Synthesis and Vasorelaxant and Platelet Antiaggregatory Activities of a New Series of 6-Halo-3-phenylcoumarins †

Elías Quezada 1, Giovanna Delogu 2*, Carmen Picciau 2, Lourdes Santana 3, Gianni Podda 2, Fernanda Borges 1, Verónica García-Morales 4, Dolores Viña 4 and Francisco Orallo 4

1 Chemistry Department, Faculty of Sciences, University Porto, 4169-007 Porto, Portugal; E-Mails: elias.quezada@usc.es (E.Q.); mfernandamborges@gmail.com (F.B.)
2 Dipartimento Farmaco Chimico Tecnologico, Universita degli Studi di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy; E-Mails: carmenpicciau@alice.it (C.P); gpodda@unica.it (G.P.)
3 Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain; E-Mail: lourdes.santana@usc.es (L.S.)
4 Department of Pharmacology, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain; E-Mails: mdolores.vina@usc.es (D.V.); francisco.orallo@usc.es (F.O.); veronica.garcia9@rai.usc.es (V.G.M.)

† In memory of Prof. Francisco Orallo.

* Author to whom correspondence should be addressed; E-Mail: delogug@unica.it; Tel.: +39-0706758566; Fax: +39-0706758553.

Received: 4 December 2009; in revised form: 21 December 2009 / Accepted: 23 December 2009 / Published: 12 January 2010

Abstract: A series of 6-halo-3-hydroxyphenylcoumarins (resveratrol-coumarins hybrid derivatives) was synthesized in good yields by a Perkin reaction followed by hydrolysis. The new compounds were evaluated for their vasorelaxant activity in intact rat aorta rings pre-contracted with phenylephrine (PE), as well as for their inhibitory effects on platelet aggregation induced by thrombin in washed human platelets. These compounds concentration-dependently relaxed vascular smooth muscle and some of them showed a platelet antiaggregatory activity that was up to thirty times higher than that shown by trans-resveratrol and some other previously synthesized derivatives.

Keywords: resveratrol; coumarin; vasorelaxant; platelet antiaggregatory activity
1. Introduction

Coumarins (or benzopyrones) are a large family of compounds of natural and synthetic origin that show numerous biological activities, including cardiovascular properties [1]. For instance, Carbochromen (3-diethylaminoethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin (Figure 1) is a potent specific coronary vasodilator that has been used for many years in the treatment of angina pectoris [2,3]. Furthermore, warfarin [3-(2-acetyl-1-phenylethyl)-4-hydroxycoumarin (Figure 1) is a coumarin with potent anticoagulant activity and a good pharmacokinetic profile [4].

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

trans-Resveratrol (t-RESV; 3,4',5-trihydroxy-trans-stilbene; Figure 1) is a natural phenolic component of Vitis vinifera L. (Vitaceae). It is abundant in the skin of the grapes and it is present in higher concentrations in red than in white wines. trans-Resveratrol has shown a number of biological activities, including protection against coronary heart disease, as a result of different effects: significant antioxidant activity, modulation of lipoprotein metabolism, and vasodilatatory and platelet antiaggregatory properties [5–8].

Because of their similar characteristics, it was of interest to design and synthesize hybrids that incorporate the nucleus of the coumarins and resveratrol molecules. In previous work, our research group had reported the vasorelaxant and platelet antiaggregatory activities of a series of coumarin-resveratrol hybrids (3-arylcoumarins), bearing hydroxy or methoxy groups on the coumarin and/or on the 3-phenyl ring. 6-Hydroxy-3-(3',5'-dihydroxyphenyl)coumarin showed vasorelaxant and platelet antiaggregatory activity higher than that of trans-resveratrol [9].

