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Bioorganic & Medicinal Chemistry 13 (2005) 5710–5716

Bioorganic & Medicinal Chemistry

Synthesis, antiproliferative, and vasorelaxing evaluations of coumarin α-methylene-γ-butyrolactones

Yeh-Long Chen,[a,*] Chih-Ming Lu,[a] Shoiw-Ju Lee,[b] Daih-Huang Kuo,[c] I-Li Chen,[c] Tai-Chi Wang[c] and Cherng-Chyi Tzeng[a]

[a]*Faculty of Medicinal and Applied Chemistry, College of Life Science, Kaohsiung Medical University, Kaohsiung City 807, Taiwan*
[b]*Division of Biotechnology and Pharmaceutical Research, National Health Research Institute, Taipei 114, Taiwan*
[c]*Department of Pharmacy, Tajen Institute of Technology, Pingtong 907, Taiwan*

Received 18 April 2005; revised 2 June 2005; accepted 2 June 2005
Available online 1 July 2005

Abstract—Certain coumarin α-methylene-γ-butyrolactones were synthesized and evaluated for antiproliferative and vasorelaxing activities. These compounds were synthesized via alkylation of hydroxycoumarins **2a–f** followed by oxidation and the Reformatsky-type condensation. The results of this study are as follows (1) for the vasorelaxing activity, coumarin-7-yl α-methylene-γ-butyrolactone **6d**, with an IC_{50} value of 9.4 μM against pig coronary arterial contraction induced by KCl, is a more active vasorelaxant than its coumarin-4-yl counterpart **6a** and its γ-methyl congener **1**. A methyl group substituted at C-4 of the coumarin-7-yl moiety reduced the vasorelaxing effect (**6d** vs **6e**) while the 3,4,8-trimethyl derivative **6f** was inactive. (2) For the antiproliferative activity, coumarin-4-yl α-methylene-γ-butyrolactone **6a**, which exhibited the most potent antiproliferative activity on the growth of MCF7, NCI-H460, and SF-268 with IC_{50} values of 6.97, 14.68, and 8.36 μM, respectively, is more cytotoxic than its coumarin-7-yl counterpart **6d** and the 6,7-dimethyl derivative **6b**. For the coumarin-7-yl derivatives, **6d** is more active than its γ-methyl congener **1**, indicating that substitution at the γ-position decreased cytotoxicity.

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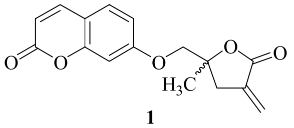
1. Introduction

The α-methylene-γ-butyrolactone moiety is a characteristic component of a large number of biologically active natural products, especially the sesquiterpene lactones.[1–4] However, the biological activity of α-methylene-γ-butyrolactones is not confined to the complex polyfunctional sesquiterpene lactones only. For example, some simple natural α-methylene-γ-butyrolactone bearing butanolides and even the parent α-methylene-γ-butyrolactone (tulipaline A) were found to have significant pharmacological activities.[5,6] Over the past few years, we were particularly interested in synthesizing α-methylene-γ-butyrolactones and evaluated their cardiovascular and cytotoxic activities.[7,8] Although the enone (O=C—C=CH_2) component in this type of lactone is essential for their biological activities, by acting as an alkylating agent through a Michael-type reaction with bionucleophiles or sulfhydryl-containing enzymes,[9] both γ-substituents of the lactone (**1**; R and aryl) also played important roles in their pharmacological properties (Fig. 1). For example, an aliphatic methyl substituent of R is more potent against high-K^+ medium, Ca^{2+}-induced and norepinephrine (NE)-induced vasoconstrictions than its phenyl substituent, which in turn is a more potent vasorelaxant than a substituted phenyl counterpart.[10] For the aryl substituent, coumarin-7-yl is superior to its coumarin-4-yl counterpart for inhibition of NE-induced vasoconstrictions in which **1** was one of the best.[10] The present report describes the preparation and antiproliferative and vasorelaxing activities of certain coumarin α-methylene-γ-butyrolactones, derivatives of **1** in which the γ-methyl substituent was removed because the more



1

Figure 1. Structure of γ-methyl-α-methylene-γ-butyrolactone.

Keywords: Coumarin; α-Methylene-γ-butyrolactones; Vasorelaxing activity; Antiproliferative activity.

* Corresponding author. Tel.: +886 7 3121101x2684; fax: +886 7 3125339; e-mail: yeloch@kmu.edu.tw

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doi:10.1016/j.bmc.2005.06.013

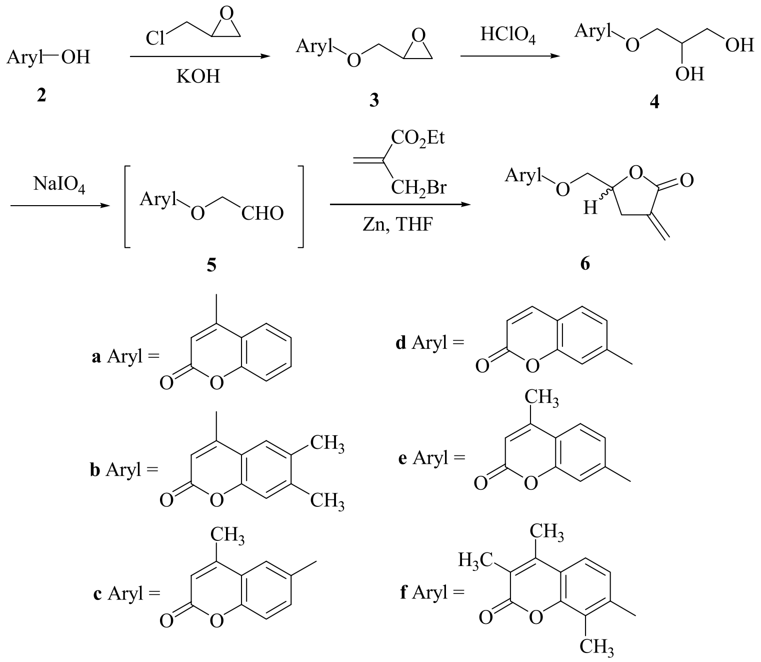
bulky the substituent at the γ-position the less the vasorelaxing activity.[10]

