Understanding the Cardioprotective Effects of Flavonols: Discovery of Relaxant Flavonols without Antioxidant Activity

Cheng Xue Qin,[†,‡] Xingqiang Chen,[†] Richard A. Hughes,[†] Spencer J. Williams,[‡] and Owen L. Woodman*[,†]

The Department of Pharmacology, University of Melbourne, Parkville, Victoria, Australia, and School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, 30 Flemington Road, University of Melbourne, Parkville, Victoria, Australia

Received March 27, 2007

3′,4′-Dihydroxyflavonol (DiOHF) is a cardioprotective flavonol that can reduce injury after myocardial ischemia and reperfusion and thus is a promising small molecule for the treatment of cardiovascular disease. Like all vasoactive flavonols reported to date, DiOHF is both relaxant and antioxidant, hindering investigation of the relative contribution of each activity for the prevention of reperfusion injury. This study investigates structure–activity relationships of variations at the 3′ and 4′ positions of the B ring of DiOHF and vasorelaxant and antioxidant activities. Relaxation of rat isolated aortic rings precontracted with KCl revealed that the most active flavonols were those with a 4′-hydroxyl group, with the opening of potassium channels as a possible contributing mechanism. For the antioxidant activity, with the exception of DiOHF, none of the flavonols investigated were able to significantly scavenge superoxide radical, and none of the three most potent vasorelaxant flavonols could prevent oxidant-induced endothelial dysfunction. The discovery of single-acting vasorelaxant flavonols without antioxidant activity, in particular 4′-hydroxy-3′-methoxyflavonol, will assist in investigating the mechanism of flavonol-induced cardioprotection.

Introduction

Cardiovascular disease, principally ischemic heart disease and stroke, is the leading cause of death worldwide.[1] The current treatments for patients with acute myocardial infarction are revascularization with thrombolytic drugs or interventional procedures (e.g., balloon angioplasty or coronary artery bypass grafting). However, the restoration of blood flow results in a unique form of myocardial damage, i.e., reperfusion injury. The manifestations of reperfusion injury include arrhythmias, reversible contractile dysfunction (such as myocardial stunning), endothelial dysfunction, and lethal cell damage.[2–5] It has been suggested that an overproduction of reactive oxygen species (ROS[a]) and intracellular calcium overload or redistribution are the most important mediators of myocardial ischemia/reperfusion (I/R) injury.[2,4,6,7] Even though there are some pharmacological interventions to limit reperfusion injury thereby preventing long-term cardiac dysfunction, such as antioxidants and calcium antagonists, none of the successful experimental treatments have demonstrated clinical efficacy.[5,8] Thus, there is a need for new approaches to the prevention and/or treatment of myocardial I/R injury.

Epidemiological studies have demonstrated a much lower morbidity and mortality due to cardiovascular disease in the French population compared to other Western Europeans, North Americans, and Australians, even though they consume a comparable diet high in fats and cholesterol. This phenomenon has been called the "French paradox".[9,10] Several studies have suggested that the effect is associated with the relatively high red wine consumption in France[9–12] and that the active component of the wine may be flavonoids, a group of polyphenolic compounds derived from grape skins.[11] Starting from this premise, previous studies in our laboratory have demonstrated that a synthetic flavonol, 3′,4′-dihydroxyflavonol (DiOHF) **1** (Figure 1), significantly reduced infarct size and injury associated with myocardial I/R in anesthetized sheep, and the level of protection by DiOHF was similar to that afforded by an interventional procedure, ischemic preconditioning, which is the most effective method to prevent myocardial I/R injury to date.[13] The beneficial effect in this study indicates that **1** has potential as an adjunctive therapeutic agent for reperfusion therapy in the treatment of myocardial infarction.[13] Like many flavonols, **1** improves vascular function, acting as an antioxidant and a vasorelaxant in rat isolated aorta, properties that might explain the ability of this compound to ameliorate reperfusion injury.[14] By itself, vasorelaxation will lead to improved perfusion, providing more effective reoxygenation of previously ischemic tissue. However, while reperfusion is a crucial step in recovery from acute myocardial infarction, it leads to a dramatic increase in the formation of ROS including superoxide, hydroxyl radical, and hydrogen peroxide, which overwhelm the body's defenses against oxidative stress. ROS cause lipid peroxidation and lead to cellular damage and death, as well as form isoprostanes, prostaglandin-like compounds resulting from the reaction of ROS with multiple unsaturated fatty acids (e.g., arachidonic acid) and which possess a variety of activities detrimental to cellular and vascular function.[15] In addition, ROS can react with the endogenous vasodilator NO, depleting available NO and leading to vasoconstriction and reduced perfusion. Thus, both

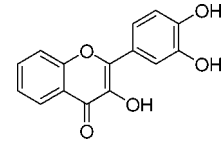


Figure 1. Structure of 3′,4′-dihydroxyflavonol **1**.

* To whom correspondence should be addressed. Phone: +61-3-9925-7305; fax, +61-3-9925-7063; e-mail, woodman@rmit.edu.au.

† The Department of Pharmacology.

‡ School of Chemistry and Bio21 Molecular Science and Biotechnology Institute.

[a] Abbreviations: DECTA, diethylthiocarbamic acid; DiOHF, 3′,4′-dihyroxyflavonol; DPPH, 1,1-diphenyl-2-picrylhydrazyl; I/R, ischemia/reperfusion; KPSS, high potassium physiological salt solution; NADPH, nicotinamide adenine dinucleotide; NBT, nitroblue tetrazolium; NMR, nuclear magnetic resonance, pEC_{50}, potency; R_{max}, maximum response; ROS, reactive oxygen species; TLC, thin layer chromatography.

10.1021/jm070352h CCC: $40.75
Published on Web 02/29/2008

the antioxidant and vasorelaxant activities of **1** might explain its cardioprotective effects. However, because these effects are simultaneously present in **1** and in all other known vasoactive flavonols, it has not been possible to determine which of these activities is required for effective cardioprotection. In this study we sought to undertake a structure–activity study with the aim to separate vasorelaxant properties from antioxidant properties, with the ultimate goal to develop single-acting antioxidant or vasorelaxant flavonols to investigate whether either or both of these activates are required to prevent reperfusion injury.

Previous studies of structure–activity relationships of vasoactive flavonols have focused on simple substituents such as hydroxyl and methoxy groups and have shown that systematic substitutions on the A ring do not correlate with antioxidant activity.[16] The 3-hydroxyl group on the C ring of flavonols is essential for the endothelium-dependent component of flavonol relaxation[16–18] but plays only a small role in flavonol-induced vasorelaxation in total.[16] On the other hand, the number and the orientation of hydroxyl groups on the B ring appear to be crucial for both the relaxant and the antioxidant activity. Flavonols with three contiguous hydroxyl groups (a pyrogallol group) enhance contraction in response to a variety of stimuli.[19] Flavonols with 2′,4′-dihydroxyl groups are weak vasodilators, and a catechol group with hydroxyls at C3′ and C4′ endows strong vasorelaxant activity.[19] When the C3′ and C4′ positions are substituted with methoxy groups, rather than hydroxyl groups, relaxation activity is abolished, underlining the importance of substituents at the 3′ and 4′ positions for vascular activity.[20] A catechol moiety is a unifying feature of the most potent flavonol scavengers of peroxyl, superoxide, and peroxynitrite radicals, as demonstrated by the enhanced inhibition of lipid peroxidation in a total oxyradical scavenging capacity assay.[20,21]

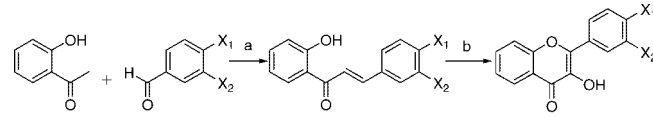
Given that much of the biological activity of vasoactive flavonols appears to reside in the substituents at the 3′ and 4′ positions, modifications at these sites offers the greatest potential to modulate the biological activity of the parent **1**. In this study, a range of flavonols were synthesized bearing a variety of substitutions at these two positions. Substituents were chosen to systematically explore the effect on vasorelaxation and antioxidant activity of electron-donating and withdrawing groups (methoxy, halogen, and trifluoromethyl groups), steric effects (hydrogen, methyl and *tert*-butyl groups), and hydrogen bond donors and acceptors (hydroxyl and methoxy groups). Eighteen flavonols [4′-substituted flavonols [H, OH, O CH_3, Cl, CF_3, CH_3, $C(CH_3)_3$], 3′-substituted flavonols (H, OH, O CH_3, Cl, CF_3, CH_3), and 4′-hydroxy-3′-substituted flavonols [H, OH, O CH_3, Br, CF_3, CH_3, $C(CH_3)_3$)] were synthesized and their vasorelaxant and antioxidant activities were evaluated in isolated rat aorta.[18,22] Surprisingly, vasorelaxant activity was found to be separable from antioxidant activity, leading to the discovery of the first single-acting vasorelaxant flavonols, compounds that should help to cast light on the mechanism of cardioprotection of flavonols.

Results and Discussion

Monosubstituted flavonols were synthesized in two steps (Scheme 1). Claisen−Schmidt condensation of various substituted benzaldehydes and 2-hydroxyacetophenone in the presence of aqueous sodium hydroxide gave the corresponding chalcones in yields of 30–60%.[23] Chalcones were converted to flavonols using the Algar−Flynn−Oyamada[24,25] reaction by treatment with alkaline hydrogen peroxide, in yields of 25–65%.[23]

The 4′-hydroxy-3′-substituted flavonols were synthesized by the four- or five-step processes shown in Scheme 2. Each of

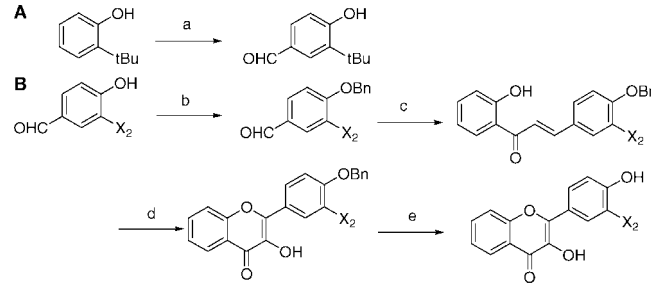
Scheme 1. Synthesis of 3′- and 4′-Monosubstituted Flavonols[a]



4′-substituted flavonols			*3′-substituted flavonols*		
	X_1	X_2		X_1	X_2
2	H	H	**2**	H	H
3	OH	H	**9**	H	OH
4	OCH_3	H	**10**	H	OCH_3
5	Cl	H	**11**	H	Cl
6	CF_3	H	**12**	H	CF_3
7	CH_3	H	**13**	H	CH_3
8	tBu	H			

[a] Reagents and conditions: (a) NaOH, EtOH, room temp; (b) H_2O_2, NaOH, EtO, 0 °C to room temp.

Scheme 2. (A) Synthesis of 3-*tert*-Butyl-4-hydroxybenzaldehyde from 2-*tert*-Butylphenol and (B) Synthesis of 4′-Hydroxy-3′-substituted Flavonols from 4-Hydroxy-3-substituted Benzaldehydes[a]



4′-hydroxy-3′-substituted flavonols		
	X_1	X_2
3	OH	H
1	OH	OH
14	OH	OCH_3
15	OH	Br
16	OH	CF_3
17	OH	CH_3
18	OH	tBu

[a] Reagents and conditions: (a) $CHCl_3$, NaOH, H_2O, 50 °C; (b) BnBr, K_2CO_3, EtOH, room temp; (c) 2-hydroxyacetophenone, dioxane, EtOH, KOH, room temp; (d) H_2O_2, dioxane, EtOH, NaOH, 0 °C to room temp; (e) HCl, AcOH, 100 °C.

the 4-hydroxy-3-substituted benzaldehydes was commercially available except 4-hydroxy-3-*tert*-butylbenzaldehyde. This benzaldehyde was synthesized from 2-*tert*-butylphenol and chloroform as a minor product of the Reimer−Tiemann reaction using conditions described by Birchall et al.[26,27] The pure product was isolated by steam distillation in a 2% yield (Scheme 2A). The 4-hydroxyl group of each benzaldehyde was protected as a benzyl ether with benzyl bromide and K_2CO_3, in yields of 60–80% (Scheme 2B).[28] The benzyl group was chosen because of its stability under the strong alkaline conditions of the Claisen−Schmidt condensation and the Algar−Flynn−Oyamada reaction and the anticipated ease of deprotection. Successful Claisen−Schmidt condensation of 4-benzyloxybenzaldehydes and 2-hydroxyacetophenone required more forcing conditions than for the monosubstituted benzaldehydes.[29] These conditions involve a prolonged reaction time, use of a higher concentration of base, and inclusion of dioxane to solubilize poorly soluble reaction intermediates.[29] The chalcones were not purified but immediately treated with alkaline hydrogen peroxide.[29] All protected flavonols were obtained in >35% yield over these two steps.

