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Synthesis and radical scavenging of novel magnolol derivatives

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Abstract

We have investigated the developdment of potential antioxidants based on magnolol, a naturally occurring biphenolic obtained from the bark of *Magnolia officinalis*. A series of aminomethylated derivatives of magnolol were synthesized under the aromatic Mannich reaction. In-vitro testing for diphenyl-*p*-picrylhydrazyl (DPPH) scavenging and chemiluminescence assays in whole cell models revealed that the pyrrolidyl-containing magnolols (**2b** (*5,5′-diallyl-3-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol*), **3a** (*5,5′-diallyl-3,3′-bis-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol*) and **4c** (*5,5′-diallyl-3-(morphorin-4-ylmethyl)-3′-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol*)) displayed promising free radical scavenging effects as compared with magnolol. The results from compound **4c** indicated that the naturally occurring component was suitable to be a lead compound toward promising antioxidants.

Introduction

Over the past decade, there has been a resurgence of interest in the discovery of natural constituents or their semi-synthetic derivatives as a source of potential pharmacological agents (Cragg et al 1997). More importantly was that most of these natural products were used in traditional medicine (Farnsworth et al 1985). Increasing evidence implies that free radicals and their related reactive oxygen-reactive species appear to be a consequence of tissue damage, exacerbating and amplifying disease pathology (Powell & Tortolani 1992). It is conceivable that free radical-related tissue damage applies to most of the neurodegenerative diseases associated with ageing (Ames et al 1993; Gutteridge 1994). Therefore, in the search of potential agents with neuroprotective effects, much attention has been paid to the discovery of hydroxylated biphenyl compounds as antioxidants, due to their great scavenging effect on oxygen-derived free radicals (Taira et al 1993; Fujita et al 1994). Magnolol (**1**, Figure 1), a naturally occurring and major biphenolic constituent of *Magnolia officinalis*, has been found to possess broad pharmacological profiles. It can relax rat vascular smooth muscle and exhibits a strong scavenging effect against hydroxyl radicals (Teng et al 1990). Recent in-vivo investigations showed that magnolol could attenuate peroxidative damage, improve survival of rats with sepsis, and protect cortical neuronal cells from chemical hypoxia in rats (Lee et al 1998; Kong et al 2000). Meanwhile, *M. officinalis*, known in Chinese folk medicine as houpo, has long been utilized for treating anxiety, cardiovascular and allergic diseases such as thrombosis and bronchial asthma. On the basis of those previous investigations and traditional effects of the herb, it was strongly conceivable that magnolol could be a suitable lead compound for the development of potent free radical scavengers as novel antioxidants.

Materials and Methods

Chemistry

All reagents were commercial materials and were used directly unless otherwise noted. Dimethylformamide was dehydrated over a 4-Å molecular sieve. NMR spectra were recorded on a Varian Gemini at 300 MHz for ^{1}H and at 75 MHz for ^{13}C. High-

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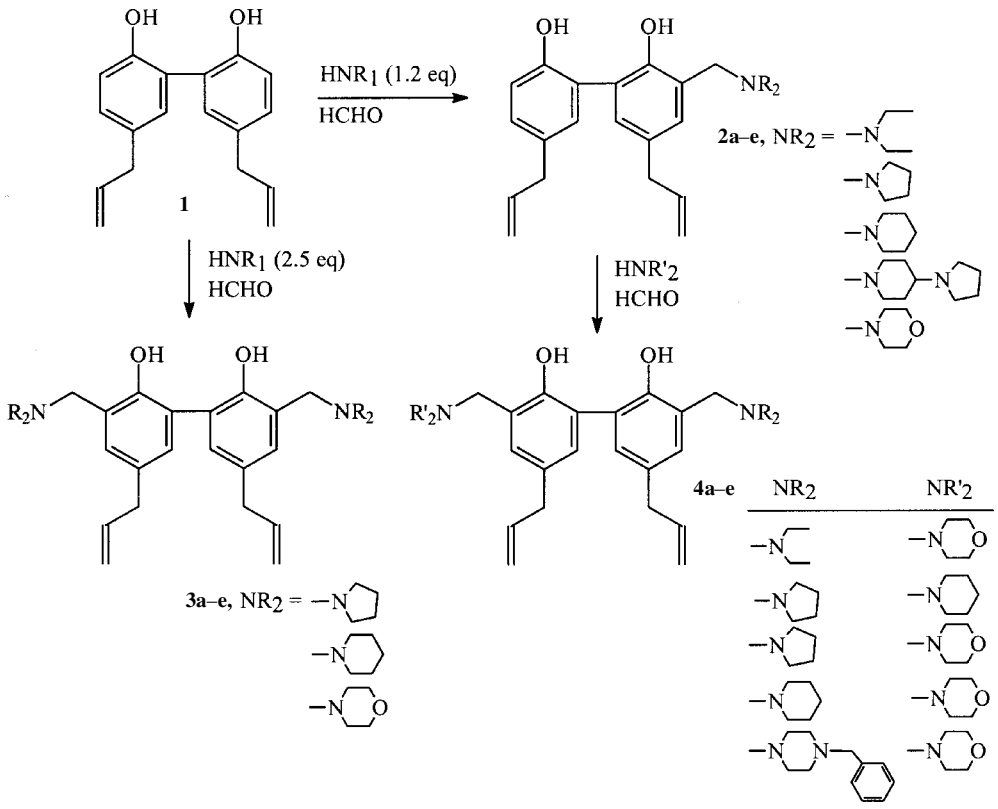

