Synthesis, antiproliferative, and vasorelaxing evaluations of coumarin \( \alpha \)-methylene-\( \gamma \)-butyrolactones

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Abstract—Certain coumarin \( \alpha \)-methylene-\( \gamma \)-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 Reformat-sky-type condensation. The results of this study are as follows (1) for the vasorelaxing activity, coumarin-7-yl \( \alpha \)-methylene-\( \gamma \)-butyrolactone 6d, with an IC\(_{50}\) value of 9.4 \( \mu M \) against pig coronary arterial contraction induced by KCl, is a more active vasorelaxant than its coumarin-4-yl counterpart 6a and its \( \gamma \)-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 \( \alpha \)-methylene-\( \gamma \)-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 \( \mu M \), 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 \( \gamma \)-methyl congener 1, indicating that substitution at the \( \gamma \)-position decreased cytotoxicity.

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1. Introduction

The \( \alpha \)-methylene-\( \gamma \)-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 \( \alpha \)-methylene-\( \gamma \)-butyrolactones is not confined to the complex polyfunctional sesquiterpene lactones only. For example, some simple natural \( \alpha \)-methylene-\( \gamma \)-butyrolactone bearing butanolides and even the parent \( \alpha \)-methylene-\( \gamma \)-butyrolactone (tulipaline A) were found to have significant pharmacological activities.5,6 Over the past few years, we were particularly interested in synthesizing \( \alpha \)-methylene-\( \gamma \)-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 \( \gamma \)-substituents of the lactone (I; 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 \( \alpha \)-methylene-\( \gamma \)-butyrolactones, derivatives of 1 in which the \( \gamma \)-methyl substituent was removed because the more

\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{figure1.png}
\caption{Structure of \( \gamma \)-methyl-\( \alpha \)-methylene-\( \gamma \)-butyrolactone.}
\end{figure}
bulky the substituent at the $\gamma$-position the less the vasorelaxing activity.\textsuperscript{10}

2. Chemistry

Preparation of $\gamma$-[2-oxo-2$H$-1-benzopyran-oxo]-methyl-$\alpha$-methylene-$\gamma$-butyrolactones 6a–f is shown in Scheme 1. Treatment of hydroxycoumarins 2a–f with KOH and epichlorohydrin provided 2-aryloxymethyl-oxiranes 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 $\gamma$-[2-oxo-2$H$-1-benzopyran-oxo]methyl-$\alpha$-methylene-$\gamma$-butyrolactones 6a–f and the $\gamma_2$-methyl counterpart 1 is given in Table 1. 7-[(2,3,4,5-Tetrahydro-4-methylene-5-oxo-2-furanyl)methoxyl]-2$H$-1-benzopyran2-one (6d), with an IC$_{50}$ value of 9.4 $\mu$M against pig coronary arterial contraction induced by KCl, is a more active vasorelaxant than its $\gamma$-methyl congener 1 (14.0 $\mu$M), indicating that the substitution at $\gamma$-position is unfavorable. Coumarin-7-yl-$\alpha$-methylene-$\gamma$-butyrolactone 6d is also a more potent vasorelaxing agent than its coumarin-4-yl counterpart 6a (35.5 $\mu$M), implying that the positional isomers of coumarin $\alpha$-methylene-$\gamma$-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 $\alpha$-methylene-$\gamma$-butyrolactones 6a–f.](image-url)

<table>
<thead>
<tr>
<th></th>
<th>3 $\mu$M</th>
<th>10$\mu$M</th>
<th>30 $\mu$M</th>
<th>100 $\mu$M</th>
<th>IC$_{50}$ ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>101.5 ± 5.2</td>
<td>103.8 ± 8.9</td>
<td>94.1 ± 7.6</td>
<td>70.0 ± 7.1</td>
<td>Nd</td>
</tr>
<tr>
<td>1</td>
<td>89.4 ± 11.0</td>
<td>43.0 ± 14.7$^b$</td>
<td>8.8 ± 8.5$^b$</td>
<td>0.0 ± 0.0$^b$</td>
<td>14.0 ± 3.7</td>
</tr>
<tr>
<td>6a</td>
<td>99.8 ± 0.5$^d$</td>
<td>63.8 ± 11.4$^c$</td>
<td>32.6 ± 11.9$^b$</td>
<td>2.8 ± 3.2$^b$</td>
<td>35.5 ± 7.4</td>
</tr>
<tr>
<td>6b</td>
<td>Nd$^e$</td>
<td>Nd</td>
<td>Nd</td>
<td>45.0 ± 8.66$^b$</td>
<td>Nd</td>
</tr>
<tr>
<td>6c</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>6d</td>
<td>84.3 ± 5.1$^d$</td>
<td>25.7 ± 9.8$^c$</td>
<td>0.0 ± 0.0$^b$</td>
<td>0.0 ± 0.0$^b$</td>
<td>9.4 ± 3.0</td>
</tr>
<tr>
<td>6e</td>
<td>100.0 ± 0.0</td>
<td>76.7 ± 5.8$^e$</td>
<td>46.0 ± 3.5$^b$</td>
<td>12.0 ± 4.0$^b$</td>
<td>39.0 ± 3.6</td>
</tr>
<tr>
<td>6f</td>
<td>Nd</td>
<td>Nd</td>
<td>Nd</td>
<td>50.5 ± 1.0</td>
<td>Nd</td>
</tr>
</tbody>
</table>