Debenzylation of protected flavonols was achieved by treatment with aqueous HCl in AcOH at 100 °C,[29] with partial

Table 1. Comparison of the Vascular Activity (Sensitivity, pEC_{50}; Maximum Response, R_{max}) and the Antioxidant Activity of 4′-Substituted, 3′-Substituted, and 4′-Hydroxy-3′-substituted Flavonols in Rat Isolated Thoracic Aorta with Intact Endothelium

			vascular activity[a]			antioxidant activity					
	X_1	X_2	*n*	pEC_{50}	R_{max} (%)	*n*	lucigenin[b]	*n*	NBT[c]	*n*	DPPH[d]
2	H	H	5	5.12 ± 0.09	91 ± 2	5	70 ± 14	3	91 ± 10	4	83 ± 9
					4′-Substituted Flavonols						
3	OH	H	5	5.33 ± 0.11	99 ± 1	5	74 ± 9	3	93 ± 11	4	32 ± 9
4	OCH_3	H	5	ND	16 ± 5	6	89 ± 17	3	91 ± 10	4	51 ± 3
5	Cl	H	6	5.09 ± 0.14	37 ± 4	6	100 ± 36	3	69 ± 8	4	93 ± 11
6	CF_3	H	5	ND	6 ± 3	6	85 ± 10	3	82 ± 17	4	90 ± 5
7	CH_3	H	5	ND	13 ± 7	6	101 ± 21	3	73 ± 6	4	76 ± 5
8	tBu	H	5	ND	10 ± 3	6	111 ± 32	3	81 ± 6	4	71 ± 4
					3′-Substituted Flavonols						
9	H	OH	5	5.49 ± 0.10	98 ± 2	6	78 ± 17	3	84 ± 10	4	87 ± 16
10	H	OCH_3	6	5.15 ± 0.05	79 ± 7	6	74 ± 16	3	97 ± 6	4	71 ± 2
11	H	Cl	6	5.17 ± 0.08	53 ± 6	5	75 ± 17	3	77 ± 11	4	87 ± 1
12	H	CF_3	5	3.94 ± 0.10	23 ± 4	6	80 ± 19	3	82 ± 23	4	89 ± 1
13	H	CH_3	5	4.13 ± 1.03	37 ± 12	6	85 ± 23	3	66 ± 17	4	80 ± 1
					4′-Hydroxy-3′-Substituted Flavonols						
1	OH	OH	5	5.30 ± 0.07	102 ± 1	5	27 ± 4	5	43 ± 6	4	36 ± 10
14	OH	OCH_3	5	5.35 ± 0.03	98 ± 2	5	106 ± 25	4	88 ± 10	4	24 ± 1
15	OH	Br	5	5.60 ± 0.07	100 ± 1	5	96 ± 37	3	95 ± 10	4	23 ± 1
16	OH	CF_3	5	4.78 ± 0.01	77 ± 5	5	109 ± 28	3	102 ± 20	4	26 ± 2
17	OH	CH_3	5	5.36 ± 0.14	98 ± 2	5	105 ± 34	3	79 ± 21	4	25 ± 1
18	OH	tBu	5	5.33 ± 0.11	38 ± 3	5	108 ± 36	3	84 ± 14	4	26 ± 2

[a] ND = not determined. [b] Superoxide levels in the presence of flavonols (10^{-5} M) expressed as percentage of control. [c] DPPH level in the presence of flavonols (10^{-4} M) expressed as percentage of control. [d] NBT level in the presence of flavonols (10^{-5} M) expressed as percentage of control.

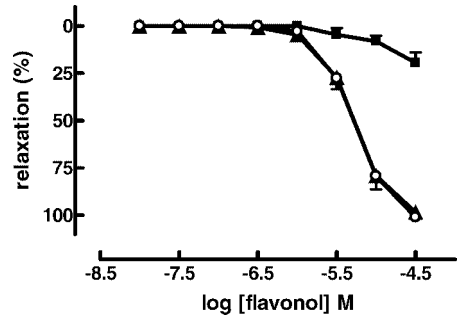


Figure 2. Examples of concentration–response curves of DiOHF **1** (○) ($n = 5$), 3′-methoxy **10** (■) ($n = 5$), and 4′-hydroxy-3′-methoxy **14** (▲) ($n = 6$) in endothelium-intact rat thoracic aortic rings. Aortic rings were precontracted with isotonic high-potassium physiological salt solution (KPSS, 30 mM KCl) to an equal level in all groups (**10**, 45 ± 3% of KPSS (123 mM KCl); **14**, 46 ± 4%; **1**, 42 ± 1%). All values are expressed as mean with standard error of the mean indicated by the vertical lines. The pEC_{50} and R_{max} values determined from the data presented are given in Table 1.

decomposition being observed for the *tert*-butyl derivative. All flavonols and intermediates synthesized in this study were purified by recrystallization.

Structure–Activity Relationships of Vascular Activity. The vascular activities of 18 flavonols were quantified using precontracted rat isolated thoracic aorta from which the potency (pEC_{50}) and maximum relaxation (R_{max}) were determined. Endothelium intact rings were precontracted with an isotonic, high potassium physiological salt solution (KPSS, 30 mM KCl) to similar levels, and the concentration–response curves to various flavonols were generated allowing quantification of the potency and efficacy.

The results for the 4′-monosubstituted flavonols (Table 1, Figure 2) indicated that substitution by most groups including Cl, OCH_3, $C(CH_3)_3$, CH_3, and CF_3 abolished vasorelaxation relative to the unsubstituted flavonols. By contrast, a 4′-hydroxyl group improved the vasorelaxant activity and was the optimum substituent among those examined. The effects of substitutions at the 3′ position were next investigated. CH_3, CF_3, and Cl substitutions at the 3′ position slightly reduced the vasorelaxation relative to the unsubstituted flavonols. The OCH_3 group did not alter the response, and the OH substitution was again the preferred substituent that maximized vascular activity. We next sought to investigate the effect of combinations of substituents and chose to maintain a 4′-hydroxyl group, which provides excellent vasorelaxant activity while investigating the effect of variation of substituents at the 3′-position. The 4′-hydroxy-3′-substituted flavonols were as active as the most potent compound described to date, **1**, except the *tert*-butyl derivative, which had a significantly lower potency and efficacy. This suggests that for flavonols with a 4′-hydroxy group in place, electron-withdrawing or -donating groups or hydrogen bond donors and/or acceptors at the 3′-position do not significantly alter the level of their vascular activity, but steric hindrance caused by a bulky *tert*-butyl group does reduce vascular activity. These results also suggest that a single B-ring hydroxyl group might be sufficient for maximal vascular activity and that a catechol group may not be as important as has been proposed previously by our group and others.[16,19] Woodman et al. proposed that a hydroxyl group at the 3′ position of a flavonol was the main determinant of the vascular activity.[22] The results of this study suggest that a hydroxyl group at either the 3′ or 4′ position is sufficient for good vasorelaxant activity. Flavonols with a similar range of substitutions at the 4′ position and with a 3′-hydroxyl could be synthesized and investigated to test this hypothesis.

Mechanisms of Flavonol-Induced Vasorelaxation. Flavonols are most commonly found to cause endothelium-independent vasorelaxation,[16,17,19,30] but Fitzpatrick et al. demonstrated that quercetin causes endothelium-dependent relaxation in rat thoracic aorta.[31] To investigate whether the flavonols under investigation here act in an endothelium-dependent manner, four of the most active flavonols (**1**, **3**, **10**, and **14**) were examined. Our data for **3**, **10**, and **14** show that the removal of the endothelium from rat aorta did not affect the efficacy or potency of the compound (Figure 3, Table 2). **1** showed a reduced sensitivity in endothelium denuded rings without affecting the maximum response. This result agrees with Chan et al., who suggested that **1** causes vasorelaxation

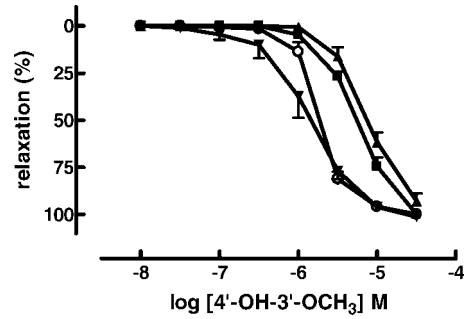


Figure 3. Role of endothelium and the effect of different contractile agents on the responses to 4′-hydroxy-3′-methoxy **14**. Comparison of concentration–response curves to **14** after active tone is induced by either U46619 or isotonic high-potassium physiological salt solutions in the presence and the absence of the endothelium (endothelium intact, U46619 induced (▼); endothelium denuded and U46619 induced (○); endothelium intact, isotonic, high-potassium physiological salt solution (KPSS, 30 mM KCl) induced (■); endothelium denuded, isotonic, high-potassium physiological salt solution (KPSS, 25 mM KCl) induced (▲)). Aortic rings were precontracted to a similar level in all groups. KCl-induced precontraction was more resistant to relaxation induced by DiOHF **1**. Removal of the endothelium did not affect the response significantly. All values are expressed as mean with standard error of the mean indicated by the vertical lines. pEC_{50} and R_{max} analysis results are summarized in Table 2.

Table 2. Comparison of the pEC_{50} and R_{max} to Different Flavonols in Endothelium-Intact (EI) and Endothelium-Denuded (EX) Thoracic Aortic Rings from Rats

	14	**1**	**3**	**10**
U46619-Induced, EI				
n^a	9	9	7	7
pEC_{50}	6.06 ± 0.23	5.87 ± 0.10	5.78 ± 0.09	5.52 ± 0.11
R_{max}	101 ± 1	101 ± 1	100 ± 0	101 ± 3
precontraction[b]	50 ± 2	53 ± 2	54 ± 3	53 ± 4
U46619-Induced, EX				
n^a	8	5	5	9
pEC_{50}	5.78 ± 0.05	5.52 ± 0.10[c]	5.50 ± 0.06	5.43 ± 0.13
R_{max}	100 ± 1	100 ± 0	102 ± 1	93 ± 2
precontraction[b]	51 ± 2	59 ± 2	53 ± 4	47 ± 5
KCl-Induced, EI [Isotonic, High-Potassium Physiological Salt Solution (KPSS, 30 mM KCl)]				
n^a	5	6	7	6
pEC_{50}	5.32 ± 0.03[c]	5.26 ± 0.03[d]	5.29 ± 0.08[d]	5.11 ± 0.05[c]
R_{max}	100 ± 1	100 ± 1	95 ± 4	87 ± 5[c]
precontraction[b]	58 ± 4	57 ± 4	50 ± 3	56 ± 6
KCl-Induced, EX [Isotonic, High-Potassium Physiological Salt Solution (KPSS, 25 mM KCl)]				
n^a	4	4	4	6
pEC_{50}	5.25 ± 0.06[d]	5.20 ± 0.10[d]	5.26 ± 0.06[d]	5.05 ± 0.08[c]
R_{max}	93 ± 4[d]	100 ± 1	92 ± 1	86 ± 5[c]
precontraction[b]	58 ± 2	60 ± 5	60 ± 6	59 ± 8

[a] n = total number of rats. [b] Level of tone expressed as percentage of contraction to KPSS (123 mM KCl). [c] Significantly different from the response of the U46619 + EI vessel ($p < 0.05$, ANOVA, Dunnett's multiple comparison test). [d] Very significantly different from the response of the U46619 + EI vessel ($p < 0.001$, ANOVA, Dunnett's multiple comparison test).

predominantly in an endothelium-independent manner but that endothelium-dependent relaxation also plays a small role.[16] The differences between **1** and other active flavonols might be due to differences in other biological activities, such as antioxidant activity, which may indirectly enhance the activity of endogenously released NO.

Several studies have demonstrated that flavonols are effective vasodilators,[16,19,31,32] but the mechanism of flavonol induced vasodilatation remains unclear. In this study, two contractile agents, isotonic high-potassium physiological salt solutions (KPSS, 30 mM/25 mM KCl) and thromboxane mimetic agent U46619, were selected to investigate the possible role of potassium channels in the vascular relaxation. To achieve the same level of precontraction, 30 mM KCl was used in endothelium intact aortic rings, whereas 25 mM KCl was used in endothelium denuded aortic rings. KCl at 25 or 30 mM partially depolarizes the aortic rings, whereas U46619 activates thromboxane A2 receptor. Our results show that the same four flavonols (i.e., **1**, **3**, **10**, **14**) exerted a more potent relaxing effect in the presence of U46619 than in the presence of 30 mM KCl (Figure 3, Table 2). This suggests that the opening of potassium channels might play a role in flavonol-induced vasorelaxation, which has been observed by other groups.[33–39] While our results suggest that these flavonols primarily cause endothelium-independent relaxation through the opening of the potassium channels, other mechanisms may also be involved, such as inhibition of enzymes involved in vascular smooth muscle contraction, e.g., protein kinase C and cAMP phosphodiesterase,[19] or interference with the utilization of Ca^{2+} in the contractile response.[16,22]

Structure–Activity Relationships of Antioxidant Activity. The antioxidant activities of the 18 flavonols were examined next using several different in vitro assays. The effect of flavonols on superoxide levels were first assessed using a cell-free colorimetric nitroblue tetrazolium chloride (NBT) assay coupled with xanthine/xanthine oxidase. The reaction of xanthine and xanthine oxidase results in production of superoxide anions that is detected by the change in color of NBT. Superoxide levels will be reduced if flavonols possess antioxidant activity. None of flavonols examined in this study were found to be as effective as the lead compound **1**[14] for superoxide scavenging when used at a concentration of 10^{-5} M (Table 1). **1** inhibited superoxide levels by 57 ± 6%, whereas most of the other flavonols inhibited by only 10–20%.

