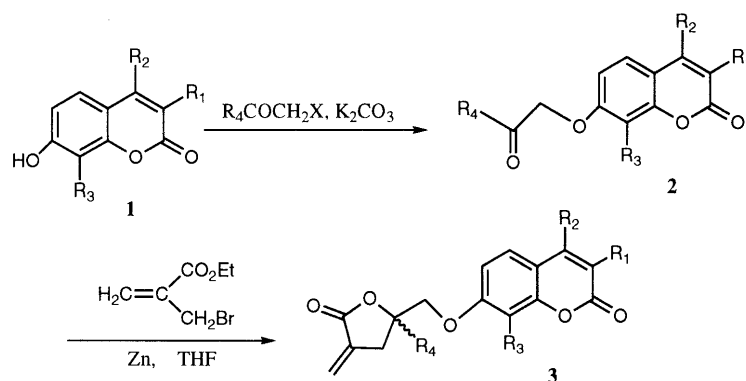
The preparation of 7-[(2,3,4,5-tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran-2-ones (**3a–c**) is illustrated in Chart 1. 7-Hydroxycoumarin (**1a**) and its derivatives were treated with potassium carbonate and chloroacetone to provide (2-oxopropoxy)-2*H*-1-benzopyran-2-ones (**2a–c**), which were then reacted with ethyl 2-(bromomethyl)acrylate and zinc powder in dry tetrahydrofuran (THF) (Reformatsky reaction) to give the target compounds, **3a–c**. Treatment of 7-hydroxycoumarins with potassium carbonate and 2-bromoacetophenone gave (2-oxo-2-phenylethoxy)-2*H*-1-benzopyran-2-ones (**2d–f**), which were reacted with ethyl 2-(bromomethyl)acrylate to afford 7-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-2*H*-1-benzopyran-2-ones (**3d–f**).

Since preliminary screening revealed that the 2-phenyl derivative **3d** was a potent inhibitor of platelet aggregation, we also prepared certain derivatives of **3d** in an attempt to optimize the antiplatelet effect. 7-Hydroxycoumarin was chosen as the starting material and underwent the same reaction sequences as described for the preparation of **3d** except that *para*-substituted 2-bromoacetophenones were used. 7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-(substituted phenyl)-2-furanyl)methoxy]-2*H*-1-benzopyran-2-ones (**3g–k**) were obtained in good overall yield.

Results and Discussion

The antiplatelet activities of 7-hydroxycoumarin and its α -methylene- γ -butyrolactone derivatives were evaluated in washed rabbit platelets. Platelet aggregation was induced by thrombin (Thr, 0.1 U/ml), arachidonic acid (AA, 100 μ M), collagen (Col, 10 μ g/ml), and platelet-activating factor (PAF, 2 nM). The final concentration of test compounds was 100 μ g/ml and the results are shown in Table 1. 7-Hydroxycoumarin (**1a**) was totally inactive. All the coumarin α -methylene- γ -butyrolactones except **3f** and **3j** were found to inhibit completely the platelet aggregation which was induced by AA, Col and PAF. Compounds **3d**, **3g**, **3i**, and **3k** also exhibited good inhibitory activity against thrombin-induced aggregation. The 50%-inhibi-

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	R ₁	R ₂	R ₃	R ₄
a	H	H	H	CH ₃
b	H	CH ₃	H	CH ₃
c	CH ₃	CH ₃	CH ₃	CH ₃
d	H	H	H	C ₆ H ₅
e	H	CH ₃	H	C ₆ H ₅
f	CH ₃	CH ₃	CH ₃	C ₆ H ₅
g	H	H	H	<i>p</i> -C ₆ H ₄ F
h	H	H	H	<i>p</i> -C ₆ H ₄ Cl
i	H	H	H	<i>p</i> -C ₆ H ₄ Br
j	H	H	H	<i>p</i> -C ₆ H ₄ C ₆ H ₅
k	H	H	H	<i>p</i> -C ₆ H ₄ OCH ₃

Chart 1

Table 1. Effect of 7-Substituted Coumarins on Platelet Aggregation (%) Induced by Thr, AA, Col and PAF in Washed Rabbit Platelets^{a)}

Comps.	Inducer			
	Thr 0.1 U/ml	AA 100 μ M	Col 10 μ g/ml	PAF 2 nM
Control	92.8 $\pm$ 1.5 (4)	87.2 $\pm$ 1.0 (6)	88.8 $\pm$ 1.5 (4)	90.3 $\pm$ 1.6 (7)
1a	80.2 $\pm$ 5.3 (3)	79.1 $\pm$ 1.9 (3) ^{b)}	81.3 $\pm$ 2.7 (3) ^{d)}	83.0 $\pm$ 0.9 (3) ^{b)}
3a	22.8 $\pm$ 6.6 (4) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3b	67.5 $\pm$ 3.9 (3) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3c	72.8 $\pm$ 6.8 (4) ^{c)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3d	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3e	80.6 $\pm$ 6.1 (3)	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3f	62.6 $\pm$ 3.5 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	20.6 $\pm$ 1.2 (3) ^{b)}	39.8 $\pm$ 13.4 (4) ^{b)}
3g	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3h	43.2 $\pm$ 12.8 (3) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
3i	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (4) ^{b)}
3j	76.1 $\pm$ 1.6 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	19.4 $\pm$ 15.9 (3) ^{c)}	60.4 $\pm$ 3.1 (3) ^{b)}
3k	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}	0.0 $\pm$ 0.0 (3) ^{b)}
Aspirin	91.9 $\pm$ 1.4 (3)	0.0 $\pm$ 0.0 (3) ^{c)}	85.4 $\pm$ 3.9 (4)	90.5 $\pm$ 1.2 (3)

a) Platelets were preincubated with DMSO (0.5%, control), aspirin (10 μ g/ml), or 7-substituted coumarins (100 μ g/ml) at 37 $^{\circ}$ C for 3 min, and then the inducer was added. Percentages of aggregation are presented as means $\pm$ standard errors of the mean (*n*). b) Significantly different from the control value at $p < 0.001$. c) Significantly different from the control value at $p < 0.01$. d) Significantly different from the control value at $p < 0.05$.