2. Chemistry

Preparation of γ-[(2-oxo-2*H*-1-benzopyranyloxy)methyl]-α-methylene-γ-butyrolactones **6a–f** is shown in Scheme 1. Treatment of hydroxycoumarins **2a–f** with KOH and epichlorohydrin provided 2-aryloxymethyloxiranes **3a–f** that were reacted with 6% perchloric acid to give 3-aryloxy-1,2-propanediols **4a–f** in good overall yields. Oxidative cleavage of **4a–f** with sodium periodate afforded 2-aryloxyacetaldehydes **5a–f** that were made to react immediately with ethyl 2-(bromomethyl)acrylate and zinc powder in dry THF (Reformatsky-type condensation) to give the desired products **6a–f**. The intermediate **5a** has also been purified and its structure confirmed by NMR spectra and elemental analysis.

3. Pharmacological results and discussion

The vasorelaxing effect of γ-[(2-oxo-2*H*-1-benzopyranyloxy)methyl]-α-methylene-γ butyrolactones **6a–f** and the C_2-methyl counterpart **1** is given in Table 1. 7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran2-one (**6d**), with an IC_{50} value of 9.4 μM against pig coronary arterial contraction induced by KCl, is a more active vasorelaxant than its γ-methyl congener **1** (14.0 μM), indicating that the substitution at γ-position is unfavorable. Coumarin-7-yl α-methylene-γ-butyrolactone **6d** is also a more potent vasorelaxing agent than its coumarin-4-yl counterpart **6a** (35.5 μM), implying that the positional isomers of coumarin α-methylene-γ-butyrolactone exhibited different vasorelaxing activities. A methyl group substituted at C-4 of the coumarin-7-yl moiety reduced vasorelaxing effect (**6d** vs **6e**). Polysubstitution on the coumarin-7-yl moiety of **6d** further decreased vasorelaxing activity in which 3,4,8-trimethyl derivative **6f** was inactive. The



Scheme 1. Synthetic pathway of coumarin α-methylene-γ-butyrolactones **6a–f**.

Table 1. The inhibitory effects of coumarin α-methylene-γ-butyrolactones on pig coronary arterial contraction induced by KCl[a]

	3 μM	10μM	30 μM	100 μM	IC_{50} (μM)
Control	101.5 ± 5.2	103.8 ± 8.9	94.1 ± 7.6	70.0 ± 7.1	Nd
1	89.4 ± 11.0	43.0 ± 14.7[b]	8.8 ± 8.5[b]	0.0 ± 0.0[b]	14.0 ± 3.7
6a	99.8 ± 0.5[d]	63.8 ± 11.4[c]	32.6 ± 11.9[b]	2.8 ± 3.2[b]	35.5 ± 7.4
6b	Nd[e]	Nd	Nd	60.6 ± 24.0	Nd
6c	Nd	Nd	Nd	45.0 ± 8.66[b]	Nd
6d	84.3 ± 5.1[d]	25.7 ± 9.8[c]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	9.4 ± 3.0
6e	100.0 ± 0.0	76.7 ± 5.8[c]	46.0 ± 3.5[b]	12.0 ± 4.0[b]	39.0 ± 3.6
6f	Nd	Nd	Nd	50.5 ± 1.0	Nd

[a] Data are expressed as means ± SEM. The IC_{50} values represent the concentration at which 50% reduction in KCl-induced tone was observed.

[b] Significantly different from control value at $P < 0.001$.

[c] Significantly different from control value at $P < 0.01$.

[d] Significantly different from control value at $P < 0.05$.

[e] Not determined.

same SAR is observed in the case of coumarin-4-yl counterpart in which **6a** exhibited an IC_{50} of 35.5 μM while **6b** was inactive.

These α-methylene-γ-butyrolactones **6a–f** were also evaluated in vitro against a panel of cell lines consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS) as described previously.[11] Compounds which reduced the growth of any one of the cell lines to 50% or less at the concentration of 4 μg/ml were subjected to further evaluation for their dose–response effects and IC_{50} measurement. The cells were treated with at least five different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the tetrazolium dye reduction assay (MTS assay), and the experiment was performed as recommended by the manufacturer (Promega, Madison, WI). The absorbance was measured at 490 nm on a Wallac 1420 VICTOR2 Multilabel counter (Perkin-Elmer, Boston, MA). The results of these assays were used to obtain the dose–response curves from which IC_{50} (μM) values were determined. An IC_{50} value represents the concentration of the tested compound at which a 50% cell growth inhibition after 3 days of incubation was produced. Results from Table 2 indicated that coumarin-4-yl α-methylene-γ-butyrolactone **6a** is more cytotoxic than its coumarin-7-yl counterpart **6d** and 6,7-dimethyl derivative **6b**. For the coumarin-7-yl derivatives, **6d** is more active than its γ-methyl congener **1**, indicating that the substitution at γ-position decreased cytotoxicity. A methyl group substituted at C-4 of the coumarin-7-yl moiety reduced cytotoxicity (**6d** > **6e**). Polysubstitution on the coumarin-7-yl moiety of **6d** further decreased antiproliferative activity in which **6f** was inactive. Among these coumarin α-methylene-γ-butyrolactones, **6a** exhibited the most potent antiproliferative activities on the growth of MCF7, NCI-H460, and SF-268 with IC_{50} values of 6.97, 14.68, and 8.36 μM, respectively.