The effect of flavonols on superoxide levels was then assessed using a tissue-based lucigenin-enhanced chemiluminescence assay, which has greater pharmacological relevance compared to cell-free assays because it uses the tissue itself to generate superoxide and demands that active antioxidants scavenge superoxide in the presence of other biological substrates.[22] Isolated rat aortic rings were incubated with NADPH as the substrate for NADPH oxidase in the vasculature, diethylthiocarbamic acid (DETCA) to inactive endogenous superoxide dismutase, and either vehicle or flavonols. Superoxide that is produced by NADPH oxidase reacts with lucigenin, leading to the emission of photons, which can be quantified to give a measure of superoxide levels.[40] **1** inhibited superoxide levels by 73 ± 4%, whereas other flavonols inhibited by up to only 30%. The findings from both of the cell-free and tissue-based systems demonstrate that the catechol group on the B ring of **1** is important for strong antioxidant activity, as others have reported.[41–43] In addition to the presence of two contiguous hydroxyl groups, the total number of hydroxyl groups might also be an important determinant of the scavenging activity.[22] Clearly, flavonols with three hydroxyl groups in total, such as **1**, exhibit good antioxidant activity but not flavonols with one or two hydroxyl groups. However, it is unclear whether the antioxidant activity of flavonols is due to their direct radical scavenging effect or inhibition of enzymes responsible for superoxide anion production, such as NADPH oxidase.[44]

The radical scavenging activity of flavonols against other free radicals, such as the organic free radical DPPH, was also examined. DPPH possesses an unpaired electron that is stabilized by resonance over the whole molecule, giving rise to a deep-

Table 3. Comparison of the Sensitivity (pEC_{50}) and Maximum Relaxation (R_{max}) to acetylcholine in the Presence of **1** ($10^{-4.5}$ M), **14** ($10^{-4.5}$ M), or Vehicle (15% DMSO, 85% Methanol) in Endothelium-Intact Rat Isolated Thoracic Aorta Exposed to Pyrogallol ($10^{-4.5}$ M) or a Combination of Xanthine (10^{-4}M)/Xanthine Oxidase (0.016 U/mL)[a]

	pyrogallol			xanthine/xanthine oxidase		
treatment	*n*	pEC_{50}	R_{max}	*n*	pEC_{50}	R_{max}
vehicle	6	6.93 ± 0.08	81 ± 5	6	7.60 ± 0.20	94 ± 2
oxidant stress	6	6.32 ± 0.23	47 ± 7^b	7	6.96 ± 0.14	47 ± 7^b
oxidative stress + **1**	6	7.19 ± 0.22	81 ± 8	6	7.26 ± 0.30	84 ± 7
oxidative stress + **14**	6	6.56 ± 0.31	42 ± 5^b	7	7.05 ± 0.28	62 ± 6^b

[a] *n* = the total number of rats. Pyrogallol: $10^{-4.5}$ M. Xanthine (10^{-4} M)/xanthine oxidase (0.016 U/mL). [b] Significantly different from the vehicle ($p < 0.05$, ANOVA, Dunnett's multiple comparison test).

violet color. When a solution of DPPH reacts with a hydrogen atom donor, it is reduced resulting in a loss of the violet color.[45] As shown in Table 1, 4′-hydroxyflavonol **3** is the most activate flavonol among the 4′-substituted flavonols in the DPPH assay. None of 3′-substituted flavonols demonstrated significant scavenging activity against the DPPH radical, and all of the 4′-hydroxy-3′-substituted flavonols could scavenge this organic radical as effectively as quercetin, as previously reported by Sim et al.[46]Owing to the delocalizaton of its free electron, DPPH is a more stable, and less reactive, radical than superoxide ion. These results indicate the importance of the orientation of phenolic hydroxyl groups on the B ring in determining the anti-DPPH efficacy, with the 4′-hydroxyl group on the B ring being required for effective scavenging of DPPH, possibly as a result of the resonance stabilization afforded to the resulting phenolic radial.

Effect of Flavonols on Vasorelaxation Stimulated by Acetylcholine in the Presence of Oxidative Stress. Preservation of NO bioactivity by superoxide scavengers depends not only on superoxide scavenging activity but also on the rate of superoxide scavenging.[47] For example, the well-known antioxidant, ascorbic acid, reacts relatively slowly with superoxide (rate $= 3.3 \times 10^{-5}$ M^{-1} s^{-1})[48] and does not improve endothelial dysfunction caused by superoxide.[47] By contrast, flavonols react almost 10 times faster with superoxide (e.g., rate $= 2.4 \times 10^{-6}$ M^{-1} s^{-1} for quercetin).[49] To determine if flavonols scavenge superoxide effectively enough to increase vascular NO bioavailability when there are high levels of superoxide, we examined the effect of relaxant responses of acetylcholine in rat isolated aortic rings subjected to oxidative stress induced by two routes, pyrogallol or xanthine/xanthine oxidase. Pyrogallol autooxidizes and generates superoxide radical directly, whereas xanthine is the substrate for xanthine oxidase and generates superoxide enzymatically. Addition of pyrogallol or xanthine/xanthine oxidase caused significant endothelial dysfunction (>50% reduction in R_{max} to acetylcholine) as shown in Figures 4 and 5 and Table 3. **1**, one of the most active flavonols reported to date,[14,22] completely reversed the reduction in relaxation caused by oxidant stress using either system, indicating a rapid reaction between the flavonol and superoxide anions, thereby preventing vascular dysfunction. This provides strong evidence that this flavonol effectively protects endothelium-derived NO from inactivation from endogenously and exogenously generated superoxide. On the other hand, **14**, which is as effective a vasorelaxant as **1**, did not improve endothelium-dependent relaxation in the presence of oxidative stress in either system, indicating that this compound cannot efficiently scavenge superoxide and that it lacks functional antioxidant activity.

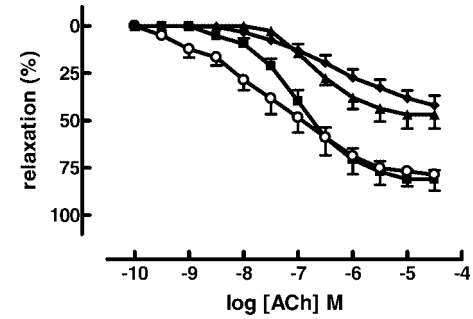


Figure 4. Effect of 4′-hydroxy-3′-methoxyflavonol **14** or DiOHF **1** on pyrogallol-induced vascular dysfunction. Concentration–responses curves to acetylcholine in the absence (■, $n = 6$) or the presence of P (pyrogallol (▲), $10^{-4.5}$ M, $n = 6$), P + **1** (pyrogallol, $10^{-4.5}$ M + **1** (○), $10^{-4.5}$ M, $n = 6$), or P + **14** (pyrogallol, $10^{-4.5}$ M + **14** (◆), $10^{-4.5}$ M, $n = 6$). Aortic rings were precontracted with U46619 to a similar level in all groups (see Table 2). Pyrogallol significantly inhibited the response to acetylcholine. This inhibitory effect is markedly reduced by **1** but not by **14**. All values are expressed as mean with standard error of the mean indicated by the vertical lines.

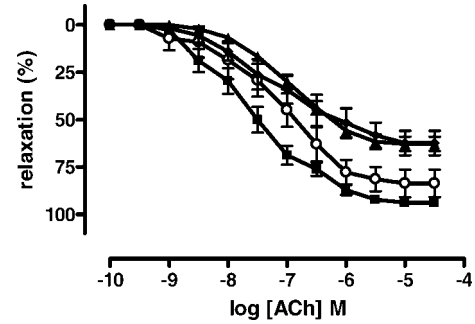


Figure 5. Effect of 4′-hydroxy-3′-methoxyflavonol **14** or DiOHF **1** on acetylcholine-induced endothelium-dependent relaxation in the presence of xanthine/xanthine oxidase: concentration–response curves to acetylcholine in the absence (■, $n = 6$) or the presence of xanthine/xanthine oxidase (▲, $n = 7$), xanthine/xanthine oxidase + **1** (○, $n = 6$), or xanthine/xanthine oxidase + **14** (◆, $n = 6$). Aortic rings were precontracted with U46619 to a similar level in all groups (see Table 2). Xanthine/xanthine oxidase significantly inhibited the response to acetylcholine. This inhibitory effect is markedly reduced by **1** but not **14**. All values are expressed as mean with standard error of the mean indicated by the vertical lines.

Conclusion

Through the synthesis and pharmacological evaluation of a series of 4′-substituted, 3′-substituted, and 4′-hydroxy-3′-substituted flavonols, we have determined the relationship between vasorelaxant and antioxidant activities and flavonol structure. Surprisingly, while vasorelaxant and antioxidant activity has been concurrent in all flavonoids studied to date, our structure–activity relationship clearly shows that these activities may be separated. The discovery that 4′-hydroxy-3′-methoxyflavonol possesses only vasorelaxant activity and lacks antioxidant properties now provides the first single-acting flavonol that maintains one activity while being devoid of the other. As such, it is now available as a small molecule to determine which of these two activities is required for cardioprotection and should cast light on the mechanisms of reper-

fusion injury and the potential for small molecule antioxidant therapeutics for the treatment of cardiovascular disease.

Experimental Section

Pharmacological Assays. All experimental procedures were performed within the guidelines of National Health and Medical Research Council of Australia and were approved by the Pharmacology and Physiology subcommittee of the University of Melbourne Animal Experimentation Ethics Committee.

Isolation of Rat Thoracic Aortic Rings. Male Sprague–Dawley rats (250–350 g) were euthanized by inhalation of 80% CO_2/20% O_2 and subsequent cervical dislocation. The chests were opened, and the thoracic aortae were isolated. After removal of the superficial connective tissue, the aorta was cut into ring segments, 2 to 3 mm in width, which were then used for relaxation assays and lucigenin chemiluminescence assays in vitro.

Relaxation by Flavonols. Aortic rings were mounted in standard 10 mL organ baths containing Krebs' bicarbonate solution (composition (mM): NaCl 118.0, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, D-glucose 11.0, $CaCl_2 \cdot 2H_2O$ 2.5). The bath medium was maintained at 37 °C with a pH of 7.4 and continuously bubbled with carbogen (5% CO_2 in O_2). When required, the endothelium was mechanically removed by gently rubbing the lumen with the tips of fine forceps. Aortic rings were equilibrated for 60 min at a resting tension of 1 g. The rings were precontracted with an isotonic, high-potassium physiological salt solution (Krebs' potassium salt solution, KPSS, 123 mM KCl) to achieve the maximum tension. After a washout of 30 min, the integrity of the endothelium was tested. Rings were precontracted with phenylephrine (PE, 10−100 nM), and only rings showing greater than 80% relaxation in response to acetylcholine (10 μM) were accepted as endothelium intact. Endothelium-denuded rings that did not relax to acetylcholine showed 100% relaxation to sodium nitroprusside (SNP, 10^{-6} M). Following testing of endothelial integrity, rings were repeatedly washed and re-equilibrated to the resting tension.

(1) Effect on KCl-Induced Precontraction. Isotonic, high-potassium physiological salt solution (KPSS, 30 mM KCl) was then used to establish a stable active force in the range of 40−60% of KPSS (123 mM KCl) induced contraction. Cumulative concentration–response curves were conducted for all 4′-substituted, 3′-substituted, 4′-hydroxyl-3′-substituted flavonols (10 nM to 30 μM) or vehicle (15% DMSO, 85% methanol). Only one concentration–response curve was obtained from each thoracic aortic ring.

(2) Effect on U4661-Induced Precontraction. To investigate the mechanism of flavonol-induced relaxation, a different contractile agent, the thromboxane mimetic 9,11-dideoxy-9α,11α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619, 1–10 nM) was used. Before precontraction, rings were incubated with the L-type Ca^{2+} channel blocker nifedipine (30 nM) to prevent spontaneous activity.[16] Cumulative concentration–response curves were obtained for the **1**, **3**, **10**, and **14**.

(3) Effect of the Removal of Endothelium on the Responses to Flavonols. In order to examine the role of endothelium in the vasorelaxation caused by flavonols, cumulative response curves to **1**, **3**, **10**, and **14** were determined in the absence and the presence of endothelium for both KPSS (25 mM for endothelium denuded rings or 30 mM KCl for endothelium intact rings) and U46619 precontracted vessels.

Antioxidant Activities of Flavonols. A. Effect of Flavonols on Free Radicals in in Vitro Assays. Several in vitro test systems were used to assess the antioxidant activities of the 18 flavonols. Effects of flavonols on superoxide levels were assessed using lucigenin-enhanced chemiluminescence assay and the colorimetric NBT (nitroblue tetrazolium chloride) assay coupled with xanthine/xanthine oxidase system. The effect on a less reactive free radical was assessed using the DPPH assay.

(1) Effects of Flavonols on Superoxide Levels in NBT Assays. The scavenging potential for superoxide radicals was also analyzed via xanthine/xanthine oxidase generating system, coupled with NBT (nitroblue tetrazolium) reduction using a modified literature procedure by Robak & Cryglewski.[50] Background was measured by a spectrophotometer (Perkin-Elmer) at 560 nm in a 96-well microplate plate containing 200 μL of 0.1 M phosphate buffer (pH 7.4), xanthine (3 mM), EDTA (3 mM), NBT (600 μM), and 4′-substituted flavonols, 3′-substituted flavonols, 4′-hydroxyl-3′-substituted flavonols (10 μM, 100% DMSO), or vehicle (5% DMSO). A total of 25 mU/mL xanthine oxidase was then added to the reaction mixture and incubated at 25 °C after a further 25 min. The absorbance of the reaction mixture was recounted and the level of superoxide was calculated using the following equation: level of superoxide (%) = $(A_{\text{flavonol+xanthine oxidase}} - A_{\text{background}})/(A_{\text{xanthine oxidase}} - A_{\text{backgroud}}) \times 100$.

