JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 653–668

Received August 2, 2000
Accepted December 14, 2000
ISSN 0022-3573

Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin-induced diabetic rat tissues

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Acknowledgements and funding: The authors are grateful to the ONO Co. in Japan and Professor K. Ohuchi at Tohoku University for epalrestat standard material support. This work was supported by grant No. 981-0716-125-2 from the Basic Research program of the Korea Science & Engineering Foundation and partly supported by the Brain Korea 21 Project.

Abstract

Aldose reductase, the key enzyme of the polyol pathway, and oxidative stress are known to play important roles in the complications of diabetes. A drug with potent inhibition of aldose reductase and oxidative stress, therefore, would be a most promising drug for the prevention of diabetic complications. The purpose of this study was to develop new compounds with these dual-effects through synthesis of chalcone derivatives and by examining the structure–activity relationships on the inhibition of rat lens aldose reductase as well as on antioxidant effects. A series of 35 flavonoid derivatives were synthesized by Winget's condensation, oxidation, and reduction of appropriate acetophenones with appropriate benzaldehydes. The inhibitory activity of these derivatives on rat lens aldose reductase and their antioxidant effects, measured using Cu^{2+} chelation and radical scavenging activities on 1,1-diphenyl-picrylhydrazyl in-vitro, were evaluated. Their effect on sorbitol accumulation in the red blood cells, lenses and sciatic nerves of streptozotocin-induced diabetic rats was also estimated. Among the new flavonoid derivatives synthesized, those with the 2′,4′-dihydroxyl groups in the A ring such as 2,4,2′,4′-tetrahydroxychalcone (**22**), 2,2′,4′-trihydroxychalcone (**11**), 2′,4′-dihydroxy-2,4-dimethylchalcone (**21**) and 3,4,2′,4′-tetrahydroxychalcone (**18**) were found to possess the highest rat lens aldose reductase inhibitory activity in-vitro, their IC50 values (concentration of inhibitors giving 50% inhibition of enzyme activity) being 1.6×10^{-7}, 3.8×10^{-7}, 4.0×10^{-7} and 4.6×10^{-7} M, respectively. All of the chalcones tested except **3**, **18**, **23** with ***o***-dihydroxy or hydroquinone moiety showed a weak free radical scavenging activity. In the in-vivo experiments, however, compound **18** with ***o***-dihydroxy moiety in the B ring showed the strongest inhibitory activity in the accumulation of sorbitol in the tissues. It also showed the strongest activity in transition metal chelation and free radical scavenging activity. Of the 35 4,2′-dihydroxyl and 2′,4′-dihydroxyl derivatives of flavonoid synthesized, including chalcone, flavone, flavanone, flavonol and dihydrochalcone, some chalcone derivatives synthesized were found to possess aldose reductase inhibition and antioxidant activities in-vitro as well as inhibition in the accumulation of sorbitol in the tissues in-vivo. 3, 4, 2′, 4′-Tetrahydroxychalcone (**18**, butein) was the most promising compound for the prevention or treatment of diabetic complications.

Introduction

Aldose reductase, the key enzyme of the polyol pathway, has been demonstrated to play an important role in the aetiology of the complications of diabetes such as neuropathy, cataract formation, nephropathy and retinopathy. It has been shown that diabetic complications can be improved by the inhibitors of aldose reductase

in experimental animals (Beyer-Mears & Cruz 1985) as well as in clinical trials (Handelsman & Turtle 1981).

There is evidence to show that diabetes is associated with increased oxidative stress. However, the source of this oxidative stress remains unclear. It has been suggested that glucose autoxidation and nonenzymatic glycation, together termed glycoxidation, are the major contributors to the increase in free radicals in the diabetic lens (Wolff & Dean 1987; Srivastata et al 1996). Endogenous protection against glycoxidation by the glutathione redox cycle is also compromised by the competing reduced nicotinamide dinucleotide phosphate (NADPH) requirement of elevated polyol pathway flux. Aldose reductase reduction of glucose to sorbitol probably contributes to oxidative stress by depleting its cofactor NADPH, which is also required for the regeneration of reduced glutathione (Lee & Chung 1999). Aldose reductase inhibitors possessing antioxidant activity would therefore seem to be desirable, because it has been suggested that increased oxidant production by metal-catalysed glucose oxidation may be an important mechanism in activation of aldose reductase (Ou et al 1996). In addition, several recent reports demonstrate induction of a large number of genes including aldose reductase on exposure of cells to oxidative stress (Spycher et al 1997; Carper et al 1999).

Naturally occurring and synthetic flavonoids have been shown to exhibit antioxidant activity (Larson 1988) as well as inhibitory effects on aldose reductase (Shin et al 1994, 1995; Iwata et al 1999). Moreover, Aida et al (1990) reported that isoliquiritigenin (4,2′,4′-trihydroxychalcone) inhibited rat lens aldose reductase. Iwata et al (1999) reported that 2,4,2′,4′-tetrahydroxychalcone and 2,2′,4′-trihydroxychalcone showed potent inhibitory activity of human aldose reductase with IC50 values (concentration of inhibitors giving 50% inhibition of enzyme activity) of 7.4×10^{-9} and 1.6×10^{-7} M, respectively.

In this study, we have evaluated new compounds with dual-effects through synthesis of flavonoid derivatives and by examining their structure–activity relationships on the inhibition of rat lens aldose reductase as well as on antioxidant effects. The effects of the synthetic derivatives on sorbitol accumulation in the tissues of diabetic rats have been investigated also.

Materials and Methods

General

Melting points (mp) were measured by a Mitamura-Riken melting point apparatus and were uncorrected. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance spectra were determined in dimethyl sulfoxide-d_6 and acetone-d_6 on a Varian Gemini 2000 spectrometer at 300 and 75 MHz, respectively. Chemical shifts were reported in value from internal tetramethylsilane. Coupling constants were expressed in Hz. Infrared (IR) spectra were measured by Jasco FT/IR-5000. High-resolution mass spectra (HRMS) were taken on a Jeol JMS-AX500WA mass spectrometer. Mass spectra (MS) were taken on a HP 5989B mass spectrometer. Cu^{2+} contents were measured on a Hitachi (Zeeman-8000) graphite furnace atomic absorption spectrophotometer (GF-AAS). Biological activities were measured on a Hitachi U-3210 spectrophotometer and Hitachi F-2000 fluorescence spectrophotometer. Thin-layer chromatographic separations were performed with silica gel 60 F_{254} plates (Merck Art. 5715) and silica gel 60 (Merck Art. 7734; 70–230 mesh), respectively. Visualization was accomplished with UV light.

Materials

Epalrestat ((E)-3-carboxymethyl-5-[(2E)-methyl-3-phenyl-propenylidene rhodanine]) was a generous gift from Ono Pharmaceutical Co., Ltd (Japan). Reduced nicotinamide dinucleotide phosphate (NADPH), sorbitol dehydrogenase, DL-glyceraldehyde, D-sorbitol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), streptozotocin, substituted acetophenones and benzaldehydes were purchased from Aldrich Inc. (USA). Unless otherwise noted, all commercially available materials were used without further purification.

Preparation of rat lens aldose reductase

Crude rat lens aldose reductase was prepared as follows: lenses were removed from Sprague-Dawley rats (250 ~ 280 g) and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to Hayman & Kinoshita (1965) and then partially purified according to Inagaki et al (1982). Partially purified enzyme with a specific activity of 6.5 mU mg^{-1} was routinely used to test enzyme inhibition. The partially purified material was separated into 1.0-mL portions and stored at −40°C.

Measurements of rat lens aldose reductase activity

Rat lens aldose reductase activities were assayed spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm over a 4-min period with

DL-glyceraldehyde as a substrate (Sato & Kador 1990). Each 1.0-mL cuvette contained equal units of enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH with or without 10 mM substrate and inhibitor. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC50) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the remaining activity.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging effect

The DPPH radical-scavenging effect was determined according to the method used by Hatano et al (1989). In microwells, 100 μL of an aqueous solution of the sample (control: 100 μL distilled water) was added to an ethanol solution of DPPH (60 μM). Seven gradual different concentrations (5–250 μM) were prepared for each sample. After mixing gently and leaving to stand for 30 min at room temperature, the optical density was determined using a microplate reader at 517 nm. The antioxidant activity of each sample was expressed in terms of IC50 (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from log-dose inhibition curves.

In-vivo experiments (sorbitol accumulation in streptozotocin-induced diabetic rat tissue)

Diabetes was induced in male Sprague-Dawley rats (220 ~ 250 g) by a single intraperitoneal injection of streptozotocin (80 mg kg^{-1}) in sodium citrate buffer (pH 4.5). Control rats were injected with the vehicle only. The animals were fed standard lab chow and water was freely available. Two weeks after the induction of diabetes, the animals were administered either compounds or vehicle alone via an intragastric tube, twice a day at a dose of 75 mg kg^{-1}/day for two weeks. Compounds were suspended in saline containing 0.5% carmellose. The animals were then killed under ether anaesthesia. The contents of sorbitol in the rat red blood cells (RBC), sciatic nerves, and lenses were determined enzymatically (Aida et al 1988).

Measurements of blood glucose

In each case a 200-μL blood sample was collected and estimated for glucose by the *o*-toluidine method (Dubowski 1962).

Cu^{2+} partitioning into *n*-octanol

Stock solutions of compounds (all at 2.5 mM) were prepared in PBS in the presence of 1 mM $CuSO_4$. Samples (1 mL) of the compounds (vehicle alone, EDTA and samples) were mixed with *n*-octanol (2 mL) and were shaken for 10 min. After centrifugation at 1000 *g*, Cu^{2+} content in the organic layer was analysed by atomic absorption spectrophotometer (Ou et al 1996).

Statistical analysis

The data are shown as the mean ± s.e.m. Significant difference was calculated by Student's *t*-test and linear regression was analysed by the least-squares method.

General procedure for the preparation of precursors

*2′,4′-Bis(methoxymethoxy)acetophenone (**A**)*

2′,4′-Dihydroxyacetophenone (12.5 g, 82.0 mmol), anhydrous potassium carbonate (76 g), acetone (300 mL) and excess chloromethylmethyl ether (7.9 mL, 102.5 mmol) were refluxed for 30 min, filtered and concentrated. The crude product, 2′-hydroxy-4′-methoxymethoxyacetophenone (**a**), was purified by column chromatography (silica gel, eluted with 20% ethyl acetate in hexane) giving a pale yellow oil in 95.6% yield. ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.58 (dd, J = 2.1, 9.0, 1H), 6.44 (dd, J = 2.1, 9.0, 1H), 6.35 (d, J = 9.0, 1H), 6.02 (s, 1H), 3.24 (s, 1H), 2.55 (s, 1H).

A sample of **a** (10.0 g, 51.0 mmol) was added to a NaH (60% in oil, 3.12 g, 130.1 mmol) suspension in 200 mL THF. This solution was stirred for 30 min in an ice bath, and then chloromethylmethyl ether (4.8 mL, 64.0 mmol) was added. After being stirred for 30 min, the reaction was quenched by the addition of 20 mL distilled water. The mixture was diluted with ethyl acetate, and then the organic phase was washed with 5% aqueous hydrochloric acid, water and brine, dried over anhydrous magnesium sulfate, filtered and concentrated. The crude product was purified by column chromatography (silica gel, eluted with 10% ethyl acetate in hexane) giving a yellow oil in 98.5% yield. ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.59 (dd, J = 2.1, 9.0, 1H), 6.52 (dd, J = 2.1, 9.0, 1H), 6.47 (d, J = 9.0, 1H), 6.02 (s,1H), 5.89 (s, 1H), 3.24 (s, 1H), 3.17 (s, 1H), 2.55 (s, 1H).