4. Conclusion

Coumarin α-methylene-γ-butyrolactones were synthesized and evaluated for antiproliferative and vasorelaxing activities. The results of this study showed that coumarin-7-yl α-methylene-γ-butyrolactone **6d** is the most potent vasorelaxing agent with an IC_{50} value of 9.4 μM against pig coronary arterial contraction induced by KCl while its coumarin-4-yl counterpart **6a** exhibited the most potent antiproliferative activities on the growth of MCF7, NCI-H460, and SF-268 with IC_{50} values of 6.97, 14.68, and 8.36 μM, respectively.

5. Experimental

5.1. General

TLC: precoated (0.2 mm) silica gel 60 F_{254} plates from EM Laboratories Inc.; detection by UV light (254 nm). All chromatographic separations were performed using silica gel (Merck 60 230–400 mesh). mp: Electrothermal IA9100 digital melting-point apparatus; uncorrected. ^1H NMR spectra: Varian-Unity-400 spectrometer at 400 MHz or Varian-Gemini-200 spectrometer at 200 MHz, chemical shifts δ in ppm with $SiMe_4$ as an internal standard (= 0 ppm), coupling constants J in Hz. Elemental analyses were carried out on a Heraeus CHN–O-Rapid elemental analyzer, and results were within ±0.4% of calculated values.

5.1.1. 6,7-Dimethyl-4-[(oxiran-2-yl)methoxy]-2*H*-1-benzopyran-2-one (3b). To a stirred solution of 4-hydroxy-6,7-dimethylcoumarin (**2b**, 3.80 g, 20 mmol) in EtOH (100 ml) was added an aqueous solution of KOH (1.25 g in 5 ml H_2O). The solution was stirred at rt for 30 min, and then epichlorohydrin (20 ml) was added. The mixture was heated at reflux for 2.5 h (TLC monitoring). Evaporation of the solvent gave a residue, which was partitioned between H_2O (80 ml) and CH_2Cl_2 (100 ml). The organic phase was washed with H_2O (80 ml), dried (Na_2SO_4), and evaporated. Crystallization of the residue from EtOH gave **3b** (4.53 g, 92%) as white solid, mp 122–123 °C. ^1H NMR ($CDCl_3$) δ : 2.31, 2.34 (each 3H, two s, 6- and 7-Me), 2.82 (1H, dd, J = 4.6, 2.6 Hz, 3′-H), 3.00 (1H, dd, J = 4.6, 4.2 Hz, 3′-H), 3.47 (1H, m, 2′-H), 4.00 (1H, dd, J = 11.2, 6.4 Hz, OCH_2), 4.44 (1H, dd, J = 11.2, 2.6 Hz, OCH_2), 5.61 (1H, s, 3-H), 7.09 (1H, s, 8-H), 7.57 (1H, s, 5-H). ^{13}C NMR ($CDCl_3$): δ 18.82, 19.83 (6- and 7-Me), 44.00 (3′-C), 48.72 (2′-C), 69.36 (OCH_2), 89.56 (3-C), 112.41 (4a-C), 116.83 (8-C), 122.51 (5-C), 132.38 (6-C), 142.26 (7-C),

Table 2. Cytotoxicity of coumarin α-methylene-γ-butyrolactones

	MCF7 (breast cancer)		NCI-H460 (lung cancer)		SF-268 (CNS cancer)	
	GI%[a]	IC_{50}[b] (μM)	GI%	IC_{50} (μM)	GI%	IC_{50} (μM)
1	49	25.52 ± 5.61	48	37.06 ± 9.90	48	38.47 ± 1.95
6a	4	6.97 ± 1.37	16	14.68 ± 3.42	10	8.36 ± 0.94
6b	32	11.08 ± 3.01	41	16.72 ± 4.76	22	21.89 ± 4.02
6c	31	12.05 ± 3.28	47	21.34 ± 3.98	27	24.59 ± 5.24
6d	38	19.57 ± 5.31	47	17.02 ± 3.93	41	20.72 ± 5.03
6e	47	22.24 ± 3.74	52	20.38 ± 4.16	55	19.76 ± 5.55
6f	70	Nd[c]	81	Nd	87	Nd

[a] Percentage of growth (GI%) of preliminary testing at 4 μg/ml concentration.

[b] Concentration necessary for 50% inhibition (IC_{50}). The values represent averages ± SD of three or more independent experiments, each with duplicate samples.

[c] Not determined.

151.30 (8a-C), 162.74 (4-C), 165.02 (2-C). Anal. Calcd for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.10; H, 5.68.