(2) Effects of Flavonols on Superoxide Levels in Lucigenin Assays. Superoxide anion generation was measured in isolated aortic segments by lucigenin-enhanced chemiluminescence. Aortic rings were prepared as described above and then placed in Krebs−HEPES buffer (composition (mM): NaCl 99.90, KCl 4.7, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 1.2, D-glucose 11.0, $NaHCO_3$ 25.0, $CaCl_2 \cdot 2H_2O$ 2.5, Na HEPES 20.0). These tissues were incubated for 45 min at 37 °C at pH 7.4 in Krebs−HEPES buffer containing diethylthiocarbamic acid (DETCA, 3 mM, to inactivate superoxide dismutase) and β-nicotinamide adenine dinucleotide phosphate (NADPH, 100 μM) as a substrate for NADPH oxidase and 4′-substituted flavonols, 3′-substituted flavonols, 4′-hydroxy-3′-substituted flavonols (10 μM, 100% DMSO), or vehicle (DMSO 1%). Background photoemission was measured by a TopCount single photocounter for 12 cycles in a 96-well Optiplate containing (300 mL) of Krebs−HEPES buffer together with bis-*N*-methylacridinium nitrate (lucigenin) (50 μM), NADPH (100 μM), 4′-substituted flavonols, 3′-substituted flavonols, 4′-hydroxy-3′-substituted flavonols (10 μM, 100% DMSO), or vehicle (DMSO 1%). After 45 min, a single ring segment of aorta was washed and added to each well and photon-emission, as a measure of superoxide production, was recounted. At the conclusion of the assay, aortic tissues were removed and dried for 48 h at 65 °C before weighing. Superoxide production was normalized to dry tissue weight.

(3) Effects of Flavonols on DPPH Levels. The free radical scavenging activity of flavonols was determined using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and measured according to the method of Sim et al.[46,51] All 4′-substituted flavonols, 3′-substituted flavonols, 4′-hydroxy-3′-substituted flavonols (30 μM) were dissolved in 0.1 mL of DMSO and then added to 0.1 mL of 0.1 mM DPPH in methanol. The mixture was allowed to stand for 10 min at room temperature in the dark, and the absorbance was measured using a microplate reader (Perkin-Elmer) at 560 nm. The level of DPPH was calculated using the following equation: level of superoxide (%) = $(A_{\text{flavonol}}/A_{\text{DPPH}}) \times 100$.

B. Effect of Flavonols on Vasorelaxation to Acetylcholine in the Presence of Oxidative Stress. To test the ability of the compounds to preserve endothelial function in the presence of oxidative stress, responses to acetylcholine were examined in the presence of pyrogallol, which auto-oxidizes to release superoxide, or xanthine/xanthine oxidase, which produces superoxide via an enzymatic pathway.[14] The two systems were chosen to indicate whether flavonols acts by enzyme inhibition or radical scavenging.

(1) Pyrogallol. Aortic rings were isolated and mounted in standard 10 mL organ baths containing Krebs bicarbonate solution as described above. After aortic rings were equilibrated, the maximum responses were established and the endothelium integrity was tested. Rings were incubated for 20 min with pyrogallol (30 μM), pyrogallol (30 μM) with **14** (30 μM, 15% DMSO, 85% methanol), pyrogallol (30 μM) with the positive control **1** (30 μM, 15% DMSO, 85% methanol), or vehicle (15% DMSO, 85% methanol). The L-type Ca^{2+} channel blocker nifedipine (30 nM) was also added to all aortic rings to prevent spontaneous activity.[16] After a stable precontraction tone [50% of KPSS (123 mM KCl)-induced contraction] with thromboxane mimetic U46619 (1–10 nM) was established, cumulative concentration–response curves to acetylcholine were determined and the effects of vehicle, **1**, and **14** in the presence of oxidative stress were compared.

(2) Xanthine/Xanthine Oxidase. Isolated aortic rings were incubated with xanthine oxidase (0.016 U/mL) for 30 min to allow it to permeate the tissue, 20 min with compound **1** (30 μM) or **14** (30 μM), and 10 min with xanthine (100 μM) before precontraction. Cumulative concentration–response curves to acetylcholine were determined, and the effect of vehicle, **1**, and **14** in the presence of oxidative stress was compared.

Drugs and Chemicals Used. Unless stated otherwise, all chemical reagents were purchased from Aldrich and were dissolved in buffer solution. 4-Hydroxy-3-trifluoromethylbenzaldehyde was supplied by Fluorochem (Derbyshire, U.K.). Acetylcholine perchlorate was obtained from BDH Chemicals (Poole, Dorset, England). 9,11-Dideoxy-9α,11α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619) was from Cayman Chemical Company (Ann Arbor, MI). DiOHF **1**, flavonol **2**, 4′-hydroxyflavonol **3**, and 3′-hydroxyflavonol **9** were from Indofine Chemical Co., Inc. (Belle Mead, NJ), and *p*-nitroblue tetrazolium chloride (NBT) was from Calbiochem (Darmstadt, Germany). U46619 was dissolved in 1 M $NaHCO_3$, and nifedipine was dissolved in absolute ethanol and subsequently in Krebs bicarbonate solution. For use in organ bath experiments, all flavonols were dissolved in 15% DMSO, 85% methanol (0.01 M stock), subsequent dilutions were made in 100% methanol (1 μM) and deionized water (0.1 and 0.01 μM). The maximum concentrations of DMSO and methanol in the organ bath were 0.15% and 0.85%, respectively. For use in the lucigenin, NBT, and DPPH assays, all flavonols were dissolved in 100% DMSO, and the maximum concentration of DMSO was 1%. DPPH was dissolved in methanol.

Data Presentation and Statistical Analysis. The results are expressed as the mean ± standard error of the mean, and *n* indicates the number of experiments (i.e., number of rats). Relaxation responses to flavonols were expressed as a percentage of the KCl or U46619 induced precontraction. Relaxation responses to acetylcholine were expressed as a percentage of U46619 induced precontraction. Concentration–response data were fitted to a sigmoidal curve using GraphPad Prism, version 4 (GraphPad Software Inc., San Diego, CA) to provide estimates of the pEC_{50} value. The calculated pEC_{50} and observed maximum response (R_{max}) were compared using the one-way analysis of variance test (ANOVA) with post hoc multiple comparisons using Newman−Keuls or Dunnett's test (GraphPad Software Inc., San Diego, CA). $P < 0.05$ was considered statistically significant.

Superoxide levels from rat aortic rings in the lucigenin assay were expressed as average counts per second normalized to dry tissue weight and calculated as a percentage of the counts in the presence of vehicle (0.1% Krebs−HEPES buffer).The superoxide levels in NBT assays was expressed as a percentage of the counts in the presence of vehicle (0.1 M phosphate buffer) minus the background, and the level of DPPH was expressed as percentage of vehicle (0.1 mM DPPH in methanol).

Synthetic Procedures and Characterization. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian Unity 400 in solutions of $CDCl_3$ or DMSO-d_6. Multiplicity abbreviations used are as follows: s = singlet, d = doublet, m = multiplet, dd = doublet of doublet, ddd = doublet of doublet of doublet, t = triplet. Thin layer chromatography (TLC) was performed with aluminum sheets precoated with Merck silica gel 60 F_{254}, using mixtures of ethyl acetate−petroleum spirits. Detection was effected by visualization in UV light. Solvents were evaporated under reduced pressure using a rotary evaporator. Melting points were obtained using a Riechert−Jung Hotstage melting point apparatus. Low-mass spectral data were obtained by triple quad electrospray using a Quattro II instrument (Melbourne University), and HRMS experiments were performed by Sally Duck at the Chemistry Department (Monash University). Elemental analyses were performed by C.M.A.S (Belmont, Victoria). HPLC analysis was performed by John Karas (University of Melbourne) using an Agilent 1200 series instrument, with UV detector set at 254 nm, and a Zorbax Eclipse XDB-C18, 4.6 mm × 150 mm, 5 μm particle size column (eluting at 1 mL/min with a 25 min gradient of 0–100% solvent B, where solvent A is 0.1% TFA in water and solvent B is 0.1% TFA in acetonitrile.

4-Hydroxy-3-*tert*-butylbenzaldehyde. Chloroform (64 mL, 799 mmol) was added dropwise with stirring to a mixture of NaOH (120 g, 3.0 mol), water (120 mL), methanol (50 mL), and 2-*tert*-butylphenol (666 mmol, 100 g) over 4 h at 50 °C. The mixture was stirred at 50 °C for another 30 min and then poured into ice/water (600 mL) and acidified to pH 5 with concentrated hydrochloric acid. The dark mixture was extracted with dichloromethane, dried ($MgSO_4$), and concentrated, and the residue was steam-distilled for 18 h until the distillate ran clear. The remaining residue was dissolved in chloroform (400 mL) and extracted with sodium hydroxide (2 M, 400 mL). The aqueous extracts were acidified with concentrated hydrochloric acid to pH 2 and extracted with chloroform (3 × 300 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and evaporated. The dark-red residue was triturated with hot petroleum spirit (10 × 100 mL), and the combined petroleum spirit extract evaporated to give a bright-red residue. The residue was recrystallized from dichloromethane and petroleum spirit to give 4-hydroxy-3-*tert*-butylbenzaldehyde as colorless crystals (1.70 g, 2%), mp 146–150 °C (lit.[27] 142 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.45 (s, 9H, $C(CH_3)_3$), 6.10 (s, 1H, OH), 6.84 (d, 1H, $J_{5,6}$ = 8.4 Hz, H5), 7.66 (dd, 1H, $J_{5,6}$ = 8.4 Hz, $J_{2,6}$ = 2.0 Hz, H6), 7.84 (d, 1H, $J_{2,6}$ = 2.0 Hz, H2), 9.86 (s, 1H, COH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.5 ($C(CH_3)_3$), 34.9 ($C(CH_3)_3$), 117.3, 129.4, 130.0, 130.6, 137.4, 161.3 (Ar,Ph), 192.5 (C=O).

General Procedure for Benzylation of 4-Benzyloxy-3-substituted Benzaldehydes. Benzyl bromide (1.05–1.40 mmol) was added to a stirred solution of 3-substituted-4-hydroxybenzaldehyde (1 mmol) and potassium carbonate (1.1–1.8 mmol) in absolute ethanol (1 mL). The reaction mixture was stirred under nitrogen for 1 or 2 days until TLC indicated consumption of the benzaldehyde. The mixture was filtered through Celite, washed with dichloromethane (3 × 1 mL), and concentrated under vacuum. The yellow residue was dissolved in dichloromethane (2 mL), washed with sodium hydroxide (1 M, 1 mL), dried ($MgSO_4$), concentrated, and recrystallized from the solvents indicated.

4-Benzyloxy-3-methoxybenzaldehyde. Vanillin (5.00 g, 32.9 mmol), potassium carbonate (5.00 g, 36.2 mmol), and benzyl bromide (4.2 mL, 34 mmol) were reacted for 24 h. Workup as for the general procedure gave a crude residue that was recrystallized to yield 4-benzyloxy-3-methoxybenzaldehyde as a colorless fine solid (4.63 g, 58%), mp 69–70 °C (EtOH, lit.[28] 62–63 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.93 (s, 3H, CH_3), 5.23 (s, 2H, CH_2), 6.97 (d, 1H, $J_{5,6}$ = 8.4 Hz, H5), 7.31–7.43 (m, 7H, Ph,H2,6), 9.81 (s, 1H, COH). The ^{1}H NMR spectrum was in agreement with that previously reported.[28]

4-Benzyloxy-3-bromobenzaldehyde. 3-Bromo-4-hydroxybenzaldehyde (2.00 g, 9.95 mmol), potassium carbonate (2.50 g, 18.1 mmol), and benzyl bromide (1.80 mL, 15.1 mmol) were reacted for 2 days. Workup as for the general procedure gave a crude residue that was recrystallized to yield 4-benzyloxy-3-bromobenzaldehyde as a colorless fine solid (2.39 g, 83%), mp 97–98 °C (Et_2O/petroleum spirit, lit.[52] 78–80 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.27 (s, 2H, CH_2Ph), 7.05 (d, 1H, $J_{5,6}$ = 8.8 Hz, H5), 7.26–7.48 (m, 5H, Ph), 7.64 (dd, 1H, $J_{5,6}$ = 8.8 Hz, $J_{2,6}$ =1.6 Hz, H6), 8.11 (d, 1H, $J_{2,6}$ = 1.6 Hz, H2), 9.84 (s, 1H, CHO). The ^{1}H NMR spectrum was in agreement with that previously reported.[52]

4-Benzyloxy-3-trifluoromethylbenzaldehyde. 4-Hydroxy-3-trifluoromethylbenzaldehyde (0.25 g, 1.31 mmol), potassium carbonate (0.68 g, 4.92 mmol), and benzyl bromide (0.40 mL, 3.34 mmol) were reacted for 2 days. Workup as for the general procedure gave a crude residue that was recrystallized to yield 4-benzyloxy-3-trifluorobenzaldehyde as a colorless solid (0.18 g, 72%), mp 83–85 °C (Et_2O/petroleum spirit, lit.[28] 81.5–82.5 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.31 (s, 2H, CH_2), 7.17 (d, 1H, $J_{5,6}$ = 8.4 Hz, H5), 7.35–7.43 (m, 5H, Ph), 8.01 (dd, 1H, $J_{5,6}$ = 8.4 Hz, $J_{2,6}$ = 2.0 Hz, H6), 8.14 (d, 1H, $J_{2,6}$ = 2.0 Hz, H2), 9.92 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ 70.4 (**C**H_2Ph), 114.6 (Ar,Ph), 117.8

(q, 1C, $J = 30.7$ Hz, CF_3), 121.8, 124.6, 127.3, 128.2, 128.6, 128.9, 129.1, 135.6, 135.9, 160.4 (Ar,Ph), 190.9 (C═O).