4-(*Methoxymethoxy*)*benzaldehyde* (***B***)

4-Hydroxybenzaldehyde (**b**) (10 g, 82.0 mmol), anhydrous potassium carbonate (76 g), acetone (300 mL)

and excess chloromethylmethyl ether (7.9 mL, 102.5 mmol) were refluxed for 30 min, filtered and concentrated. The crude product, 4-(methoxymethoxy)benzaldehyde (**B**), was purified by column chromatography (silica gel, eluted with 10% ethyl acetate in hexane) giving a yellow oil in 98.8% yield. ^{1}H NMR (acetone-d_6, 300 MHz) δ 9.87 (s, 1H), 7.70 (dd, J = 2.4, 8.7, 2H), 6.96 (dd, J = 2.4, 8.7, 2H), 6.02 (s, 2H), 3.24 (s, 3H).

General procedure for the preparation of 2′-hydroxychalcone (1–24)

We synthesized 24 chalcones using Winget's condensation of appropriate acetophenones with appropriate aromatic aldehyde (Winget et al 1969). For the synthesis of compounds possessing a 2′,4′- or 3,4-dihydroxyl group, the hydroxyl group of the component was protected with methoxymethyl using chloromethylmethyl ether (Figure 1) (Rall et al 1976).

*2′,4′-Bis(methoxymethoxy)chalcone (**10c**)*
To a mixture of 2′,4′-bis(methoxymethoxy)acetophenone (**A**) (2.0 g, 9.4 mmol) and 50% aqueous potassium hydroxide solution (5.3 mL, as KOH: 2.64 g, 47.0 mmol) in 20 mL 95% ethanol was added benzaldehyde (1.72 g, 9.43 mmol). After being stirred for 5 h at room temperature, the reaction was quenched with distilled water. The resulting solution was acidified with 1 M HCl, and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous magnesium sulfate and concentrated. The compound **10c** was obtained in 91.8% isolated yield. A single crystallization from ethyl acetate–hexane gave the target material as a pale yellow plate. ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.21 (d, J = 8.7, 1H), 7.97 (d, J = 15.3, 1H), 7.87 (d, J = 15.3, 1H), 7.85 (dd, J = 2.4, 9.0, 2H), 7.4–7.5 (m, 1H) 6.64 (dd, J = 2.4, 8.7, 1H), 6.62 (dd, J = 2.1, 9.0, 2H), 6.57 (d, J = 8.7, 1H).

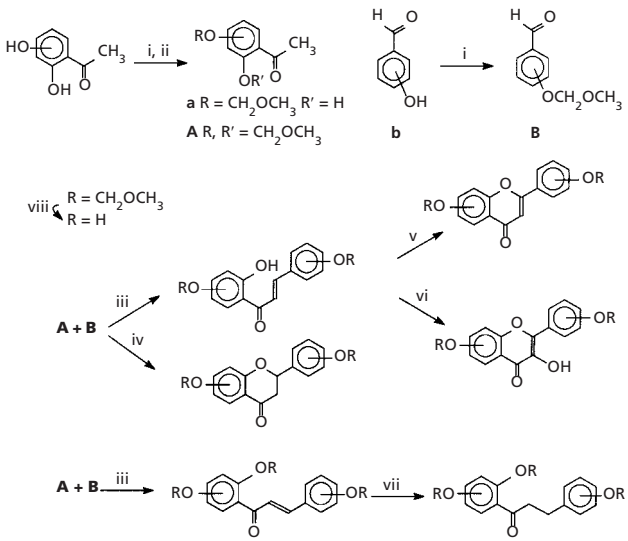


Figure 1 Preparation of hydroxylated flavonoids. i. CH_3OCH_2Cl, K_2CO_3, acetone, reflux. ii. CH_3OCH_2Cl, THF, NaH, 0 °C. iii. KOH, 95% ethyl hydroxide. iv. SiO_2, H_3BO_3, piperidine, DMF. v. I_2, DMSO, reflux. vi. Methyl hydroxide, 30% H_2O_2, NaOH. vii. ethyl hydroxide, 10% Pd/C. viii. 3 M HCl, methanol, reflux.

*2′,4′-Dihydroxychalcone (**10**)*
To a stirred solution of **10c** (2.83 g, 8.63 mmol) in 20 mL methanol was added 5 mL 3 M HCl. After 3 h, the reaction was concentrated and diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and evaporated. This crude solid was purified by column chromatography (silica gel, eluted with 38% ethyl acetate in hexane) to **10** (0.47 g, 1.97 mmol, 22.9%) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 13.32 (s, 1H), 8.12 (d, J = 8.7, 1H), 7.94 (d, J = 15.3, 1H), 7.35 (d, J = 15.3, 1H), 7.44 (dd, J = 2.1, 8.7, 1H), 7.3–7.4 (m, 3H), 6.51 (dd, J = 2.1, 8.4, 1H), 6.48 (dd, J = 2.1, 8.4, 1H), 6.39 (d, J = 2.1, 1H). ^{13}C NMR (acetone-d_6): δ 116.1 (C-1′), 159.6 (C-2′), 103.0 (C-3′), 164.5 (C-4′), 107.6 (C-5′), 132.3 (C-6′), 187.1 (C=O), 123.7 (C-α), 142.1 (C-β), 134.6 (C-1), 126.2 (C-2,6), 128.4 (C-3,5), 127.7 (C-4). TLC, R_f 0.5 (5:3 hexane/EtOAc); IR (KBr) 3449, 1635 cm^{-1}; EI-MS (electron impact mass spectrophotometry) (70 eV) m/z 240 (M^+, 100), 163 (74), 137 (47), 103 (28), 77 (37) (Wollen-weber & Seigler 1982).

*4,2′-Dihydroxychalcone (**1**)*
2′-Hydroxyacetophenone (5.35 g, 35.2 mmol) and chloromethylmethyl ether (5.26 g, 68.4 mmol) were treated as in **a** to give crude 2′-methoxymethoxyacetophenone (**1a**).

A mixture of crude **1a** (0.63 g, 3.70 mmol), **B** (0.61 g, 3.70 mmol), and potassium hydroxide (1.04 g, 18.5 mmol) was treated as in **10c** to give **1** (0.26 g, 2.63 mmol, 22.9%) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 12.32 (s, 1H), 8.25 (dd, J = 2.4, 8.7,1H), 7.92 (d, J = 15.3, 1H), 7.84 (d, J = 15.3, 1H), 7.82 (d, J = 9.0, 2H), 7.5–7.6 (m, 1H) 7.41 (dd, J = 2.4, 8.7, 1H), 7.06 (d, J = 9.0, 2H), 6.9–7.0 (m, 1H). ^{13}C NMR (acetone-d_6): δ 127.4 (C-1′), 164.4 (C-2′), 116.1 (C-3′), 136.0 (C-4′), 122.0 (C-5′), 132.1 (C-6′), 205.8

(C═O), 120.9 (C-α), 146.6 (C-β), 127.3 (C-1), 128.9 (C-2), 116.8 (C-3), 161.3 (C-4), 116.8 (C-5), 127.3 (C-6). TLC, R_f 0.54 (5:3 hexane/EtOAc); IR (KBr) 3320, 1637 cm^{-1}; EI-MS (70 eV) m/z 240 (M^+, 22), 146 (38), 121 (100), 119 (20), 107 (34), 91 (32) (Bhartiya & Gupta 1982).

*4,2′,4′-Trihydroxychalcone (**2**)*

2′,4′-Dihydroxyacetophenone (5.35 g, 35.2 mmol) and chloromethylmethyl ether (5.26 g, 68.4 mmol) were treated as in **a** to give crude 2′,4′-bis(methoxymethoxy)acetophenone (**2a**). A mixture of crude **2a** (4.5 g, 18.75 mmol), **B** (3.42 g, 18.75 mmol) and potassium hydroxide (5.26 g, 93.8 mmol) was treated as in **10c** to give **2** (0.66 g, 2.58 mmol, 17.4%) as a pale brown needle. ^{1}H NMR (acetone-d_6) δ: 12.87 (s, 1H), 8.09 (d, J = 8.4, 1H), 7.79 (d, J = 15.3, 1H), 7.77 (d, J = 15.3, 1H), 7.72 (d, J = 8.7, 2H), 6.92 (d, J = 8.7, 2H), 6.36 (d, J = 2.1, 1H), 6.43 (dd, J = 2.1, 8.4, 1H). ^{13}C NMR (acetone-d_6): δ 116.5 (C-1′), 159.7 (C-2′), 103.1 (C-3′), 164.1 (C-4′), 108.8 (C-5′), 132.1 (C-6′), 187.6 (C═O), 123.5 (C-α), 142.9 (C-β), 127.5 (C-1), 127.6 (C-2,6), 115.3 (C-3,5), 156.3 (C-4). TLC, R_f 0.4 (1:1 hexane/EtOAc); IR (KBr) 3200, 1630 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 44), 137 (53), 120 (130), 91 (17) (Grasselli & Ritchey 1975).

*4,2′,5′-Trihydroxychalcone (**3**)*

2′,5′-Dihydroxyacetophenone (0.42 g, 2.75 mmol) and chloromethylmethyl ether (0.72 g, 9.3 mmol) were treated as in **a** to give crude 2′,5′-bis(methoxymethoxy)acetophenone (**3a**). A mixture of crude **3a** (0.5 g, 2.08 mmol), **B** (0.38 g, 2.08 mmol) and potassium hydroxide (0.58 g, 10.4 mmol) was treated as in **10c** to give **3** (0.15 g, 0.59 mmol, 21.1%) as a brown needle. ^{1}H NMR (acetone-d_6) δ: 12.87 (s, 1H), 8.11 (d, J = 2.1, 1H), 7.87 (d, J = 15.3, 1H), 7.56 (d, J = 15.3, 1H), 7.58 (d, J = 8.7, 2H), 7.09 (d, J = 8.7, 2H), 6.91 (dd, J = 2.1, 8.4, 1H), 6.81 (d, J = 8.4, 1H). ^{13}C NMR (acetone-d_6): δ 125.3 (C-1′), 146.4 (C-2′), 116.7 (C-3′), 132.0 (C-4′), 119.4 (C-5′), 132.1 (C-6′), 206.2 (C═O), 119.4 (C-α), 132.1 (C-β), 125.4 (C-1), 125.5 (C-2,6), 115.5 (C-3,5), 146.4 (C-4). TLC, R_f 0.46 (1:1 hexane/EtOAc); IR (KBr) 3364, 1610 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 61), 137 (100), 120 (63), 105 (23), 91 (63) (Bhartiya & Gupta 1982).

*4,2′-Dihydroxy-4′-methoxychalcone (**4**)*

2′-Hydroxy-4′-methoxyacetophenone (0.43 g, 2.61 mmol) and chloromethylmethyl ether (0.42 g, 5.39 mmol) were treated as in **a** to give crude 2′-methoxymethoxy-4′-methoxyacetophenone (**4a**). A mixture of crude **4a** (0.66 g, 3.11 mmol), **B** (0.56 g, 3.11 mmol) and potassium hydroxide (0.87 g, 15.6 mmol) was treated as in **10c** to give **4** (0.17 g, 0.67 mmol, 24.4%) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 12.67 (s, 1H), 8.16 (d, J = 8.4, 1H), 7.76 (d, J = 15.3, 1H), 7.74 (d, J = 15.3, 1H), 7.72 (d, J = 8.7, 2H), 6.93 (d, J = 8.7, 2H), 6.49 (d, J = 2.1, 1H), 6.52 (dd, J = 2.1, 8.4, 1H), 3.87 (s, 3H). ^{13}C NMR (acetone-d_6): δ 116.7 (C-1′), 160.4 (C-2′), 115.9 (C-3′), 169.2 (C-4′), 116.6 (C-5′), 132.7 (C-6′), 206.2 (C═O), 128.9 (C-α), 139.2 (C-β), 131.1 (C-1), 131.8 (C-2,6), 118.5 (C-3,5), 157.0 (C-4), 56.0 (OCH_3). TLC, R_f 0.51 (5:3 hexane/EtOAc); IR (KBr) 3244, 1630 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 95), 177 (33), 151 (100), 120 (36) (Severi et al 1996).

