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# Synthesis and Vasorelaxant Activity of New Coumarin and Furocoumarin Derivatives

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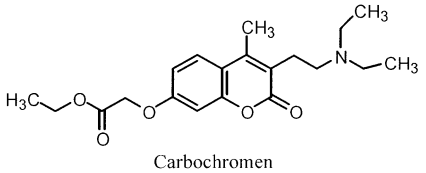
**Abstract**—We have synthesized a new series of coumarins and furocoumarins and evaluated their vasorelaxant activity in rat aorta rings pre-contracted with noradrenaline or by depolarisation with high KCl. The new furocoumarins relax smooth vascular muscle with a profile similar to that of khellin (a furochromone that directly relaxes smooth muscle) and at a greater potency, suggesting that these compounds have a potential interest for the development of new and more efficient vasodilator drugs. © 2002 Elsevier Science Ltd. All rights reserved.

Several coumarin derivatives have been shown to posses cardiovascular properties. Many of them are selective coronary vasodilators,[1–3] an effect that may be related to a $Ca^{2+}$-antagonistic activity.[4] Carbochromen (3-diethylaminoethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin; Fig. 1) is a potent specific coronary vasodilator[5,6] which has been used for many years in the treatment of angina pectoris. Although the exact mechanism of action remains still unknown, it has been reported that carbochromen coronary effects could be mediated by an increased release of prostaglandins.[7] Khellin (2-methyl-5,8-dimethoxyfurochromone; Fig. 1) is an active principle obtained from *Ammi visnaga* L. with strong vasodilator and spasmolytic activities.[8,9] It probably decreases the availability of $Ca^{2+}$ required for smooth muscle activation acting at multiple sites.[10]

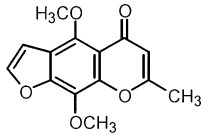
For several years, we have been working on the synthesis and evaluation of the pharmacological activity of new series of compounds, including coumarin derivatives.[11,12] We have here synthesised several coumarin analogues (**1**–**3**) (Scheme 1) structurally related to carbochromen and several furocoumarin derivatives (**4**–**6**) (Scheme 1), which can be considered isosters of khellin. In continuation of our research on the development of new vasodilator drugs with greater activity and fewer side effects,[13,14] in the work described herein we measured the vasorelaxant activity of these compounds in rat aorta rings pre-contracted with noradrenaline or by depolarisation with high KCl.

## Results and Discussion

The compounds **1**, **4** and **5** (Scheme 1) were previously synthesised from resorcinol or 2-methoxyresorcinol[15] and the synthesis of the compounds **2**, **3** and **6** was performed starting from pyrogallol, according to the




Carbochromen




Khellin

**Figure 1.** Chemical structure of carbochromen and khellin.

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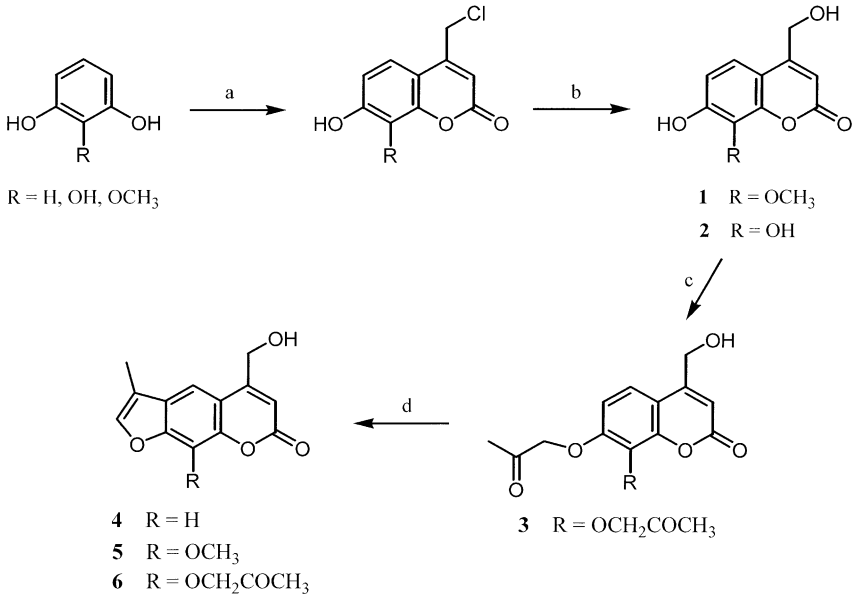