4-Benzyloxy-3-methylbenzaldehyde. 4-Hydroxy-3-methylbenzaldehyde (0.93 g, 6.20 mmol), potassium carbonate (1.54 g, 11.1 mmol), and benzyl bromide (1.1 mL, 9.2 mmol) were reacted for 40 h. Workup as for the general procedure gave a crude residue that was recrystallized to yield 4-benzyloxy-3-methylbenzaldehyde as a brown fine solid (0.84 g, 56%), mp 51–53 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.33 (s, 3H, CH_3), 5.18 (s, 2H, CH_2), 6.99 (d, 1H, $J_{5,6} = 8.4$ Hz, H5), 7.35–7.45 (m, 5H, Ph), 7.73–7.70 (m, 2H, H2,H6), 9.87 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.6 (CH_3), 70.2 (CH_2), 111.1, 127.3, 128.2, 128.3, 128.8, 129.8, 130.7, 131.8, 136.5, 162.1 (Ar,Ph), 191.3 (C═O).

4-Benzyloxy-3-*tert*-butylbenzaldehyde. 4-Hydroxy-3-*tert*-butylbenzaldehyde (0.75 g, 4.21 mmol), potassium carbonate (1.05 g, 7.57 mmol), and benzyl bromide (0.8 mL, 6.3 mmol) were reacted for 24 h. Workup as for the general procedure gave a crude residue that was recrystallized to yield 4-benzyloxy-3-*tert*-butylbenzaldehyde as a yellow fine solid (0.76 g, 68%), mp 87–88 °C (Et_2O/petroleum spirit). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.42 (s, 9H, C(**C**H_3)$_3$), 5.21 (s, 2H, CH_2), 7.04 (d, 1H, $J_{5,6} = 8.0$ Hz, H5), 7.35–7.46 (m, 5H, Ph), 7.71 (dd, 1H, $J_{5,6} = 8.0$ Hz, $J_{2,6} = 2.0$ Hz, H6), 7.97 (d, 1H, $J_{2,6} = 2.0$ Hz, H2), 9.88 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.7 (C(**C**H_3)$_3$), 35.3 (**C**(CH$_3$)$_3$), 70.7 (CH_2), 112.4, 127.6, 128.4, 128.5, 128.9, 129.7, 130.8, 136.3, 139.3, 162.8 (Ar,Ph), 191.6 (C═O).

General Procedure for Sequential Claisen−Schmidt Condensation of 2-Hydroxyacetophenone and Various Substituted Benzaldehydes and Algar−Flynn−Oyamada Reaction. Method A. 2-Hydroxyacetophenone (1 mmol) was added to a suspension of the aldehyde (1.0–1.5 mmol) in ethanol (2 mL) and aqueous NaOH (1 mL of 25.2 g/100 mL). The mixture was stirred at room temperature for 5 h or overnight. The reaction mixture was cooled on ice, and aqueous AcOH (30%) was added until the mixture was acidic. The mixture was stirred for an additional 30 min at 0 °C, and the solid precipitate was collected by filtration. Recrystallization from the indicated solvent afforded the pure chalcone. Hydrogen peroxide (30%, 0.25 mL) was then added to an ice-cold suspension of the chalcone (1 mmol) in ethanol (5 mL) and 1 M NaOH (2 mL). The mixture was allowed to warm to room temperature and was stirred overnight. The mixture was acidified with 1 M HCl until slightly acidic, and the precipitate formed was collected by filtration. Recrystallization from the indicated solvent afforded the flavonol.

Method B. Method B is as for method A except for the workup of the Claisen−Schmidt condensation reaction. The reaction mixture was extracted with ethyl acetate and washed with saturated $NaHCO_3$, saturated NaCl and water. The organic layer was dried ($MgSO_4$) and evaporated to dryness to afford the crude chalcone.

Method C. KOH solution (40% w/v, 0.8 mL) was added dropwise to a suspension of 4-benzyloxy-3-substituted benzaldehyde (1 mmol) and 2-hydroxyacetophenone (1 mmol) in ethanol (2 mL) and dioxane (2 mL) cooled on ice. The reaction mixture was allowed to warm to room temperature, and stirring was continued for 70 h. Dichloromethane (13 mL) was added, and the organic layer was washed with water (3 × 2 mL), dried ($MgSO_4$) and concentrated in vacuo. The yellow oily residue was dissolved in dioxane (4 mL), ethanol (10 mL), and NaOH solution (5.4% w/v, 3 mL) and was treated dropwise with H_2O_2 solution (30%, 0.4 mL). The reaction mixture was stirred in an ice bath for 2 h and subsequently at room temperature overnight, resulting in a yellow suspension. The suspension was acidified with 2 M HCl (3 mL), and the resultant precipitate was collected by filtration and washed with water (16 mL). The crude product was recrystallized from the solvents indicated.

4′-Methoxyflavonol (4). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 4-methoxybenzaldehyde (1.72 mL, 15.0 mmol) were reacted for 5 h according to method A to afford 2′-hydroxy-4-methoxychalcone as yellow needles (1.42 g, 56%), mp 93–94 °C (EtOH, lit.[53] 89–91 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.85 (s, 3H, CH_3), 6.90–6.95 (m, 1H, H4′), 6.94 (d, 2H, $J = 7.9$ Hz, H3,5), 7.00 (d, 1H, $J_{3',4'} = 7.9$ Hz, H3′), 7.47 (t, 1H, $J_{4',5'} = J_{5',6'} = 7.4$ Hz, H5′), 7.53 (d, 1H, $J_{trans} = 15.5$ Hz, C═CH), 7.62 (d, 2H, $J = 7.9$ Hz, H2,6), 7.88 (d, 1H, $J_{trans} = 15.5$ Hz, C═CH), 7.89–7.92 (m, 1H, H6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.5 (OCH_3), 115.6, 118.6, 119.6, 119.8, 121.2, 128.4, 130.6, 131.6, 137.2, 146.4, 163.1, 164.6 (Ar, Ph), 194.7 (C═O). Treatment of 2′-hydroxy-4-methoxychalcone (1.00 g, 3.93 mmol) according to method A afforded the flavonol as pale-yellow needles (536 mg, 51%), mp 230–231.5 °C (EtOH, lit.[54] 231–232 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.88 (s, 3H, Me), 6.93 (br s, OH), 7.04 (d, 2H, $J = 6.9$ Hz, H3′,5′), 7.39 (ddd, 1H, $J_{5,6} = 5.8$ Hz, $J_{6,7} = 5.4$ Hz, $J_{6,8} = 0.6$ Hz, H6), 7.57 (br d, 1H, $J_{7,8} = 6.2$ Hz, H8), 7.68 (ddd, 1H, $J_{5,7} = 1.2$ Hz, $J_{6,7} = 5.4$ Hz, $J_{7,8} = 6.2$ Hz, H7), 8.22 (d, 2H, $J = 6.9$ Hz, H2′,6′), 8.23 (d, 1H, $J_{5,6} = 5.4$ Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 56.0 (CH_3), 114.5, 118.8, 122.1, 124.5, 124.9, 125.7, 130.2, 133.9, 139.0, 155.5, 161.3 (Ar,Ph), 173.7 (C═O). LRMS (ESI^-) *m/z*: 268.1 [$C_{16}H_{12}O_4$ $(M + H)^+$ requires 268.1]. Anal. RP-HPLC $t_R = 16.6$ min (purity 96%).

4′-Chloroflavonol (5). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 4-chlorobenzaldehyde (2.11 g, 15.0 mmol) were reacted for 5 h according to method A to afford 4-chloro-2′-hydroxychalcone as yellow needles (1.37 g, 65%), mp 149–150 °C (EtOH, lit.[53] 149–150 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm 6.94 (dd, 1H, $J_{4',5'} = J_{5',6'} = 7.7$ Hz, H5′), 7.03 (d, 1H, $J_{3',4'} = 7.7$ Hz, H3′), 7.40 (d, 2H, $J = 8.5$ Hz, H3,5), 7.50 (dd, $J_{3',4'} = J_{4',5'} = 7.7$ Hz, H4′), 7.58 (d, 2H, $J = 8.5$ Hz, H2,6), 7.61 (d, 1H, $J_{trans} = 15.5$ Hz, C═CH), 7.85 (d, 1H, $J_{trans} = 15.5$ Hz, C═CH), 7.09 (d, 1H, $J_{5',6'} = 7.7$ Hz, H6′), 12.81 (s, OH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 118.9, 119.2, 120.1, 120.8, 129.6, 129.9, 130.0, 133.3, 136.8, 137.1, 144.2, 163.9 (Ar,Ph), 193.7 (C═O). Treatment of the crude 4′-chloro-2′-hydroxychalcone (1.64 g) according to method A afforded the flavonol as pale-yellow needles (597 mg, 22%), mp 198.5–200 °C (EtOH, lit.[54] 203–205 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.09 (br s, OH), 7.42 (dd, 1H, $J_{5,6} = J_{6,7} = 8.0$ Hz, H6), 7.49 (d, 2H, $J = 8.8$ Hz, H3′,5′), 7.57 (d, 1H, $J_{7,8} = 8.8$ Hz, H8), 7.71 (ddd, 1H, $J_{5,7} = 1.6$ Hz, $J_{6,7} = 8.0$, $J_{7,8} = 8.8$ Hz, H7), 8.21 (d, 2H, $J = 8.8$ Hz, H2′,6′), 8.24 (dd, 1H, $J_{5,7} = 1.6$, $J_{5,6} = 8.0$ Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ 118.9, 122.0, 125.0, 125.8, 129.2, 129.9, 130.8, 134.2, 135.8, 140.1, 144.8, 155.5 (Ar,Ph), 174.0 (C═O). LRMS (ESI^-) *m/z*: 272.0 [$C_{15}H_9ClO_3$ $(M + H)^+$ requires 272.0]. Anal. RP-HPLC $t_R = 18.4$ min (purity 99%).

4′-Trifluoromethylflavonol (6). 2-Hydroxyacetophenone (1.10 mL, 9.0 mmol) and 4-trifluoromethylbenzaldehyde (0.80 mL, 5.97 mmol) according to method A afforded the crude chalcone as yellow needles. Treatment of the crude 4-trifluoromethyl-2′-hydroxychalcone (561 mg) according to method A afforded the flavonol as pale-yellow needles (293 mg, 43% over two steps), mp 190–191 °C (ethyl acetate/petroleum spirit, lit.[55] 181 °C). ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 7.12 (br s, OH), 7.46 (t, 1H, $J_{6,7} = J_{5,6} = 8.0$ Hz, H6), 7.63 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.2$ Hz, H3′,5′), 7.81–7.74 (m, 2H, H7,8), 8.28 (d, 1H, $J_{5,6} = 8.0$ Hz, H5), 8.40 (d, 2H, $J_{2',3'} = J_{5',6'} = 8.2$ Hz, H2′,6′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 118.5, 121.3, 122.7, 124.7, 124.9, 125.4, 128.2 (Ar,Ph), 129.2 (q, 1C, J 31.9 Hz, CF_3), 134.1, 135.3, 140.1, 143.3, 154.6 (Ar), 173.2 (C═O). LRMS (ESI^-) *m/z*: 306.1 [$C_{16}H_9F_3O_3$ $(M + H)^+$ requires 306.1]. Anal. RP-HPLC $t_R = 18.8$ min (purity 99%).

4′-Methylflavonol (7). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 4-methylbenzaldehyde (1.77 mL, 15.0 mmol) were reacted for 5 h according to method A to afford 2′-hydroxy-4-methylchalcone as yellow needles (1.54 g, 65%), mp 115–117 °C (EtOH, lit.[53] 114–115 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.39 (s, 3H, CH_3), 6.93 (dd, 1H, $J_{4',5'} = 7.2$ Hz, $J_{5',6'} = 8.0$ Hz, H5′), 7.01 (d, 1H, $J_{3',4'} = 8.4$ Hz, H3′), 7.23 (d, 1H, $J = 8.0$ Hz, H3,5), 7.48 (ddd, 1H, $J_{3',4'} = 8.8$ Hz, $J_{4',5'} = 7.2$, $J_{5',6'} = 8.0$ Hz, H4′), 7.55 (d, 2H, $J = 8.0$ Hz, H2,6), 7.61 (d, 1H, $J_{trans} = 15.6$ Hz, C═CH), 7.89 (d, 1H, $J_{trans} = 15.6$ Hz, C═CH), 7.92 (dd, 1H, $J_{4',6'} = 1.6$ Hz, $J_{5',6'} = 8.0$ Hz, H6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.7 (CH_3), 119.6, 119.9, 120.0, 121.1, 129.8, 130.7, 130.9, 132.9, 137.3, 142.7, 146.6, 164.6 (Ar,Ph), 194.8 (C═O). Treatment of 2′-hydroxy-4-methylchalcone (1.00 g, 4.20 mmol) according to method A afforded the flavonol as pale-yellow needles (475 mg, 45%),

mp 190–192 °C (EtOH, lit.[54] 196–198 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.42 (s, 3H, CH_3), 7.95 (br s, OH), 7.33 (d, 2H, J = 8.0 Hz, H3′,5′), 7.40 (dd, 1H, $J_{5,6} = J_{6,7}$ = 6.7 Hz, H6), 7.77 (d, 1H, $J_{7,8}$ = 8.3 Hz, H8), 7.68 (ddd, 1H, $J_{5,7}$ = 1.8 Hz, $J_{6,7}$ = 6.7, $J_{7,8}$ = 8.3 Hz, H7), 8.24 (d, 2H, J = 8.0 Hz, H2′,6′), 8.23 (dd, 1H, $J_{5,6}$ = 7.8 Hz, $J_{5,7}$ = 1.8 Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ 21.3 (CH_3), 118.0, 121.2, 124.1, 125.0, 127.6, 128.5, 129.0, 133.1, 138.7, 139.8, 145.4, 154.8 (Ar), 173.1 (C═O). LRMS (ESI^-) m/z: 252.1 [$C_{16}H_{12}O_3$ $(M + H)^+$ requires 252.1]. Anal. RP-HPLC t_R = 17.8 min (purity 99%).