*4,2′-Dihydroxy-5′-methoxychalcone (**5**)*

2′-Hydroxy-5′-methoxyacetophenone (0.28 g, 1.67 mmol) and chloromethylmethyl ether (0.42 g, 5.40 mmol) were treated as in **a** to give crude 5′-methoxy-2′-methoxymethoxyacetophenone (**5a**). A mixture of crude **5a** (0.64 g, 3.01 mmol), **B** (0.50 g, 3.01 mmol) and potassium hydroxide (0.85 g, 15.05 mmol) was treated as in **10c** to give **5** (0.18 g, 0.71 mmol, 24.1%) as a yellow powder. ^{1}H NMR (acetone-d_6) δ: 13.12 (s, 1H), 8.05 (d, J = 2.1, 1H), 7.78 (d, J = 15.3, 1H), 6.94 (d, J = 15.3, 1H), 7.75 (d, J = 8.7, 2H), 7.36 (d, J = 8.7, 2H), 6.85 (d, J = 8.4, 1H), 6.39 (dd, J = 2.1, 8.4, 1H), 3.92 (s, 3H). ^{13}C NMR (acetone-d_6): δ 124.9 (C-1′), 150.8 (C-2′), 117.2 (C-3′), 121.3 (C-4′), 155.2 (C-5′), 116.7 (C-6′), 187.0 (C═O), 123.3 (C-α), 142.8 (C-β), 127.5 (C-1), 127.6 (C-2,6), 115.6 (C-3,5), 156.5 (C-4), 54.7 (OCH_3). TLC, R_f 0.59 (5:3 hexane/EtOAc); IR (KBr) 3254, 1637 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 24), 167 (31), 149 (83), 79 (79), 57 (100); HR-MS (EI) exact mass for $C_{16}H_{14}O_4$ (M^+) 270.0892, found 270.0868.

*4,2′-Dihydroxy-6′-methoxychalcone (**6**)*

2′-Hydroxy-6′-methoxyacetophenone (0.31 g, 1.83 mmol) and chloromethylmethyl ether (0.42 g, 5.40 mmol) were treated as in **a** to give crude 2′-methoxymethoxy-6′-methoxyacetophenone (**6a**). A mixture of crude **6a** (0.66 g, 1.85 mmol), **B** (0.56 g, 3.11 mmol) and potassium hydroxide (0.87 g, 15.6 mmol) was treated as in **10c** to give **6** (0.14 g, 0.55 mmol, 28.9%) as a brown needle. ^{1}H NMR (acetone-d_6) δ: 12.62 (s, 1H), 7.86 (d, J = 15.1, 1H), 7.74 (d, J = 15.1, 1H), 7.53 (d, J = 8.7, 2H), 6.98 (d, J = 8.7, 2H), 6.63 (brd, J = 8.4, 1H), 7.29 (t, J = 8.4, 1H), 6.41 (brd, J = 8.4, 1H), 3.97(s, 3H). ^{13}C NMR (acetone-d_6): δ 116.8 (C-1′), 147.7 (C-2′), 117.6 (C-3′), 133.1 (C-4′), 116.7 (C-5′), 164.6 (C-6′), 205.9 (C═O), 147.6 (C-α), 117.5 (C-β), 121.1 (C-1),

121.2 (C-2,6), 116.7 (C-3,5), 139.5 (C-4), 57.1 (OCH_3). TLC, R_f 0.35 (5:3 hexane/EtOAc); IR (KBr) 3331, 1666 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 100), 177 (38), 151 (98), 120 (32) (Severi et al 1996).

*4,2′-Dihydroxy-5′-methylchalcone (**7**)*

2′-Hydroxy-5′-methylacetophenone (0.34 g, 2.28 mmol) and chloromethylmethyl ether (0.54 g, 7.02 mmol) were treated as in **a** to give crude 2′-methoxymethoxy-5′-methylacetophenone (**7a**). A mixture of crude **7a** (0.64 g, 3.30 mmol), **B** (0.61 g, 3.3 mmol) and potassium hydroxide (0.92 g, 16.5 mmol) was treated as in **10c** to give **7** (0.12 g, 0.45 mmol, 19.8%) as a pale red powder. ^{1}H NMR (acetone-d_6) δ: 13.23 (s, 1H), 8.13 (s, 1H), 7.88 (d, J = 15.3, 1H), 7.76 (d, J = 15.3, 1H), 7.38 (d, J = 8.7, 2H), 7.13 (d, J = 8.7, 2H), 6.87 (d, J = 8.4, 1H), 6.93 (d, J = 8.4, 1H), 2.86 (s, 3H). ^{13}C NMR (acetone-d_6): δ 123.8 (C-1′), 155.5 (C-2′), 116.1 (C-3′), 136.4 (C-4′), 130.8 (C-5′), 131.8 (C-6′), 187.0 (C═O), 123.9 (C-α), 142.8 (C-β), 127.4 (C-1), 127.6 (C-2,6), 115.1 (C-3,5), 156.5 (C-4), 20.3 (CH_3). TLC, R_f 0.56 (5:3 hexane/EtOAc); IR (KBr) 3350, 1637 cm^{-1}; EI-MS (70 eV) m/z 254 (M^+, 1.3), 150 (15), 135 (100), 107 (12), 77 (15); HR-MS (EI) exact mass for $C_{16}H_{14}O_3$ (M^+) 254.0943, found 254.0962.

*5′-Chloro-4,2′-dihydroxychalcone (**8**)*

2′-Hydroxy-5′-chloroacetophenone (2.0 g, 2.69 mmol) and chloromethylmethyl ether (0.54 g, 7.02 mmol) were treated as in **a** to give crude 5′-chloro-2′-methoxymethoxyacetophenone (**8a**). A mixture of crude **8a** (0.64 g, 3.30 mmol), **B** (0.61 g, 3.30 mmol) and potassium hydroxide (0.92 g, 16.5 mmol) was treated as in **10c** to give **8** (0.13 g, 0.46 mmol, 17.3%) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 13.15 (s, 1H), 8.24 (d, J = 2.1, 1H), 7.93 (d, J = 15.3, 1H), 7.88 (d, J = 15.3, 1H), 7.79 (d, J = 8.7, 2H), 7.50 (d, J = 8.7, 2H), 6.98 (d, J = 8.4, 1H), 6.92 (dd, J = 2.1, 8.4, 1H). ^{13}C NMR (acetone-d_6): δ 125.1 (C-1′), 162.9 (C-2′), 118.2 (C-3′), 132.7 (C-4′), 127.9 (C-5′), 131.0 (C-6′), 190.9 (C═O), 125.0 (C-α), 146.6 (C-β), 127.3 (C-1), 127.6 (C-2,6), 115.4 (C-3,5), 161.2 (C-4). TLC, R_f 0.43 (5:3 hexane/EtOAc); IR (KBr) 3368, 1637 cm^{-1}; EI-MS (70 eV) m/z 274 (M^+, 35), 155 (27), 120 (100), 107 (16); HR-MS (EI) exact mass for $C_{15}H_{11}O_3Cl$ (M^+) 274.0397, found 274.0372.

*5′-Bromo-4,2′-dihydroxychalcone (**9**)*

5′-Bromo-2′-hydroxyacetophenone (0.53 g, 2.45 mmol) and chloromethylmethyl ether (0.42 g, 5.39 mmol) were treated as in **a** to give crude 5′-bromo-2′-methoxymethoxyacetophenone (**9a**). A mixture of crude **9a** (0.64 g, 3.30 mmol), **B** (0.71 g, 3.30 mmol) and potassium hydroxide (0.92 g, 16.5 mmol) was treated as in **10c** to give **9** (0.18 g, 0.55 mmol, 22.5%) as a yellow plate. ^{1}H NMR (acetone-d_6) δ: 12.56 (s, 1H), 8.38 (d, J = 2.4, 1H), 7.94 (d, J = 15.3, 1H), 7.81 (d, J = 15.3, 1H), 7.66 (d, J = 8.7, 2H), 7.01 (d, J = 8.7, 2H), 6.92 (d, J = 8.7, 1H), 6.95 (dd, J = 2.4, 8.7, 1H). ^{13}C NMR (acetone-d_6): δ 126.1 (C-1′), 157.5 (C-2′), 118.4 (C-3′), 139.0 (C-4′), 116.2 (C-5′), 134.4 (C-6′), 187.7 (C═O), 123.2 (C-α), 142.4 (C-β), 127.7 (C-1), 127.6 (C-2,6), 115.4 (C-3,5), 156.5 (C-4). TLC, R_f 0.46 (5:3 hexane/EtOAc); IR (KBr) 3427, 1637 cm^{-1}; EI-MS (70 eV) m/z 318 (M^+, 47), 201 (25), 147 (10), 120 (100), 91 (31); HR-MS (EI) exact mass for $C_{15}H_{11}O_3Br$ (M^+) 317.9892, found 317.9868.

*2,2′,4′-Trihydroxychalcone (**11**)*

2-Hydroxybenzaldehyde (0.99 g, 8.09 mmol) and chloromethylmethyl ether (1.3 g, 17.1 mmol) were treated as in **b** to give crude 2-methoxymethoxybenzaldehyde (**11b**).

A mixture of **A** (2.0 g, 9.4 mmol), crude **11b** (1.72 g, 9.4 mmol) and potassium hydroxide (2.64 g, 47.0 mmol) was treated as in **10c** to give **11** (0.35 g, 1.38 mmol, 17.0%) as a red needle. ^{1}H NMR (acetone-d_6) δ: 13.12 (s, 1H), 8.04 (d, J = 8.7, 1H), 8.25 (d, J = 15.1, 1H), 7.95 (d, J = 15.1, 1H), 6.95 (d, J = 2.1, 1H), 7.54 (dd, J = 2.1, 8.7, 1H), 7.2–7.3 (m, 1H), 7.74 (d, J = 8.7, 1H), 6.37 (d, J = 8.7, 1H), 6.47 (d, J = 2.1, 1H). ^{13}C NMR (acetone-d_6): δ 116.2 (C-1′), 164.4 (C-2′), 103.6 (C-3′), 167.4 (C-4′), 108.7 (C-5′), 132.6 (C-6′), 192.9 (C═O), 129.8 (C-α), 140.9 (C-β), 122.6 (C-1), 157.8 (C-2), 116.9 (C-3), 129.8 (C-4), 120.7 (C-5), 127.4 (C-6). TLC, R_f 0.34 (5:3 hexane/EtOAc); IR (KBr) 3393, 1630 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 49), 147 (10), 137 (100), 118 (19), 91 (83) (Geissman & Clinton 1946).

*2′,4′-Dihydroxy-4-methoxychalcone (**12**)*

A mixture of **A** (3.60 g, 16.94 mmol), 4-methoxybenzaldehyde (2.27 g, 16.94 mmol) and potassium hydroxide (5.84 g, 104.0 mmol) was treated as in **10c** to give **12** (1.10 g, 4.08 mmol, 24.1%) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 13.22 (s, 1H), 8.12 (d, J = 8.7, 1H), 7.80 (d, J = 15.3, 1H), 7.70 (d, J = 15.3, 1H), 7.49 (d, J = 8.7, 2H), 7.01 (d, J = 8.7, 2H), 6.36 (d, J = 2.1, 1H), 6.46 (dd, J = 2.1, 8.7, 1H), 3.84 (s, 3H). ^{13}C NMR (acetone-d_6): δ 115.7 (C-1′), 159.3 (C-2′), 103.2 (C-3′), 164.3 (C-4′), 108.1 (C-5′), 132.2 (C-6′), 187.3 (C═O),

123.4 (C-α), 142.7 (C-β), 127.2 (C-1), 127.3 (C-2,6), 114.0 (C-3,5), 161.2 (C-4), 56.8 (OCH_3). TLC, R_f 0.55 (5:3 hexane/EtOAc); IR (KBr) 3422, 1635 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 67), 163 (12), 134 (77), 121 (100); HR-MS (EI) exact mass for $C_{16}H_{14}O_4$ (M^+) 270.0892, found 270.0881.