Based on this, and with the aim of improving the vasorelaxant and platelet antiaggregatory activities of resveratrol-coumarin hybrids and establishing a relationship between the structure and activity for this type of compounds we have studied the effects of substitution on the 3-arylcoumarin moiety with groups showing different steric and electronics effects. In this paper we report the synthesis and evaluation of a new series of 3-arylcoumarins in which the hydroxy group in the 6 position has been changed for a halogen group, and different positions of hydroxyl group in 3-phenyl ring were explored.
2. Results and Discussion

The 3-phenylcoumarins 6-11 [10] were prepared from the conveniently substituted phenylacetic acids 1-3, the appropriate salicylaldehyde 4, 5 and dicyclohexylcarbodiimide (DCC) by a Perkin reaction [11–13] in dimethylsulfoxide (DMSO). These reactions gave 46%, 40%, 27%, 29%, 31% and 33% yield, respectively. Hydrolysis of the methoxy groups [14] by treatment with HI in acetic acid/acetic anhydride gave the hydroxy derivatives 12, 13, 14, 15, 16 and 17 [15–16], in 28%, 70%, 60%, 73%, 94% and 57% yield, respectively (Scheme 1).

Scheme 1. General synthetic route to obtain compounds 6-17.

Reagents and conditions: (a) DCC, DMSO, 110 °C, 12h; (b) HI/AcOH/Ac2O, 0 °C to rt, 3 h.

The vasorelaxant effects of compounds 12-17 were studied on pre-contracted rat aortic rings with endothelium [6]. The cumulative addition of t-RESV or the new compounds (1-100 μM) caused a concentration-dependent relaxation of the contractions induced by phenylephrine (PE, 1 μM) in intact rat aortic rings. The corresponding IC50 values are shown in Table 1.
Table 1. Vasorelaxant activity (IC50 in μM) of tested compounds.

| Compound | PE (1 μM) |
|----------|-----------|
| 12       | 36.63 ± 2.46* |
| 13       | 57.63 ± 3.87* |
| 14       | **         |
| 15       | 48.79 ± 3.27* |
| 16       | 46.67 ± 3.13* |
| 17       | **         |
| t-RESV   | 3.12 ± 0.26 |

* P < 0.01 versus the corresponding IC50 values of t-RESV; ** Inactive at 100 μM (highest concentration tested). At higher concentrations compounds precipitate.

All evaluated compounds resulted less efficient than t-RESV in relaxing the contractions induced by PE. Furthermore, substitution with a p-hydroxy group in the 3-phenyl ring (compounds 14 and 17) leads to inactive compounds. Platelet aggregation studies were also performed. Compounds 12, 13 and 17 inhibited platelet aggregation more effectively than t-RESV when thrombin (0.25 U/mL) was used as the stimulating agent (Table 2).

Table 2. Antiplatelet activity (IC50 in μM) for tested compounds.

| Compound | Thrombin (0.25 U/mL) |
|----------|---------------------|
| 12       | 91.36 ± 6.13*       |
| 13       | 6.41 ± 2.15*        |
| 14       | **                  |
| 15       | **                  |
| 16       | **                  |
| 17       | 20.1 ± 1.35*        |
| t-RESV   | 195.50 ± 13.82      |

* P < 0.01 versus the corresponding IC50 values of t-RESV; ** Inactive at 100 μM (highest concentration tested). At higher concentrations compounds precipitate.

These results indicate that the variation of the position of the hydroxyl group on the 3-phenyl and the substitution with different halogen groups in the 6 position of the coumarin ring, in this type of molecules (resveratrol-coumarins hybrid), can give derivatives with a significantly higher pharmacological potency than t-RESV. This is the case for compound 13, which has a platelet antiaggregatory activity that is more than 32 times higher than that of t-RESV.

3. Experimental

3.1. General

Melting points were determined in a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus, and are uncorrected. Infrared (IR) spectra were recorded in a Perkin-Elmer 1640FT spectrometer (KBr disks, ν in cm-1).13C and 1H spectra of samples approximately 10% solutions in chloroform-d, were recorder at room temperature in 5 mm o.d. tubes. TMS was used as internal
standard, chemical shifts are expressed in ppm (δ), J in Hz. NMR spectra were recorded with a Bruker AMX 500 (1H-, 500 MHz; 13C-, 125 MHz) instrument. Mass spectra were obtained using a Hewlett-Packard 5988A spectrometer (70 eV). Silica gel (35-60 mesh) was used for flash chromatography (FC). Analytical TLC was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). Elemental analyses were performed with a Perkin-Elmer 240B microanalyser. Phenylacetic acids 1-3, salicylaldehydes 4, 5, hydriodic acid (HI), acetic acid (AcOH), acetic anhydride (Ac₂O), dicyclohexylcarbodiimide (DCC) and dimethylsulfoxide (DMSO) were commercially available (Aldrich).

