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## Design, synthesis, and vasorelaxant and platelet antiaggregatory activities of coumarin–resveratrol hybrids

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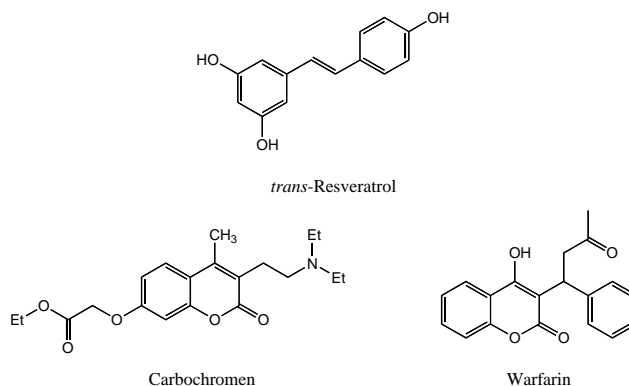
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**Abstract**—We have synthesized the coumarin–resveratrol hybrid **4** and its dimethoxy derivative **3** by a very direct synthetic route involving a Pechmann procedure. Compound **4** has also been synthesized by an alternative route (Perkin), which also allowed the synthesis of compounds **9–13**. In addition, we have evaluated the potential vasorelaxant activity of the new compounds in endothelium-containing rat aorta rings pre-contracted with noradrenaline, as well as the inhibitory effects on platelet aggregation induced by thrombin in washed human platelets. The compounds reported here relaxed vascular smooth muscle and inhibited platelet aggregation with a profile similar to that of *trans*-resveratrol (*t*-RESV) and, in some cases, showed activity higher than that of the natural compound. This is the case for compound **13**, which has a vasorelaxant activity that is twice as high as that of *t*-resveratrol and a platelet antiaggregant activity that is six times higher. These results suggest that these novel compounds may have potential as structural templates for the design and subsequent development of new vasodilatory and platelet antiaggregatory drugs.  
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A number of large-scale epidemiological studies have suggested that prolonged and moderate consumption of red wine is associated with a very low incidence of cardiovascular diseases, notably coronary heart disease.<sup>1</sup> The cardioprotective effects of red wine appear to be independent of alcohol content, and over the last few years numerous studies have been carried out with the aim of identifying the components responsible. Although these compounds have yet to be conclusively identified, the principal candidates are a number of polyphenolic compounds including *trans*-resveratrol (*t*-RESV; *trans*-3,4',5-trihydroxystilbene; Fig. 1). *t*-RESV is a natural phenolic component of *Vitis vinifera* L. (Vitaceae), is mainly abundant in the skin of the grapes, and is present in higher concentrations in red than in white wines.<sup>2</sup> This natural compound has shown a number of biological activities including antiinflammatory and anticancer properties.<sup>3–6</sup> Several studies within the last



**Figure 1.** Chemical structures of *trans*-resveratrol, carbochromen, and warfarin.

few years have demonstrated that *t*-RESV may protect against coronary heart disease as a result of different effects, including significant antioxidant activity, modulation of lipoprotein metabolism, and vasodilatory and platelet antiaggregatory properties.<sup>7–10</sup> *t*-RESV (the *E* isomer) readily undergoes photochemical isomerization