Table 2. IC₅₀ Values of 7-Substituted Coumarins on Platelet Aggregation Induced by AA (100 μ M) and PAF (2 nM)

Compounds	IC ₅₀ (μ M)	
	AA	PAF
3a	127	> 150
3b	113	147
3c	> 150	> 150
3d	3.65	16.36
3e	13.96	> 150
3f	10.09	> 150
3g	3.71	16.08
3h	4.23	39.29
3i	7.61	33.26
3j	14.25	> 150
3k	8.99	33.25

tory concentrations (IC₅₀) for aggregation induced by AA and PAF are given in Table 2. The 7-[(2,3,4,5-tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran-2-ones (3a–c), which have an aliphatic methyl substituent at the lactone C₂, were less active against AA-induced aggregation than their C₂-phenyl counterparts (3d–f). The IC₅₀ values among 3a, 3b, and 3c are comparable, indicating that the methyl substituent

tions on the coumarin did not affect the antiplatelet activity of these compounds. However, in comparing **3d**–**f** and **3g**–**k**, any methyl substitution on the coumarin led to a sharp decrease in the inhibitory activity on PAF-induced platelet aggregation. Compounds **3g**–**k**, which possess a substituted phenyl at the C₂ position, were found to inhibit AA- and PAF- induced aggregation. This finding is important, because most of the antiplatelet agents are rather specific. For example, aspirin inhibits platelet aggregation induced by AA, but not that induced by Thr, Col or PAF. The poor inhibitory potency of **3j** implies that a bulky substituent on the C₂ phenyl moiety reduced the antiplatelet potency. In contrast, **3d** (unsubstituted phenyl) and **3g** (*p*-fluorophenyl) are two of the most potent inhibitors of platelet aggregation. Compounds **3h** (*p*-chlorophenyl), **3i** (*p*-bromophenyl), and **3k** (*p*-methoxyphenyl) were somewhat less active in these assays.

Experimental

Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. The ultraviolet absorption spectra were obtained on a Beckman UV-Visible spectrophotometer. Infrared spectra were recorded on a Hitachi 260-30 spectrophotometer. Nuclear magnetic resonance (¹H and ¹³C) spectra were obtained with a Varian Gemini-200 spectrometer. Chemical shifts are expressed in parts per million (δ) with tetramethylsilane as an internal standard. Thin-layer chromatography was run on precoated (0.2 mm) Silica gel 60 F-254 plates manufactured by EM Laboratories, Inc., and short-wave length ultraviolet light (254 nm) was used to detect the UV-absorbing spots. Elemental analyses were carried out on a Heraeus CHN-O-Rapid elemental analyzer.

7-(2-Oxopropoxy)-2H-1-benzopyran-2-one (2a) Potassium carbonate (5.53 g, 40 mmol) and chloroacetone (1.38 g, 15 mmol) were added to a solution of 7-hydroxycoumarin (**1a**) (1.62 g, 10 mmol) in acetone (20 ml). The resulting mixture was refluxed for 4 h (monitored by TLC). Evaporation of the solvent gave a residue, which was poured into ice water (50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford **2a** (2.10 g, 96%) as white needles. mp 165–167 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1709, 1620. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 308 (4.14), 244 (3.52). ¹H-NMR (CDCl₃) δ: 2.31 (3H, s, CH₃), 4.65 (2H, s, OCH₂), 6.29 (1H, d, *J* = 9.5 Hz, 3-H), 6.76 (1H, d, *J* = 2.4 Hz, 8-H), 6.88 (1H, dd, *J* = 8.6, 2.5 Hz, 6-H), 7.42 (1H, d, *J* = 8.6 Hz, 5-H), 7.65 (1H, d, *J* = 9.5 Hz, 4-H). ¹³C-NMR (CDCl₃) δ: 26.56 (C-3'), 72.98 (C-1'), 101.87 (C-8), 112.59 (C-3), 113.39 (C-4a), 113.87 (C-6), 129.11 (C-5), 143.16 (C-4), 155.75 (C-8a), 160.71, 160.84 (C-2, C-7), 203.52 (C-2'). Anal. Calcd for C₁₂H₁₀O₄: C, 66.05; H, 4.62. Found: C, 65.98; H, 4.61.

4-Methyl-7-(2-oxopropoxy)-2H-1-benzopyran-2-one (2b) Compound **2b** was prepared from 7-hydroxy-4-methylcoumarin by the same procedure as used for **2a** in 70% yield, mp 147–149 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1710, 1619. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 308 (4.13), 243 (3.45). ¹H-NMR (CDCl₃) δ: 2.31 (3H, s, 3'-CH₃), 2.41 (3H, d, *J* = 1.2 Hz, 4-CH₃), 4.65 (2H, s, OCH₂), 6.17 (1H, q, *J* = 1.2 Hz, 3-H), 6.76 (1H, d, *J* = 2.5 Hz, 8-H), 6.89 (1H, dd, *J* = 8.8, 2.6 Hz, 6-H), 7.54 (1H, d, *J* = 8.8 Hz, 5-H). ¹³C-NMR (CDCl₃) δ: 18.67 (4-Me), 26.57 (C-3'), 72.95 (C-1'), 101.87 (C-8), 112.35, 112.61 (C-3, C-6), 114.50 (C-4a), 125.92 (C-5), 152.31 (C-4), 155.16 (C-8a), 160.54, 160.97 (C-2, C-7), 203.61 (C-2'). Anal. Calcd for C₁₃H₁₂O₄: C, 67.23; H, 5.21. Found: C, 67.05; H, 5.29.