3.2. Chemistry

The studied compounds are all known and were prepared by a traditional Perkin reaction carried out in refluxing dimethylsulfoxide (DMSO) between conveniently substituted phenylacetic acids 1-3 and the corresponding salicylaldehyde 4, 5, using dicyclohexylcarbodiimide (DCC) as dehydrating agent (Scheme 1) [11–13]. Hydrolysis of methoxy groups [14], by treatment with HI in acetic acid/acetic anhydride gave the desired hydroxyl derivatives 12-17 [15–16].

6-Chloro-3-(2’-methoxy)phenylcoumarin (6). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent; Mp: 177–179 ºC; IR (KBr): 2920, 1705, 1610, 1302, 1125, 780 cm⁻¹; ¹H-NMR (CDCl₃) δ (ppm): 3.83 (s, 3H, CH₃O), 7.00 (d, J = 8.2 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 7.22 (d, J = 8.2 Hz, 1H), 7.41 (m, 3H), 7.60 (m, 1H), 7.66 (s, 1H); ¹³C-NMR (CDCl₃) δ (ppm): 55.7, 111.3, 116.7, 118.2, 120.6, 121.0, 126.9, 130.0, 130.4, 130.7, 131.0, 133.8, 140.2, 152.2, 156.9, 157.1; MS m/z (%): 288 ([M+2]+, 33), 286 (M+, 100), 269(16), 251 (8), 240 (5), 193 (21), 152 (16), 118 (14); Anal. Calcd for C₁₆H₁₁ClO₃: C, 67.03; H, 3.87. Experimental: C, 67.25; H, 3.99.

6-Chloro-3-(3’-methoxy)phenylcoumarin (7). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent; Mp: 144–146 ºC; IR (KBr): 2925, 1700, 1615, 1300, 1115, 775 cm⁻¹; ¹H-NMR (CDCl₃) δ (ppm): 3.85 (s, 3H, CH₃O), 6.96 (dd, J = 2.5; 8.2 Hz, 1H), 7.25 (m, 2H), 7.35 (m, 2H), 7.48 (m, 2H), 7.72 (s, 1H); ¹³C-NMR (CDCl₃) δ (ppm): 55.32, 114.2, 114.8, 117.8, 120.6, 120.8, 127.0, 129.2, 129.5, 129.7, 131.3, 135.5, 138.5, 151.8, 159.5, 159.8; MS m/z (%): 288 ([M+2]+, 29), 286 (M+, 100), 258(30), 215 (20), 201 (5), 152 (7); Anal. Calcd for C₁₆H₁₁ClO₃: C, 67.03; H, 3.87. Experimental: C, 67.20; H, 3.95.

6-Chloro-3-(4’-methoxy)phenylcoumarin (8). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent; Mp: 194–196 ºC; IR (KBr): 2918, 1710, 1614, 1300, 1117, 779 cm⁻¹; ¹H-NMR (CDCl₃) δ (ppm): 3.85 (s, 3H, CH₃O), 6.99 (d, J = 8.9 Hz, 2H), 7.29 (d, J = 8.0 Hz, 1H), 7.48 (m, 2H), 7.66 (d, J = 8.9 Hz, 2H), 7.71 (s, 1H); ¹³C-NMR (CDCl₃) δ (ppm): 55.4, 113.9 (2C), 117.7, 118.3, 120.3, 121.1, 126.8, 129.8 (2C), 130.8, 136.9, 141.1, 151.9, 156.3, 158.2; MS m/z (%): 288 ([M+2]+, 37), 286 (M+, 100), 258(13), 243 (16), 180 (3), 152 (5); Anal. Calcd for C₁₆H₁₁ClO₃: C, 67.03; H, 3.87. Experimental: C, 67.10; H, 3.99.
6-Bromo-3-(2'-methoxy)phenylcoumarin (9). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent; Mp: 183–185 ºC; IR (KBr): 2909, 1709, 1615, 1305, 1120, 783 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) (ppm): 3.82 (s, 3H, CH\(_3\)O), 6.99 (d, \(J = 7.5\) Hz, 1H), 7.03 (d, \(J = 7.4\) Hz, 1H), 7.24 (d, \(J = 8.4\) Hz, 1H), 7.36 (m, 2H), 7.60 (m, 2H), 7.66 (s, 1H); \(^1\)C-NMR (CDCl\(_3\)) \(\delta\) (ppm): 55.7, 111.3, 116.7, 118.2, 120.6, 121.0, 123.5, 127.6, 130.0, 130.5, 130.7, 133.8, 140.2, 152.5, 157.1, 159.6; MS m/z (%): 332 ([M+2]+, 100), 330 (M\(^+\), 97), 315 (23), 237 (32), 152 (15), 118 (16); Anal. Calcd for C\(_{16}\)H\(_{11}\)BrO\(_3\): C, 58.03; H, 3.35. Experimental: C, 58.25; H, 3.49.