**Keywords:** *trans*-Resveratrol; Coumarin; Vasorelaxant; Platelet antiaggregatory activity.

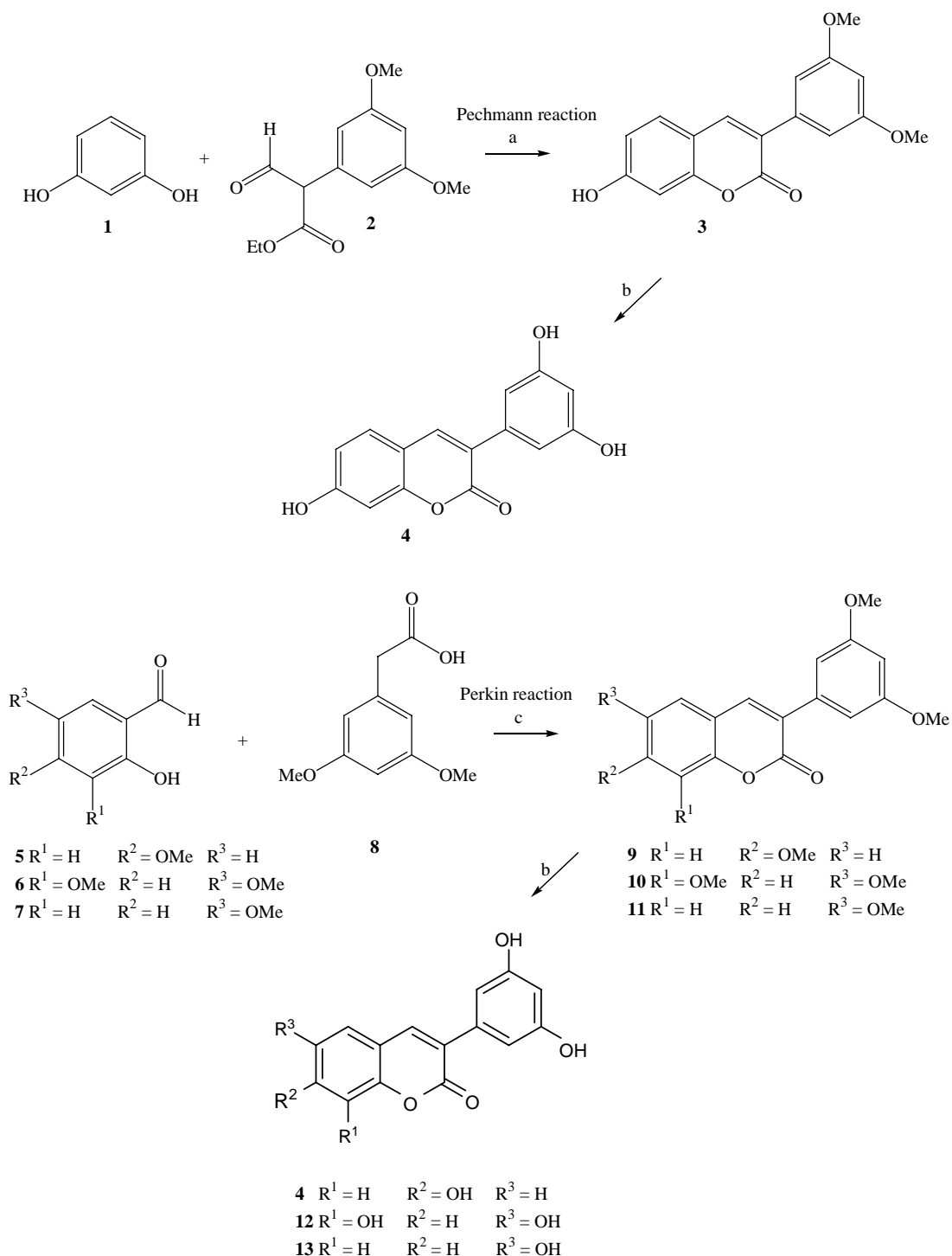
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of the central bond, which leads to partial transformation to the *cis*-isomer (*Z*-isomer)—the pharmacological properties of which have been less widely studied.

On the other hand, coumarins are a large group of compounds that have been reported to possess a wide range of biological activities, including cardiovascular properties.<sup>11,12</sup> For instance, carbochromen (3-diethylaminoethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin) is a potent specific coronary vasodilator that has been used

for many years in the treatment of angina pectoris. Furthermore, warfarin [3-(2-acetyl-1-phenylethyl)-4-hydroxycoumarin] is a coumarin with potent anticoagulant activity and a good pharmacokinetic profile (Fig. 1).

Bearing in mind the cardioprotective effects of *t*-RESV and certain coumarins, as well as our previous experience working with both kinds of substances, we designed the synthesis of a hybrid molecule (compound **4**) in which the 3,4-double bond of the coumarin nucleus



**Scheme 1.** Reagents and conditions: (a) 12 M  $H_2SO_4$ , rt, 12 h; (b) 57% HI/AcOH/Ac<sub>2</sub>O, 0 °C → rt, 3 h; (c) DCC, DMSO, 110 °C, 12 h.

fixes the *trans* disposition of the *t*-RESV-type double bond. In this way, the proposed 3-arylcoumarin **4** has the three hydroxyl groups in the same positions as those in the *t*-RESV fragment. A series of derivatives was also synthesized in which hydroxy groups were incorporated in positions contiguous to the hydroxy group on the benzene ring of compound **4**. The aim of this exercise was to establish a relationship between the structure and activity for this type of compound.