5.1.2. 4-Methyl-6-[(oxiran-2-yl)methoxy]-2*H*-1-benzopyran-2-one (3c). From 6-hydroxy-4-methylcoumarin (**2c**) as described for **3b**: yield 87%, mp 125–127 °C. ^{1}H NMR ($CDCl_3$): δ 2.41 (3H, d, *J* = 1.2 Hz, 4-Me), 2.79 (1H, dd, *J* = 4.8, 2.8 Hz, 3′-H), 2.94 (1H, dd, *J* = 4.8, 4.0 Hz, 3′-H), 3.38 (1H, m, 2′-H), 3.97 (1H, dd, *J* = 11.2, 6.0 Hz, OCH_2), 4.34 (1H, dd, *J* = 11.2, 3.2 Hz, OCH_2), 6.29 (1H, q, *J* = 1.2 Hz, 3-H), 7.09 (1H, d, *J* = 2.8 Hz, 5-H), 7.13 (1H, dd, *J* = 8.8, 2.8 Hz, 7-H), 7.26 (1H, d, *J* = 8.8 Hz, 8-H). ^{13}C NMR ($CDCl_3$): δ 18.63 (4-Me), 44.49 (3′-C), 50.09 (2′-C), 69.66 (OCH_2), 109.10 (5-C), 115.52 (8-C), 117.96 (3-C), 119.26 (7-C), 120.47 (4a-C), 148.17 (8a-C), 151.90 (4-C), 154.86 (6-C), 160.82 (2-C). Anal. Calcd for $C_{13}H_{12}O_4$: C, 67.24; H, 5.21. Found: C, 67.09; H, 5.23.

5.1.3. 3,4,8-Trimethyl-7-[(oxiran-2-yl)methoxy]-2*H*-1-benzopyran-2-one (3f). From 7-hydroxy-3,4,8-trimethylcoumarin (**2f**) as described for **3b**: yield 84%, mp 138–140 °C. ^{1}H NMR ($CDCl_3$): 2.20, 2.35, 2.38 (9H, three s, 3-, 4-, and 8-Me), 2.76 (1H, dd, *J* = 5.2, 2.8 Hz, 3′-H), 2.93 (1H, dd, *J* = 5.2, 4.4 Hz, 3′-H), 3.36 (1H, m, 2′-H), 3.94 (1H, dd, *J* = 11.2, 6.0 Hz, OCH_2), 4.32 (1H, dd, *J* = 11.2, 2.8 Hz, OCH_2), 6.78 (1H, d, *J* = 8.8 Hz, 6-H), 7.42 (1H, d, *J* = 8.8 Hz, 5-H). ^{13}C NMR ($CDCl_3$): 8.21, 13.08, 14.99 (3-, 4-, and 8-Me), 43.50 (3′-C), 49.12 (2′-C), 70.78 (OCH_2), 107.88 (8-C), 114.00 (4a-C), 114.22 (6-C), 119.51 (3-C), 121.86 (5-C), 146.30 (4-C), 151.10 (8a-C), 158.53 (7-C), 162.61 (2-C). Anal. Calcd for $C_{15}H_{16}O_4$: C, 69.22; H, 6.20. Found: C, 59.12; H, 6.38.

5.1.4. 4-(2,3-Dihydroxypropoxy)-6,7-dimethyl-2*H*-1-benzopyran-2-one (4b). A solution of **3b** (2.46 g, 10 mmol) in 6% perchloric acid (50 ml) was stirred at rt for 16 h. Then, the pH was adjusted to 8 with $NaHCO_3$, and the solution was extracted continuously with CH_2Cl_2 for 24 h. Removal of the solvent gave **4b** (1.43 g, 58%) as a viscous liquid which solidified upon standing. mp 130–131 °C. ^{1}H NMR (DMSO-d_6): 2.80 (6H, two s, 6.7-Me), 3.49 (2H, m, 3′-H), 3.89 (1H, m, 2′-H), 4.07 (1H, dd, *J* = 10.8, 6.0 Hz, 1′-C), 4.20 (1H, dd, *J* = 10.8, 3.6 1′-H), 4.78 (1H, t, *J* = 6.0 Hz, 3′-OH), 5.19 (1H, d, *J* = 5.2 Hz, 2′-OH), 5.77 (1H, s, 3-H), 7.20 (1H, s, 8-H), 7.62 (1H, s, 5-H). ^{13}C NMR (DMSO-d_6): 18.78, 19.68 (6-, 7-Me), 62.22 (3′-C), 69.34 (2′-C), 71.02 (1′-C), 89.50 (3-C), 112.78 (4a-C), 116.73 (8-C), 122.80 (5-C), 132.52 (6-C), 142.41 (7-C), 151.15 (8a-C), 161.96 (4-C), 165.52 (2-C). Anal. Calcd for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10. Found: C, 63.37; H, 6.04.

The same procedure was used to convert each of the compounds **3c**, **3d**,[12] **3e**,[13] and **3f**–**4c**, **4d**, **4e**, and **4f**, respectively.

5.1.5. 6-(2,3-Dihydroxypropoxy)-4-methyl-2*H*-1-benzopyran-2-one (4c). Yield 52%, mp 98–99 °C. ^{1}H NMR (DMSO-d_6): 2.41 (3H, d, *J* = 1.2 Hz, 4-Me), 3.46 (2H, m, 3′-H), 3.80 (1H, m, 2′-H), 3.94 (1H, dd, *J* = 10.0, 6.4 Hz, 1′-H), 4.07 (1H, dd, *J* = 10.0, 4.0 Hz, 1′-H), 4.69 (1H, t, *J* = 6.0 Hz, 3′-OH), 4.97 (1H, d, *J* = 5.2 Hz, 2′-OH), 6.36 (1H, q, *J* = 1.2 Hz, 3-H), 7.21 (2H, m, 5-, 7-H), 7.30 (1H, d, *J* = 8.8 Hz, 8-H). ^{13}C NMR (DMSO-d_6): 18.13 (4-Me), 62.63 (3′-C), 69.96 (2′-C), 70.38 (1′-C), 108.85 (5-C), 114.62 (8-C), 117.41 (3-C), 119.55 (7-C), 120.06 (4a-C), 147.14 (8a-C), 152.97 (4-C), 155.12 (6-C), 159.86 (2-C). Anal. Calcd for $C_{13}H_{14}O_5$: C, 62.40; H, 5.64. Found: C, 62.47; H, 5.68.

