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Original Article

# Does nature provide the best therapeutic options? Synthesis and anti-inflammatory activity of a naturally occurring homoisoflavanone and its enantiomer

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ABSTRACT

The Hyacinthaceae family is one of the most important plant families across the eastern seaboard of Africa. The (*R*)**-5** enantiomer of a homoisoflavanone with anti-inflammatory activity was previously isolated from members of this family, namely *Drimiopsis burkei* Bak. and *Scilla nervosa* (Burch.) Jessop. However, the activity of the (*S*)**-5** enantiomer is unknown. In this paper, we report the synthesis and structural elucidation, *in vivo* anti-inflammatory activity and *in vitro* cytotoxic properties of both the (*R*)**-5** and (*S*)**-5** enantiomers and the racemate. The enantiomers and racemate exhibited a relatively short duration of action and activity similar to that of the known non-steroidal anti-inflammatory drug, diclofenac. The naturally occurring enantiomer exhibited the least cytotoxicity.



## 1. Introduction

A homoisoflavanone, (3*R*)-5,7-dimethoxy-(4′-hydroxybenzyl)-4-chromanone of the compound (*R*)**-5**, was previously isolated from *Scilla nervosa* (Burch.) Jessop[1] as well as from *Drimiopsis burkei* Bak.[2] The traditional use of *S. nervosa* for rheumatic fever indicates possible anti-inflammatory properties of its constituents.[3] Subsequent studies showed strong inhibition of prostaglandin synthesis in microsomal cells by the isolated homoisoflavanone, supporting the traditional use of *S. nervosa*.[4] Studies indicate that stereoselectivity plays an important role in the anti-inflammatory activities of non-steroidal anti-inflammatory drugs.[5] The decision to employ either a racemate or a pure enantiomer for therapeutic purposes is usually based on the diverse mechanisms of actions of the enantiomers.[6] The absolute configuration at C-3 position of a series of naturally occurring homoisoflavanones was investigated using circular dichroism.[2,7] The (*R*)-configuration was established for all of these compounds.[2,7] Therefore, the anti-inflammatory activity of the naturally occurring (*R*)**-5** enantiomer is known, but the activity of the (*S*)**-5** enantiomer and racemate is unknown. A study of the anti-inflammatory

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activity of both the enantiomers could provide an answer to the question whether nature truly provides the best therapeutic options.

## 2. Materials and methods

### 2.1. General experimental procedures

All reagents were obtained from Aldrich chemicals suppliers and solvents were obtained from a commercial supplier and used without further purification. All reaction mixtures were magnetically stirred and monitored by TLC using Kieselgel 60 F254 obtained from Merck (Darmstadt, Germany). $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AVANCE III at 400 MHz with $CDCl_3$ as internal reference. The value for chemical shift ($\delta$) is given in ppm and coupling constants ($J$) in Hertz (Hz). Melting points were recorded with a Mel-Temp melting point apparatus in open capillaries and are uncorrected. Optical rotations were measured at room temperature in chloroform using a Perkin Elmer Polarimeter-Model 341. High-resolution mass spectroscopy (HRMS) data was recorded on a Waters Micromass Q-Tof Micro mass spectrometer with a lock spray source.

### 2.2. Synthesis and structural elucidation of homoisoflavanones

#### 2.2.1. 3-(3,5-Dimethoxyphenoxy) propanoic acid 2

Synthetic procedure, $^1$H and $^{13}$C NMR data were previously reported[8]; mass $m/z = 227$ $(M + 1)^+$. $R_f = 0.24$ on silicagel with ethyl acetate/hexane (30:70).

#### 2.2.2. 5,7-Dimethoxy-4-chromanone 3

Synthetic procedure, $^1$H and $^{13}$C NMR data were previously reported[8]; mass $m/z = 209$ $(M + 1)^+$. $R_f = 0.54$ on silicagel with ethyl acetate/hexane (30:70).