4′-*tert*-Butylflavonol (8). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 4-*tert*-butylbenzaldehyde (2.51 mL, 15.0 mmol) were reacted for 5 h according to method A to afford 4′-*tert*-butyl-2′-hydroxychalcone as yellow needles (2.07 g, 74%), mp 87.5–88 °C (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.37 (s, 9H, $C(CH_3)_3$), 6.96 (dd, 1H, $J_{4′,5′}$ = 7.0 Hz, $J_{5′,6′}$ = 7.0 Hz, H5′), 7.04 (d, 1H, $J_{3′,4′}$ = 8.5 Hz, H3′), 7.47 (d, 2H, J = 8.0 Hz, H3,5), 7.51 (ddd, $J_{3′,4′}$ = 8.5 Hz, $J_{4′,5′}$ = 7.0 Hz, $J_{4′,6′}$ = 1.5 Hz, H4′), 7.62 (d, 2H, J = 8.0 Hz, H2,6), 7.65 (d, 1H, J_{trans} = 16.0 Hz, C═CH), 7.94 (d, 1H, J_{trans} = 16.0 Hz, C═CH), 7.95 (dd, 1H, $J_{4′,6′}$ = 1.5 Hz, $J_{5′,6′}$ = 7.0 Hz, H6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.2 (CH_3), 36.1 (**C**$(CH_3)_3$), 119.9, 120.3, 121.1, 127.1, 129.7, 130.7, 132.9, 137.3, 146.6, 155.8, 164.7 (Ar, C═C), 194.9 (C═O). Treatment of 4-*tert*-butyl-2′-hydroxychalcone (561 mg, 2.00 mmol) according to method A afforded the flavonol as pale-yellow needles (365 mg, 62%), mp 175–177 °C (EtOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 1.38 (s, 9H, $C(CH_3)_3$), 6.96 (br s, OH), 7.40 (dd, 1H, $J_{5,6}$ = 5.7 Hz, $J_{6,7}$ = 5.4 Hz, H6), 7.55 (d, 2H, J = 6.4 Hz, H3′,5′), 7.57 (d, 1H, $J_{7,8}$ = 6.6 Hz, H8), 7.68 (ddd, $J_{5,7}$ =1.2 Hz, $J_{6,7}$ = 5.4 Hz, $J_{7,8}$ = 6.6 Hz, H7), 8.18 (d, 2H, J = 6.4 Hz, H2′,6′), 8.24 (dd, 1H, $J_{5,6}$ = 5.7 Hz, $J_{5,7}$ = 1.2 Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.2 (CH_3), 36.0 (**C**$(CH_3)_3$), 119.3, 121.8, 125.5, 126.5, 126.6, 128.7, 129.3, 134.5, 139.3, 146.4, 154.7, 156.4 (Ar), 174.4 (C═O). LRMS (ESI^-) m/z: 294.1 [$C_{19}H_{18}O_3$ $(M + H)^+$ requires 294.1]. Anal. RP-HPLC t_R = 21.0 min (purity 96%).

3′-Methoxyflavonol (10). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 3-methoxybenzaldehyde (1.82 mL, 15.0 mmol) were reacted overnight according to method A to afford 3-methoxy-2′-hydroxychalcone as yellow needles (1.49 g, 59%), mp 92–94 °C (EtOH, lit.[56] 94–96 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 3.87 (s, 3H, CH_3), 6.95 (dd, 1H, $J_{4′,5′} = J_{5′,6′}$ = 8.0 Hz, H5′), 6.99 (dd, 1H, $J_{4,6}$ = 2.4 Hz, $J_{5,6}$ = 8.0 Hz, H6), 7.03 (d, 1H, $J_{3′,4′}$ = 8.4 Hz, H3′), 7.17 (s, 1H, H2), 7.27 (d, 1H, $J_{4,5}$ = 8.0 Hz, H4), 7.36 (dd, 1H, $J_{4,5} = J_{5,6}$ = 8.0 Hz, H5), 7.51 (m, 1H, H4′), 7.64 (d, 1H, J_{trans} = 15.6 Hz, C═CH), 7.89 (d, 1H, J_{trans} = 15.6 Hz, C═CH), 7.92 (dd, 1H, $J_{4′,6′}$ = 1.2, $J_{5′,6′}$ = 8.0 Hz, H6′). Treatment of 3-methoxy-2′-hydroxychalcone (1.03 g, 4.00 mmol) according to method A afforded the flavonol as pale-yellow needles (530 mg, 49%), mp 131 °C (EtOH, lit.[57] 131 °C). ^{1}H NMR ($CDCl_3$, 500 MHz) δ ppm 3.92 (s, 3H, CH_3), 7.03–7.06 (m, 2H, OH, H4′), 7.42–7.49 (m, 2H, H5′,6), 7.61 (d, 1H, $J_{7,8}$ = 8.0 Hz, H8), 7.73 (ddd, 1H, $J_{6,7} = J_{7,8}$ = 8.2 Hz, $J_{5,7}$ = 1.6 Hz, H7), 7.84 (dd, 1H, $J_{2′,4′} = J_{2′,6′}$ = 2.4 Hz, H2′), 7.88 (d, 1H, $J_{5′,6′}$ = 8.0 Hz, H6′), 8.27 (dd, 1H, $J_{5,7}$ = 1.6 Hz, $J_{5,6}$ = 8.4 Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.2 (CH_3), 113.4, 115.2, 118.5, 120.0, 121.2, 124.6, 124.8, 129.6, 132.5, 133.7, 139.2, 146.8, 154.5, 159.1 (Ar,Ph), 173.0 (C═O). LRMS (ESI^-) m/z: 268.1 [$C_{16}H_{12}O_4$ $(M + H)^+$ requires 268.0]. Anal. RP-HPLC t_R = 16.7 min (purity 99%).

3′-Chloroflavonol (11). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 3-chlorobenzaldehyde (1.70 mL, 15.0 mmol) were reacted overnight according to method A to afford 3-chloro-2′-hydroxychalcone as yellow needles (0.76 g, 29%), mp 106 °C (EtOH, lit.[56] 105–106 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 6.97 (dd, 1H, $J_{4′,5′} = J_{5′,6′}$ = 8.0 Hz, H4′), 7.06 (dd, 1H, $J_{3′,5′}$ = 1.0 Hz, $J_{3′,4′}$ = 8.0 Hz, H3′), 7.38–7.41 (m, 2H, H4,5), 7.52–7.54 (m, 2H, H5′,6), 7.66 (d, 1H, J_{trans} = 15.6 Hz, C═CH), 7.67 (s, 1H, H2), 7.85 (d, 1H, J_{trans} = 15.6 Hz, C═CH), 7.93 (dd, 1H,$J_{4′,6′}$ = 1.6 Hz, $J_{5′,6′}$ = 8.0 Hz, H6′). Treatment of 3-chloro-2′-hydroxychalcone (1.03 g, 4.00 mmol) according to method A afforded the flavonol as pale-yellow needles (0.37 g, 34%), mp 158–160 °C (EtOH, lit.[58] 158–160 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 7.11 (br s, 1H, OH), 7.41–7.51 (m, 3H, H4′,5′,6), 7.62 (d, 1H, $J_{7,8}$ = 8.8 Hz, H8), 7.75 (ddd, 1H, $J_{5,7}$ = 1.6 Hz, $J_{6,7} = J_{7,8}$ = 8.0 Hz, H7), 8.19–8.28 (m, 3H, H2′,5,6′). ^{13}C NMR (100 MHz, $CDCl_3$) δ 118.5, 121.2, 124.7, 124.8, 126.0, 127.1, 129.5, 130.5, 133.3, 133.9, 139.6, 143.3, 154.6 (Ar,Ph), 173.1 (C═O). LRMS (ESI^+) m/z: 272.0 [$C_{15}H_9ClO_4$ $(M + H)^+$ requires 272.0]. Anal. RP-HPLC t_R = 18.3 min (purity 98%).

3′-Trifluoromethylflavonol (12). 2-Hydroxyacetophenone (0.50 mL, 4.15 mmol) and 3-trifluoromethylbenzaldehyde (0.40 mL, 2.99 mmol) according to method A afforded the crude 3-trifluoromethyl-2′-hydroxychalcone. Treatment of the chalcone (461 mg, 1.58 mmol) according to method A afforded the flavonol as pale-yellow needles (203 mg, 22% over two steps), mp 171–172 °C (ethyl acetate/petroleum spirit). ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm 7.45–7.51 (m, 1H, H4′), 7.79–7.89 (m, 5H, OH, H5′,7,6, 6′), 8.11 (d, 1H, $J_{7,8}$ = 8.0 Hz, H8), 8.49 (d, 1H, $J_{5,6}$ = 8.0 Hz, H5), 8.56 (s, H2′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 119.3, 122.0, 123.4, 124.7, 125.4, 125.5, 126.1, 126.9 (Ar,Ph), 130.0 (q, 1C, J = 34.3 Hz, CF_3), 131.9, 133.1, 134.7, 140.5, 144.0, 155.3 (Ar,Ph), 173.8 (C═O). LRMS (ESI^-) m/z: 306.1 [$C_{16}H_9F_3O_3(M + H)^+$ requires 306.1]. Anal. RP-HPLC t_R = 18.5 min (purity 99%).

3′-Methylflavonol (13). 2-Hydroxyacetophenone (1.20 mL, 10.0 mmol) and 3-methylbenzaldehyde (1.41 mL, 12.0 mmol) were reacted overnight according to method B to afford the crude 3-methoxy-2′-hydroxychalcone as orange needles. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.42 (s, 3H, CH_3), 6.96 (dd, 1H, $J_{4′,5′} = J_{5′,6′}$ = 8.4 Hz, H5′), 7.04 (d, 1H, $J_{3′,4′}$ = 8.4 Hz, H3′), 7.25–7.27 (m, 1H, H4), 7.34 (dd, 1H, $J_{4,5} = J_{5,6}$ = 8.4 Hz, H5), 7.47–7.51 (m, 3H, H2,4′,6), 7.66 (d, 1H, J_{trans} = 15.6 Hz, C═CH), 7.91 (d, 1H, J_{trans} = 15.6 Hz, C═CH), 7.95 (dd, 1H, $J_{4′,6′}$ = 1.6, $J_{5′,6′}$ = 8.4 Hz, H6′). Treatment of 3-methyl-2′-hydroxychalcone (1.89 g, 7.50 mmol) according to method A afforded the flavonol as pale-yellow needles (1.18 g, 47% over two steps), mp 146–148 °C (EtOH, lit.[59] 146–148 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm 2.48 (s, 3H, CH_3), 6.99 (br s, 1H, OH), 7.29–7.31 (m, 1H, H6′), 7.41–7.45 (m, 2H, H4′,5′), 7.62 (ddd, 1H, $J_{7,8}$ = 8.5 Hz, $J_{6,8}$ = 2.0 Hz, $J_{5,8}$ = 0.5 Hz, H8), 7.72 (ddd, 1H, $J_{6,7} = J_{7,8}$ = 8.5 Hz, $J_{5,7}$ = 2.0 Hz, H7), 8.07–8.08 (m, 2H, H2′,6′), 8.26 (ddd, 1H, $J_{5,8}$ = 0.5 Hz, $J_{5,7}$ = 2.0 Hz, $J_{5,6}$ = 8.0 Hz, H5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9 (CH_3), 119.1, 122.0, 125.2, 125.5, 125.7, 128.6, 129.1, 131.2, 131.9, 134.4, 138.3, 139.7, 146.0, 155.27 (Ar,Ph), 173.6 (C═O). LRMS (ESI^-) m/z: 252.1 [$C_{16}H_{12}O_3$ $(M + H)^+$ requires 252.1]. Anal. RP-HPLC t_R = 17.7 min (purity 99%).

4′-Benzyloxy-3′-methoxyflavonol. 4-Benzyloxy-3-methoxybenzaldehyde (5.74 g, 23.7 mmol) and 2-hydroxyacetophenone (2.85 mL, 23.7 mmol) according to method C gave 4′-benzyloxy-3′-methoxyflavonol as bright-yellow crystals (3.91 g, 44%), mp 187–190 °C (EtOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.99 (s, 3H, CH_3), 5.24 (s, 2H, CH_2), 7.01 (d, 1H, $J_{5′,6′}$ = 8.4 Hz, H5′), 7.32 (t, 1H, J = 7.2 Hz, H4″), 7.37–7.41 (m, 3H, H6,3″,5″), 7.46 (d, 2H, J = 7.2 Hz, H2″,6″), 7.55 (d, 1H, $J_{7,8}$ = 8.4 Hz, H8), 7.67 (ddd, 1H, $J_{6,7} \approx J_{7,8}$ = 8.4, $J_{5,7}$ = 1.6 Hz, H7), 7.81 (dd, 1H, $J_{2′,6′}$ = 2.0, $J_{5′,6′}$ = 8.4 Hz, H6′), 7.87 (d, 1H, $J_{2′,6′}$ = 2.0 Hz, H2′), 8.23 (dd, 1H, $J_{5,6}$ = 8.0, $J_{5,7}$ = 1.6 Hz, H5). ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.1 (CH_3), 70.7 (CH_2), 111.2, 113.2, 118.1, 120.6, 121.3, 123.9, 124.4, 125.3, 127.2, 128.0, 128.6, 133.3, 136.5, 137.7, 145.1, 149.3, 149.8, 155.1 (Ar,Ph), 173.0 (C═O). LRMS (ESI^+) m/z: 375.1 [$C_{23}H_{18}O_5$ $(M + H)^+$ requires 375.1]. Anal. Calcd for $C_{23}H_{18}O_5$: C, 73.79; H, 4.85. Found: C, 73.79; H, 4.79%.