5.1.6. 7-(2,3-Dihydroxypropoxy)-2*H*-1-benzopyran-2-one (4d). Yield 61%, mp 123–124 °C [lit.[14] 122–124 °C]. ^{1}H NMR (DMSO-d_6): 3.45 (2H, m, 3′-H), 3.81 (1H, m, 2′-H), 3.98 (1H, dd, *J* = 10.0, 6.0 Hz, 1′-H), 4.11 (1H, dd, *J* = 10.0, 4.0 Hz, 1′-H), 4.71 (1H, t, *J* = 6.0 Hz, 3′-OH), 5.02 (1H, d, *J* = 5.2 Hz, 2′-OH), 6.28 (1H, d, *J* = 9.2 Hz, 3-H), 6.96 (2H, m, 6-, 8-H), 7.62 (1H, d, *J* = 8.4 Hz, 5-H), 7.98 (1H, d, *J* = 9.2 Hz, 4-H). ^{13}C NMR (DMSO-d_6): 62.48 (3′-C), 69.74 (2′-C), 70.32 (1′-C), 101.19 (8-C), 112.25 (4a-C), 112.38, 112.70 (3-, 6-C), 129.42 (5-C), 144.28 (4-C), 155.33 (8a-C), 160.26, 161.98 (2-, 7-C). Anal. Calcd for $C_{12}H_{12}O_5$: C, 61.02; H, 5.12. Found: C, 60.90; H, 5.15.

5.1.7. 7-(2,3-Dihydroxypropoxy)-4-methyl-2*H*-1-benzopyran-2-one (4e). Yield 53%, mp 99–100 °C [lit.[15] 108–110 °C]. ^{1}H NMR (DMSO-d_6): 2.38 (3H, d, *J* = 1.2 Hz, 4-Me), 3.46 (2H, m, 3′-H), 3.81 (1H, m, 2′-H), 3.94 (1H, dd, *J* = 10.4, 6.0 Hz, 1′-H), 4.11 (1H, dd, *J* = 10.0, 4.0 Hz, 1′-H), 4.72 (1H, t, *J* = 5.6 Hz, 3′-OH), 5.02 (1H, d, *J* = 5.2 Hz, 2′-OH), 6.18 (1H, q, *J* = 1.2 Hz, 3-H), 6.97 (2H, m, 6-, 8-H), 7.66 (1H, d, *J* = 8.8 Hz, 5-H). ^{13}C NMR (DMSO-d_6): 18.07 (4-Me), 62.51 (3′-C), 69.77 (2′-C), 70.31 (1′-C), 101.21 (8-C), 111.05, 112.38 (3-, 6-C), 113.02 (4a-C), 126.37 (5-C), 153.35 (4-C), 154.68 (8a-C), 160.13, 161.89 (2-, 7-C). Anal. Calcd for $C_{13}H_{14}O_5$: C, 62.40; H, 5.64. Found: C, 62.02; H, 5.65.

5.1.8. 7-(2,3-Dihydroxypropoxy)-3,4,8-trimethyl-2*H*-1-benzopyran-2-one (4f). Yield 51%, mp 136–137 °C. ^{1}H NMR (DMSO-d_6): 2.05, 2.18, 2.31 (9H, three s, 3-, 4-, and 8-Me), 3.50 (2H, m, 3′-H), 3.85 (1H, m, 2′-H), 3.98 (1H, dd, *J* = 9.8, 5.6 Hz, 1′-H), 4.09 (1H, dd, *J* = 9.8, 4.4 Hz, 1′-H), 4.71 (1H, t, *J* = 5.6 Hz, 3′-OH), 5.01 (1H, d, *J* = 5.0 Hz, 2′-OH), 6.98 (1H, d, *J* = 8.8 Hz, 6-H), 7.53 (1H, d, *J* = 8.8 Hz, 5-H). ^{13}C NMR (DMSO-d_6): 7.97, 12.85, 14.85 (3-, 4- and 8-Me), 62.62 (3′-C), 69.96 (2′-C), 70.23 (1′-C), 108.06 (8-C), 112.15 (4a-C), 113.63 (6-C), 117.49 (3-C), 123.00 (5-C), 146.87 (4-C), 150.37 (8a-C), 158.34 (7-C), 161.27 (2-C). Anal. Calcd for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52. Found: C, 64.59; H, 6.52.

5.1.9. 2-(2-Oxo-2*H*-1-benzopyran-4-yloxy)acetaldehyde (5a). To a stirred solution of **4a** (1.89 g, 8 mmol) in dioxane (25 ml) was added an aqueous solution of $NaIO_4$ (3.42 g, 16 mmol in 60 ml H_2O). The solution was stirred at rt for 24 h, and then H_2O (100 ml) was added. The mixture was extracted with CH_2Cl_2 (75 ml × 3), the organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (silica gel, EtOAc:hexane, 1:4) yielding **5a** (0.98 g, 60%) as a white powder. mp 158 °C (dec). ^{1}H NMR ($CDCl_3$): 4.81 (2H, s, 1′-H), 5.61 (1H, s, 3-H), 7.33 (2H, m, 6-, 8-H), 7.60 (1H, m, 7-H), 7.93 (1H, dd, *J* = 7.6, 1.2 Hz, 5-H), 9.89 (1H, s, CHO). ^{13}C NMR ($CDCl_3$): 72.54 (1′-C), 91.53 (3-C), 115.04 (4a-C), 116.92 (8-C), 123.07 (5-C), 124.24 (6-C), 132.97 (7-C), 153.40 (8a-C), 162.23 (4-C), 164.50 (2-C), 194.67 (CHO). Anal. Calcd for $C_{11}H_8O_4$: C, 64.71; H, 3.95. Found: C, 64.62; H, 4.06.