3,4,8-Trimethyl-7-(2-oxopropoxy)-2H-1-benzopyran-2-one (2c) Compound **2c** was prepared from 7-hydroxy-3,4,8-trimethylcoumarin by the same procedure as used for **2a** in 90% yield, mp 173–175 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1742, 1698, 1604. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 313 (4.11), 247 (3.60). ¹H-NMR (CDCl₃) δ: 2.20 (3H, s, 3'-CH₃), 2.33 (3H, s, 3'-CH₃), 2.37 (3H, s, 4-CH₃), 2.39 (3H, s, 8-CH₃), 4.62 (2H, s, OCH₂), 6.65 (1H, d, *J* = 8.8 Hz, 6-H), 7.40 (1H, d, *J* = 9.0 Hz, 5-H). ¹³C-NMR (CDCl₃) δ: 8.40 (Me), 13.22 (Me), 15.09 (Me), 26.70 (C-3'), 73.41 (C-1'), 107.22 (C-8), 114.28 (C-4a), 115.26 (C-6), 119.46 (C-3), 122.23 (C-5), 146.07 (C-4), 151.29 (C-8a), 157.19 (C-7), 162.36 (C-2), 205.19 (C-2'). Anal. Calcd for C₁₅H₁₆O₄: C, 69.22; H, 6.20. Found: C, 69.03; H, 6.17.

7-(2-Oxo-2-phenylethoxy)-2H-1-benzopyran-2-one (2d) 2-Bromoacetophenone (1.99 g, 10 mmol) and potassium carbonate (5.53 g, 40 mmol)

were added to a solution of **1a** (1.62 g, 10 mmol) in acetone (60 ml). The mixture was refluxed for 3 h (monitored by TLC). Evaporation of the solvent gave a residue, which was poured into ice water (50 ml). The resulting solid was collected and crystallized from ethyl acetate to afford **2d** (1.53 g, 73%) as needles, mp 163–165 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1728, 1702, 1627. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 320 (4.16), 249 (4.13). ¹H-NMR (CDCl₃) δ: 5.39 (2H, s, OCH₂), 6.27 (1H, d, *J* = 9.5 Hz, 3-H), 6.80 (1H, d, *J* = 2.4 Hz, 8-H), 6.93 (1H, dd, *J* = 8.6, 2.5 Hz, 6-H), 7.40 (1H, d, *J* = 8.6 Hz, 5-H), 7.49–7.68 (4H, m, 4-H and Ar-H), 7.97–8.02 (2H, m, Ar-H). ¹³C-NMR (CDCl₃) δ: 70.59 (C-1'), 101.93 (C-8), 112.91 (C-3), 113.18 (C-4a), 113.72 (C-6), 127.99, 128.97, 134.16, 134.23 (Ar-Cs), 129.03 (C-5), 143.23 (C-4), 155.68 (C-8a), 161.01, 161.05 (C-2, C-7), 193.07 (C-2'). Anal. Calcd for C₁₇H₁₂O₄: C, 72.85; H, 4.32. Found: C, 72.98; H, 4.35.

4-Methyl-7-(2-oxo-2-phenylethoxy)-2H-1-benzopyran-2-one (2e) 2-Bromoacetophenone (1.99 g, 10 mmol) and potassium carbonate (5.53 g, 40 mmol) were added to a solution of 7-hydroxy-4-methylcoumarin (1.76 g, 10 mmol) in acetone (60 ml). The mixture was refluxed for 3 h (monitored by TLC). After cooling, the solvent was evaporated, and the residue was poured into ice water (100 ml) and extracted with CH₂Cl₂ (75 ml × 2). The extracts were combined, washed with brine, dried over Na₂SO₄, and then evaporated to give a solid residue, which was crystallized from ethyl acetate to afford **2e** (2.86 g, 97%), mp 167–168 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1718, 1702, 1614. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 315 (4.14), 249 (4.10). ¹H-NMR (CDCl₃) δ: 2.39 (3H, d, *J* = 1.2 Hz, 4-CH₃), 5.39 (2H, s, OCH₂), 6.14 (1H, q, *J* = 1.2 Hz, 3-H), 6.80 (1H, d, *J* = 2.6 Hz, 8-H), 6.95 (1H, dd, *J* = 8.8, 2.6 Hz, 6-H), 7.49–7.63 (4H, m, 5-H and Ar-H), 7.96–8.02 (2H, m, Ar-H). ¹³C-NMR (CDCl₃) δ: 18.65 (4-Me), 70.58 (C-1'), 101.91 (C-8), 112.42, 112.64 (C-3, C-6), 114.34 (C-4a), 125.78 (C-5), 128.01, 129.02, 134.18, 134.24 (Ar-Cs), 152.38 (C-4), 155.10 (C-8a), 160.90, 161.04 (C-2 and C-7), 193.17 (C-2'). Anal. Calcd for C₁₈H₁₄O₄: C, 73.46; H, 4.79. Found: C, 73.28; H, 4.77.

7-(2-Oxo-2-phenylethoxy)-3,4,8-trimethyl-2H-1-benzopyran-2-one (2f) Compound **2f** was prepared from 7-hydroxy-3,4,8-trimethylcoumarin by the same procedure as used for **2e** in 52% yield, mp 196–198 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1710, 1699, 1608. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 318 (4.16), 249 (4.16). ¹H-NMR (CDCl₃) δ: 2.18 (3H, s, 3-CH₃), 2.34 (3H, s, 4-CH₃), 2.38 (3H, s, 8-CH₃), 5.38 (2H, s, OCH₂), 6.70 (1H, d, *J* = 9.0 Hz, 6-H), 7.35 (1H, d, *J* = 8.8 Hz, 5-H), 7.47–7.68 (3H, m, Ar-H), 7.97–8.03 (2H, m, Ar-H). ¹³C-NMR (CDCl₃) δ: 8.42 (Me), 13.20 (Me), 15.07 (Me), 71.26 (C-1'), 107.70 (C-8), 114.59 (C-6), 115.13 (C-4a), 119.28 (C-3), 122.02 (C-5), 128.15, 128.90, 134.06, 134.41 (Ar-Cs), 146.12 (C-4), 151.29 (C-8a), 157.59 (C-7), 162.47 (C-2), 194.25 (C-2'). Anal. Calcd for C₂₀H₁₈O₄: C, 74.52; H, 5.63. Found: C, 74.45; H, 5.60.