$^a$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 6\(a\) exhibited an IC\(_{50}\) of 35.5 \(\mu M\) while 6\(b\) was inactive.

These \(\alpha\)-methylene-\(\gamma\)-butyrolactones 6\(a\)-\(f\) 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. Compounds which reduced the growth of any one of the cell lines to 50\% or less at the concentration of 4 \(\mu M\) 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\(_{50}\) (\(\mu M\)) values were determined. An IC\(_{50}\) value represents the concentration of the tested compound at which a 50\% growth of any one of the cell lines to 50\% or less was induced by KCl while its coumarin-4-yl counterpart 6\(a\) 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 \(\mu M\), 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. \(^1\)H NMR spectra: Varian-Unity-400 spectrometer at 400 MHz or Varian-Gemini-200 spectrometer at 200 MHz, chemical shifts \(\delta\) 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 \(\pm 0.4\%\) of calculated values.

### 5.1.1. 6,7-Dimethyl-4-[\{(oxiran-2-yl)methoxy\}-2H-1-benzopyran-2-one (3b).

For the coumarin-7-yl derivation, 6\(d\) is more active than its coumarin-4-yl congener 1, indicating that the substitution at \(\gamma\)-position decreased cytotoxicity. A methyl group substituted at C-4 of the coumarin-7-yl moiety reduced cytotoxicity (6\(d\) \(>\) 6\(e\)). Polysubstitution on the coumarin-7-yl moiety of 6\(d\) further decreased antiproliferative activity in which 6\(f\) was inactive. Among these coumarins \(\alpha\)-methylene-\(\gamma\)-butyrolactones, 6\(a\) 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 \(\mu M\), respectively.

### 4. Conclusion

Coumarin \(\alpha\)-methylene-\(\gamma\)-butyrolactones were synthesized and evaluated for antiproliferative and vasorelaxing activities. The results of this study showed that coumarin-7-yl \(\alpha\)-methylene-\(\gamma\)-butyrolactone 6\(d\) is the most potent vasorelaxing agent with an IC\(_{50}\) value of 9.4 \(\mu M\) against pig coronary arterial contraction induced by KCl while its coumarin-4-yl counterpart 6\(a\) exhibited the most potent antiproliferative activities.

### Table 2. Cytotoxicity of coumarin \(\alpha\)-methylene-\(\gamma\)-butyrolactones

<table>
<thead>
<tr>
<th></th>
<th>MCF7 (breast cancer)</th>
<th>NCI-H460 (lung cancer)</th>
<th>SF-268 (CNS cancer)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI%(^a)</td>
<td>IC(_{50})(^b) ((\mu M))</td>
<td>GI%</td>
</tr>
<tr>
<td>1</td>
<td>49</td>
<td>25.52 ± 5.61</td>
<td>48</td>
</tr>
<tr>
<td>6(a)</td>
<td>14</td>
<td>6.97 ± 1.37</td>
<td>16</td>
</tr>
<tr>
<td>6(b)</td>
<td>32</td>
<td>11.08 ± 3.01</td>
<td>41</td>
</tr>
<tr>
<td>6(c)</td>
<td>31</td>
<td>12.05 ± 3.28</td>
<td>47</td>
</tr>
<tr>
<td>6(d)</td>
<td>38</td>
<td>19.57 ± 5.31</td>
<td>47</td>
</tr>
<tr>
<td>6(e)</td>
<td>47</td>
<td>22.24 ± 3.74</td>
<td>52</td>
</tr>
<tr>
<td>6(f)</td>
<td>70</td>
<td>Nd</td>
<td>81</td>
</tr>
</tbody>
</table>