5.1.10. 4-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran2-one (6a). *Method A.* To a solution of **5a** (0.82 g, 4 mmol) in dry THF (60 ml) were added activated Zn powder (0.41 g, 4.8 mmol), hydroquinone (8 mg), and ethyl 2-(bromomethyl)acrylate (0.98 g, 5 mmol). The mixture was refluxed under N_2 for 20 h (TLC monitoring). After cooling, it was poured into ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml × 3). The CH_2Cl_2 extract was washed with brine, dried, and evaporated to give a viscous liquid that was purified by column chromatography (silica gel, EtOAc:hexane, 1:1) and crystallization from EtOH was performed to give **6a** (0.72 g, 66%) as a white solid. mp 128–130 °C. UV λ_{max} nm (log ε): 305 (3.72), 276 (3.93), 266 (3.96), 230 (3.68) (in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 3.01 (1H, ddt, *J* = 17.2, 5.2, 2.6 Hz, 3′-H), 3.26 (1H, ddt, *J* = 17.2, 8.8, 3.0 Hz, 3′-H), 4.23 (1H, dd, *J* = 10.4, 4.4 Hz, OCH_2), 4.39 (1H, dd, *J* = 10.4, 2.8 Hz, OCH_2), 5.04 (1H, m, 2′-H), 5.80 (1H, t, *J* = 2.6 Hz, CH_2=C(4′)), 6.40 (1H, t, *J* = 3.0 Hz, CH_2=C(4′)), 5.71 (1H, s, 3-H), 7.30 (2H, m, 6-, 8-H), 7.57 (1H, m, 7-H-C), 7.87 (1H, dd, *J* = 7.9, 1.3 Hz, 5-H). ^{13}C NMR ($CDCl_3$): 29.52 (3′-C), 69.99 (OCH_2), 73.40 (2′-C), 91.04 (3-C), 115.09 (4a-C), 116.82 (8-C), 122.85 (vinylic-C), 123.04 (5-C), 124.12 (6-C), 132.74 (7-C), 133.15 (4′-C), 153.28 (8a-C), 160.34 (4-C), 164.94 (2-C), 169.30 (5′-C). Anal. Calcd for $C_{15}H_{12}O_5$ 0.25 H_2O: C, 65.09; H, 4.55. Found: C, 65.04; H, 4.78.

5.1.11. 4-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran2-one (6a). *Method B.* To a stirred solution of **4a**[7a] (1.18 g, 5 mmol) in dioxane (25 ml) was added an aqueous solution of $NaIO_4$ (3.42 g, 20 mmol in 60 ml H_2O). The solution was stirred at rt for 24 h, and then H_2O (100 ml) was added. The mixture was extracted with CH_2Cl_2 (75 ml × 3), the organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was dissolved in dry THF (100 ml), which was added to activated Zn powder (0.43 g, 5 mmol), hydroquinone (10 mg), and ethyl 2-(bromomethyl)acrylate (1.18 g, 6 mmol). The mixture was refluxed under N_2 for 30 h (TLC monitoring). After cooling, it was poured into ice-cold 5% HCl solution (300 ml) and extracted with CH_2Cl_2 (75 ml × 3). The CH_2Cl_2 extract was washed with brine, dried, and evaporated to give a viscous liquid that was purified by column chromatography (silica gel, EtOAc:hexane, 1:1) and crystallization from EtOH was performed to give **6a** (0.79 g, 58% yield) as a white solid. mp 128–130 °C. UV λ_{max} nm (log ε): 305 (3.72), 276 (3.93), 266 (3.96), 230 (3.68) (in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 3.01 (1H, ddt, *J* = 17.2, 5.2, 2.6 Hz, 3′-H), 3.26 (1H, ddt, *J* = 17.2, 8.8, 3.0 Hz, 3′-H), 4.23 (1H, dd, *J* = 10.4, 4.4 Hz, OCH_2), 4.39 (1H, dd, *J* = 10.4, 2.8 Hz, OCH_2), 5.04 (1H, m, 2′-H), 5.80 (1H, *t*, *J* = 2.6 Hz, CH_2=C(4′)), 6.40 (1H, t, *J* = 3.0 Hz, CH_2=C(4′)), 5.71 (1H, s, 3-H). 7.30 (2H, m, 6-, 8-H), 7.57 (1H, m, 7-H-C), 7.87 (1H, dd, *J* = 7.9, 1.3 Hz, 5-H), ^{13}C NMR ($CDCl_3$): 29.52 (3′-C), 69.99 (OCH_2), 73.40(2′-C), 91.04 (3-C), 115.09 (4a-C), 116.82 (8-C), 122.85 (vinylic-C), 123.04 (5-C), 124.12 (6-C), 132.74 (7-C), 133.15 (4′-C), 153.28 (8a-C), 160.34 (4-C), 164.94 (2-C), 169.30 (5′-C). Anal. Calcd for $C_{15}H_{12}O_5$ 0.25 H_2O: C, 65.09; H, 4.55. Found: C, 65.04; H, 4.78.