7-[2-(4-Fluorophenyl)-2-oxoethoxy]-2H-1-benzopyran-2-one (2g) 2-Chloro-4'-fluoroacetophenone (0.86 g, 5 mmol), potassium carbonate (2.76 g, 20 mmol) and potassium iodide (0.16 g, 1 mmol) were added to a solution of **1a** (0.81 g, 5 mmol) in acetone (50 ml). The mixture was refluxed for 3 h (monitored by TLC). Evaporation of the solvent gave a residue, which was poured into ice water (30 ml). The resulting solid was collected and crystallized from ethyl acetate to afford **2g** (1.25 g, 84%), mp 196–197 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1720, 1619. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 319 (4.02), 250 (4.00). ¹H-NMR (DMSO-*d*₆) δ: 5.74 (2H, s, OCH₂), 6.31 (1H, d, *J* = 9.5 Hz, 3-H), 7.03 (1H, dd, *J* = 8.6, 2.4 Hz, 6-H), 7.10 (1H, d, *J* = 2.4 Hz, 8-H), 7.39–7.48 (2H, m, Ar-H), 7.65 (1H, d, *J* = 8.6 Hz, 5-H), 8.00 (1H, d, *J* = 9.5 Hz, 4-H), 8.10–8.17 (2H, m, Ar-H). ¹³C-NMR (DMSO-*d*₆) δ: 70.78 (C-1'), 101.88 (C-8), 112.94, 113.12, 113.14 (C-3, C-4a, C-6), 116.18 (*J* = 22.0 Hz), 131.28 (*J* = 9.7 Hz), 147.32 (*J* = 3.2 Hz), 165.82 (*J* = 246.4 Hz) (Ar-Cs), 129.71 (C-5), 144.52 (C-4), 155.55 (C-8a), 160.50, 161.41 (C-2, C-7), 192.67 (C-2'). Anal. Calcd for C₁₇H₁₁FO₄: C, 68.46; H, 3.72. Found: C, 68.23; H, 3.69.

7-[2-(4-Chlorophenyl)-2-oxoethoxy]-2H-1-benzopyran-2-one (2h) Compound **2h** was prepared from 2-bromo-4'-chloroacetophenone by the same procedure as used for **2g** in 83% yield, mp 192–194 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1718, 1615. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ε): 319 (4.12), 258 (4.22). ¹H-NMR (DMSO-*d*₆) δ: 5.74 (2H, s, OCH₂), 6.31 (1H, d, *J* = 9.5 Hz, 3-H), 7.03 (1H, dd, *J* = 8.6, 2.5 Hz, 6-H), 7.11 (1H, d, *J* = 2.4 Hz, 8-H), 7.62–7.69 (3H, m, 5-H, Ar-H), 7.98–8.08 (3H, m, 4-H, Ar-H). ¹³C-NMR (DMSO-*d*₆) δ: 70.85 (C-1'), 101.89 (C-8), 112.96, 113.11, 113.14 (C-3, C-4a, C-6), 129.21, 129.71, 130.13, 133.17, 139.06 (Ar-Cs, C-5), 144.51 (C-4), 155.55 (C-8a), 160.50, 161.37 (C-2, C-7), 193.13 (C-2'). Anal. Calcd for C₁₇H₁₁ClO₄·0.25H₂O: C, 63.96; H, 3.63. Found: C, 64.09; H, 3.49.

7-[2-(4-Bromophenyl)-2-oxoethoxy]-2H-1-benzopyran-2-one (2i) Compound **2i** was prepared from 2-bromo-4'-bromoacetophenone by

the same procedure as used for **2g** in 71% yield, mp 175–177 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1699, 1615. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 319 (4.22), 267 (4.33). $^1\text{H-NMR}$ (CDCl_3) δ : 5.33 (2H, s, OCH_2), 6.27 (1H, d, $J=9.5$ Hz, 3-H), 6.79 (1H, d, $J=2.6$ Hz, 8-H), 6.91 (1H, dd, $J=8.6$, 2.5 Hz, 6-H), 7.40 (1H, d, $J=8.6$ Hz, 5-H), 7.64 (1H, d, $J=9.2$ Hz, 4-H), 7.65–7.70 (2H, m, Ar-H), 7.83–7.89 (2H, m, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 70.64 (C-1'), 101.92 (C-8), 112.86 (C-3), 113.38 (C-4a), 113.81 (C-6), 129.04, 129.58, 132.39, 132.87 (Ar-Cs, C-5), 143.17 (C-4), 155.69 (C-8a), 160.86 (C-2, C-7), 192.47 (C-2'). *Anal.* Calcd for $\text{C}_{17}\text{H}_{11}\text{BrO}_4$: C, 56.85; H, 3.09. Found: C, 56.81; H, 3.09.

7-[2-Oxo-2-(4-phenylphenyl)ethoxy]-2H-1-benzopyran-2-one (2j) Compound **2j** was prepared from 2-bromo-4'-phenylacetophenone by the same procedure as used for **2g** in 89% yield, mp 187–188 °C; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1729, 1700, 1626. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 271 (4.38). $^1\text{H-NMR}$ (CDCl_3) δ : 5.41 (2H, s, OCH_2), 6.27 (1H, d, $J=9.5$ Hz, 3-H), 6.83 (1H, d, $J=2.6$ Hz, 8-H), 6.95 (1H, dd, $J=8.6$, 2.5 Hz, 6-H), 7.40 (1H, d, $J=8.6$ Hz, 5-H), 7.45–7.67 (6H, m, 4-H, 4'-phenylic H), 7.73–7.78 (2H, m, Ar-H), 8.05–8.09 (2H, m, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 70.68 (C-1'), 101.97 (C-8), 112.93 (C-3), 113.28 (C-4a), 113.70 (C-6), 127.32, 127.62, 128.57, 128.65, 129.00, 129.07, 132.81, 139.54, 147.00 (Ar-Cs, C-5), 143.23 (C-4), 155.71 (C-8a), 160.94, 161.10 (C-2, C-7), 192.47 (C-2'). *Anal.* Calcd for $\text{C}_{23}\text{H}_{16}\text{O}_4$: C, 76.54; H, 4.61. Found: C, 76.64; H, 4.42.