*4-Bromo-2′,4′-dihydroxychalcone (**13**)*

A mixture of **A** (5.0 g, 20.8 mmol), 4-bromobenzaldehyde (2.58 mL, 20.8 mmol) and potassium hydroxide (5.84 g, 104.0 mmol) was treated as in **10c** to give **13** (2.67 g, 8.36 mmol, 35.8 %) as a yellow plate. ^{1}H NMR (acetone-d_6) δ: 13.57 (s, 1H), 8.14 (d, J = 8.7, 1H), 7.98 (d, J = 15.3, 1H), 7.74 (d, J = 15.3, 1H), 7.81 (d, J = 8.7, 2H), 7.64 (d, J = 8.7, 2H), 6.38 (d, J = 2.1, 1H), 6.47 (dd, J = 2.1, 8.7, 1H). ^{13}C NMR (acetone-d_6): δ 116.4 (C-1′), 159.1 (C-2′), 103.1 (C-3′), 164.2 (C-4′), 108.0 (C-5′), 132.4 (C-6′), 187.4 (C═O), 123.6 (C-α), 142.3 (C-β), 133.9 (C-1), 128.4 (C-2,6), 131.7 (C-3,5), 122.3 (C-4). TLC, R_f 0.5 (1:1 hexane/EtOAc); IR (KBr) 3447, 1637 cm^{-1}; EI-MS (70 eV) m/z 317 (M^+, 14), 181 (37), 137 (29), 102 (100); HR-MS (EI) exact mass for $C_{15}H_{11}O_3Br$ (M^+) 317.9892, found 317.9866.

*2′,4′-Dihydroxy-4-nitrochalcone (**14**)*

A mixture of **A** (2.5 g, 9.18 mmol), 4-nitrobenzaldehyde (1.38 g, 9.18 mmol) and potassium hydroxide (2.58 g, 45.9 mmol) was treated as in **10c** to give **14** (0.14 g, 0.49 mmol, 8.84 %) as a brown powder. ^{1}H NMR (acetone-d_6) δ 13.78 (s, 1H), 8.32 (d, J = 8.7, 1H), 8.18 (d, J = 15.3, 1H), 7.95 (d, J = 15.3, 1H), 8.14 (d, J = 8.7, 2H), 8.29 (d, J = 8.7, 2H), 6.60 (s, 1H), 6.64 (d, J = 8.7, 1H). ^{13}C NMR (acetone-d_6): δ 115.2 (C-1′), 164.4 (C-2′), 103.8 (C-3′), 165.5 (C-4′), 108.9 (C-5′), 134.6 (C-6′), 203.9 (C═O), 124.1 (C-α), 133.6 (C-β), 147.7 (C-1), 127.6 (C-2,6), 123.9 (C-3,5), 152.9 (C-4). TLC, R_f 0.48 (5:3 hexane/EtOAc); IR (KBr) 3395, 1628 cm^{-1}; EI-MS (70 eV) m/z 285 (M^+, 17), 163 (20), 137 (100), 77 (33) (Jorge & Sbarbati-Nudelman 1986).

*2′,4′-Dihydroxy-4-methylchalcone (**15**)*

A mixture of **A** (1.0 g, 4.16 mmol), 4-methylbenzaldehyde (0.50 g, 4.16 mmol) and potassium hydroxide (1.17 g, 20.8 mmol) was treated as in **10c** to give **15** (0.10 g, 0.39 mmol, 16.0 %) as a yellow powder. ^{1}H NMR (acetone-d_6) δ: 13.08 (s, 1H), 8.12 (d, J = 8.7, 1H), 7.88 (d, J = 15.3, 1H), 7.67 (d, J = 15.3, 1H), 7.85 (d, J = 8.7, 2H), 7.26 (d, J = 8.7, 2H), 6.37 (d, J = 2.1, 1H), 6.47 (dd, J = 2.1, 8.4, 1H), 2.35 (s, 3H). ^{13}C NMR (acetone-d_6): δ 116.2 (C-1′), 159.3 (C-2′), 103.3 (C-3′), 163.5 (C-4′), 108.5 (C-5′), 132.6 (C-6′), 189.0 (C═O), 123.4 (C-α), 141.8 (C-β), 131.9 (C-1), 126.1 (C-2,6), 129.1 (C-3,5), 136.9 (C-4), 19.8 (CH_3). TLC, R_f 0.59 (5:3 hexane/EtOAc); IR (KBr) 3267, 1631 cm^{-1}; EI-MS (70 eV) m/z 254 (M^+, 100), 163 (61), 137 (47), 118 (45), 91 (22); HR-MS (EI) exact mass for $C_{16}H_{14}O_3$ (M^+) 254.0943, found 254.0976.

*2′,4′-Dihydroxy-4-dimethylaminochalcone (**16**)*

A mixture of **A** (1.0 g, 4.16 mmol), 4-dimethylaminobenzaldehyde (0.62 g, 4.16 mmol) and potassium hydroxide (1.17 g, 20.8 mmol) was treated as in **10c** to give **16** (0.24 g, 0.86 mmol, 25.24 %) as a dark brown needle. ^{1}H NMR (acetone-d_6) δ: 12.42 (s, 1H), 7.86 (d, J = 8.7, 1H), 7.82 (d, J = 15.3, 1H), 7.67 (d, J = 15.3, 1H), 7.34 (d, J = 8.7, 2H), 6.39 (d, J = 8.7, 2H), 6.32 (d, J = 2.1, 1H), 6.37 (dd, J = 2.1, 8.7, 1H), 3.04 (s, 3H), 3.03(s, 3H). ^{13}C NMR (acetone-d_6): δ 116.7 (C-1′), 159.5 (C-2′), 103.6 (C-3′), 165.4 (C-4′), 107.9 (C-5′), 132.9 (C-6′), 187.9 (C═O), 123.8 (C-α), 142.0 (C-β), 124.4 (C-1), 127.1 (C-2,6), 113.0 (C-3,5), 143.7 (C-4). TLC, R_f 0.5 (5:3 hexane/EtOAc); IR (KBr) 3294, 1660 cm^{-1}; EI-MS (70 eV) m/z 283 (M^+, 49), 147 (100), 134 (84), 77 (15); HR-MS (EI) exact mass for $C_{17}H_{17}O_3N$ (M^+) 283.1208, found 283.1263.

*2′,4′-Dihydroxy-3,4-dimethoxychalcone (**17**)*

A mixture of **A** (5.0 g, 20.8 mmol), 3,4-dimethoxybenzaldehyde (3.46 g, 20.8 mmol) and potassium hydroxide (5.84 g, 104.0 mmol) was treated as in **10c** to give **17** (0.52 g, 1.73 mmol, 11.3 %) as a yellow plate. ^{1}H NMR (acetone-d_6) δ: 12.98 (s, 1H), 8.08 (d, J = 8.1, 1H), 7.88 (d, J = 15.1, 1H), 7.68 (d, J = 15.1, 1H), 7.52 (d, J = 8.1, 1H), 7.01 (dd, J = 2.1, 8.1, 1H), 7.26 (d, J = 2.1, 1H), 6.36 (d, J = 2.1, 1H), 6.44 (dd, J = 2.1, 8.4, 1H), 3.87 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (acetone-d_6): δ 116.9 (C-1′), 159.2 (C-2′), 103.2 (C-3′), 164.3 (C-4′), 108.6 (C-5′), 132.2 (C-6′), 187.2 (C═O), 122.9 (C-α), 141.9 (C-β), 128.2 (C-1), 112.8 (C-2), 147.5 (C-3), 146.8 (C-4), 115.0 (C-5), 119.5 (C-6), 54.5 (OCH_3), 54.2 (OCH_3). TLC, R_f 0.56 (5:3 hexane/EtOAc); IR (KBr) 3423, 1637 cm^{-1}; EI-MS (70 eV) m/z 300 (M^+, 73), 191 (100), 137 (73), 102 (81); HR-MS (EI) exact mass for $C_{17}H_{16}O_5$ (M^+) 300.3024, found 300.3052.

*3,4,2′,4′-Tetrahydroxychalcone (**18**)*

3, 4-Dihydroxybenzaldehyde (1.11 g, 8.16 mmol) and chloromethylmethyl ether (1.82 g, 23.9 mmol) were

treated as in **b** to give crude 3, 4-bis(methoxymethoxy)benzaldehyde (**18b**). A mixture of **A** (2.5 g, 9.18 mmol), crude **18b** (2.0 g, 9.18 mmol) and potassium hydroxide (2.58 g, 45.9 mmol) was treated as in **10c** to give **18** (0.54 g, 1.97 mmol, 21.5%) as a pale red needle. ^{1}H NMR (acetone-d_6) δ: 13.75 (s, 1H), 8.10 (d, J = 8.4, 1H), 7.74 (d, J = 15.3, 1H), 7.50 (d, J = 15.3, 1H), 7.17 (dd, J = 2.1, 8.4, 1H), 6.81 (d, J = 2.1, 1H), 7.12 (dd, J = 2.1, 8.4, 1H), 6.35 (d, J = 8.4, 1H), 6.45 (d, J = 2.1, 1H). ^{13}C NMR (acetone-d_6): δ 116.1 (C-1′), 163.3 (C-2′), 110.9 (C-3′), 134.0 (C-4′), 114.4 (C-5′), 118.9 (C-6′), 206.4 (C═O), 118.1 (C-α), 146.1 (C-β), 129.2 (C-1), 114.4 (C-2), 145.3 (C-3), 146.2 (C-4), 130.2 (C-5), 126.7 (C-6). TLC, R_f 0.43 (1:1 hexane/EtOAc); IR (KBr) 3335, 1637 cm^{-1}; EI-MS (70 eV) m/z 272 (M^+, 100), 163 (26), 150 (38), 137 (94) (Price 1939).

*3,4-Dichloro-2′,4′-dihydroxychalcone (**19**)*

A mixture of **A** (1.0 g, 4.16 mmol), 3,4-dichlorobenzaldehyde (0.74 g, 4.16 mmol) and potassium hydroxide (1.17 g, 20.8 mmol) was treated as in **10c** to give **19** (0.43 g, 1.39 mmol, 33.6%) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 12.56 (s, 1H), 8.17 (d, J = 8.4, 1H), 8.05 (d, J = 15.3, 1H), 7.71 (d, J = 15.3, 1H), 8.11 (dd, J = 2.1, 8.4, 1H), 7.65 (d, J = 8.4, 1H), 7.81 (d, J = 2.1, 1H), 6.38 (d, J = 2.1, 1H), 6.47 (dd, J = 2.1, 8.4, 1H). ^{13}C NMR (acetone-d_6): δ 115.9 (C-1′), 159.0 (C-2′), 103.4 (C-3′), 163.5 (C-4′), 108.9 (C-5′), 132.1 (C-6′), 187.8 (C═O), 123.1 (C-α), 142.0 (C-β), 134.4 (C-1), 128.0 (C-2), 134.1 (C-3), 133.4 (C-4), 130.2 (C-5), 126.7 (C-6). TLC, R_f 0.36 (5:3 hexane/EtOAc); IR (KBr) 3200, 1630 cm^{-1}; EI-MS (70 eV) m/z 310 (M^+, 46), 163 (100), 136 (78), 108 (37), 81 (24); HR-MS (EI) exact mass for $C_{16}H_{14}O_3Cl$ (M^+) 324.0320, found 324.0312.