3-Phenylcoumarin **3**<sup>13</sup> was prepared in 42% yield from  $\beta$ -oxoester **2**<sup>14</sup> and resorcinol (**1**) by a Pechmann condensation<sup>15,16</sup> in 12 M H<sub>2</sub>SO<sub>4</sub>. The 3-phenylcoumarins **9**,<sup>17</sup> **10**,<sup>18</sup> and **11**<sup>19</sup> were prepared from **5**, **6**<sup>20</sup> and **7**, respectively, and 3,5-dimethoxyphenylacetic (**8**) and dicyclohexylcarbodiimide (DCC) by a Perkin reaction<sup>21,22</sup> in dimethylsulfoxide (DMSO). These reactions gave 31%, 47%, and 20% yield, respectively. Hydrolysis of the methoxy groups<sup>23</sup> in **3** gave trihydroxy derivative **4**<sup>24</sup> by treatment with HI in acetic acid/acetic anhydride to give the desired product in 65% yield. A similar procedure was used in the hydrolysis of compounds **9**, **10**, and **11** to give compounds **4**,<sup>24</sup> **12**,<sup>25</sup> and **13**<sup>26</sup> in 67%, 79%, and 61% yield, respectively (Scheme 1).

The effects of compounds **3–4** and **9–13** were studied on pre-contracted rat aortic rings with endothelium.<sup>8,27</sup> The cumulative addition of *t*-RESV and the new compounds (1–100  $\mu$ M) caused a concentration-dependent relaxation of the contractions induced by noradrenaline (NA, 1  $\mu$ M) in intact rat aortic rings. The corresponding IC<sub>50</sub> values are shown in Table 1.<sup>28</sup>

Compounds **3–4** and **9–12** were less efficient than *t*-RESV in relaxing the contractions induced by NA. However, compound **13** was found to be more potent than *t*-RESV in the vasorelaxation assays carried out when IC<sub>50</sub> = 10.55  $\mu$ M.

Platelet aggregation studies were also performed.<sup>29</sup> In addition, the new compounds (in a similar way to *t*-RESV) exhibited antiplatelet activity when thrombin (0.25 U/mL) was used as the stimulating agent, albeit at higher concentrations. Compounds **3**, **4**, and **13** inhibited thrombin-induced platelet aggregation more effectively than *t*-RESV (Table 2).<sup>28</sup>

Compound **13** showed vasorelaxant and platelet antiaggregation activity higher than that of *t*-RESV and compound **4** is structurally the most similar to *t*-RESV in

**Table 1.** Vasorelaxant activity (IC<sub>50</sub> in  $\mu$ M) of tested compounds

Compound	Noradrenaline (NA, 1 $\mu$ M)
<i>t</i> -RESV	19.95 $\pm$ 1.64
<b>3</b>	39.80 $\pm$ 2.94*
<b>4</b>	38.92 $\pm$ 2.61*
<b>9</b>	52.48 $\pm$ 3.86*
<b>10</b>	>100*
<b>11</b>	42.65 $\pm$ 2.98*
<b>12</b>	95.5 $\pm$ 7.06*
<b>13</b>	10.55 $\pm$ 0.73*

\*  $P < 0.01$  versus the corresponding IC<sub>50</sub> values of *t*-RESV.

**Table 2.** Antiplatelet activity (IC<sub>50</sub> in  $\mu$ M) for tested compounds

Compound	Thrombin (0.25 U/mL)
<i>t</i> -RESV	195.50 $\pm$ 13.82
<b>3</b>	105.00 $\pm$ 9.98*
<b>4</b>	62.30 $\pm$ 4.80*
<b>9</b> <sup>a</sup>	>100*
<b>10</b> <sup>a</sup>	>100*
<b>11</b> <sup>a</sup>	>25*
<b>12</b>	285.00 $\pm$ 29.2*
<b>13</b>	30.10 $\pm$ 1.1*

<sup>a</sup> Not determined by precipitation of the product.

\*  $P < 0.01$  versus the corresponding IC<sub>50</sub> values of *t*-RESV.

terms of the positions of the hydroxy groups. These results indicate that variation of the positions of the hydroxy groups in this type of molecule can give derivatives with a significantly higher pharmacological potency than *t*-RESV. This is the case for compound **13**, which has a vasorelaxant activity that is twice as high as that of *trans*-resveratrol and a platelet antiaggregant activity that is six times higher. The studied compounds with hydroxyl groups, like resveratrol, show a slightly greater activity than that of similar compounds with methoxy groups.