7-[2-(4-Methoxyphenyl)-2-oxoethoxy]-2H-1-benzopyran-2-one (2k) Compound **2k** was prepared from 2-bromo-4'-methoxyacetophenone by the same procedure as used for **2g** in 76% yield, mp 159–161 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1718, 1700, 1620. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 315 (4.22), 271 (4.27). $^1\text{H-NMR}$ (CDCl_3) δ : 3.90 (3H, s, OCH_3), 5.33 (2H, s, OCH_2), 6.26 (1H, d, $J=9.5$ Hz, 3-H), 6.79 (1H, d, $J=2.6$ Hz, 8-H), 6.92 (1H, dd, $J=8.6$, 2.5 Hz, 6-H), 6.97–7.02 (2H, m, Ar-H), 7.39 (1H, d, $J=8.6$ Hz, 5-H), 7.63 (1H, d, $J=9.5$ Hz, 4-H), 7.96–8.00 (2H, m, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 55.61 (Me), 70.45 (C-1'), 101.92 (C-8), 112.95 (C-3), 113.18 (C-4a), 113.57 (C-6), 114.22, 127.16, 130.42, 164.36 (Ar-Cs), 128.95 (C-5), 143.28 (C-4), 155.69 (C-8a), 161.00, 161.20 (C-2, C-7), 191.58 (C-2'). *Anal.* Calcd for $\text{C}_{18}\text{H}_{14}\text{O}_5$: C, 69.67; H, 4.55. Found: C, 69.63; H, 4.57.

7-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-2H-1-benzopyran-2-one (3a) Activated zinc powder (0.255 g, (3.9 mmol), hydroquinone (6 mg), and ethyl 2-(bromomethyl)acrylate (0.78 g, 4 mmol) were added to a solution of **2a** (0.655 g, 3 mmol) in dry THF (60 ml). The mixture was refluxed under a nitrogen atmosphere for 36 h (monitored by TLC). After cooling, it was poured into ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml $\times$ 3). The dichloromethane extracts were combined and washed with brine, dried over Na_2SO_4 , and then evaporated to give a solid residue, which was crystallized from ethyl acetate to afford **3a**: Yield 80%, mp 123–124 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1755, 1727, 1626; UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 312 (4.18), 243 (3.58). $^1\text{H-NMR}$ (CDCl_3) δ : 1.58 (3H, s, 2'- CH_3), 2.79 (1H, dt, $J=17.2$, 2.8 Hz, 3'-H), 3.18 (1H, dt, $J=17.2$, 2.6 Hz, 3'-H), 3.99, 4.08 (2H, AB type, $J=9.7$ Hz, OCH_2), 5.69 (1H, t, $J=2.6$ Hz, vinylic H), 6.26 (1H, d, $J=9.5$ Hz, 3-H), 6.29 (1H, t, $J=2.9$ Hz, vinylic H), 6.76–6.84 (2H, m, 6 and 8-H), 7.38 (1H, d, $J=8.4$ Hz, 5-H), 7.65 (1H, d, $J=9.5$ Hz, 4-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 24.11 (Me), 36.60 (C-3'), 73.18 (C-1'), 81.05 (C-2'), 101.65 (C-8), 112.78 (C-3), 113.17 (C-4a), 113.63 (C-6), 122.32 (vinylic C), 128.96 (C-5), 135.06 (C-4'), 143.28 (C-4), 155.69 (C-8a), 160.93, 161.18 (C-2, C-7), 169.39 (C-5'). *Anal.* Calcd for $\text{C}_{16}\text{H}_{14}\text{O}_5$: C, 67.13; H, 4.93. Found: C, 66.95; H, 5.10.

The same procedure was used to convert compounds **2b–k** to **3b–k**, respectively.

7-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-4-methyl-2H-1-benzopyran-2-one (3b) Yield, 76%; mp 135–137 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1758, 1705, 1616. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 320 (3.99), 242 (3.35). $^1\text{H-NMR}$ (CDCl_3) δ : 1.58 (3H, s, 2'- CH_3), 2.40 (3H, d, $J=1.2$ Hz, 4- CH_3), 2.79 (1H, dt, $J=17.1$, 2.8 Hz, 3'-H), 3.18 (1H, dt, $J=17.2$, 2.6 Hz, 3'-H), 3.99, 4.08 (2H, AB type, $J=9.8$ Hz, OCH_2), 5.69 (1H, t, $J=2.6$ Hz, vinylic H), 6.16 (1H, q, $J=1.2$ Hz, 3-H), 6.30 (1H, t, $J=2.9$ Hz, vinylic H), 6.78 (1H, d, $J=2.5$ Hz, 8-H), 6.83 (1H, dd, $J=8.7$, 2.5 Hz, 6-H), 7.50 (1H, d, $J=8.6$ Hz, 5-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 18.67 (Me), 24.14 (2'-Me), 36.63 (C-3'), 73.14 (C-1'), 81.06 (C-2'), 101.68 (C-8), 112.46, 112.48 (C-3, C-6), 114.26 (C-4a), 122.34 (vinylic C), 125.72 (C-5), 135.04 (C-4'), 152.38 (C-4), 155.11 (C-8a), 160.02, 160.05 (C-2, C-7), 169.39 (C-5'). *Anal.* Calcd for $\text{C}_{17}\text{H}_{16}\text{O}_5$: C, 67.99; H, 5.37. Found: C, 67.87; H, 5.63.