*3-Fluoro-2′,4′-dihydroxy-4-methoxychalcone (**20**)*

A mixture of **A** (1.0 g, 4.16 mmol), 3-fluoro-4-methoxybenzaldehyde (0.68 g, 4.06 mmol) and potassium hydroxide (1.17 g, 20.8 mmol) was treated as in **10c** to give **20** (0.48 g, 1.67 mmol, 30.6%) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 12.53 (s, 1H), 8.15 (d, J = 8.4, 1H), 7.87 (d, J = 15.3, 1H), 7.60 (d, J = 15.3, 1H), 7.84 (s, 1H), 7.18 (dd, J = 2.1, 8.4, 1H), 7.49 (d, J = 8.4, 1H), 6.37 (d, J = 2.1, 1H), 6.46 (d, J = 8.4, 1H), 3.80 (s, 3H). ^{13}C NMR (acetone-d_6): δ 116.5 (C-1′), 159.1 (C-2′), 103.2 (C-3′), 164.6 (C-4′), 107.9 (C-5′), 132.5 (C-6′), 187.4 (C═O), 123.8 (C-α), 142.5 (C-β), 128.8 (C-1), 114.2 (C-2), 147.6 (C-3), 148.2 (C-4), 115.6 (C-5), 122.8 (C-6), 57.5 (OCH_3). TLC, R_f 0.55 (1:1 hexane/EtOAc); IR (KBr) 3250, 1640 cm^{-1}; EI-MS (70 eV) m/z 288 (M^+, 89), 179 (91), 151 (100), 137 (75); HR-MS (EI) exact mass for $C_{16}H_{13}O_4F$ (M^+) 288.0798, found 288.0785.

*2′,4′-Dihydroxy-2,4-dimethylchalcone (**21**)*

A mixture of **A** (1.0 g, 4.17 mmol), 2,4-dimethylbenzaldehyde (0.56 g, 4.17 mmol) and potassium hydroxide (1.27 g, 20.8 mmol) was treated as in **10c** to give **21** (0.24 g, 0.91 mmol, 29.4%) as a brown needle. ^{1}H NMR (acetone-d_6) δ: 12.89 (s, 1H), 7.47 (d, J = 8.4, 1H), 8.17 (d, J = 15.3, 1H), 7.39 (d, J = 15.3, 1H), 6.81 (d, J = 2.1, 1H), 6.89 (dd, J = 2.1, 8.4, 1H), 7.06 (dd, J = 2.1, 8.4, 1H), 6.39 (d, J = 8.4, 1H), 6.48 (d, J = 2.1, 1H), 2.43 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (acetone-d_6): δ 115.5 (C-1′), 164.4 (C-2′), 103.7 (C-3′), 166.9 (C-4′), 108.6 (C-5′), 132.6 (C-6′), 192.8 (C═O), 127.4 (C-α), 142.3 (C-β), 132.6 (C-1), 137.6 (C-2), 129.0 (C-3), 139.1 (C-4), 127.4 (C-5), 126.9 (C-6), 21.3 (CH_3), 19.7 (CH_3). TLC, R_f 0.52 (5:3 hexane/EtOAc); mp 110–112°C (CH_3OH); IR (KBr) 3410, 1630 cm^{-1}; EI-MS (70 eV) m/z 268 (M^+, 52), 250 (100), 163 (29), 137 (66), 115 (49); HR-MS (EI) exact mass for $C_{17}H_{16}O_3$ (M^+) 268.1099, found 268.1065.

*2,4,2′,4′-Tetrahydroxychalcone (**22**)*

2, 4-Dihydroxybenzaldehyde (2.19 g, 15.9 mmol) and chloromethylmethyl ether (2.48 g, 34.2 mmol) were treated as in **b** to give crude 2,4-bis(methoxymethoxy)benzaldehyde (**22b**). A mixture of **A** (4.92 g, 20.4 mmol), crude **22b** (4.6 g, 20.4 mmol) and potassium hydroxide (5.72 g, 102.0 mmol) was treated as in **10c** to give **22** (1.24 g, 4.56 mmol, 28.6%) as a pale red needle. ^{1}H NMR (acetone-d_6) δ: 12.56 (s, 1H), 8.02 (d, J = 8.4, 1H), 8.22 (d, J = 15.3, 1H), 7.78 (d, J = 15.3, 1H), 6.51 (d, J = 2.1, 1H), 7.66 (d, J = 8.4, 1H), 7.74 (dd, J = 2.1, 8.4, 1H), 6.34 (d, J = 2.1, 1H), 6.43 (dd, J = 2.1, 8.4, 1H). ^{13}C NMR (acetone-d_6): δ 116.8 (C-1′), 165.6 (C-2′), 108.8 (C-3′), 164.3 (C-4′), 110.9 (C-5′), 133.6 (C-6′), 195.2 (C═O), 125.0 (C-α), 149.4 (C-β), 113.9 (C-1), 162.5 (C-2), 103.0 (C-3), 162.5 (C-4), 108.7 (C-5), 129.4 (C-6). TLC, R_f 0.35 (1:3 hexane/EtOAc); IR (KBr) 3366, 1624 cm^{-1}; EI-MS (70 eV) m/z 272 (M^+, 24), 167 (31), 149 (83), 79 (79), 55 (100) (Delle-Monache et al 1995).

*2,5,2′,4′-Tetrahydroxychalcone (**23**)*

2,5-Dihydroxybenzaldehyde (0.36 g, 2.64 mmol) and chloromethylmethyl ether (0.46 g, 5.98 mmol) were treated as in **b** to give crude 2,5-bis(methoxymethoxy)benzaldehyde (**23b**). A mixture of **A** (1.80 g, 4.08 mmol), crude **23b** (0.9 g, 4.08 mmol) and potassium

hydroxide (2.85 g, 20.4 mmol) was treated as in **10c** to give **23** (0.17 g, 0.63 mmol, 23.9 %) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 13.87 (s, 1H), 8.07 (d, J = 8.4, 1H), 8.20 (d, J = 15.3, 1H), 7.86 (d, J = 15.3, 1H), 6.82 (d, J = 8.4, 1H), 7.24 (d, J = 8.4, 1H), 7.97 (s, 1H), 6.36 (dd, J = 2.1, 8.4, 1H), 6.47 (dd, J = 2.1, 8.4, 1H). ^{13}C NMR (acetone-d_6): δ 116.1 (C-1′), 159.4 (C-2′), 103.6 (C-3′), 164.7 (C-4′), 108.6 (C-5′), 131.7 (C-6′), 187.9 (C═O), 123.4 (C-α), 142.9 (C-β), 123.5 (C-1), 147.6 (C-2), 117.0 (C-3), 116.3 (C-4), 149.8 (C-5), 114.8 (C-6). TLC, R_f 0.45 (1:3 hexane/EtOAc); IR (KBr) 3391, 1637 cm^{-1}; EI-MS (70 eV) m/z 272 (M^+, 14), 137 (100), 107 (20), 81 (33), 55 (53); HR-MS (EI) exact mass for $C_{15}H_{12}O_5$ (M^+) 272.0685, found 272.0639.

*2,3,4,2′,4′-Pentahydroxychalcone (**24**)*

2, 3, 4-Trihydroxybenzaldehyde (0.38 g, 2.44 mmol) and chloromethylmethyl ether (0.58 g, 7.32 mmol) were treated as in **b** to give crude 2, 3, 4-Tris(methoxymethoxy)benzaldehyde (**24b**). A mixture of **A** (1.0 g, 4.16 mmol), crude **24b** (1.20 g, 4.16 mmol) and potassium hydroxide (1.17 g, 20.8 mmol) was treated as in **10c** to give **24** (0.11 g, 0.39 mmol, 15.9 %) as a yellow needle. ^{1}H NMR (acetone-d_6) δ: 13.33 (s, 1H), 8.01 (d, J = 8.4, 1H), 8.18 (d, J = 15.3, 1H), 7.81 (d, J = 15.3, 1H), 7.22 (d, J = 8.4, 1H), 7.76 (dd, J = 2.1, 8.4, 1H), 6.30 (d, J = 8.4, 1H), 6.38 (d, J = 2.1, 1H). ^{13}C NMR (acetone-d_6): δ 116.9 (C-1′), 160.3 (C-2′), 103.0 (C-3′), 165.5 (C-4′), 108.3 (C-5′), 132.5 (C-6′), 187.1 (C═O), 123.3 (C-α), 142.7 (C-β), 116.1 (C-1), 143.6 (C-2), 131.6 (C-3), 145.1 (C-4), 109.6 (C-5), 121.6 (C-6). TLC, R_f 0.35 (1:3 hexane/EtOAc); IR (KBr) 3422, 1625 cm^{-1}; EI-MS (70 eV) m/z 288 (M^+, 21), 137 (100), 179 (62), 151 (32), 109 (20); HR-MS (EI) exact mass for $C_{15}H_{12}O_6$ (M^+) 288.0634, found 288.0629.

General procedure for the preparation of 2′-hydroxydihydrochalcone (25–27)

*4,2′,5′-Trihydroxydihydrochalcone (**25**)*

A solution of 4,2′,5′-Tris(methoxymethoxy)chalcone (0.5 g, 1.45 mmol) in ethyl hydroxide (20 mL) was hydrogenated at 1 atm for 4 h in the presence of 10 % palladium on charcoal (5 mg) at room temperature. The catalyst was removed by filtration through Celite and washed with ethyl hydroxide (100 mL). The combined filtrate was evaporated to afford crude 4,2′,5′-Tris-(methoxymethoxy)-dihydrochalcone (**25c**), which was dissolved in methyl hydroxide (20 mL). To this solution was added 2 mL 3 M HCl. After 3 h, the reaction was concentrated and diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and evaporated. This crude solid was purified by column chromatography (silica gel, eluted with 10 % ethyl acetate in hexane) to **25** (0.16 g, 0.63 mmol, 43.5 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 11.80 (s, 1H), 8.02 (s, 1H), 6.74 (d, J = 8.7, 1H), 6.65 (d, J = 8.7, 1H), 7.33 (d, J = 8.4, 2H), 6.99 (d, J = 8.4, 2H), 3.26 (t, J = 7.3, 2H), 2.92 (t, J = 7.3, 2H). ^{13}C NMR (acetone-d_6): δ 126.0 (C-1′), 150.0 (C-2′), 117.0 (C-3′), 121.5 (C-4′), 149.8 (C-5′), 117.2 (C-6′), 196.7 (C═O), 41.5 (C-α), 29.9 (C-β), 132.8 (C-1), 129.3 (C-2), 115.6 (C-3), 154.5 (C-4), 115.6 (C-5), 129.3 (C-6). TLC, R_f 0.48 (5:3 hexane/EtOAc); IR (KBr) 3434, 1643 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 18), 149 (42), 137 (100), 129 (26), 119 (40); HR-MS (EI) exact mass for $C_{15}H_{14}O_4$ (M^+) 258.0892, found 258.0839.

*2,2′,4′-Trihydroxydihydrochalcone (**26**)*

A mixture of 2,2′,4′-Tris(methoxymethoxy)chalcone (0.5 g, 1.45 mmol), 10 % palladium on charcoal (5 mg) was treated as in **25c** to give **26** (93 mg, 0.36 mmol, 24.6 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 12.82 (s, 1H), 7.83 (d, J = 8.4, 1H), 6.41 (d, J = 8.4, 1H), 6.32 (d, J = 2.1, 1H), 7.73 (dd, J = 2.1, 8.4, 1H), 6.7–6.8 (m, 1H), 7.02 (dd, J = 2.1, 8.4, 1H), 7.15 (dd, J = 2.1, 8.4, 1H), 3.23 (t, J = 7.2, 2H), 2.94 (t, J = 7.2, 2H). ^{13}C NMR (acetone-d_6): δ 117.8 (C-1′), 158.1 (C-2′), 101.9 (C-3′), 162.8 (C-4′), 107.9 (C-5′), 131.7 (C-6′), 198.1 (C═O), 42.7 (C-α), 19.9 (C-β), 127.4 (C-1), 156.7 (C-2), 115.4 (C-3), 127.1 (C-4), 121.0 (C-5), 128.7 (C-6). TLC, R_f 0.62 (5:3 hexane/EtOAc); IR (KBr) 3477, 1649 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 27), 137 (100), 149 (54), 121 (14), 107 (26); HR-MS (EI) exact mass for $C_{15}H_{14}O_4$ (M^+) 258.0892, found 258.0870.