\(^a\) Percentage of growth (GI\%) of preliminary testing at 4 \(\mu M\) 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.
5.1.5. 6-(2,3-Dihydroxypropoxy)-4-methyl-2-benzopyran-2-one (3c). From 6-hydroxy-4-methylcoumarin (2c) as described for 3b: yield 87%, mp 125–127 °C. ^1H NMR (CDCl3): 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, OCH2), 4.34 (1H, dd, J = 11.2, 3.2 Hz, OCH3), 6.29 (1H, q, J = 1.2 Hz, 4-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). ^13C NMR (CDCl3): δ 18.63 (4-Me), 44.49 (3'-C), 50.09 (2'-C), 69.66 (OCH2), 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 C13H12O4: C, 70.24; H, 5.21. Found: C, 67.09; H, 5.23.

5.1.6. 7-(2,3-Dihydroxypropoxy)-2H-1-benzopyran-2-one (4d). Yield 61%, mp 123–124 °C [lit.12, 122–124 °C]. ^1H NMR (DMSO-d6): δ 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). ^13C NMR (DMSO-d6): δ 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 C13H12O2: C, 61.02; H, 5.12. Found: C, 60.90; H, 5.15.

5.1.7. 7-(2,3-Dihydroxypropoxy)-4-methyl-2H-1-benzopyran-2-one (4e). Yield 53%, mp 99–100 °C [lit.13, 108–110 °C]. ^1H NMR (DMSO-d6): δ 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). ^13C NMR (DMSO-d6): δ 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 C13H16O3: C, 62.40; H, 5.64. Found: C, 62.02; H, 5.65.

5.1.8. 7-(2,3-Dihydroxypropoxy)-3,4,8-trimethyl-2H-1-benzopyran-2-one (4f). Yield 51%, mp 136–137 °C. ^1H NMR (DMSO-d6): δ 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). ^13C NMR (DMSO-d6): δ 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 C13H16O3: C, 64.74; H, 5.62. Found: C, 64.59; H, 5.62.

5.1.9. 2-(2-Oxo-2H-1-benzopyran-4- yl)acetaldehyde (5a). To a stirred solution of 4a (1.89 g, 8 mmol) in dioxane (25 ml) was added an aqueous solution of NaIO4 (3.42 g, 16 mmol in 60 ml H2O). The solution was stirred at rt for 24 h, and then H2O (100 ml) was added. The mixture was extracted with CH2Cl2 (75 ml x 3), the organic phase was washed with brine, dried (Na2SO4), and evaporated. The residue was purified by column chromatography.
chromatography (silica gel, EtOAc:hexane, 1:4) yielding 5a (0.98 g, 60%) as a white powder. mp 158 °C (dec). 1H NMR (CDCl3): 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). 13C NMR (CDCl3): 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), 194.20 (CHO). Anal. Caled for C11H12O4: 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]-2H-1-benzopyran-2-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), a solution of 5a poured into ice-cold 5% HCl solution (300 ml) and 5a 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 λmax nm (log ε): 305 (3.72), 276 (3.93), 266 (3.96), 230 (3.68) (in CH2Cl2). 1H NMR (CDCl3): 2.27, 2.33 (6H, two s, 6-, 7-Me), 2.62 (1H, ddt, J = 39.2, 2.8 Hz, OCH2), 4.93 (1H, ddt, J = 10.4, 4.4 Hz, OCH2), 4.39 (1H, dd, J = 10.4, 8.8, and 2.8 Hz, 3'-H), 4.19 (1H, dd, J = 10.4, 4.4 Hz, OCH2), 4.35 (1H, dd, J = 10.4, 2.8 Hz, OCH2), 5.03 (1H, m, 2-H), 5.60 (1H, s, 3-H), 5.80 (1H, t, J = 2.4 Hz, CH2=C(4’)), 6.40 (1H, t, J = 3.0 Hz, CH2=C(4’)), 6.71 (1H, s, 3-H), 7.30 (2H, m, 6-, 8-H), 7.57 (1H, m, 7-H), 8.77 (1H, dd, J = 7.9, 1.3 Hz, 5-H). 13C NMR (CDCl3): 29.52 (3-C), 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), 194.20 (CHO). Anal. Caled for C11H12O4: C, 64.71%; H, 3.95. Found: C, 64.62; H, 4.06.