7-[(2,3,4,5-Tetrahydro-2-methyl-4-methylene-5-oxo-2-furanyl)methoxy]-3,4,8-trimethyl-2H-1-benzopyran-2-one (3c) Yield, 82%; mp 163–165 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1751, 1699, 1603. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 319 (4.18),

246 (3.71). $^1\text{H-NMR}$ (CDCl_3) δ : 1.60 (3H, s, 2'- CH_3), 2.18 (3H, s, 3- CH_3), 2.22 (3H, s, 8- CH_3), 2.36 (3H, s, 4- CH_3), 2.81 (1H, dt, $J=17.2$, 2.9 Hz, 3'-H), 3.23 (1H, dt, $J=17.2$, 2.5 Hz, 3'-H), 3.99, 4.09 (2H, AB type, $J=9.7$ Hz, OCH_2), 5.69 (1H, t, $J=2.5$ Hz, vinylic H), 6.30 (1H, t, $J=2.9$ Hz, vinylic H), 6.75 (1H, d, $J=8.8$ Hz, 6-H), 7.38 (1H, d, $J=8.8$ Hz, 5-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 8.16 (Me), 13.20 (Me), 15.08 (Me), 24.20 (2'-Me), 36.76 (C-3'), 73.44 (C-1'), 81.25 (C-2'), 107.27 (C-8), 114.11 (C-6), 115.04 (C-4a), 119.26 (C-3), 122.14 (C-5, vinylic C), 135.30 (C-4'), 146.13 (C-4), 151.11 (C-8a), 157.57 (C-7), 162.39 (C-2), 169.49 (C-5'). *Anal.* Calcd for $\text{C}_{19}\text{H}_{20}\text{O}_5$: C, 69.50; H, 6.14. Found: C, 69.22; H, 6.11.

7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-2H-1-benzopyran-2-one (3d) Yield, 78%; mp 105–106 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1758, 1719, 1616. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 321 (4.22), 243 (3.62). $^1\text{H-NMR}$ (CDCl_3) δ : 3.24 (1H, dt, $J=17.0$, 2.9 Hz, 3'-H), 3.66 (1H, dt, $J=17.0$, 2.5 Hz, 3'-H), 4.18, 4.23 (2H, AB type, $J=10.3$ Hz, OCH_2), 5.71 (1H, t, $J=2.5$ Hz, vinylic H), 6.24 (1H, d, $J=9.5$ Hz, 3-H), 6.31 (1H, t, $J=2.9$ Hz, vinylic H), 6.72 (1H, d, $J=2.4$ Hz, 8-H), 6.78 (1H, dd, $J=8.5$, 2.4 Hz, 6-H), 7.35 (1H, d, $J=8.5$ Hz, 5-H), 7.40–7.52 (5H, m, Ar-H), 7.62 (1H, d, $J=9.5$ Hz, 4-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 37.32 (C-3'), 74.43 (C-1'), 83.91 (C-2'), 101.80 (C-8), 112.76 (C-3), 113.21 (C-4a), 113.62 (C-6), 121.96 (vinylic C), 125.02, 128.72, 128.89, 134.60 (Ar-Cs), 128.95 (C-5), 139.23 (C-4'), 143.25 (C-4), 155.60 (C-8a), 160.90, 161.04 (C-2, C-7), 169.12 (C-5'). *Anal.* Calcd for $\text{C}_{21}\text{H}_{16}\text{O}_5$: C, 72.41; H, 4.63. Found: C, 72.30; H, 4.67.

7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-4-methyl-2H-1-benzopyran-2-one (3e) Yield, 85%; mp 168–169 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1773, 1717, 1609. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 320 (4.21), 242 (3.55). $^1\text{H-NMR}$ (CDCl_3) δ : 2.38 (3H, d, $J=1.2$ Hz, 4- CH_3), 3.23 (1H, dt, $J=17.0$, 2.9 Hz, 3'-H), 3.66 (1H, dt, $J=17.0$, 2.4 Hz, 3'-H), 4.18, 4.25 (2H, AB type, $J=10.3$ Hz, OCH_2), 5.71 (1H, t, $J=2.5$ Hz, vinylic H), 6.13 (1H, q, $J=1.1$ Hz, 3-H), 6.31 (1H, t, $J=2.9$ Hz, vinylic H), 6.72 (1H, d, $J=2.5$ Hz, 8-H), 6.80 (1H, dd, $J=8.8$, 2.5 Hz, 6-H), 7.37–7.52 (6H, m, 5-H, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 18.66 (Me), 37.36 (C-3'), 74.42 (C-1'), 83.93 (C-2'), 101.86 (C-8), 112.49 (C-3, C-6), 114.33 (C-4a), 121.96 (vinylic C), 125.71 (C-5), 125.05, 128.73, 128.91, 134.61 (Ar-Cs), 139.90 (C-4'), 152.33 (C-4), 155.06 (C-8a), 160.90, 160.99 (C-2, C-7), 169.11 (C-5'). *Anal.* Calcd for $\text{C}_{22}\text{H}_{18}\text{O}_5$: C, 72.92; H, 5.01. Found: C, 72.83; H, 5.16.

7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-phenyl-2-furanyl)methoxy]-3,4,8-trimethyl-2H-1-benzopyran-2-one (3f) Yield, 73%; mp 142–143 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1771, 1698, 1604. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 309 (4.17), 258 (3.96). $^1\text{H-NMR}$ (CDCl_3) δ : 2.18 (3H, s, 3- CH_3), 2.22 (3H, s, 8- CH_3), 2.34 (3H, s, 4- CH_3), 3.27 (1H, dt, $J=17.0$, 3.0 Hz, 3'-H), 3.71 (1H, dt, $J=16.8$, 2.5 Hz, 3'-H), 4.17, 4.22 (2H, AB type, $J=10.0$ Hz, OCH_2), 5.73 (1H, t, $J=2.5$ Hz, vinylic H), 6.34 (1H, t, $J=3.0$ Hz, vinylic H), 6.69 (1H, d, $J=8.9$ Hz, 6-H), 7.34 (1H, d, $J=8.8$ Hz, 5-H), 7.39–7.54 (5H, m, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 8.20 (Me), 13.19 (Me), 15.07 (Me), 37.43 (C-3'), 74.74 (C-1'), 84.06 (C-2'), 107.30 (C-8), 114.19 (C-6), 115.11 (C-4a), 119.27 (C-3), 121.99 (vinylic C), 122.08 (C-5), 125.07, 128.67, 128.86, 134.77 (Ar-Cs), 140.17 (C-4'), 146.10 (C-4), 151.12 (C-8a), 157.51 (C-7), 162.38 (C-2), 169.13 (C-5'). *Anal.* Calcd for $\text{C}_{24}\text{H}_{22}\text{O}_5$: C, 73.83; H, 5.68. Found: C, 73.61; H, 5.74.