*2,4,2′,4′-Tetrahydroxydihydrochalcone (**27**)*

A mixture of 2,4,2′,4′-tetra(methoxymethoxy)chalcone (0.5 g, 1.24 mmol) and 10 % palladium on charcoal (5 mg) was treated as in **25c** to give **27** (0.18 g, 0.65 mmol, 52.8 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 12.84 (s, 1H), 7.82 (d, J = 8.7, 1H), 6.33 (d, J = 8.7, 1H), 6.23 (d, J = 8.7 1H), 6.93 (d, J = 8.7, 1H), 6.41 (s, 1H), 3.16 (t, J = 7.2, 2H), 2.87 (t, J = 7.2, 2H). ^{13}C NMR (acetone-d_6): δ117.2 (C-1′), 158.8 (C-2′), 102.8 (C-3′), 163.1 (C-4′), 108.2 (C-5′), 131.4 (C-6′), 197.6 (C═O), 41.8 (C-α), 19.7 (C-β), 120.0 (C-1), 158.1 (C-2), 102.8 (C-3), 155.9 (C-4), 108.2 (C-5), 130.7 (C-6). TLC, R_f 0.46 (5:3 hexane/EtOAc); IR (KBr) 3447, 1644 cm^{-1}; EI-MS (70 eV) m/z 272 (M^+, 41), 165 (29), 138 (29), 137 (100), 123 (14); HR-MS (EI) exact mass for $C_{15}H_{14}O_5$ (M^+) 274.0841, found 274.0829.

General procedure for the preparation of flavanone (28–30)

*6,4′-Dihydroxyflavanone (**28**)*

2′-Hydroxy-5′-methoxymethoxyacetophenone (0.98 g, 5 mmol) and 4-methoxymethoxy-benzaldehyde (0.82 g, 5 mmol) were added to a mixture of H_3BO_3 (0.46 g, 7.5 mmol), piperidine (0.11 g, 1.25 mmol) and SiO_2 (2.5 g) in DMF (20 mL). The content was stirred and heated at 120°C under nitrogen for 6–12 h. The mixture was then cooled to room temperature and diluted with acetone (20 mL) and filtered. The SiO_2 was rinsed with acetone (10 mL) three times. The acetone solution was combined with the filtrate and evaporated at reduced pressure to dryness to afford crude 6,4′-di(methoxymethoxy)flavanone (**28c**). To a stirred solution of **28c** (1.07 g, 3.1 mmol) in 20 mL methanol was added 5 mL 3 M HCl. After 3 h, the reaction was concentrated and diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and evaporated. This crude solid was purified by column chromatography (silica gel, eluted with 38 % ethyl acetate in hexane) to **28** (0.56 g, 2.19 mmol, 43.8 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.98 (s, 1H), 6.88 (d, J = 8.7, 1H), 6.78 (d, J = 8.7, 1H), 7.37 (d, J = 8.7, 2H), 7.05 (d, J = 8.7, 2H), 5.41 (dd, J = 2.7, 13.2, 1H), 3.39 (dd, J = 13.2, 16.8, 1H), 3.32 (dd, J = 2.7, 16.8, 1H). ^{13}C NMR (acetone-d_6): δ 72.9 (C-2), 48.8 (C-3), 197.6 (C-4), 116.4 (C-5), 148.8 (C-6), 120.7 (C-7), 115.5 (C-8), 133.5 (C-1′), 128.7 (C-2′), 115.9 (C-3′), 156.2 (C-4′), 115.9 (C-5′), 128.7 (C-6′). TLC, R_f 0.49 (5:3 hexane/EtOAc); IR (KBr) 1611, 1219 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 29), 137 (100), 107 (28), 93 (17), 75 (14); HR-MS (EI) exact mass for $C_{15}H_{12}O_4$ (M^+) 256.0736, found 256.0719.

*7,2′-Dihydroxyflavanone (**29**)*

A mixture of 4′-methoxymethoxy-2′-hydroxyacetophenone (0.59 g, 3.03 mmol) and 2-methoxymethoxybenzaldehyde (0.5 g, 3.03 mmol) was treated as in **28c** to give **29** (0.26 g, 1.03 mmol, 34.1 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 8.06 (d, J = 8.7 1H), 6.43 (dd, J = 2.1, 8.7, 1H), 6.31 (d, J = 8.4, 1H), 7.85 (d, J = 2.1, 1H), 6.8–7.0 (m, 1H), 7.15 (dd, J = 2.1, 8.4, 1H), 7.27 (dd, J = 2.1, 8.4, 1H), 5.78 (dd, J = 2.7, 12.3, 1H), 3.00 (dd, J = 12.3, 17.1, 1H), 2.81 (dd, J = 2.7, 17.1, 1H). ^{13}C NMR (acetone-d_6): δ 62.3 (C-2), 48.9 (C-3), 196.4 (C-4), 130.1 (C-5), 106.8 (C-6), 161.7 (C-7), 101.1 (C-8), 128.1 (C-1′), 156.1 (C-2′), 115.1 (C-3′), 128.8 (C-4′), 121.3 (C-5′), 128.1 (C-6′). TLC, R_f 0.36 (5:3 hexane/EtOAc); IR (KBr) 1612, 1227 cm^{-1}; EI-MS (70 eV) m/z 256 (M^+, 44), 137 (100), 107 (36), 93 (14), 75 (21); HR-MS (EI) exact mass for $C_{15}H_{12}O_4$ (M^+) 256.0736, found 256.0718.

*7,2′,4′-Trihydroxyflavanone (**30**)*

A mixture of 4′-methoxymethoxy-2′-hydroxyacetophenone (0.5 g, 2.56 mmol) and 2,4-di(methoxymethoxy)-benzaldehyde (0.57 g, 2.56 mmol) was treated as in **28c** to give **30** (0.1 g, 0.37 mmol, 14.3 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.99 (d, J = 8.7, 1H), 6.40 (d, J = 8.7, 1H), 6.33 (s, 1H), 7.71 (d, J = 8.7, 1H), 7.34 (d, J = 8.7, 1H), 6.85 (s, 1H), 5.75 (dd, J = 2.4, 12.8, 1H), 3.42 (dd, J = 12.8, 17.1, 1H), 2.89 (dd, J = 2.4, 17.1, 1H). ^{13}C NMR (acetone-d_6): δ 62.7 (C-2), 49.1 (C-3), 197.8 (C-4), 130.6 (C-5), 107.2 (C-6), 162.3 (C-7), 101.3 (C-8), 120.7 (C-1′), 157.5 (C-2′), 103.1 (C-3′), 157.6 (C-4′), 108.5 (C-5′), 130.1 (C-6′). ^{13}C NMR (acetone-d_6): δ 168.8 (C-2), 94.9 (C-3), 187.0 (C-4), 117.6 (C-5), 152.0 (C-6), 122.2 (C-7), 118.9 (C-8), 127.5 (C-1′), 127.6 (C-2′), 115.6 (C-3′), 156.5 (C-4′), 115.6 (C-5′), 127.6 (C-6′). TLC, R_f 0.43 (5:3 hexane/EtOAc); IR (KBr) 1622, 1221 cm^{-1}; EI-MS (70 eV) m/z 272 (M^+, 36), 137 (100), 124 (72), 107 (21), 100 (20) (Fukai et al 1996).

General procedure for the preparation of flavone (31–33)

*6,4′-Dihydroxyflavone (**31**)*

To a solution of 2′-hydroxy-4,5′-di(methoxymethoxy)-chalcone (400 mg, 1.16 mmol) in DMSO (5 mL) at 150°C was added iodine (15 mg, 0.06 mmol) with stirring. After being stirred for 0.5 h, the mixture was poured into a cold solution of $Na_2S_2O_3$ (1.0 g) and KOH (1.0 g) in water (50 mL). The resulting solution was diluted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous magnesium sulfate and concentrated. The compound **31c** was obtained in 50.8 % isolated yield. To a stirred solution of **31c** (0.20 g, 0.59 mmol) in 20 mL methanol was added 5 mL 3 M HCl. After 3 h, the reaction was concentrated and diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and evaporated. This crude solid was purified by column chromatography (silica gel, eluted with 38 % ethyl acetate in hexane) to **31** (65 mg, 0.25 mmol, 22.8 %) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.93 (d, J = 2.1, 1H), 6.98 (d, J = 8.7, 1H), 6.65 (dd, J = 2.1, 8.7, 1H), 6.54 (s, 1H), 7.58 (d, J = 8.4, 2H), 7.29 (d, J = 8.4, 2H). ^{13}C NMR (acetone-d_6): δ 167.9 (C-2), 93.6 (C-3), 189.2 (C-4), 117.6 (C-5), 152.0 (C-6), 122.2 (C-7), 118.9 (C-8), 127.5 (C-1′), 127.6 (C-2′), 114.6 (C-3′), 156.5 (C-4′), 115.6 (C-5′), 127.1 (C-6′). TLC, R_f 0.49 (1:3 hexane/

EtOAc); IR (KBr) 1601, 1221 cm^{-1}; EI-MS (70 eV) m/z 254 (M^+, 24), 137 (100), 121 (46), 107 (72), 93 (49) (Singh & Singh 1985).

*7,2′-Dihydroxyflavone (**32**)*

2′-Hydroxy-2,4′-di(methoxymethoxy)chalcone (0.5 g, 1.45 mmol) was treated as in **31c** to give **32** (78.7 mg, 0.31 mmol, 21.6%) as a pale brown needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.98 (d, J = 8.4, 1H), 7.14 (s, 1H), 6.51 (d, J = 8.4, 1H), 7.61 (d, J = 8.4, 1H), 6.9–7.0 (m, 1H), 7.13 (dd, J = 2.1, 8.4, 1H), 7.38 (d, J = 8.4 1H), 6.56 (s, 1H). ^{13}C NMR (acetone-d_6): δ 168.8 (C-2), 94.9 (C-3), 187.0 (C-4), 131.8 (C-5), 110.4 (C-6), 163.8 (C-7), 104.7 (C-8), 122.1 (C-1′), 155.0 (C-2′), 115.6 (C-3′), 129.1 (C-4′), 121.0 (C-5′), 128.4 (C-6′). TLC, R_f 0.44 (1:3 hexane/EtOAc); IR (KBr) 1602, 1219 cm^{-1}; EI-MS (70 eV) m/z 254 (M^+, 46), 137 (100), 121 (24), 107 (19), 93 (41) (Ahmad et al 1991).

*7,2′,4′-Trihydroxyflavone (**33**)*

2′-Hydroxy-2,4,4′-Tris(methoxymethoxy)chalcone (0.5 g, 1.24 mmol) was treated as in **31c** to give **33** (0.14 g, 0.54 mmol, 43.2%) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 8.05 (d, J = 8.4, 1H), 6.41 (dd, J = 2.1, 8.4, 1H), 6.34 (d, J = 2.1, 1H), 7.57 (d, J = 8.7, 1H), 6.54 (dd, J = 2.1, 8.4, 1H), 6.51 (d, J = 2.1, 1H), 6.32 (s, 1H). ^{13}C NMR (acetone-d_6): δ 132.4 (C-2), 121.9 (C-3), 189.0 (C-4), 131.4 (C-5), 111.6 (C-6), 163.1 (C-7), 103.6 (C-8), 114.7 (C-1′), 156.4 (C-2′), 102.8 (C-3′), 157.9 (C-4′), 108.2 (C-5′), 129.0 (C-6′). TLC, R_f 0.39 (1:3 hexane/EtOAc); IR (KBr) 1602, 1210 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 29), 137 (100), 123 (24), 109 (16), 93 (29); HR-MS (EI) exact mass for $C_{15}H_{10}O_5$ (M^+) 270.0528, found 270.0512.