#### 2.2.3. (E)-5,7-Dimethoxy-3-(4′-hydroxybenzylidene)-4-chromanone 4

Synthetic procedure, $^1$H and $^{13}$C NMR data were previously reported.[8] HRMS calcd for $C_{18}H_{17}O_4$ $[M + H]^+$ 297.1049, found 297.1121; $R_f = 0.58$ on silicagel with ethyl acetate/hexane (30:70).

#### 2.2.4. 5,7-Dimethoxy-3-(4′-hydroxybenzyl)-4-chromanone (R,S)-5

To a solution of 5,7-dimethoxy-3-(4′-hydroxybenzylidene)-4-chromanone (1.0 g, 3.2 mmol) in a mixture of anhydrous MeOH/THF (1:1, 20 ml) at a temperature of 0 °C, Pd/c (0.4 g, 3.8 mmol) was added portion wise. $H_2$ gas was passed through the stirred mixture at room temperature for 0.5 h after which it was filtered through celite and concentrated under reduced pressure. The residue obtained after evaporation of the solvent was chromatographed over a silicagel column using mixture of ethyl acetate/hexane (20:80) as eluent to produce the homoisoflavanone (R,S)-**5**. Yield 68%; $R_f = 0.43$ (20:80 ethyl acetate/hexane); mp 174–176 °C; light yellow powder; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.65 (1H, dd, $J$ = 10.4, 13.5 Hz, H-9a), 2.68–2.70 (1H, m, H-3), 3.15 (1H, dd, $J = 4.1$, 13.4 Hz, H-9b), 3.81 (3H, s, Ar-$OCH_3$-7), 3.86 (3H, s, Ar–$OCH_3$-5), 4.12 (1H, dd, $J = 4.2$, 7.0 Hz, H-2a), 4.27 (1H, dd, $J = 3.9$, 11.2 Hz, H-2b), 6.06 (1H, s, H-8), 6.07 (1H, s, H-6), 6.80 (2H, d, $J = 8.4$ Hz, H-2′,6′), 7.07 (2H, d, $J = 8.4$ Hz, H-3′,5′); $^{13}$C NMR (100 MHz, $CDCl_3$) 32.1 ($CH_2$, C-9), 48.6 (CH, C-3), 55.0 ($OCH_3$, C-7), 55.8 ($OCH_3$, C-5), 68.8 ($CH_2$, C-2), 92.8 (CH, C-8), 93.2 (CH, C-6), 130.2 (CH, C-2′,6′), 105.4 (C, C-4a), 115.5 (CH, C-3′,5′), 130.4 (C, C-1′), 154.7 (C, C-4′), 162.8 (C, C-7), 165.0 (C, C-8a), 165.7 (C, C-5), 191.9 (C, C-4); HRMS (EI) calcd for $C_{18}H_{19}O_5$ 315.1154, found 315.1224.