7-[(2-(4-Fluorophenyl)-2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2H-1-benzopyran-2-one (3g) Yield, 86%; mp 157–159 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1771, 1712, 1613. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 320 (4.18), 253 (3.82). $^1\text{H-NMR}$ (CDCl_3) δ : 3.21 (1H, dt, $J=17.0$, 3.0 Hz, 3'-H), 3.65 (1H, dt, $J=17.0$, 2.5 Hz, 3'-H), 4.14, 4.21 (2H, AB type, $J=10.2$ Hz, OCH_2), 5.73 (1H, t, $J=2.5$ Hz, vinylic H), 6.26 (1H, d, $J=9.5$ Hz, 3-H), 6.33 (1H, t, $J=3.0$ Hz, vinylic H), 6.73 (1H, d, $J=2.5$ Hz, 8-H), 6.78 (1H, dd, $J=8.5$, 2.5 Hz, 6-H), 7.09–7.18 (2H, m, Ar-H), 7.36 (1H, d, $J=8.6$ Hz, 5-H), 7.44–7.51 (2H, m, Ar-H), 7.63 (1H, d, $J=9.5$ Hz, 4-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 37.41 (C-3'), 74.36 (C-1'), 83.45 (C-2'), 101.81 (C-8), 112.76 (C-3), 113.32 (C-4a), 113.82 (C-6), 115.91 ($J=21.9$ Hz), 127.02 ($J=8.2$ Hz), 135.73 ($J=3.6$ Hz), 162.73 ($J=248.7$ Hz) (Ar-Cs), 122.32 (vinylic C), 128.96 (C-5), 134.31 (C-4'), 143.17 (C-4), 155.65 (C-8a), 160.85, 160.93 (C-2, C-7), 168.87 (C-5'). *Anal.* Calcd for $\text{C}_{21}\text{H}_{15}\text{FO}_5$: C, 68.01; H, 4.21. Found: C, 68.19; H, 4.08.

7-[(2-(4-Chlorophenyl)-2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2H-1-benzopyran-2-one (3h) Yield, 89%; mp 202–204 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1771, 1708, 1608. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 322 (4.13), 244 (3.58). $^1\text{H-NMR}$ (CDCl_3) δ : 3.19 (1H, dt, $J=16.9$, 2.9 Hz, 3'-H), 3.64 (1H, dt, $J=17.0$, 2.5 Hz, 3'-H), 4.14, 4.21 (2H, AB type, $J=10.1$ Hz, OCH_2), 5.73 (1H, t, $J=2.5$ Hz, vinylic H), 6.27 (1H, d, $J=9.5$ Hz, 3-H),

6.33 (1H, t, $J=2.9$ Hz, vinylic H), 6.73 (1H, d, $J=2.4$ Hz, 8-H), 6.78 (1H, dd, $J=8.5, 2.4$ Hz, 6-H), 7.36 (1H, d, $J=8.5$ Hz, 5-H), 7.39–7.43 (4H, br s, Ar-H), 7.63 (1H, d, $J=9.5$ Hz, 4-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 37.33 (C-3'), 74.19 (C-1'), 88.38 (C-2'), 101.81 (C-8), 112.75 (C-3), 113.35 (C-4a), 113.86 (C-6), 122.49 (vinylic C), 126.56, 129.13, 134.13, 134.84 (Ar-Cs), 128.98 (C-5), 138.39 (C-4'), 143.16 (C-4), 155.66 (C-8a), 160.84, 160.88 (C-2, C-7), 168.80 (C-5'). *Anal.* Calcd for $\text{C}_{21}\text{H}_{15}\text{ClO}_5 \cdot 0.25\text{H}_2\text{O}$: C, 65.12; H, 4.70. Found: C, 64.93; H, 3.93.

7-[[2-(4-Bromophenyl)-2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furan-yl]methoxy]-2H-1-benzopyran-2-one (3i) Yield, 76%; mp 206–208 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1770, 1710, 1610. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 321 (4.20), 243 (3.68). $^1\text{H-NMR}$ (CDCl_3) δ : 3.18 (1H, dt, $J=16.9, 2.9$ Hz, 3'-H), 3.64 (1H, dt, $J=16.9, 2.5$ Hz, 3'-H), 4.14, 4.20 (2H, AB type, $J=10.1$ Hz, OCH_2), 5.73 (1H, t, $J=2.5$ Hz, vinylic H), 6.27 (1H, d, $J=9.5$ Hz, 3-H), 6.33 (1H, t, $J=2.9$ Hz, vinylic H), 6.73 (1H, d, $J=2.4$ Hz, 8-H), 6.78 (1H, dd, $J=8.5, 2.4$ Hz, 6-H), 7.33–7.40 (3H, m, 5-H, Ar-H), 7.54–7.65 (3H, m, 4-H, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 37.28 (C-3'), 74.10 (C-1'), 83.38 (C-2'), 101.80 (C-8), 112.73 (C-3), 113.34 (C-4a), 113.84 (C-6), 122.50 (vinylic C), 122.95, 126.83, 132.07, 134.06 (Ar-Cs), 128.96 (C-5), 138.91 (C-4'), 143.14 (C-4), 155.64 (C-8a), 160.86, 160.92 (C-2, C-7), 168.74 (C-5'). *Anal.* Calcd for $\text{C}_{21}\text{H}_{15}\text{BrO}_5 \cdot 0.25\text{H}_2\text{O}$: C, 58.42; H, 3.62. Found: C, 58.53; H, 3.63.