6-Bromo-3-(3'-methoxy)phenylcoumarin (10). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent; Mp: 148-150 ºC; IR (KBr): 2898, 1700, 1620, 1300, 1110, 789 cm\(^{-1}\); \(^1\)H-NMR (CDCl\(_3\)) \(\delta\) (ppm): 3.85 (s, 3H, CH\(_3\)O), 6.96 (dd, \(J = 2.3; 8.2\) Hz, 1H), 7.20 (m, 2H), 7.36 (s, 1H), 7.60 (d, \(J = 8.3\) Hz, 1H), 7.75 (s, 1H), 7.85 (s, 1H); \(^1\)C-NMR (CDCl\(_3\)) \(\delta\) (ppm): 55.2, 114.1, 114.6, 116.9, 118.0, 120.7, 121.0, 129.0, 129.4, 130.0, 133.9, 135.3, 152.1, 159.3, 159.6; MS m/z (%): 332 ([M+2]+, 100), 330 (M\(^+\), 28), 302 (34), 195 (10), 180 (10), 152 (17); Anal. Calcd for C\(_{16}\)H\(_{11}\)BrO\(_3\): C, 58.03; H, 3.35. Experimental: C, 58.15; H, 3.50.

6-Bromo-3-(4'-methoxy)phenylcoumarin (11). Purified by chromatography using 9:1 hexane/ethyl acetate as eluent; Mp: 205–206 ºC; IR (KBr): 3500, 2920, 1709, 1613, 1300, 1120, 781 cm\(^{-1}\); \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 6.86 (m, 2H), 7.23 (m, 2H), 7.39 (d, \(J = 8.8\) Hz, 1H), 7.78 (dd, \(J = 2.2; 8.8\) Hz, 1H), 7.97 (s, 1H), 8.00 (s, 1H), 9.64 (bs, 1H, 1OH). \(^1\)C-NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 115.7, 116.0, 118.2, 118.7, 121.2, 121.8, 127.1, 129.9, 130.3, 130.7, 133.8, 140.6, 140.7, 152.1, 155.1. MS m/z (%): 274 ([M+2]+, 23), 272 (M\(^+\), 100), 244(35), 180 (19), 152 (20), 118 (10). Anal. Calcd for C\(_{15}\)H\(_9\)ClO\(_3\): C, 66.07; H, 3.33. Experimental: C, 66.25; H, 3.42.

6-Chloro-3-(2'-hydroxy)phenylcoumarin (12). Purified by chromatography using 8:2 hexane/ethyl acetate as eluent; Mp: 225–227 ºC. IR (KBr): 3500, 2920, 1705, 1613, 1300, 1120, 780 cm\(^{-1}\). \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 6.86 (m, 2H), 7.23 (m, 2H), 7.39 (d, \(J = 8.8\) Hz, 1H), 7.78 (dd, \(J = 2.3; 8.8\) Hz, 1H), 7.97 (s, 1H), 8.00 (s, 1H), 9.64 (bs, 1H, 1OH). \(^1\)C NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 115.7, 116.0, 118.2, 118.7, 121.2, 121.8, 127.1, 129.9, 130.3, 130.7, 133.8, 140.6, 140.7, 152.1, 155.1. MS m/z (%): 274 ([M+2]+, 23), 272 (M\(^+\), 100), 244(35), 180 (19), 152 (20), 118 (10). Anal. Calcd for C\(_{15}\)H\(_9\)ClO\(_3\): C, 66.07; H, 3.33. Experimental: C, 66.25; H, 3.42.