4′-Benzyloxy-3′-bromoflavonol. 4-Benzyloxy-3-bromobenzaldehyde (1.00 g, 3.43 mmol) and 2-hydroxyacetophenone (0.41 mL, 3.43 mmol) according to method C gave 4′-benzyloxy-3′-methoxyflavonol as bright-yellow crystals (0.50 g, 35%), mp 204–208 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 5.25 (s, 2H, CH_2), 6.90 (br s, OH), 7.06 (d, 1H, $J_{5′,6′}$ = 8.8 Hz, H5′), 7.34 (t, 1H, J = 7.6 Hz, H4″), 7.38–7.42 (m, 3H, H6,3″,5″), 7.48 (d, 2H, J = 8.8 Hz, H2″,6″), 7.58 (d, 1H, $J_{7,8}$ = 8.0 Hz, H8), 7.70 (ddd, 1H, $J_{6,7} \approx J_{7,8}$ = 8.0 Hz, $J_{5,7}$ = 1.6 Hz, H7), 8.20–8.24 (m, 2H, H5,6′), 8.47 (d, 1H, $J_{2′,6′}$ = 2.0 Hz, H2′). ^{13}C NMR (400 MHz, DMSO-d_6) δ 70.63 (CH_2), 114.0, 114.0, 118.6, 121.7, 124.7, 125.2, 125.7, 127.7, 128.3, 128.7, 128.8, 132.4, 133.8, 136.5, 139.2,

144.1, 154.8, 155.7 (Ar,Ph), 173.2 (C═O). LRMS (ESI^+) *m/z*: 423.3 [$C_{22}H_{15}BrO_4$ (M + H)$^+$ requires 423.0]. Anal. Calcd for $C_{22}H_{15}BrO_4$: C, 62.43; H, 3.57. Found: C, 62.50; H, 3.41%.

4′-Benzyloxy-3′-trifluoromethylflavonol. 4-Benzyloxy-3-trifluoromethylbenzaldehyde (0.42 g, 1.49 mmol) and 2-hydroxyacetophenone (0.18 mL, 1.49 mmol) according to method C gave 4′-benzyloxy-3′-trifluoromethylflavonol as bright-yellow crystals (0.25 g, 41%), mp 165–166 °C (THF/petroleum spirit). ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 5.31 (s, 2H, CH_2), 6.91 (br s, OH), 7.17 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H5′), 7.34 (d, 1H, $J_{5,6}$ = 6.3 Hz, H6), 7.35–7.43 (m, 5H, H2″,3″,4″,5″,6″), 7.60 (d, 1H, $J_{7,8}$ = 7.8 Hz, H8), 7.71 (d, 1H, $J_{7,8}$ = 7.8 Hz, H7), 8.24 (d, 1H, $J_{5',6'}$ = 7.8 Hz, H6′), 8.57 (d, 1H, $J_{5,6}$ = 6.3 Hz, H5), 8.51 (s, 1H, H2′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 70.8 (CH_2), 115.1 (Ar,Ph), 118.0 (q, 1C, J = 30.0 Hz, CF_3), 119.1, 122.0, 124.3, 125.4, 125.4, 125.6, 126.9, 127.0, 127.8, 128.9, 129.3, 129.3, 133.9, 134.4, 136.7, 139.4, 144.6, 155.1, 157.4 (Ar,Ph), 173.5 (C═O).

4′-Benzyloxy-3′-methylflavonol. 4-Benzyloxy-3-methylbenzaldehyde (0.75 g, 3.32 mmol) and 2-hydroxyacetophenone (0.40 mL, 3.32 mmol) according to method C gave 4′-benzyloxy-3′-methylflavonol as bright-yellow crystals (0.50 g, 42%), mp 199–202 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.29 (s, 3H, CH_3), 5.23 (s, 2H, CH_2), 7.20 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H5′), 7.32–7.50 (m, 6H, H6,2″,3″,4″,5″,6″), 7.74–7.80 (m, 2H, H7,8), 8.05–8.10 (m, 3H, H5,2′,6′), 9.40 (br s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.4 (CH_3), 69.4 (CH_2), 111.8, 118.4, 120.4, 123.4, 124.6, 124.8, 126.2, 127.4, 127.5, 128.0, 128.6, 129.8, 133.6, 137.1, 138.3, 145.8, 154.5, 157.7 (Ar,Ph), 172.7 (C═O). LRMS (ESI^+) *m/z*: 358.4 [$C_{23}H_{18}O_5$ (M + H)$^+$ requires 358.4]. Anal. Calcd for $C_{23}H_{18}O_4$: C, 77.08; H, 5.06. Found: C, 77.05; H, 5.06%.

4′-Benzyloxy-3′-*tert*-butylflavonol. 4-Benzyloxy-3-*tert*-butylbenzaldehyde (0.57 g, 2.11 mmol) and 2-hydroxyacetophenone (0.21 mL, 2.11 mmol) according to method C gave 4′-benzyloxy-3′-*tert*-butylflavonol as brown crystals (0.47 g, 55%), mp 163–164 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.41 (s, 9H, $C(CH_3)_3$), 5.26 (s, 2H, CH_2), 7.28 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H5′), 7.36 (t, 1H, J = 7.2 Hz, H4″), 7.42–7.48 (m, 3H, H6,3″,5″), 7.53 (d, 2H, J = 7.6 Hz, H2″,6″), 7.75 (d, 1H, $J_{7,8}$ = 8.4 Hz, H8), 7.79 (ddd, 1H, $J_{6,7} \approx J_{7,8}$ = 8.4 Hz, $J_{5,7}$ = 1.2 Hz, H7), 8.07–8.11 (m, 2H, H5,6′), 8.22 (d, 1H, $J_{2',6'}$ = 2.4 Hz, H2′). ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.5 (**C**$(CH_3)_3$), 34.8 (**C**$(CH_3)_3$), 69.8 (CH_2), 112.8, 118.4, 121.4, 123.2, 124.5, 124.8, 126.2, 127.4, 127.8, 128.0, 128.6, 133.5, 136.8, 137.2, 138.2, 146.1, 154.4, 158.3 (Ar, Ph), 172.6 (C═O). Anal. Calcd for $C_{26}H_{24}O_4$: C, 77.98; H, 6.04. Found: C, 78.01; H, 6.09%.

General Procedure for Debenzylation of 4′-Benzyloxy-3′-substituted Flavonols. A suspension of protected flavonol (1 mmol) in HCl (36%, 25 mL) and acetic acid (100%, 25 mL) was heated at 100 °C for 2 h. The solvent was evaporated under reduced pressure, and the product was recrystallized from the solvents indicated.

4′-Hydroxy-3′-methoxyflavonol (14). 4′-Benzyloxy-3′-methoxyflavonol (1.00 g, 2.67 mmol) gave a yellow solid after recrystallization (0.48 g, 63%), mp 249–251 °C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.79 (s, 3H, CH_3), 6.84 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H5′), 7.22 (t, 1H, $J_{6,7} \approx J_{5,6}$ = 8.4 Hz, H6), 7.41 (d, 1H, $J_{7,8}$ = 8.4 Hz, H8), 7.51 (t, 1H, $J_{6,7} \approx J_{7,8}$ = 8.4 Hz, H7), 7.63 (dd, 1H, $J_{2',6'}$ = 2.0, $J_{5',6'}$ = 8.8 Hz, H6′), 7.68 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H2′), 8.03 (d, 1H, $J_{5,6}$ = 8.4 Hz, H5), 8.40 (br s, OH). ^{13}C NMR (100 MHz, DMSO-d_6 + $CDCl_3$) δ 55.8 (CH_3), 111.6, 115.4, 118.0, 121.3, 121.8, 122.4, 124.0, 124.8, 132.9, 138.0, 145.8, 147.3, 148.7, 154.5 (Ar,Ph), 172.6 (C═O). Anal. Calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.62; H, 4.23%. LRMS (ESI^+) *m/z*: 284.3 [$C_{16}H_{12}O_5$ (M + H)$^+$ requires 284.3]. Anal. RP-HPLC t_R = 13.5 min (purity 96%).

3′-Bromo-4′-hydroxyflavonol (15). 4′-Benzyloxy-3′-bromoflavonol (0.39 g, 0.92 mmol) gave a brown solid after recrystallization (0.15 g, 50%), mp 252–254 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.13 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H5′), 7.46 (t, 1H, $J_{6,7} \approx J_{5,6}$ = 7.2 Hz, H6), 7.51–7.80 (m, 2H, H7,8), 8.09 (d, 2H, H5,6′), 8.40 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H2′), 9.60 (br s, OH), 11.05 (br s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 109.5, 116.2, 118.4, 121.3, 123.6, 124.5, 124.7, 128.4, 132.2, 133.6, 138.2, 144.3, 154.4, 155.6 (Ar,Ph), 172.6 (C═O). Anal. Calcd for $C_{15}H_9BrO_4$: C, 54.08; H, 2.72. Found: C, 54.04; H, 2.80%. LRMS (ESI^+) *m/z*: 333.1 [$C_{15}H_9BrO_4$ (M + H)$^+$ requires 333.1]. Anal. RP-HPLC t_R = 15.2 min (purity 97%).

4′-Hydroxy-3′-trifluoromethylflavonol (16). 4′-Benzyloxy-3′-trifluoromethylflavonol (0.20 g, 0.49 mmol) gave a brown solid after recrystallization (100 mg, 64%), mp 205–206 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.20 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H5′), 7.46 (t, 1H, $J_{6,7} \approx J_{5,6}$ = 7.6 Hz, H6), 7.76–7.77 (m, 2H, H7,8), 8.08 (d, 1H, $J_{5',6'}$ = 8.8 Hz, H6′), 8.29 (d, 1H, $J_{5,6}$ = 7.6 Hz, H5), 8.43 (s, 1H, H2′), 9.62 (br s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 115.6 (CF_3, 1C, J = 29.2 Hz), 117.3, 118.4, 121.3, 121.8, 122.5, 124.6, 124.7, 126.4, 132.9, 133.6, 138.3, 144.5, 154.4, 157.2 (Ar, Ph), 172.7 (C═O). LRMS (ESI^+) *m/z*: 322.2 [$C_{16}H_9F_3O_4$ (M + H)$^+$ requires 322.2]. Anal. RP-HPLC t_R = 15.8 min (purity 99%).

4′-Hydroxy-3′-methylflavonol (17). 4′-Benzyloxy-3′-methylflavonol (0.39 g, 1.08 mmol) gave a brown solid after recrystallization (0.15 g, 52%), mp 255–257 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.22 (s, 3H, CH_3), 6.95 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H5′), 7.45 (t, 1H, $J_{6,7} \approx J_{5,6}$ = 8.1 Hz, H6), 7.73–7.80 (m, 2H, H7,8), 7.96 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H6′), 8.00 (s, 1H, H2′), 8.09 (d, 1H, $J_{5,6}$ = 8.1 Hz, H5), 9.28 (br s, OH), 10.02 (br s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.8 (CH_3), 115.3, 118.9, 122.0, 122.5, 124.8, 125.1, 125.4, 127.8, 130.9, 134.0, 138.5, 146.9, 155.1, 158.0 (Ar,Ph), 173.1 (C═O). Anal. Calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.59; H, 4.58%. LRMS (ESI^+) *m/z*: 268.2 [$C_{16}H_{12}O_4$ (M + H)$^+$ requires 268.2]. Anal. RP-HPLC t_R = 14.6 min (purity 98%).

4′-Hydroxy-3′-*tert*-butylflavonol (18). 4′-Benzyloxy-3′-*tert*-butylflavonol (0.39 g, 0.97 mmol) gave a brown solid after recrystallization (0.052 g, 17%), mp 203–207 °C (THF/petroleum spirit). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9H, $C(CH_3)_3$), 6.96 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H5′), 7.45 (t, 1H, $J_{6,7} \approx J_{5,6}$ = 8.0 Hz, H6), 7.73 (d, 1H, $J_{7,8}$ = 8.0 Hz, H8), 7.78 (ddd, 1H, $J_{7,8}$ = 8.0 Hz, $J_{5,7}$ = 1.6 Hz, H7), 7.83 (d, 1H, $J_{5',6'}$ = 8.4 Hz, $J_{2',6'}$ = 2.4 Hz, H6′), 8.00 (dd, 1H, $J_{5,6}$ = 8.0 Hz, $J_{5,7}$ = 1.6 Hz, H5), 8.12 (dd, 1H, $J_{2',6'}$ = 2.4 Hz, H2′), 9.27 (br s, OH), 10.11 (br s, OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.3 (C(**C**$H_3)_3$), 34.6 (**C**$(CH_3)_3$), 116.3, 118.3, 121.4, 121.6, 124.5, 124.7, 126.6, 133.4, 135.3, 137.7, 146.7, 154.4, 157.8 (Ar,Ph), 172.5 (C═O). HRMS (ESI^+) *m/z*: 333.1095 [$C_{19}H_{18}NaO_4$ (M + Na)$^+$ requires 333.1103]. Anal. RP-HPLC t_R = 17.9 min (purity 97%).

Acknowledgment. This work was supported by the Shepherd Foundation, the Australian Research Council, and by University of Melbourne MIFRS and MIRS Scholarships to C.X.Q.