The same procedure was used to convert each of the compounds **4b–f** to **6b–f**, respectively.

5.1.12. 6,7-Dimethyl-4-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran-2-one (6b). Yield 58%, mp 154–155 °C. UV λ_{max} nm (log ε): 313 (3.88), 281 (4.02), 270 (4.04), 232 (3.87) (in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 2.27, 2.33 (6H, two s, 6-,7-Me), 3.09 (1H, ddt, *J* = 17.2, 4.8, 2.4 Hz, 3′-H), 3.25 (1H, ddt, *J* = 17.2, 8.8, and 2.8 Hz, 3′-H), 4.19 (1H, dd, *J* = 10.4, 4.4 Hz, OCH_2), 4.35 (1H, dd, *J* = 10.4, 2.8 Hz, OCH_2), 5.03 (1H, m, 2′-H), 5.60 (1H, s, 3-H), 5.80 (1H, t, *J* = 2.4 Hz, CH_2=C(4′)), 6.40 (1H, t, *J* = 2.8 Hz, CH_2=C(4′)), 7.09 (1H, s, 8-H), 7.38 (1H, s, 5-H). ^{13}C NMR ($CDCl_3$): 19.31, 20.26 (6-, 7-Me), 29.64 (3′-C), 69.94 (OCH_2), 73.51 (2′-C), 90.08 (3-C), 112.59 (4a-C), 117.29 (8-C), 122.76 (vinylic-C), 122.82 (5-C), 132.92 (6-C), 133.38 (4′-C) 142.88 (7-C), 151.73 (8a-C), 162.89 (4-C), 165.20 (2-C), 169.35 (5′-C). Anal. Calcd for $C_{17}H_{16}O_5$ 0.25 H_2O: C, 66.99; H, 5.56. Found: C, 67.05; H, 5.50.

5.1.13. 4-Methyl-6-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1benzopyran-2-one (6c). Yield 54%, mp 157–158 °C. UV λ_{max} nm (log ε): 337 (3.69), 272 (4.06), 232 (4.07) (in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 2.41 (3H, d, *J* = 1.2 Hz, 4-Me), 3.01 (1H, ddt, *J* = 17.2, 5.2, 2.6 Hz, 3′-H), 3.16 (1H, ddt, *J* = 17.2, 8.8, 3.0 Hz, 3′-H), 4.15 (1H, dd, *J* = 10.4, 4.4 Hz, OCH_2), 4.22 (1H, dd, *J* = 10.4, 4.0 Hz, OCH_2), 4.91 (1H, m, 2′-H), 5.73 (1H, t, *J* = 2.6 Hz, CH_2=C(4′)), 6.31 (2H, m, CH_2=C(4′) and 3-H), 7.04 (1H, d, *J* = 3.0 Hz, 5-H), 7.10 (1H, dd, *J* = 8.8, 3.0 Hz, 7-H), 7.27 (1H, d, *J* = 8.8 Hz, 8-H). ^{13}C NMR ($CDCl_3$): 18.67 (4-Me), 29.64 (3′-C), 70.01 (OCH_2), 74.40 (2′-C), 109.17 (5-C), 115.74 (8-C), 118.13 (3-C), 119.11 (7-C), 120.57 (4a-C), 122.70 (vinylic-C), 133.52 (4′-C), 148.42 (8a-C), 151.71 (4-C), 154.56(6-C), 160.70 (2-C), 169.63 (5′-C). Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 66.96; H, 4.97.

5.1.14. 7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1-benzopyran2-one (6d). Yield 57%, mp 129 °C. UV λ_{max} nm (log ε): 320 (4.12), 232 (3.67) (in CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 3.00 (1H, ddt, *J* = 17.2, 5.6, 2.6 Hz, 3′-H), 3.17 (1H, ddt, *J* = 17.2, 8.8, 3.0 Hz, 3′-H), 4.14 (1H, dd, *J* = 10.4, 4.0 Hz, OCH_2), 4.24 (1H, dd, *J* = 10.4, 3.0 Hz, OCH_2), 4.93 (1H, m, 2′-H), 5.74 (1H, *t*, *J* = 2.6 Hz, CH_2=C(4′)), 6.28 (1H, d, $J = 9.2$ Hz, 3-H), 6.32 (1H, t, $J = 3.0$ Hz, CH_2=C(4′)), 6.79 (1H, d, $J = 2.8$ Hz, 8-H), 6.84 (1H, dd, $J = 8.8$, 2.8 Hz, 6-H), 7.39 (1H, d, $J = 8.8$ Hz, 5-H), 7.64 (1H, d, $J = 9.6$ Hz, 4-H). ^{13}C NMR ($CDCl_3$): 29.57 (3′-C), 69.42 (OCH_2), 74.09 (2′-C), 101.56 (8-C), 112.80 (3-C), 113.15 (4a-C), 113.65 (6-C), 122.74 (vinylic-C), 128.93 (5-C), 133.38 (4′-C), 143.20 (4-C), 155.68 (8a-C), 160.88, 161.12 (2-, 7-C), 169.57 (5′-C). Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.18; H, 4.44. Found: C, 65.86; H, 4.70.