6-Chloro-3-(3'-hydroxy)phenylcoumarin (13). Purified by chromatography using 8:2 hexane/ethyl acetate as eluent; Mp: 221–223 ºC; IR (KBr): 3502, 2925, 1701, 1614, 1310, 1110, 785 cm\(^{-1}\). \(^1\)H-NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 6.82 (dd, \(J = 2.2; 8.0\) Hz, 1H), 7.10 (m, 2H), 7.25 (m, 1H), 7.44 (d, \(J = 8.8\) Hz, 1H), 7.61 (dd, \(J = 2.5; 8.8\) Hz, 1H), 7.86 (d, \(J = 2.5\) Hz, 1H), 8.14 (s, 1H), 9.60 (bs, 1H, 1OH). \(^1\)C-NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 115.5, 115.9, 117.8, 119.2, 120.9, 127.6, 128.0, 128.2, 129.3, 131.1, 135.5, 139.1, 151.5, 157.1, 159.2; MS m/z (%): 274 ([M+2]+, 21), 272 (M\(^+\), 100), 244(48), 180 (14), 152 (23), 118 (10); Anal. Calcd for C\(_{15}\)H\(_9\)ClO\(_3\): C, 66.07; H, 3.33. Experimental: C, 66.30; H, 3.45.

6-Chloro-3-(4'-hydroxy)phenylcoumarin (14). Purified by chromatography using 8:2 hexane/ethyl acetate as eluent; Mp: 241–243 ºC; IR (KBr): 3500, 2918, 1708, 1610, 1296, 1119, 783 cm\(^{-1}\). \(^1\)H-NMR
Molecules 2010, 15 276

(DMSO-$d_6$) $\delta$ (ppm): 6.84 (d, $J = 8.6$ Hz, 2H), 7.44 (d, $J = 8.8$ Hz, 1H), 7.58 (m, 3H), 7.84 (d, $J = 2.5$ Hz, 1H), 8.09 (s, 1H), 9.81 (bs, 1H, 1OH); $^{13}$C-NMR (DMSO-$d_6$) $\delta$ (ppm): 115.1 (2C), 117.7, 121.1, 124.8, 127.2, 127.8, 128.1, 129.9 (2C), 130.6, 137.1, 151.2, 158.2, 159.5; MS m/z (%): 274 ([M+2]$^+$, 20), 272 (M$^+$, 100), 244(74), 180 (15), 152 (40), 118 (5); Anal. Calcd for C$_{15}$H$_9$ClO$_3$: C, 66.07; H, 3.33. Experimental: C, 66.22; H, 3.47.

6-Bromo-3-(2'-hydroxy)phenylcoumarin (15). Purified by chromatography using 8:2 hexane/ethyl acetate as eluent; Mp: 230–232 ºC; IR (KBr): 3510, 2918, 1709, 1620, 1310, 1120, 780 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$ (ppm): 6.87 (m, 2H), 7.23 (m, 2H), 7.40 (d, $J = 8.8$ Hz, 1H), 7.74 (dd, $J = 2.3$; 8.8 Hz, 1H), 7.99 (s, 1H), 8.02 (s, 1H), 9.64 (bs, 1H, 1OH); $^{13}$C-NMR (DMSO-$d_6$) $\delta$ (ppm): 115.7, 116.0, 118.2, 121.2, 127.1, 129.9, 130.3, 130.7, 133.8, 140.6, 152.1, 155.1, 158.8; MS m/z (%): 318 ([M+2]$^+$, 47), 316 (M$^+$, 71), 300(8), 288 (40), 180 (54), 152 (100); Anal. Calcd for C$_{15}$H$_9$BrO$_3$: C, 56.81; H, 2.86. Experimental: C, 56.95; H, 3.00.