In conclusion, the new molecules synthesized have been characterized as agents with remarkable vasorelaxant effects in intact rat aorta and also have significant human platelet antiaggregatory activity. The new compounds show a pharmacological profile similar to that of *t*-RESV and, therefore, have a promising future as vasodilators and platelet antiaggregatory drugs.

Further experiments are in progress aimed at providing new data to clarify the precise mechanism by which coumarin–resveratrol hybrids produce their characteristic vasorelaxant and platelet antiaggregatory effects.

## Acknowledgments

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18. 6,8-Dimethoxy-3-(3',5'-dimethoxyphenyl)coumarin (**10**). Purified by chromatography using CH<sub>2</sub>Cl<sub>2</sub> as eluent. Mp: 160–161 °C. IR (KBr): 2944, 2845, 1720, 1601, 1389, 1112, 1061, 846 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 3.82 (s, 6H, CH<sub>3</sub>O-C3' + CH<sub>3</sub>O-C5'), 3.84 (s, 3H, CH<sub>3</sub>O-C6), 3.93 (CH<sub>3</sub>O-C8), 6.49 (m, 2H, H-5 + H-4'), 6.67 (d, *J* = 2.6 Hz, 1H, H-7), 6.84 (d, *J* = 2.4 Hz, 2H, H-2' + H-6'), 7.72 (s, 1H, H-4). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ (ppm): 55.9 (2CH<sub>3</sub>O), 56.2, 56.7, 100.5, 101.5, 103.5, 107.2 (2C), 120.4, 129.2, 137.1, 138.7, 140.6, 148.3, 156.8, 160.3, 161.1 (2C). MS *m/z* (%): 343 ([M+1]<sup>+</sup>, 4), 342 (M<sup>+</sup>, 22), 341 (100), 213 (7), 313 (18), 270 (26). Anal. Calcd for C<sub>19</sub>H<sub>18</sub>O<sub>6</sub>: C, 66.66; H, 5.30. Experimental: C, 66.58; H, 5.58.
19. 6-Methoxy-3-(3',5'-dimethoxyphenyl)coumarin (**11**). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent. Mp: 154–155 °C. IR (KBr): 2948, 2856, 1722, 1608, 1384, 1107, 1065, 846 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ (ppm): 3.82 (s, 6H, CH<sub>3</sub>O-C3' + CH<sub>3</sub>O-C5'), 3.85 (s, 3H, CH<sub>3</sub>O-C6), 6.50 (t, *J* = 2.0 Hz, 1H, H-4'), 6.84 (d, *J* = 2.0 Hz, 2H, H-2' + H-6'), 6.95 (d, *J* = 2.8 Hz, 1H, H-5), 7.10 (dd, *J* = 9.0; 2.8 Hz, 1H, H-7), 7.28 (d, *J* = 9.0 Hz, 1H, H-8), 7.75 (s, 1H, H-4). <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ (ppm): 55.4, 55.7 (2CH<sub>3</sub>O), 101.0, 106.7 (2C), 109.8, 117.3, 119.2, 119.8, 128.4, 136.5, 139.8, 147.9, 156.0, 160.4, 160.6 (2C). MS *m/z* (%): 313 ([M+1]<sup>+</sup>, 20), 312 (M<sup>+</sup>, 100), 284 (16), 241 (13), 213 (8), 198 (7). Anal. Calcd. for C<sub>18</sub>H<sub>16</sub>O<sub>5</sub>: C, 69.22; H, 5.16. Experimental: C, 69.28; H, 5.28.
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25. 6,8-Dihydroxy-3-(3',5'-dihydroxyphenyl)coumarin (**12**). Purified by chromatography using 7:3 hexane/ethyl acetate as eluent and then recrystallization from CH<sub>3</sub>CN. Mp: 299 °C (dec). IR (KBr): 3534, 2912, 1709, 1623, 1310, 1130, 778 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 6.25 (t, *J* = 2.0 Hz, 1H, H-4'), 6.52 (d, *J* = 2.6 Hz, 1H, H-5), 6.55 (d, *J* = 2.2 Hz, 2H, H-2' + H-6'), 6.58 (d, *J* = 2.6 Hz, 1H, H-7), 7.96 (s, 1H, H-4), 9.35 (br s, 3H, 3OH), 10.11 (br s, 1H, OH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 102.8, 103.0, 106.5, 106.8 (2C), 120.4, 127.1, 135.4, 136.5, 140.6, 144.9, 153.8, 158.1 (2C), 159.6. MS *m/z* (%): 287 ([M+1]<sup>+</sup>, 17), 286 (M<sup>+</sup>, 100), 258 (75), 217 (32), 189 (6), 131 (4). Anal. Calcd. for C<sub>15</sub>H<sub>10</sub>O<sub>6</sub>: C, 62.94; H, 3.52. Experimental: C, 62.88; H, 3.60.
26. 6-Hydroxy-3-(3',5'-dihydroxyphenyl)coumarin (**13**). Purified by chromatography using 7:3 hexane/ethyl acetate as eluent and then recrystallization from CH<sub>3</sub>CN. Mp: 326 °C (dec). IR (KBr): 3533, 2922, 1705, 1629, 1300, 1129, 786 cm<sup>-1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 6.25 (t, *J* = 1.8 Hz, 1H, H-4'), 6.55 (d, *J* = 2.1 Hz, 2H, H-2' + H-6'), 7.00 (dd, *J* = 8.9; 2.8 Hz, 1H, H-7), 7.08 (d, *J* = 2.7 Hz, 1H, H-5), 7.23 (d, *J* = 8.9 Hz, 1H, H-8), 8.04 (s, 1H, H-4), 9.38 (br s, 2H, 2OH), 9.75 (br s, 1H, OH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ (ppm): 102.8, 106.8 (2C), 112.6, 116.6, 119.6, 120.0, 127.1, 136.4, 140.2, 146.3, 153.8, 158.1 (2C), 159.7. MS *m/z* (%): 271 ([M+1]<sup>+</sup>, 16), 270 (M<sup>+</sup>, 100), 242 (93), 213 (18), 185 (8), 139 (7). Anal. Calcd. for C<sub>15</sub>H<sub>10</sub>O<sub>5</sub>: C, 66.67; H, 3.73. Experimental: C, 66.72; H, 3.86.
27. Vascular rings were prepared from aortae of male Wistar rats weighing 230–270 g.<sup>8</sup> After an equilibration period of at least 1 h, isometric contractions induced by NA (1 μM) were obtained. When contraction of the tissue in response to the corresponding vasoconstrictor agent had stabilized (after about 20 min), cumulatively increasing concentrations of the tested compounds were added to the bath at 15–20 min intervals (the time needed to obtain steady-state relaxation). Control tissues were subjected to the same procedures simultaneously, but in this case omitting the compounds and adding the vehicle [appropriate dilutions of dimethylsulfoxide (DMSO)].
28. Results shown in the tables are expressed as means ± SEM from five experiments. Means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's post