5.1.15. 4-Methyl-7-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]-2*H*-1benzopyran-2-one (6e). Yield 56%, mp 105–107 °C. UV λ_{max} nm (log ε): 318 (4.14), 232 (3.73) (in CH_2Cl_2). ^1H NMR ($CDCl_3$): 2.40 (3H, d, $J = 1.2$ Hz, 4-Me), 2.99 (1H, ddt, $J = 17.6$, 5.6, 2.4 Hz, 3′-H), 3.17 (1H, ddt, $J = 17.6$, 8.8, 2.8 Hz, 3′-H), 4.15 (1H, dd, $J = 10.4$, 4.4 Hz, OCH_2), 4.24 (1H, dd, $J = 10.4$, 3.6 Hz, OCH_2), 4.93 (1H, m, 2′-H), 5.74 (1H, t, $J = 2.4$ Hz, CH_2=C(4′)), 6.15 (1H, d, $J = 1.2$ Hz, 3-H), 6.32 (1H, t, $J = 2.8$ Hz, CH_2=C(4′)), 6.79 (1H, d, $J = 2.4$ Hz, 8-H), 6.86 (1H, dd, $J = 8.8$, 2.4 Hz, 6-H), 7.50 (1H, d, $J = 8.8$ Hz, 5-H). ^{13}C NMR ($CDCl_3$): 18.62 (4-Me), 29.59 (3′-C), 69.41 (OCH_2), 74.14 (2′-C), 101.59 (8-C), 112.42, 112.50 (3-, 6-C), 114.24 (4a-C), 122.72 (vinylic-C), 125.72 (5-C), 133.42 (4′-C), 152.37 (4-C), 155.09 (8a-C), 160.98, 161.01 (2-, 7-C), 169.59 (5′-C). Anal. Calcd for $C_{16}H_{14}O_5$ 0.5 H_2O: C, 65.08; H 5.13. Found: C, 65.38; H, 5.04.

5.1.16. 3,4,8-Trimethyl-7-[(2,3,4,5-tetrahydro-4-methylene-5-oxo-2-furanyl)methoxy]2*H*-1-benzopyran-2-one (6f). Yield 49%, mp 152–154 °C. UV λ_{max} nm (log ε): 316 (4.18), 256 (3.67), 231 (3.77) (in CH_2Cl_2). ^1H NMR ($CDCl_3$): 2.18, 2.23, 2.36 (9H, three s, 3-, 4-, 8-Me), 3.05 (1H, ddt, $J = 17.2$, 4.8, and 2.6 Hz, 3′-H), 3.19 (1H, ddt, $J = 17.2$, 8.8, and 2.8 Hz, 3′-H), 4.17 (1H, dd, $J = 10.4$, 3.6 Hz, OCH_2), 4.27 (1H, dd, $J = 10.4$, 3.6 Hz, OCH_2), 4.95 (1H, m, 2′-H), 5.74 (1H, t, $J = 2.6$ Hz, CH_2=C(4′)), 6.33 (1H, t, $J = 2.8$ Hz, CH_2=C(4′)), 6.78 (1H, d, $J = 9.0$ Hz, 6-H), 7.40 (1H, d, $J = 9.0$ Hz, 5-H). ^{13}C NMR ($CDCl_3$): 8.12, 13.17, 15.04 (3-, 4-, 8-Me), 29.71 (3′-C), 69.61 (OCH_2), 74.32 (1′-C), 107.37 (8-C), 114.28 (6-C), 115.10 (4a-C), 119.30 (3-C), 122.11 (vinylic-C), 122.50 (5-C), 133.63 (3′-C), 146.04 (4-C), 151.14 (8a-C), 157.14 (7-C), 162.34 (2-C), 169.70 (5′-C). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.47; H, 5.81.

5.2. Pharmacology

Porcine hearts were obtained from a local abattoir within 30 min of slaughter and were transported to the laboratory in Krebs–Henseleit (KH) buffer solution with the following composition (mM): NaCl (120), KCl (5.2), KH_2PO_4 (1), $MgSO_4$ (1.3), $CaCl_2$ (2.5), $NaHCO_3$ (15.5), glucose (11.3), and pyruvate (1). pH value of the KH buffer solution was adjusted to 7.4. The right coronary and anterior descending branches of the left coronary arteries were dissected from each heart and stored overnight at 4 °C in an oxygenated KH buffer solution. On one of the two following days, these arteries were cleaned of any remaining connective tissue and cut into 3-mm rings. Coronary arterial rings were suspended in organ bath filled with 20 ml KH solution. The bath solution was gassed with a mixture of 95% O_2 and 5% CO_2, and the temperature was maintained at 37 °C throughout the experiment. Each ring was suspended by two fine stainless-steel wire clips; one clip was anchored inside the organ bath and the other was connected to a force transducer (model FT03, Grass Instrument, USA). Isometric tension was measured by Cyber 380 and Digidata 1320A (Axon Instrument, USA) and recorded in computer.

Tissues were allowed to equilibrate for a minimum of 1 h before testing was begun. An amount of 30 mM KCl was then poured into the organ chamber to contract these rings. When the contraction reached a stable plateau (usually 15 min), testing compounds (100 μM) were added in the organ bath to screen the activity of relaxation. Concentration–response curves for some more potent compounds were established by cumulative addition at doses of 3, 10, 30, and 100 μM.

Contractile responses were calculated using the difference between resting tension and maximum tension developed in response to KCl-stimulation. Data are expressed as mean ± SEM from a number ($n = 4$–6) of experiments. Statistical analysis were performed using paired Student's t test; $P \leq 0.05$ was considered significant.

Acknowledgments

Financial support of this work by the National Science Council of the Republic of China is gratefully acknowledged. We also thank the National Center for High-Performance Computing for providing computer resources and chemical database services.

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