6-Bromo-3-(3'-hydroxy)phenylcoumarin (16). Purified by chromatography using 8:2 hexane/ethyl acetate as eluent; Mp: 219–221 ºC; IR (KBr): 3505, 2919, 1709, 1622, 1312, 1123, 780 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$ (ppm): 6.82 (dd, $J = 2.0$; 7.7 Hz, 1H), 7.10 (m, 2H), 7.25 (m, 1H), 7.38 (d, $J = 8.8$ Hz, 1H), 7.73 (dd, $J = 2.3$; 8.8 Hz, 1H), 8.00 (d, $J = 2.3$ Hz, 1H), 8.14 (s, 1H), 9.59 (bs, 1H, 1OH); $^{13}$C-NMR (DMSO-$d_6$) $\delta$ (ppm): 115.5, 115.9, 116.1, 118.1, 119.2, 121.4, 128.0, 129.3, 130.6, 133.9, 135.5, 139.0, 151.9, 157.1, 159.1; MS m/z (%): 318 ([M+2]$^+$, 46), 316 (M$^+$, 79), 288 (46), 180 (19), 152 (100); Anal. Calcd for C$_{15}$H$_9$BrO$_3$: C, 56.81; H, 2.86. Experimental: C, 56.98; H, 3.01.

6-Bromo-3-(4'-hydroxy)phenylcoumarin (17). Purified by chromatography using 8:2 hexane/ethyl acetate as eluent; Mp: 226–228 ºC; IR (KBr): 3520, 2939, 1710, 1622, 1302, 1133, 784 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$ (ppm): 6.84 (d, $J = 8.8$ Hz, 2H), 7.34 (d, $J = 9.1$ Hz, 1H), 7.54 (d, $J = 8.8$ Hz, 2H), 7.66 (m, 1H), 7.99 (s, 1H), 8.09 (s, 1H), 9.81 (bs, 1H, 1OH); $^{13}$C-NMR (DMSO-$d_6$) $\delta$ (ppm): 115.1 (2C), 116.0, 118.0, 121.6, 124.8, 127.7, 129.9 (2C), 130.2, 133.3, 137.0, 151.6, 158.2, 159.4; MS m/z (%): 318 ([M+2]$^+$, 31), 317 ([M+1]$^+$, 100), 316 (M$^+$, 40), 288 (39), 180 (14), 152 (46); Anal. Calcd for C$_{15}$H$_9$BrO$_3$: C, 56.81; H, 2.86. Experimental: C, 56.87; H, 3.0.

3.3. Vasorelaxant activity

Vascular rings were prepared from aortae of male or female Wistar rats weighing 230–270 g. After an equilibration period of at least 1 h, isometric contractions induced by PE (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 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 procedure simultaneously, but in this case omitting the compounds and adding the vehicle [appropriate dilutions of dimethylsulfoxide (DMSO)]. 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 platelet are expressed as IC50 (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 compounds and $Y = \%$ of pharmacological response.

3.4. Antiplatelet activity

*Preparation of washed platelets.* Washed human platelets were prepared from blood anticoagulated with citrate-phosphate-dextrose, which was obtained from Centro de Transfusion 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 400 g for 9 min. The upper layer containing platelet (platelet-rich plasma) was removed and centrifuged at 400 g for 9 min. The resulting platelet pellet was recovered, resuspended with washing buffer, and centrifuged again at 1,000 g 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; MgCl₂, 0.5 mM; NaHCO₃, 5 mM; glucose, 10 mM; pH 7.4) to afford a cell density of 2.5–3.5 × 10⁸ 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 platelet at 37 °C for 5 min. Stimulus (thrombin) was then added to induce platelet aggregation and the light transmission was monitored over 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.

4. Conclusions

In conclusion, some of the new synthesized molecules have been characterized as agents with remarkable human platelet antiaggregatory activity and significant vasorelaxant effects in intact rat aorta. Further experiments are in progress aimed at providing new data to clarify the precise mechanism by which coumarin-resveratrol hybrid derivatives produce their characteristic vasorelaxant and platelet antiaggregatory effects.

**Acknowledgements**

We thank Progetto di Ricerca Scientifica 2007-Università di Cagliari and Fondazione del Banco di Sardegna, Spanish Ministerio de Sanidad y Consumo (FISS PI061537, PI061457), Xunta de Galicia (Spain; INCITE08PXIB203022PR) and Spanish Ministerio de Ciencia e Innovación (FISS PS09/00618). D. Vina acknowledges sponsorships for a tenure-track research position at the University of Santiago de Compostela from the Isidro Parga Pondal Programme of the “Dirección Xeral de Investigación e Desenvolvemento, Xunta de Galicia”. E. Quezada thanks for a postdoctoral grant from FCT (Portugal). Verónica García-Morales thanks for a predoctoral grant (FPU, AP2008-
02609, Spanish Ministerio de Ciencia e Innovación). C. Picciau thanks for a predoctoral grant Master & Back-Regione Sardegna.