7-[[2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-(4-phenylphenyl)-2-furan-yl]methoxy]-2H-1-benzopyran-2-one (3j) Yield, 79%; mp 135–137 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1769, 1708, 1613. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 322 (4.18), 253 (4.36). $^1\text{H-NMR}$ (CDCl_3) δ : 3.27 (1H, dt, $J=17.0, 2.9$ Hz, 3'-H), 3.68 (1H, dt, $J=17.0, 2.4$ Hz, 3'-H), 4.21, 4.28 (2H, AB type, $J=10.2$ Hz, OCH_2), 5.73 (1H, t, $J=2.4$ Hz, vinylic H), 6.26 (1H, d, $J=9.5$ Hz, 3-H), 6.33 (1H, t, $J=2.9$ Hz, vinylic H), 6.76 (1H, d, $J=2.5$ Hz, 8-H), 6.81 (1H, dd, $J=8.5, 2.5$ Hz, 6-H), 7.34–7.69 (11H, m, 4-, 5-H, Ar-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 37.35 (C-3'), 74.39 (C-1'), 83.82 (C-2'), 101.85 (C-8), 112.81 (C-3), 113.26 (C-4a), 113.76 (C-6), 122.10 (vinylic C), 125.53, 127.12, 127.59, 127.76, 128.90, 138.72, 140.10, 141.75 (Ar-Cs), 128.91 (C-5), 143.17 (C-4), 155.67 (C-8a), 160.78, 161.04 (C-2, C-7), 169.05 (C-5'). *Anal.* Calcd for $\text{C}_{27}\text{H}_{20}\text{O}_5 \cdot 0.25\text{H}_2\text{O}$: C, 75.60; H, 4.82. Found: C, 75.54; H, 4.81.

7-[[2,3,4,5-Tetrahydro-2-(4-methoxyphenyl)-4-methylene-5-oxo-2-furan-yl]methoxy]-2H-1-benzopyran-2-one (3k) Yield, 72%; mp 148–150 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1778, 1714, 1615. UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (log ϵ): 320 (4.05), 245 (3.85). $^1\text{H-NMR}$ (CDCl_3) δ : 3.21 (1H, dt, $J=17.0, 2.9$ Hz, 3'-H), 3.62 (1H, dt, $J=17.0, 2.6$ Hz, 3'-H), 3.84 (3H, s, OCH_3), 4.12, 4.21 (2H, AB type, $J=10.2$ Hz, OCH_2), 5.70 (1H, t, $J=2.5$ Hz, vinylic H), 6.24–6.32 (2H, m, 3-H, vinylic H), 6.73 (1H, d, $J=2.4$ Hz, 8-H), 6.79 (1H, dd, $J=8.5, 2.4$ Hz, 6-H), 6.92–7.00 (2H, m, Ar-H), 7.34–7.42 (3H, m, 5-H, Ar-H), 7.63 (1H, d, $J=9.6$ Hz, 4-H). $^{13}\text{C-NMR}$ (CDCl_3) δ : 37.31 (C-3'), 55.39 (Me), 74.50 (C-1'), 83.78 (C-2'), 101.81 (C-8), 112.83 (C-3), 113.22 (C-4a), 113.71 (C-6), 114.25, 126.40, 131.78, 159.81 (Ar-Cs), 121.88 (vinylic C), 128.92 (C-5), 134.77 (C-4'), 143.19 (C-4), 155.67 (C-8a), 160.90, 161.09 (C-2, C-7), 169.16 (C-5'). *Anal.* Calcd for $\text{C}_{22}\text{H}_{18}\text{O}_6 \cdot 0.25\text{H}_2\text{O}$: C, 69.01; H, 4.87. Found: C, 68.93; H, 4.83.

Antiplatelet Evaluation A) Preparation of Platelet Aggregation Inducers: 1) Bovine thrombin, obtained from Parke Davis Co., was dissolved in 50% (v/v) glycerol to give a stock solution of 100 NIH units/ml. 2) Collagen (type I, bovine Achilles tendon), obtained from Sigma Chemical Co., was homogenized in 25 mM acetic acid and stored (1 mg/ml) at -70°C . 3) PAF (1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine), purchased from Sigma, was dissolved in chloroform and diluted into 0.1% bovine serum albumin in saline solution immediately prior to use. 4) Arachidonic acid, purchased from Sigma, was dissolved in deionized water.

B) Preparation of Platelets: Platelet suspension was prepared from

EDTA-anticoagulated platelet-rich plasma according to the washing procedures described previously.¹⁷⁾ Platelets were counted by a Hemalaser 2 (Sebia, France) and adjusted to a concentration of 4.5×10^8 platelets/ml. Platelet pellets were finally suspended in Tyrode's buffer (pH 7.4) of the following composition: NaCl (136.8 mM), KCl (2.8 mM), NaHCO_3 (11.9 mM), MgCl_2 (2.1 mM), NaH_2PO_4 (0.33 mM), CaCl_2 (1 mM), glucose (11.2 mM) containing 0.35% bovine serum albumin.

C) Platelet Aggregation and ATP Release Reaction: Aggregation was measured by the turbidimetry method as described by O'Brien.¹⁸⁾ ATP released from platelets was detected by the bioluminescence method of DeLuca and McElory.¹⁹⁾ Aggregation and ATP release were measured simultaneously in a Lumi-aggregometer (model 1020B, Payton, Canada) connected to two dual-channel recorders. Platelet preparations were stirred at 900 rpm. When dimethyl sulfoxide (DMSO) was used as the solvent, its final concentration was fixed at 0.5% (v/v). For the calculation of percentage aggregation, the absorbance of the platelet suspension was designated as 0% aggregation and the absorbance of platelet-free Tyrode's solution as 100% aggregation. The 50%-inhibitory concentration (IC_{50}) for aggregation was calculated using CA-Cricket Graph III for five or six dose-effect levels.

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