#### 2.2.5. 5,7-Dimethoxy-3-(4′-hydroxybenzyl)-4-chromanol (R,R)-6/(R,S)-6

$NaBH_4$ (0.3 g, 9.5 mmol) was added portion wise to a solution of 5,7-dimethoxy-3-(4-hydroxybenzyl)-4-chromanone (1.0 g, 3.1 mmol) in anhydrous MeOH (15 ml) at a temperature of 0 °C under nitrogen atmosphere. The mixture was then allowed to reach room temperature and stirred for 1 h. The reaction mixture was quenched with water and extracted with ethyl acetate (3 × 30). The organic layer was washed with brine, dried over magnesium sulphate, and concentrated under reduced pressure to produce a viscous oil mixture of (R,R)-**6** and (R,S)-**6**. The residue obtained after evaporation of the solvent was chromatographed over a silicagel column using mixture of ethyl acetate/hexane (30:70) as eluent to produce an oily syrup at an overall yield of 88%. Compound (R,R)-**6**; $R_f$ = 0.48 (30:70 ethyl acetate/hexane); oily syrup; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.08–2.15 (1H, m, H-3), 2.58 (1H, dd, $J = 2.6$, 7.2 Hz, H-9a), 2.85 (1H, dd, $J$ = 2.6, 7.2 Hz, H-9b), 3.78 (3H, s, Ar–$OCH_3$-5), 3.83 (3H, s, Ar-$OCH_3$-7), 3.99 (2H, d, $J = 8.2$ Hz, H-2a & 2b), 4.66 (1H, d, $J = 2.5$ Hz, H-4), 5.99 (1H, d, $J = 7.1$ Hz, H-8), 6.01 (1H, d, $J = 7.1$ Hz, H-6), 6.76 (2H, d, $J = 8.2$ Hz, H-3′,5′), 7.12 (2H, d, $J = 8.0$ Hz, H-2′,6′); $^{13}$C NMR (100 MHz, $CDCl_3$) 31.9 ($CH_2$, C-9), 40.1 (CH, C-3), 55.3 ($OCH_3$, C-7), 55.4 ($OCH_3$, C-5), 59.6 (CH, C-4), 65.2 ($CH_2$, C-2), 91.3 (CH, C-6), 93.0 (CH, C-8), 106.6 (C, C-4a), 115.2 (CH, C-3′,5′), 130.2 (C, C-1′), 131.6 (CH, C-2′,6′), 153.8 (C, C-4′), 155.9 (C, C-5), 159.2 (C, C-8a), 161.1 (C, C-7); mass $m/z = 317$ $(M + 1)^+$.

Compound (R,S)-**6**; $R_f = 0.45$ (30:70 ethyl acetate/hexane); oily syrup; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.12-2.18 (1H, m, H-3), 2.40 (1H, dd, $J = 2.9$, 7.9 Hz, H-9a), 2.55 (1H, dd, $J = 2.9$, 7.9 Hz, H-9b), 3.76 (3H, s, Ar–$OCH_3$-5), 3.81 (3H, s, Ar–$OCH_3$-7), 3.90 (1H, dd, $J = 1.8$, 1.8 Hz, H-2a), 4.07 (1H, dd, $J = 1.9$, 2.0 Hz, H-2b), 4.62 (1H, s, H-4), 6.06 (1H, d, $J = 3.9$ Hz, H-6), 6.07 (1H, d, $J = 3.9$ Hz, H-8), 6.74 (2H, d, $J = 8.3$ Hz, H-3′,5′), 7.04 (2H, d, $J = 8.3$ Hz, H-2′,6′); $^{13}$C NMR (100 MHz, $CDCl_3$) 33.6 ($CH_2$, C-9), 40.5 (CH, C-3), 55.3 ($OCH_3$, C-7), 55.5 ($OCH_3$, C-5), 62.9 (CH, C-4), 64.3 ($CH_2$, C-2), 91.8 (CH, C-6), 93.2 (CH, C-8), 104.9 (C, C-4a), 115.3 (CH, C-3′,5′), 130.2 (C, C-1′), 131.2 (CH, C-2′,6′), 154.2 (C, C-4′), 155.8 (C, C-5), 159.8 (C, C-8a), 161.0 (C, C-7); mass $m/z = 317$ $(M + 1)^+$.

#### 2.2.6. 5,7-Dimethoxy-3-(4′-hydroxybenzyl)-4-chromanone (R)-5 or (S)-5

To a mixture of either (R,R)-**6** or (R,S)-**6** respectively (0.1 g, 1.0 mmol) in acetic acid (4 ml) was added $CrO_3$ (0.16 g, 5.0 mmol). The reaction mixture was stirred at room temperature and allowed to stand for 0.5 h. The solvent was evaporated and extracted with ethyl acetate (2 × 15 ml). The organic layer was washed with brine (2 × 15 ml) and dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed over a silicagel column using a mixture of ethyl acetate/hexane (20:80) as eluent to