**Scheme 1.** Reagents and conditions: (a) ethyl 4-chloroacetate/$H_2SO_4$; (b) $H_2O$; (c) $ClCH_2COCH_3/K_2CO_3/(CH_3)_2CO$; (d) NaOH.

pathways shown in Scheme 1. Treatment of pyrogallol with ethyl 4-chloroacetate in concentrated $H_2SO_4$ led to the 4-chloromethyl-7,8-dihydroxycoumarin in 65% yield involving a Pechmann condensation, and further hydrolysis of this halogen derivative giving 4-hydroxymethylcoumarin **2**[16] in 88% yield. The bis(acetonyl) derivative **3**[17] was obtained in 48% yield by a reaction of the dihydroxycoumarin **2** with chloroacetone in acetone solution in the presence of the potassium carbonate. The synthesis of the furane ring was performed by treatment of the compound **3** in alkaline solution, and the final acidification led to the psoralen derivative **6**[18] in 41% yield.

In order to study their vasorelaxant activity, rat thoracic aorta rings were pre-contracted with noradrenaline (NA) or high KCl, and the effects of cumulative concentrations of the new compounds were compared to those of carbochromen and khellin.[19] NA (10 μM) or KCl (60 mM) produced a sustained contraction in the rat isolated aortic rings with functional endothelium. In control rings, the maximal tensions (mg) reached were 2033.5±229.5 and 2367.6±325.4, respectively ($n=5$), and these contractile effects were maintained without significant tension changes for at least 90 min.[20]

Khellin (0.1 μM–1 mM), the furocoumarins **4**, **5**, **6** (0.1–100 μM) and the coumarin derivative **1** (0.1–100 μM) relaxed in a concentration-dependent manner the NA- or high KCl-induced contractions (Fig. 2). Differences between $IC_{50}$ values[21] for each compound against NA and $K^+$ were not significant and the order of potency was **5** > **6** ~ **4** > khellin > **1** (Table 1). This non-specific concentration-dependent vasorelaxant activity of khellin in rat aorta is in good agreement with previous reports.[10]

On the other hand, carbochromen (0.1 μM–1 mM) and the new coumarins **2** and **3** (0.1–100 μM) did not significantly modify the contractile effect induced by NA or high KCl (Fig. 2). Other authors have reported a relaxation of KCl-depolarised large coronary arteries by carbochromen,[22] a

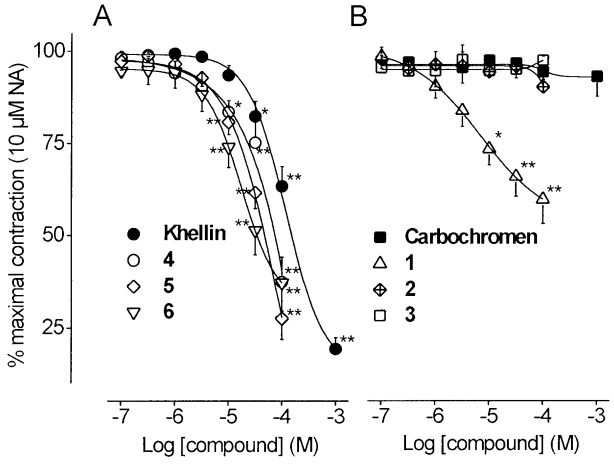


**Figure 2.** Effects of **4**, **5**, **6** (A) and **1**, **2**, **3** (B) on the contractions induced by noradrenaline (10 μM) in rat aorta rings. Similar results were obtained for 60 mM KCl. Data given as mean±SEM ($n=5$). Level of statistical significance: $^{**}P<0.01$ and $^{*}P<0.05$ with respect to the maximal tension.