Supporting Information Available: Table indicating results from HPLC purity analysis and/or combustion analysis of compounds **1**−**18**, HPLC traces for compounds **1**−**18**, and ^{1}H and ^{13}C NMR spectra for compounds **4**–**8** and **10**–**18**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *The World Health Report 2002*; WHO: Geneva, 2005; pp 186–191; www.who.int/whr/2002/en/whr2002_annex2.pdf.

(2) Park, J. L.; Lucchesi, B. R. Mechanisms of myocardial reperfusion injury. *Ann. Thorac. Surg.* **1999**, *68*, 1905–1912.

(3) Carden, D. L.; Granger, D. N. Pathophysiology of ischaemia-reperfusion injury. *J. Pathol.* **2000**, *190*, 255–266.

(4) Moens, A. L.; Claeys, M. J.; Timmermans, J. P.; Vrints, C. J. Myocardial ischemia/reperfusion-injury, a clinical view on a complex pathophysiological process. *Int. J. Cardiol.* **2005**, *100*, 179–190.

(5) Verma, S.; Fedak, P. W. M.; Weisel, R. D.; Butany, J.; Rao, V.; et al. Fundamentals of reperfusion injury for the clinical cardiologist. *Circulation* **2002**, *105*, 2332–2336.

(6) Jennings, R. B.; Steenbergen, C., Jr.; Reimer, K. A. Myocardial Ischaemia and Reperfusion. In *Cardiovascular Pathology: Clinicopathologic Correlations and Pathogenetic Mechanisms*; Schoen, F. J., Gimbrone, M. A., Jr.; Williams and Wilkins: Baltimore, MD, 1995; pp 47−80,

(7) Maxwell, S. R. J.; Lip, G. Y. H. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. *Int. J. Cardiol.* **1997**, *58*, 95–117.
(8) Wang, Q. D.; Pernow, J.; Sjoquist, P. O.; Ryden, L. Pharmacological possibilities for protection against myocardial reperfusion injury. *Cardiovasc. Res.* **2002**, *55*, 25–37.
(9) Balkau, B.; Eschwege, F.; Eschwege, E. Ischemic heart disease and alcohol-related causes of death: a view of the French paradox. *Ann. Epidemiol.* **1997**, *7*, 490–497.
(10) Renaud, S.; de Lorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523–1526.
(11) Hertog, M. G.; Feskens, E. J.; Hollman, P. C.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **1993**, *342*, 1007–1011.
(12) Sampson, L.; Rimm, E.; Hollman, P. C.; de Vries, J. H.; Katan, M. B. Flavonol and flavone intakes in U.S. health professionals. *J. Am. Diet. Assoc.* **2002**, *102*, 1414–1420.
(13) Wang, S.; Dusting, G. J.; May, C. N.; Woodman, O. L. 3′,4′-Dihydroxyflavonol reduces infarct size and injury associated with myocardial ischaemia and reperfusion in sheep. *Br. J. Pharmacol.* **2004**, *142*, 443–452.
(14) Chan, E. C.; Drummond, G. R.; Woodman, O. L. 3′,4′-Dihydroxyflavonol enhances nitric oxide bioavailability and improves vascular function after ischemia and reperfusion injury in the rat. *J. Cardiovasc. Pharmacol.* **2003**, *42*, 727–735.
(15) Poli, G.; Leonarduzzi, G.; Biasi, F.; Chiarpotto, E. Oxidative stress and cell signalling. *Curr. Med. Chem.* **2004**, *11*, 1163–1182.
(16) Chan, E. C.; Pannangpetch, P.; Woodman, O. L. Relaxation to flavones and flavonols in rat isolated thoracic aorta: mechanism of action and structure−activity relationships. *J. Cardiovasc. Pharmacol.* **2000**, *35*, 326–333.
(17) Duarte, J.; Perez Vizcaino, F.; Utrilla, P.; Jimenez, J.; Tamargo, J.; et al. Vasodilatory effects of flavonoids in rat aortic smooth muscle. Structure−activity relationships. *Gen. Pharmacol.* **1993**, *24*, 857–862.
(18) Woodman, O. L.; Chan, E. Vascular and anti-oxidant actions of flavonols and flavones. *Clin. Exp. Pharmacol. Physiol.* **2004**, *31*, 786–790.
(19) Herrera, M. D.; Zarzuelo, A.; Jimenez, J.; Marhuenda, E.; Duarte, J. Effects of flavonoids on rat aortic smooth muscle contractility: structure−activity relationships. *Gen. Pharmacol.* **1996**, *27*, 273–277.
(20) Ratty, A. K.; Das, N. P. Effects of flavonoids on nonenzymatic lipid peroxidation: structure−activity relationship. *Biochem. Med. Metab. Biol.* **1988**, *39*, 69–79.
(21) Dugas, A. J., Jr.; Castaneda-Acosta, J.; Bonin, G. C.; Price, K. L.; Fischer, N. H.; et al. Evaluation of the total peroxyl radical-scavenging capacity of flavonoids: structure−activity relationships. *J. Nat. Prod.* **2000**, *63*, 327–331.
(22) Woodman, O. L.; Meeker, W. F.; Boujaoude, M. Vasorelaxant and antioxidant activity of flavonols and flavones: structure−activity relationships. *J. Cardiovasc. Pharmacol.* **2005**, *46*, 302–309.
(23) Raval, A. A.; Shah, N. M. Studies in chalcones and related compounds derived from 2-hydroxy-5-acetamiacetophenone: Part I. Synthesis of 2-hydroxy-5′-acetamionchalcones and 6-aminoflavanones. *J. Org. Chem.* **1956**, *21*, 1408–1411.
(24) Algar, J.; Flynn, J. P. A new method for the synthesis of flavonols. *Proc. R. Ir. Acad.* **1934**, *42B*, 1–8.
(25) Oyamada, B. A new general method for the synthesis of the derivatives of flavonols. *J. Chem. Soc. Jpn.* **1934**, *55*, 1256–1261.
(26) Reimer, K.; Tiemann, F. Upon the reaction of chloroform with alkaline phenolate. *Ber.* **1876**, *9*, 1268.
(27) Birchall, G. R.; Harney, D. W. G.; Forsyth, B. A. Substituted Benzylidenemalononitriles. Australian Patent 75-7617337, 1979.
(28) Pouysegu, L.; Avellan, A. V.; Quideau, S. Iodine(III)-mediated generation of nitrogen-tethered orthoquinol acetates for the construction of oxygenated indole, quinoline, and phenanthridine alkaloid motifs. *J. Org. Chem.* **2002**, *67*, 3425–3436.
(29) Van Acker, F. A.; Hageman, J. A.; Haenen, G. R.; Van Der Vijgh, W. J.; Bast, A.; et al. Synthesis of novel 3,7-substituted-2-(3′,4′-dihydroxyphenyl)flavones with improved antioxidant activity. *J. Med. Chem.* **2000**, *43*, 3752–3760.
(30) Flesch, M.; Schwarz, A.; Bohm, M. Effects of red and white wine on endothelium-dependent vasorelaxation of rat aorta and human coronary arteries. *Am. J. Physiol.* **1998**, *275*, H1183–H1190.
(31) Fitzpatrick, D. F.; Hirschfield, S. L.; Coffey, R. G. Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am. J. Physiol.* **1993**, *265*, H774–H778.
(32) Chen, C. K.; Pace-Asciak, C. R. Vasorelaxing activity of resveratrol and quercetin in isolated rat aorta. *Gen. Pharmacol.* **1996**, *27*, 363–366.
(33) Scholz, E. P.; Zitron, E.; Kiesecker, C.; Luck, S.; Thomas, D.; et al. Inhibition of cardiac HERG channels by grapefruit flavonoid naringenin: implications for the influence of dietary compounds on cardiac repolarisation. *Naunyn-Schmeideberg's Arch. Pharmacol.* **2005**, *371*, 516–525.
(34) Dambros, M.; de Jongh, R.; van Koeveringe, G. A.; van Deutekom, M.; De Mey, J. G. R.; et al. Multiple-signaling pathways are involved in the inhibitory effects of galangin on urinary bladder contractility. *Neurourol. Urodynam.* **2005**, *24*, 369–373.
(35) Calderone, V.; Chericoni, S.; Martinelli, C.; Testai, L.; Nardi, A.; et al. Vasorelaxing effects of flavonoids: investigation on the possible involvement of potassium channels. *Naunyn-Schmeideberg's Archi. Pharmacol.* **2004**, *370*, 290–298.
(36) Koh, D. S.; Reid, G.; Vogel, W. Effect of the flavoid phloretin on Ca^{2+}-activated K^+ channels in myelinated nerve-fibers of *Xenopus-laevis*. *Neurosci. Lett.* **1994**, *165*, 167–170.
(37) Rouzaireduboi s, B.; Gerard, V.; Dubois, J. M. Involvement of K^+ channels in the quercetin-induced inhibition of neuroblastoma cell-growth. *Pfluegers Arch.* **1993**, *423*, 202–205.
(38) Leal, L.; Costa, M. F.; Marcia, P. D.; Barroso, V. M.; Silveira, E. R.; et al. Mechanisms underlying the relaxation induced by isokaempferide from *Amburana cearensis* in the guinea-pig isolated trachea. *Life Sci.* **2006**, *79*, 98–104.
(39) Petrault, O.; Bastide, M.; Cotelle, N.; Gele, P.; Gautier, S.; et al. The neuroprotective effect of the antioxidant flavonoid derivate di-*tert*-butylhydroxyphenyl is parallel to the preventive effect on post-ischemic Kir2.x impairment but not to post-ischemic endothelial dysfunction. *Naunyn-Schmeideberg's Arch. Pharmacol.* **2004**, *370*, 395–403.
(40) Munzel, T.; Afanas'ev, I. B.; Kleschyov, A. L.; Harrison, D. G. Detection of superoxide in vascular tissue. *Arterioscler., Thromb., Vasc. Biol.* **2002**, *22*, 1761–1768.
(41) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749–760.
(42) Burda, S.; Oleszek, W. Antioxidant and antiradical activities of flavonoids. *J. Agric. Food Chem.* **2001**, *49*, 2774–2779.
(43) Bors, W.; Heller, W.; Michel, C.; Saran, M. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343–355.
(44) Cotelle, N. Role of flavonoids in oxidative stress. *Curr. Top. Med. Chem.* **2001**, *1*, 569–590.
(45) Kaur, I. P.; Geetha, T. Screening methods for antioxidants−a review. *Mini-Rev. Med. Chem.* **2006**, *6*, 305–312.
(46) Sim, G. S.; Lee, B. C.; Cho, H. S.; Lee, J. W.; Kim, J. H.; et al. Structure activity relationship of antioxidative property of flavonoids and inhibitory effect on matrix metalloproteinase activity in UVA-irradiated human dermal fibroblast. *Arch. Pharmacal Res.* **2007**, *30*, 290–298.
(47) Jackson, T. S.; Xu, A.; Vita, J. A.; Keaney, J. F., Jr. Ascorbate prevents the interaction of superoxide and nitric oxide only at very high physiological concentrations. *Circ. Res.* **1998**, *83*, 916–922.
(48) Gotoh, N.; Niki, E. Rates of interactions of superoxide with vitamin E, vitamin C and related compounds as measured by chemiluminescence. *Biochim. Biophys. Acta* **1992**, *1115*, 201–207.
(49) Tournaire, C.; Croux, S.; Maurette, M.-T.; Beck, I.; Hocquaux, M.; et al. Antioxidant activity of flavonoids: efficiency of singlet oxygen (1[Delta]g) quenching. *J. Photochem. Photobiol., B* **1993**, *19*, 205–215.
(50) Robak, J.; Gryglewski, R. J. Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol.* **1988**, *37*, 837–841.
(51) Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.
(52) Fuji, K.; Ichikawa, K.; Node, M.; Fujita, E. Hard acid and soft nucleophile system. New efficient method for removal of benzyl protecting group. *J. Org. Chem.* **1979**, *44*, 1661–1664.
(53) Liu, M.; Wilairat, P.; Croft, S. L.; Tan, A. L.; Go, M. L. Structure−activity relationships of antileishmanial and antimalarial chalcones. *Bioorg. Med. Chem.* **2003**, *11*, 2729–2738.
(54) Smith, M. A.; Neumann, R. M.; Webb, R. A. A modification of Algar−Flynn−Oyamada preparation of flavonols. *J. Heterocycl. Chem.* **1968**, *5*, 425–426.
(55) Sriastava, K.; SuKhwal, S.; Ashawa, A.; L, V. B. Synthesis and reactions of some chalcones containing a $-CF_3$ group. *J. Indian Chem. Soc.* **1996**, *74*, 573–574.
(56) Batchelor, J. F.; Bauer, D. J.; Hodson, H. F.; Selway, J. W. T.; Young, D. A. B. Amino-Substituted Flavans Useful as Anti-Viral Agents (U.K.). Cont-in-part of U.S. Ser. No. 127,465, 1984; 13 pp.
(57) Shaw, B. L.; Simpson, T. H. Chelate systems. III. Infrared spectra of flavanones and flavones. *J. Chem. Soc. Jpn.* **1955**, 655–658.
(58) Chen, F. C.; Shu, H. S. Synthesis of haloflavonols. *Taiwan Yaoxue Zazhi* **1953**, *5*, 49–52.
(59) Strandjord, A. J. G.; Smith, D. E.; Barbara, P. F. Structural effects on the proton-transfer kinetics of 3-hydroxyflavones. *J. Chem. Phys.* **1985**, *89*, 2362–2366.

JM070352H