General procedure for the preparation of flavonol (34, 35)

*6,4′-Dihydroxyflavonol (**34**)*

2′-Hydroxy-4,5′-di(methoxymethoxy)chalcone(144 mg, 0.4 mmol) was dissolved in ethanol (1.5 mL). Sodium hydroxide (1 M, 0.4 mL) and hydrogen peroxide (0.5 g) were added, and the mixture was heated at 90°C for 35 min. The cooled mixture was diluted with water (5 mL) and acidified with hydrochloric acid (1 M). A precipitate was collected and recrystallized from methanol. The compound **34c** was obtained in 60.0% isolated yield. To a stirred solution of **34c** (86.4 mg, 0.24 mmol) in 20 mL methanol was added 5 mL 3 M HCl. After 3 h, the reaction was concentrated and diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, filtered and evaporated. This crude solid was purified by column chromatography (silica gel, eluted with 38% ethyl acetate in hexane) to **34** (52.8 mg, 0.19 mmol, 48.7%) as a yellow needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.78 (d, J = 2.1, 1H), 6.90 (d, J = 8.4, 1H), 6.79 (d, J = 8.4, 1H), 7.33 (d, J = 8.4, 2H), 7.04 (d, J = 8.4, 2H). ^{13}C NMR (acetone-d_6): δ 134.1 (C-2), 122.1 (C-3), 187.7 (C-4), 117.6 (C-5), 153.4 (C-6), 123.6 (C-7), 117.9 (C-8), 126.9 (C-1′), 127.3 (C-2′), 114.9 (C-3′), 155.9 (C-4′), 114.9 (C-5′), 127.3 (C-6′). TLC, R_f 0.36 (1:3 hexane/EtOAc); IR (KBr) 1603, 1214 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 19), 137 (100), 121 (29), 109 (19), 93 (18); HR-MS (EI) exact mass for $C_{15}H_{11}O_5$ (M^+) 271.0606, found 271.0639.

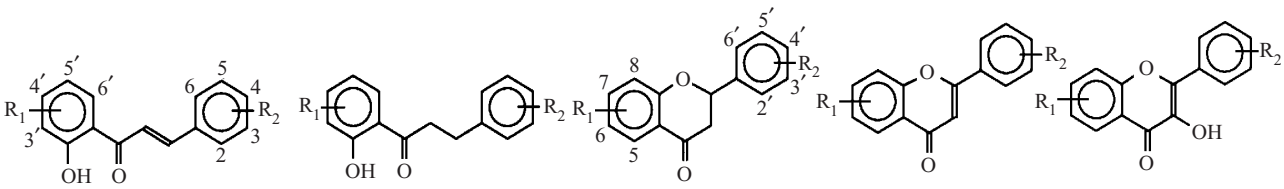
*7,2′-Dihydroxyflavonol (**35**)*

2′-Hydroxy-2,4′-di(methoxymethoxy)chalcone (0.5 g, 1.45 mmol) was treated as in **34c** to give **35** (0.23 g, 0.84 mmol, 57.8%) as a pale brown needle. ^{1}H NMR (acetone-d_6, 300 MHz) δ: 7.87 (d, J = 8.4, 1H), 6.43 (d, J = 8.4, 1H), 6.38 (s, 1H), 7.73 (d, J = 8.4, 1H), 6.8–7.0 (m, 1H), 6.64 (dd, J = 2.1, 8.4, 1H), 7.13 (d, J = 8.4 1H). ^{13}C NMR (acetone-d_6): δ 133.5 (C-2), 122.6 (C-3), 187.0 (C-4), 130.6 (C-5), 110.2 (C-6), 162.9 (C-7), 104.2 (C-8), 121.4 (C-1′), 154.5 (C-2′), 115.1 (C-3′), 128.4 (C-4′), 120.7 (C-5′), 127.6 (C-6′). TLC, R_f 0.46 (1:6 hexane/EtOAc); IR (KBr) 1601, 1219 cm^{-1}; EI-MS (70 eV) m/z 270 (M^+, 41), 137 (100), 121 (37), 109 (24), 93 (31); HR-MS (EI) exact mass for $C_{15}H_{11}O_5$ (M^+) 271.0606, found 271.0612.

Results and Discussion

The chemical structures of the synthesized flavonoid derivatives, their yields and the physicochemical data are shown in Materials and Methods. Known flavonoids are indicated by supplementary reference and flavonoids without reference are new compounds (Table 1). The structures of new flavonoid derivatives were elucidated by ^{1}H and ^{13}C NMR spectra. Their high-resolution mass spectra (M^+) were within $\pm$0.9 millimass unit of the calculated values. The geometry of chalcones was proved *trans* by ^{1}H NMR spectrum in which the coupling constant between the two vinylic protons appearing at 6.94–7.95 and 7.18–8.22 ppm were approximately 15 Hz.

Structure–activity relationships among flavonoids were estimated by their effects on rat lens aldose reductase using DL-glyceraldehyde as a substrate and by their free radical scavenging effect on DPPH (Table 1). The inhibitory activities on the enzyme were expressed as percent inhibition at 1.0 μM and as IC50 values. The



1 – 24 25 – 27 28 – 30 31 – 33 34, 35

Table 1 The physicochemical and biological data of flavonoid derivatives.

Compound	R_1	R_2	mp (°C) (lit. mp)[c]	% yield	Formula	Rat lens aldose reductase[a] IC50 (μM)	DPPH[b] IC50 (μM)
1	H	4-OH	160–161 (162–162.5)	22.9[d]	$C_{15}H_{12}O_3$	4.25	64.8 ± 4.3
2	4′-OH	4-OH	188–190 (187–188)	17.4[d]	$C_{15}H_{12}O_4$	0.40	54.7 ± 3.2
3	5′-OH	4-OH	191–192 (191–192)	21.1[d]	$C_{15}H_{12}O_4$	5.39	20.7 ± 3.6
4	4′-OCH_3	4-OH	150–153 (151–154)	24.4[e]	$C_{16}H_{14}O_4$	5.24	85.4 ± 6.9
5	5′-OCH_3	4-OH	158–160	24.1[e]	$C_{16}H_{14}O_4$	3.18	88.5 ± 5.1
6	6′-OCH_3	4-OH	175–177 (171–174)	28.9[e]	$C_{16}H_{14}O_4$	3.27	31.5 ± 2.5
7	5′-CH_3	4-OH	96–97	19.8[d]	$C_{16}H_{14}O_3$	4.38	32.8 ± 2.6
8	5′-Cl	4-OH	155–157	17.3[d]	$C_{15}H_{11}O_3Cl$	1.01	79.2 ± 9.2
9	5′-Br	4-OH	135–137	22.5[d]	$C_{15}H_{11}O_3Br$	2.15	88.9 ± 4.6
10	4′-OH	H	145–148 (149–150)	22.9[d]	$C_{15}H_{12}O_3$	1.01	85.6 ± 5.8
11	4′-OH	2-OH	194–196 (193–194)	17.0[d]	$C_{15}H_{12}O_4$	0.38	48.2 ± 6.2
12	4′-OH	4-OCH_3	130–133	24.1[e]	$C_{16}H_{14}O_4$	0.93	85.9 ± 5.9
13	4′-OH	4-Br	162–164	35.8[e]	$C_{15}H_{11}O_3Br$	1.12	145.9 ± 9.8
14	4′-OH	4-NO_2	219–222 (223–225)	8.74[d]	$C_{15}H_{11}O_5N$	3.21	198.6 ± 6.9
15	4′-OH	4-CH_3	137–139	16.0[d]	$C_{16}H_{14}O_3$	0.81	230.1 ± 12.3
16	4′-OH	4-$N(CH_3)_2$	144–146	25.2[d]	$C_{17}H_{17}O_3N$	1.15	189.5 ± 10.5
17	4′-OH	3,4-diOCH_3	112	11.3[e]	$C_{17}H_{16}O_5$	1.05	89.6 ± 9.5
18	4′-OH	3,4-diOH	214–216 (211–213)	21.5[e]	$C_{15}H_{12}O_5$	0.45	7.40 ± 0.8
19	4′-OH	3,4-diCl	103–107	33.6[e]	$C_{15}H_{10}O_3Cl_2$	1.21	150.6 ± 10.4
20	4′-OH	3-F,4-OCH_3	146–148	30.6[e]	$C_{16}H_{13}O_4F$	0.91	187.6 ± 6.8
21	4′-OH	2,4-diCH_3	110–112	29.4[e]	$C_{17}H_{16}O_3$	0.40	190.2 ± 7.9
22	4′-OH	2,4-diOH	> 320 (> 320)	28.6[e]	$C_{15}H_{12}O_5$	0.16	38.2 ± 3.2
23	4′-OH	2,5-diOH	156–158	23.9[e]	$C_{15}H_{12}O_5$	0.58	20.5 ± 2.4
24	4′-OH	2,3,4-triOH	164–169	15.9[e]	$C_{15}H_{12}O_6$	0.75	30.4 ± 3.6
25	4′-OH	4-OH	214–217	43.5[e]	$C_{15}H_{14}O_4$	> 10.0	10.2 ± 1.2
26	4′-OH	2-OH	261–267	24.6[e]	$C_{15}H_{14}O_4$	0.62	11.8 ± 0.9
27	4′-OH	2,4-diOH	295–297	52.8[d]	$C_{15}H_{14}O_5$	2.95	12.7 ± 1.1
28	6-OH	4′-OH	236–238	43.8[f]	$C_{15}H_{12}O_4$	> 10.0	87.8 ± 0.7
29	7-OH	2′-OH	202–204	34.1[d]	$C_{15}H_{12}O_4$	1.66	97.5 ± 8.7
30	7-OH	2′,4′-diOH	221–223 (225–226)	14.3[d]	$C_{15}H_{12}O_5$	2.29	107.5 ± 0.9
31	6-OH	4′-OH	> 320 (> 320)	22.8[f]	$C_{15}H_{10}O_4$	5.25	85.9 ± 8.7
32	7-OH	2′-OH	320 (> 320)	21.6[f]	$C_{15}H_{10}O_4$	> 10.0	121.5 ± 10.2
33	7-OH	2′,4′-diOH	140–142	43.2[f]	$C_{15}H_{10}O_5$	0.35	98.2 ± 7.8
34	6-OH	4′-OH	203–206	48.7[e]	$C_{15}H_{11}O_5$	> 10.0	13.8 ± 8.5
35	7-OH	2′-OH	230–232	57.8[e]	$C_{15}H_{11}O_5$	> 10.0	32.5 ± 2.7

[a]Rat lens aldose reductase activities were determined by measuring the decrease in absorption of NADPH at 340 nm in a reaction mixture containing 100 mM phosphate buffer (pH 6.3), 0.3 M ammonium sulphate, 1 mM EDTA, 0.2 mM NADPH, and 10 mM DL-glyceraldehyde in a final volume of 1 mL. [b]In microwells, 100 μL of an aqueous solution of the sample (control: 100 μL distilled water) was added to an ethanolic solution of DPPH (60 μM). Seven concentrations were prepared for each sample. After mixing gently and leaving to stand for 30 min at room temperature, the optical density was determined using a microplate reader at 517 nm. [c]Parenthesis numbers are literature melting points of known flavonoids. Solvent used for recrystallization: [d]methanol, [e]cyclohexane–ethyl acetate, [f]ethanol–water.

rat lens aldose reductase assay demonstrated that a hydroxyl group in position C-4′ was important to aldose reductase inhibition activity. This finding led to the study of the activity of compounds **1**–**9**. While compound **2**, which bears only the 4′-hydroxyl, was active (IC50 = 0.40 μM), the corresponding methoxylated compounds **4** and **3** (5′-hydroxyl), hydroxylated in a different position, were considerably less active (IC50 = 5.24 and 5.39 μM, respectively) on rat lens aldose reductase.

Given that the pK_a value (< 7.47) (Rastelli et al 2000) of compound **2** approached the pH of the enzymatic assay, these compounds probably act in their anionic forms, in line with the general view (Bors & Saran 1987) regarding the importance of the presence of anionic forms on the inhibition of aldose reductase. In support of this hypothesis, the methoxylated compound **4**, in which proton dissociation was prevented, was found to be much less active than **2**.