**Table 1.** $IC_{50}$ values (μM) for the vasorelaxation induced by khellin, carbochromen, the coumarin (**1**–**3**) and the furocoumarin derivatives (**4**–**6**) in rat aorta rings pre-contracted with NA or extracellular high KCl concentration

| Compd | NA (10 μM) | KCl (60 mM) |
|---|---|---|
| Khellin | 309.0±9.0 | 316.1±6.0 |
| Carbochromen | >1000 | >1000 |
| **1** | 933.3±16.0* | 1010.0±20.1* |
| **2** | >1000 | >1000 |
| **3** | >1000 | >1000 |
| **4** | 68.4±1.3* | 54.0±1.9* |
| **5** | 42.0±1.1* | 42.6±1.8* |
| **6** | 52.6±0.9* | 93.3±2.0* |

Each value represents the mean±SEM from five experiments. Level of statistical significance: $^{*}P<0.05$ with respect to values for khellin.

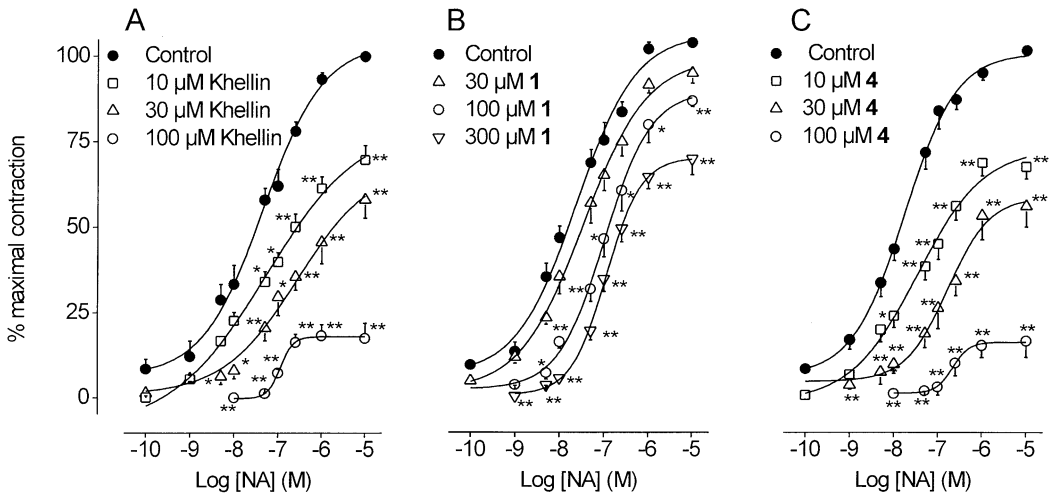


**Figure 3.** Cumulative concentration–response curves for noradrenaline in the absence or presence of (A) khellin, (B) compound **1** and (C) compound **4** in rat aorta rings. Each point represents the mean value ± SEM ($n = 5$). Level of statistical significance with respect to control curves: $^{**}P < 0.01$ and $^{*}P < 0.05$.

difference that may be explained by a high selectivity of the drug. Similarly, a selective coronary vasodilatation has been described for other coumarin derivatives such as cloricromene (8-chloro-3-[2-(diethylamino)ethyl]-4-methyl-7-ethoxycarbonylmethoxycoumarin), originally called AD6.[1]

Additionally, the effects of two of the new compounds (**1** and **4**) were compared to those induced by khellin on the cumulative concentration–response curves obtained with NA at 60 min intervals.[23] NA (0.1 nM–10 μM) elicited concentration-dependent contractions in rat aortic rings (Fig. 3). The $pD_2$ and the maximal tension (mg) values were 6.07 ± 0.07 and 2790 ± 130, respectively ($n = 15$).

Khellin (10, 30 and 100 μM), compound **1** (30, 100 and 300 μM) and compound **4** (10, 30 and 100 μM) antagonised non-competitively the NA-induced contractions, shifting the concentration–response curve for the agonist to the right with a decrease of the maximal effect (Fig. 3). The $PD'_2$ values[24] obtained were 4.46 ± 0.15, 2.57 ± 0.08 and 4.73 ± 0.13, respectively ($n = 5$).

## Conclusion

Compounds **1**, **4**, **5** and **6** have been characterised as agents with clear vasorelaxant effects on isolated rat aorta, although further studies are needed to determine the mechanisms underlying such activity. The novel furocoumarin compounds show a pharmacological profile similar to that of khellin, a greater potency and, therefore, a promising future as antihypertensive and/or spasmolytic agents.