hoc test. The inhibitory effects of the tested compounds in rat aorta and human platelets are expressed as  $IC_{50}$  (concentrations that produce a 50% inhibition) estimated by least-squares linear regression, using the program Origin 5.0, with  $X = \log$  molar concentration of the tested compound and  $Y = \%$  of pharmacological response.

29. *Preparation of washed platelets.* Washed human platelets were prepared from blood anticoagulated with citrate–phosphate–dextrose, which was obtained from Centro de Transfusión de Galicia (Santiago de Compostela, Spain). Bags containing buffy coat from individual donors were diluted with the same volume of washing buffer (NaCl, 120 mM; KCl, 5 mM; trisodium citrate, 12 mM; glucose, 10 mM; sucrose, 12.5 mM; pH 6) and centrifuged at 400g for 9 min. The upper layer containing platelets (platelet-rich plasma) was removed and centrifuged at 1000g for 18 min. The resulting platelet pellet was recovered, resuspended with washing buffer, and centrifuged again at

1000g for 15 min. Finally, the platelet pellet from this step was resuspended in a modified Tyrode–HEPES buffer (HEPES, 10 mM; NaCl, 140 mM; KCl, 3 mM;  $MgCl_2$ , 0.5 mM;  $NaHCO_3$ , 5 mM; glucose, 10 mM; pH 7.4) to afford a cell density of  $3\text{--}3.5 \times 10^8$  platelet/mL. The calcium concentration in the extracellular medium was 2 mM. *Platelet aggregation studies.* Platelet aggregation was measured using a dual channel aggregometer (Chrono-log, Havertown, PA, USA). Each tested compound, dissolved in DMSO, was incubated with washed platelets at 37 °C for 5 min. Stimulus (thrombin) was then added to induce platelet aggregation and the light transmission was monitored over a 5 min period. Platelet aggregation is measured as the maximum change in light transmission during this period. The 100% aggregation value was obtained when vehicle (DMSO) was added instead of the compounds. The final DMSO concentration was below 1% (v/v) in all cases.