References and Notes

1. Borges, F.; Roleira, F.; Milhazes, N.; Santana, L.; Uriarte, E. Simple coumarins and analogues in medicinal chemistry: Occurrence, synthesis and biological activity. *Curr. Med. Chem.* 2005, 12, 887–916.

2. Fiedler, V.B.; Scholtholt, J. Effects of carbocromene on myocardial oxygen consumption in isolated dog hearts. *J. Pharmacol. Exp. Ther.* 1981, 217, 306–313.

3. Opherk, D.; Schuler, G.; Waas, W.; Dietz, R.; Kubler, W. Intravenous carbochromen: A potent and effective drug for estimation of coronary dilatory capacity. *Eur. Heart J.* 1990, 11, 342–347.

4. Cao, Y.G.; Liu, X.Q.; Chen, Y.C.; Hao, K.; Wang, G.J. Warfarin maintenance dose adjustment with indirect pharmacodynamic model in rats. *Eur. J. Pharm. Sci.* 2007, 30, 175–180.

5. Bradamante, S.; Barenghi, L.; Villa, A. Cardiovascular protective effects of resveratrol. *Cardiovasc. Drug Rev.* 2004, 22, 169–188.

6. Orallo, F.; Alvarez, E.; Camina, M.; Leiro, J.M.; Gomez, E.; Fernandez, P. The possible implication of trans-Resveratrol in the cardioprotective effects of long-term moderate wine consumption. *Mol. Pharmacol.* 2002, 61, 294–302.

7. Wu, J.M.; Wang, Z.R.; Hsieh, T.C.; Bruder, J.L.; Zou, J.G.; Huang, Y.Z. Mechanism of cardioprotection by resveratrol, a phenolic antioxidant present in red wine. *Int. J. Mol. Med.* 2001, 8, 3–17.

8. Hao, H.D.; He, L.R. J. Mechanisms of cardiovascular protection by resveratrol. *Med. Food* 2004, 7, 290–298.

9. Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yanez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. Design, synthesis, and vasorelaxant and platelet antiaggregatory activities of coumarin-resveratrol hybrids. *Bioorg. Med. Chem. Lett.* 2006, 16, 257–261.

10. Oda, N.; Yoshida, Y.; Nagai, S.; Ueda, T.; Sakakibara, J. Synthesis of coumarins by Nucleophilic Denitrocyclization Reaction. *Chem. Pharm. Bull.* 1987, 35, 1796–1802.

11. Hans, N.; Singhi, M.; Sharma, V.; Grover, S.K. A novel one-step synthesis of 3-phenyl-, 4-methyl-3-phenyl- and 3-phenyl-4-styrylcoumarins using DCC-DMSO. *Indian J. Chem.* 1996, 35B, 1159–1162.

12. Perkin, W.H. On the artificial production of coumarin and formation of its homologues. *J. Chem. Soc.* 1868, 21, 53–63.

13. Perkin, W.H. On the formation of coumarin and of cinnamic and of other analogous acids from the aromatic aldehydes. *1877, 31*, 388–427.

14. Begala, M.; Delogu, G.; Maccioni, E.; Podda, G.; Tocco, G.; Quezada, E.; Uriarte, E.; Fedrigo, M.A.; Favretto, D.; Traldi, P. Electrospray ionisation tandem mass spectrometry in the characterisation of isomeric benzofurocoumarin. *Rapid Commun. Mass Spectrom.* 2001, 15, 1000–1010.

15. Walter, R.; Zimmer, H.; Purcell, T.C. Synthesis and cyclization reactions of 3-(2-hydroxybenzylidene)-2(3H)-coumaranones. *J. Org. Chem.* 1966, 31, 3854–3857.
16. Mimai, M.; Suzuki, Y.; Hamma, N.; Murayama, E.; Aono, S. Coumarin derivatives. JP Pat. 49054371, 1974.

Sample Availability: Contact the authors.

© 2010 by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/3.0/).