2′,4′-Dihydroxychalcone derivatives (**10**–**24**), except compound **14**, with a 4-nitro group in the B-ring when used at a concentration of 10^{-6} M reduced rat lens aldose reductase activity by more than 45%. The compounds possessing the highest aldose reductase inhibitory activity were 2,4,2′,4′-tetrahydroxychalcone (**22**, IC50 = 0.16 μM), 2,2′,4′-trihydroxychalcone (**11**, IC50 = 0.38 μM), 2′,4′-dihydroxy-2,4-dimethylchalcone (**21**, IC50 = 0.4 μM) and 3,4,2′,4′-tetrahydroxychalcone (**18**, IC50 = 0.45 μM) for DL-glyceraldehyde, which were slightly less potent than epalrestat (IC50 = 0.08 μM), a known potent aldose reductase inhibitor. At present, only epalrestat, which reached the Japanese market in 1992, is available for clinical trials (Costantino et al 1999).

Antioxidant or free radical scavenging activity of flavonoids has been demonstrated to be related to the number and position of free hydroxyl groups, which could act through their hydrogen donating capability (Bors & Saran 1987; Cotelle et al 1992). The DPPH system is a stable radical-generating procedure (Hatano et al 1989). It can accommodate a large number of samples in a short time and is sensitive enough to detect active principles at low concentrations, and so it was used in this study for primary screening of the free-radical-scavenging activities of 24 synthetic chalcones. Unfortunately, the chalcones tested showed a weak free radical scavenging activity except for **3**, **18**, **23** which had an *o*-dihydroxy or hydroquinone moiety. The presence of a single hydroxy group in the B ring of compounds **1**, **2**, **4**–**9** and **11** in place of the *o*-dihydroxy (**18**) and hydroquinone (**23**) structure caused a marked reduction in the free radical scavenging activity, attesting to the fundamental significance of this structural arrangement. These results suggested that an isolated hydroxyl group on the B ring made no contribution to the free radical scavenging activity. Moreover, when the free hydroxyl group was methoxylated as it was in compounds **5** or **17**, the scavenging activity was significantly decreased.

Table 2 Bodyweight and blood glucose concentrations of diabetic rats.

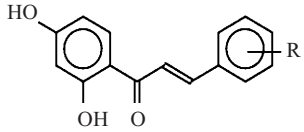
Group	Bodyweight (g)	Blood glucose (mg dL^{-1})
Normal (6)	289 ± 8	123 ± 3
Diabetic control (7)	179 ± 6	459 ± 23
Diabetic + epalrestat (6)	185 ± 9	469 ± 15
Diabetic + **2** (6)	192 ± 5	453 ± 26
Diabetic + **11** (6)	175 ± 9	476 ± 20
Diabetic + **18** (6)	191 ± 7	492 ± 19
Diabetic + **21** (6)	176 ± 8	453 ± 23
Diabetic + **22** (6)	174 ± 6	468 ± 10

Values are mean ± s.e.m. The number of animals in each group is given in parentheses.

As shown in the DPPH assay (Table 1), 3,4,2′,4′-tetrahydroxychalcone (**18**) acted as a free radical scavenger with a potency (IC50 = 7.40 ± 0.8 μM) similar to α-tocopherol, a chain-breaking antioxidant.

In the case of other the flavonoids, compounds **25**, **26** and **27** (reduction from **3**, **11** and **22**, respectively) exerted significant scavenging effect on DPPH free radical, while the inhibitory activities on aldose reductase were insignificant. Compounds **28**–**30**, flavanones, showed weak activity in both assay systems. In the case of flavones **31** and **33** (not **32**) the inhibition on aldose reductase showed a similar activity so long as the substitution pattern was the same, but radical-scavenging effect showed weak activity. The flavonols **34** and **35** exhibited slightly stronger radical-scavenging effects on DPPH. This showed that the hydroxyl group at C-3 in flavone and the saturation of α, β bond of a compound enhanced its scavenging effects on free radical DPPH (Dziedzic et al 1985; Yokozawa et al 1998).

An in-vivo study showed the efficacy of synthetic chalcones, in comparison with epalrestat, on sorbitol accumulation in the tissues of diabetic rats. The diabetes was confirmed by measurement of blood glucose. Although there were no significant differences in body weights or blood glucose concentrations between the rats treated with synthetic chalcones and diabetic control rats (Table 2), the mean sorbitol contents of the



	R
2	4-OH
11	2-OH
18	3,4-diOH
21	3,4-diOCH$_3$
22	2,4-diOH

Table 3 The effects of synthetic chalcones and epalrestat on the accumulation of sorbitol in the tissues of diabetic rats.

Group	RBC (nmol (g haemoglobin)$^{-1}$)[a] [% inhibition]	Sciatic nerve (nmol (mg wet wt)$^{-1}$)[a] [% inhibition]	Lens (nmol (mg wet wt)$^{-1}$)[a] [% inhibition]
Normal (6)	74.5 ± 6.5	0.14 ± 0.01	0.2 ± 0.01
Diabetic control (7)	163.6 ± 5.7$^{\#\#\#}$	0.47 ± 0.03$^{\#\#\#}$	18.9 ± 2.3$^{\#\#\#}$
Diabetic + epalrestat (6)	114.3 ± 7.3 [55.3]**	0.38 ± 0.02 [27.2]*	15.8 ± 1.2 [16.6]
Diabetic + **2** (6)	127.5 ± 4.7 [40.5]**	0.29 ± 0.02 [54.5]**	16.5 ± 1.3 [12.8]
Diabetic + **11** (6)	145.3 ± 8.0 [20.5]	0.28 ± 0.04 [57.2]**	15.1 ± 1.5 [20.3]
Diabetic + **18** (6)	115.5 ± 6.8 [54.0]**	0.24 ± 0.02 [69.7]***	14.7 ± 1.3 [22.5]*
Diabetic + **21** (6)	124.0 ± 9.8 [44.4]**	0.42 ± 0.03 [15.1]	14.6 ± 1.2 [23.0]*
Diabetic + **22** (6)	113.7 ± 6.6 [56.0]**	0.30 ± 0.03 [51.5]*	15.1 ± 1.0 [20.3]

Animals were given the vehicle alone, synthetic compounds (**2**, **12**, **19**, **22**, **23**) or epalrestat via intragastric tubing twice a day at a dose of 75 mg kg^{-1}/day for two weeks. The number of animals in each group is given in parenthesis. [a]Mean ± s.e.m. % Inhibition = (the sorbitol contents of control – the sorbitol contents of sample)/(the sorbitol contents of control – the sorbitol contents of normal) × 100. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control. ###$P < 0.001$ compared with normal.

RBC, sciatic nerves and lenses were observed to alter significantly, as shown in Table 3. In diabetic control rats, the sorbitol contents of the RBC (163.6 ± 5.7 nmol (g haemoglobin)$^{-1}$, $P < 0.001$ compared with normal) was much higher than in normal rats (74.5 ± 6.5 nmol (g haemoglobin)$^{-1}$). In diabetic rats treated with chalcones (**2**, **11**, **18**, **21**, **22**) or epalrestat, the accumulation of sorbitol in the RBC was significantly inhibited by 40.5 % ($P < 0.01$ compared with control), 20.5 %, 54.0 % ($P < 0.01$), 44.4 % ($P < 0.01$), 56.0 % ($P < 0.01$), and 55.3 % ($P < 0.01$), respectively. The sorbitol content of the sciatic nerves in diabetic rats was shown to be 0.47 nmol (mg wet wt)$^{-1}$ ($P < 0.001$ compared with normal), which was significantly higher than that of the normal rats (0.14 nmol (mg wet wt)$^{-1}$). Following treatment with chalcones **2**, **11** and **22**, the accumulation of sorbitol in the sciatic nerves was significantly inhibited by 54.5 % ($P < 0.01$ compared with diabetic control), 57.2 % ($P < 0.01$) and 51.5 % ($P < 0.05$), respectively. Compounds **2**, **11** and **22** except **21** (15.1 %) suppressed the accumulation of sorbitol in sciatic nerves stronger than epalrestat (27.2 %, $P < 0.05$ compared with control). Among the five chalcones tested, 3,4,2′,4′-tetrahydroxychalcone (**18**) (69.7 %, $P < 0.001$) was the most potent suppressor of the accumulation of sorbitol in sciatic nerves. In the lens, compounds **2**, **11**, **18**, **21**, **22** and epalrestat decreased sorbitol contents by 12.8, 20.3, 22.5, 23.0, 20.3 and 16.6 %, respectively.

Previous reports suggested that glucose-induced oxidative stress played a causative role in induction of aldose reductase in sensitive tissues exposed to long-term hyperglycaemia (Tawata et al 1992) and that increased oxidant production by metal-catalysed glucose oxidation may be an important mechanism in activation of aldose reductase (Ou et al 1996). Therefore, antioxidant and selective metal-chelating agents would be a useful tool in the experimental approach for the prevention of induction and activation of aldose reductase.

The abilities of the chalcones tested as transition-metal-chelators in-vitro are shown in Figure 1. The chalcones **11**, **18** and **22** enhanced partitioning of copper ion into *n*-octanol by 1.43 ± 0.08, 2.32 ± 0.09 and 1.26 ± 0.05 μM, respectively. They were more effective in partitioning copper ions into *n*-octanol than the hydrophilic metal chelator EDTA (1.25 ± 0.05 μM). Among the five chalcones, 2′,3,4,4′-tetrahydroxychalcone (**18**), with *o*-dihydroxy, was the highest transition-metal-chelator and free radical scavenger (Figure 2 and Table 1).

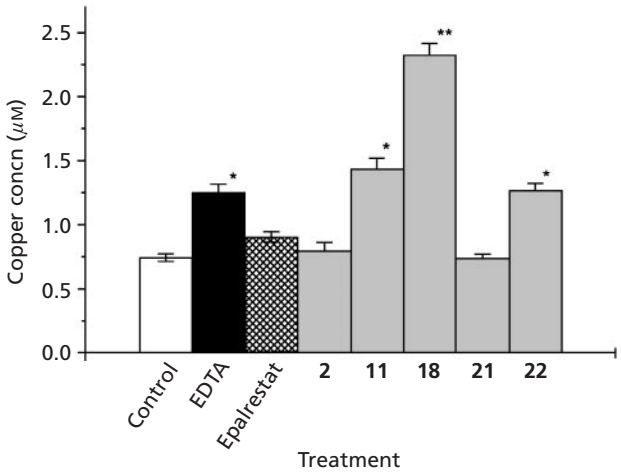


Figure 2 Effect of chalcones on Cu^{2+} partitioning into *n*-octanol. Stock solutions of compounds (all at 2.5 mM) were prepared in PBS in the presence of 1 mM $CuSO_4$. Samples (1 mL) of the compounds were mixed with *n*-octanol (2 mL) and were shaken for 10 min. After centrifugation at 1000 *g*, Cu^{2+} content in the organic layer was analysed by atomic absorption spectrophotometry. Data represent the mean ± s.d. of triplicate samples. $*P < 0.05$, $**P < 0.01$ compared with control). Control, vehicle alone; EDTA, positive control.

3,4,2′,4′-Tetrahydroxychalcone (**18**, butein) has been isolated from *Dalbergia odorifera*. It has been reported to be an inhibitor of xanthine oxidase and exhibit various antioxidant effects such as free radical scavenging, metal ion chelation and protection from lipid peroxidation in rat brain and human low density lipoprotein (Cheng et al 1998).

In conclusion, from in-vitro and in-vivo data there are strong indications that the antioxidant effect by some synthetic chalcone derivatives, as well as inhibition of aldose reductase, plays an important role in the inhibition of the accumulation of sorbitol in the tissues in streptozotocin-induced diabetic rats. As a result, we have presented some new chalcone derivatives as a group of potent sorbitol suppressors in the tissues of diabetic rats, in which 3,4,2′,4′-tetrahydroxychalcone (**18**) is the most promising compound for further investigation.

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