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]-2H-1-benzopyran-2-one (6b). Yield 58%, mp 154–155 °C. UV λmax nm (log ε): 313 (3.88), 281 (4.02), 270 (4.04), 232 (3.87) (in CH2Cl2). 1H NMR (CDCl3): 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, OCH2), 4.35 (1H, dd, J = 10.4, 2.8 Hz, OCH2), 5.03 (1H, m, 2-H), 5.60 (1H, s, 3-H), 5.80 (1H, t, J = 2.4 Hz, CH2=C(4’)), 6.40 (1H, t, J = 2.8 Hz, CH2=C(4’)), 7.09 (1H, s, 8-H), 7.38 (1H, s, 5-H). 13C NMR (CDCl3): 29.52 (3-C), 69.99 (OCH2), 73.40 (2’-C), 91.04 (3-C), 115.09 (4a-C), 116.82 (8-C), 122.85 (vinyl-c-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. Caled for C11H10O2.5H2O: 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]-2H-1-benzopyran-2-one (6c). Yield 54%, mp 157–158 °C. UV λmax nm (log ε): 337 (3.69), 272 (4.06), 232 (4.07) (in CH2Cl2). 1H NMR (CDCl3): 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, OCH2), 4.22 (1H, dd, J = 10.4, 4.0 Hz, OCH2), 4.91 (1H, m, 2'-H), 5.73 (1H, t, J = 2.6 Hz, CH2=C(4’)), 6.31 (2H, m, CH2=CH(3’-C) and 3-H), 7.04 (1H, d, J = 3.0 Hz, 5-H), 7.10 (1H, d, J = 8.8, 3.0 Hz, 7-H), 7.27 (1H, d, J = 8.8 Hz, 8-H). 13C NMR (CDCl3): 18.67 (4-Me), 29.64 (3’-C), 70.01 (OCH2), 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 (vinyl-c-C), 133.52 (4’-C), 148.42 (8a-C), 151.71 (4-C), 154.56 (6-C), 160.70 (2-C), 169.30 (5’-C). Anal. Caled for C11H14O2C: 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]-2H-1-benzopyran-2-one (6d). Yield 57%, mp 129 °C. UV λmax nm (log ε): 320 (4.12), 232 (3.67) (in CH2Cl2). 1H NMR (CDCl3): 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, OCH2), 4.24 (1H, dd, J = 10.4, 3.0 Hz, OCH2), 4.93 (1H, m, 2’-H), 5.74 (1H, t, J = 2.6 Hz, CH2=C(4’)),
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 λmax nm (log ε): 318 (4.14), 232 (3.73) (in CH2Cl2). 1H NMR (CDCl3): 2.40 (3H, d, J = 1.2 Hz, 3-H), 2.99 (1H, ddt, J = 17.2, 6.5, 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, OCH2), 4.24 (1H, dd, J = 10.4, 3.6 Hz, OCH2), 4.93 (1H, m, 2'-H), 5.74 (1H, t, J = 2.4 Hz, CH2=C(4')), 6.15 (1H, d, J = 1.2 Hz, 3-H), 6.32 (1H, t, J = 2.8 Hz, CH2=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). 13C NMR (CDCl3): 18.62 (4-Me), 29.59 (3C, 4a-C), 169.59 (5-C), 17.40 (8a-C), 128.93 (5-C), 133.38 (4-C), 146.04 (4a-C), 122.72 (vinylic-C), 125.72 (5-C), 133.42 (3-C), 114.24 (4a-C), 122.72 (vinylic-C), 122.72 (4-C), 107.37 (8-C), 114.28 (6-C), 115.10 (4a-C), 133.63 (3-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 (3-C), 150.59 (8a-C), 169.59 (5'-C). Anal. Caled for C19H18O5: C, 65.86; H, 4.70. 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-1benzopyran-2-one (6f).

Yield 49%, mp 152–154 °C. UV λmax nm (log ε): 316 (4.18), 256 (3.67), 231 (3.77) (in CH2Cl2). 1H NMR (CDCl3): 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, OCH2), 4.27 (1H, dd, J = 10.4, 3.6 Hz, OCH2), 4.95 (1H, m, 2'-H), 5.74 (1H, t, J = 2.6 Hz, CH2=C(4')), 6.33 (1H, t, J = 2.8 Hz, CH2=C(4')), 6.78 (1H, d, J = 9.0 Hz, 6-H), 7.40 (1H, d, J = 9.0 Hz, 5-H). 13C NMR (CDCl3): 8.12, 13.17, 15.04 (3-, 4-, 8- Me), 29.71 (3-C), 69.61 (OCH2), 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. Caled for C19H18O5: 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), KH2PO4 (1), MgSO4 (1.3), CaCl2 (2.5), NaHCO3 (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% O2 and 5% CO2, 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 ≤ 0.05 was considered significant.

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References and notes