## Acknowledgements

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16. 4-Hydroxymethyl-7,8-dihydroxycoumarin (**2**). Purified by recrystallisation in EtOH. Mp: 285 °C. IR (KBr): 3431, 3183, 1683, 1595, 1350, 1318, 1077 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$) δ: 10.02 (s, 1H, OH), 9.29 (s, 1H, OH), 7.00 (d, 1H, $J = 8.65$ Hz, H-5), 6.77 (d, 1H, $J = 8.65$ Hz, H-6),

6.23 (s, 1H, H-3), 5.55 (bs, 1H, OH), 4.67 (s, 2H, $CH_2$) ppm. Anal. calcd for $C_{10}H_8O_5$: C, 57.69; H, 3.87. Found: C, 57.04; H, 3.92.

17. 7,8-Bis(acetonyloxy)-4-hydroxymethylcoumarin (**3**). Purified by chromathography using 2:1 toluene/ethyl acetate as eluent and then recrystallisation in EtOH–hexane. Mp: 165 °C. IR (KBr): 3402, 1725, 1704, 1682, 1567, 1508, 1430, 1301, 1111 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$) δ: 7.34 (d, 1H, $J=8.99$ Hz, H-5), 6.96 (d, 1H, $J=8.99$ Hz, H-6), 6.32 (s 1H, H-3), 5.62 (bs, 1H, OH), 5.02 (s, 2H, $CH_2CO$), 4.75 (s, 2H, $CH_2CO$), 4.70 (s, 2H, $C\underline{H}_2OH$), 2.23 (s, 3H, $CH_3$), 2.15 (s, 3H, $CH_3$) ppm. Anal. calcd for $C_{16}H_{16}O_7$: C, 59.99; H, 5.03. Found: C, 60.05; H, 5.14.

18. 8-Acetonyloxy-4-hydroxymethylpsoralen (**6**). Purified by chromathography using 2:1 toluene/ethyl acetate as eluent and then recrystallisation in EtOH–hexane. Mp: 220 °C. IR (KBr): 3462, 1723, 1590, 1362, 1101 $cm^{-1}$. $^1H$ NMR (DMSO-$d_6$) δ: 7.84 (s, 1H, H-5′), 7.55 (s, 1H, H-5), 6.44 (s, 1H, H-3), 5.69 (bs, 1H, OH), 5.18 (s, 2H, $CH_2CO$), 4.83 (s, 2H, $C\underline{H}_2OH$), 2.22 (s, 3H, $CH_3$), 2.20 (s, 3H, $CH_3$) ppm. Anal. calcd for $C_{16}H_{14}O_6$: C, 63.57; H, 4.67. Found: C, 63.74; H, 4.69.

19. Rat aorta rings with endothelium were obtained, transferred to an organ bath and the contractions studies were done as described previously.[14] Khellin, carbochromen and the synthesised compounds were dissolved daily in de-ionised water from dimethyl sulfoxide (DMSO) stock solutions kept at −20 °C. The final concentration of DMSO in the bath never exceeded 0.1%.

20. Results shown in the text and figures are expressed as means ± SEM. Significant differences between two means ($P<0.05$ or $P<0.01$) were determined by Student's two-tailed test for paired or unpaired data, where appropriate.

21. In functional studies, contractile responses to vasoconstrictor agents are expressed as a percentage of the maximal contraction obtained. From the cumulative concentration–response curves for the relaxant effects of carbochromen, khellin or the new compounds, the 50% inhibitory concentrations ($IC_{50}$) were calculated using a sigmoidal curve-fitting analysis programme (Origin 5.0).

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23. Cumulative concentration–response curves and vasoconstrictor agent $PD_2$ values (negative $\log_{10}$ of the molar concentration of agonist required to elicit 50% of the maximal response) were obtained according to Van Rossum, J. M. *Arch. Int. Pharmacodyn. Ther.* **1963**, *143*, 299.

24. Antagonist $PD'_2$ values (negative $\log_{10}$ of the molar concentration of antagonist required to cause a 50% depression of the maximal response of the agonist) were calculated as described in Orallo.[13]