Figure 1 Synthesis of aminomethylated derivatives of magnolol (**1**).

resolution mass spectra were obtained on a JEOL J.M.S.-300 spectrometer. Reactions were followed by thin-layer chromatography (TLC) on Merck (0.2 mm) aluminum-packed precoated silica gel plates (60 F_{254}). Chromatography refers to flash chromatography on silica gel (silica gel 60, 230–400 mesh ASTM, E. Merck). Melting points were recorded on a Thomas Hoover capillary melting point apparatus in open capillary tubes and are uncorrected.

Magnolol (5,5′-diallyl-biphenyl-2,2′-diol, 1)

The dried stem bark of *M. officinalis* (1.0 kg) was soaked in anhydrous ethanol at room temperature for three days. Concentration of the solvent gave a dark brown syrup alcoholic extract (300 g), which was taken to silica gel chromatography (n-hexane/EtOAc = 20:1 → 15:1 as eluents). The desired magnolol was obtained (4.5 g) as a white solid: mp 99–101°C (lit. 100–102°C (Fujita et al 1972)); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13–6.92 (6H, m, Ar-H), 6.05–5.93 (2H, m, 2 CH=), 5.14–5.07 (4H, m, 2 $=CH_2$), 3.37 (4H, d, J = 6.6 Hz, 2 Ar-CH_2); ^{13}CNMR (75 MHz, $CDCl_3$) δ 151.8, 138.1, 131.8, 130.5, 124.3, 117.2, 116.4, 40.0; UV λ_{max} (EtOH) nm (ϵ): 290 (5370); FABMS (NBA as matrix): m/z $[M+H]^+$ 266.1.

Preparation of aminomethylated magnolols: procedure A

To a secondary amine (1 equiv.) was added a formaldehyde solution (36%, 1.5 equiv.) at 0°C. The mixture was stirred for 1 h, and then a solution of magnolol (1 equiv.) in methanol (20 mL) or ethanol (20 mL) was added. The resulting mixture was heated under reflux for 2 h and the solvent was evaporated to give a pale yellow oil. The residue was diluted with ethyl acetate and washed with water, aqueous carbonate, and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in-vacuo to give an oil, which was purified by flash chromatography on silica gel (n-hexane/EtOAc = 3:1 to 1:1 to 1:3) to afford the respective aminomethylated magnolol.

Preparation of symmetrical aminomethylated magnolols: procedure B

To a secondary amine was added formaldehyde solution (36%) under an ice bath. The mixture was stirred for 1 h, and then a solution of magnolol or mono-aminomethylated magnolol in methanol was added. The resulting mixture was heated under reflux for two days and the solvent was evaporated to give a yellow oil. The residue was diluted with ethyl acetate and washed with water, aqueous carbonate, and brine. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in-vacuo to give an oil, which was purified by flash chromatography on silica gel (n-hexane/EtOAc = 3:1, 1:1 then EtOAc/MeOH = 95:5) to afford the title compound.

5,5′-diallyl-3-diethylaminomethyl-biphenyl-2,2′-diol (2a)

According to procedure A, reaction of magnolol (0.8 g, 3.0 mmol), diethylamine (0.31 mL, 3.0 mmol) and a formaldehyde solution (0.26 mL) in ethanol (30 mL) after 5 h, aqueous workup, and chromatography on silica gel afforded the title compound (0.82 g, 78%) as a pale yellow solid: mp 93–95°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.10 (3H, m, Ar-H), 7.00 (1H, s Ar-H), 6.84 (1H, s, Ar-H), 6.10–5.95 (2H, m, 2 CH═), 5.15–5.00 (4H, m, 2 ═CH_2), 3.90 (2H, s, Ar-CH_2-N), 3.39 (2H, d, J = 6.7 Hz, Ar-CH_2), 3.35 (2H, d, J = 6.7 Hz, Ar-CH_2), 2.73 (4H, q, J = 7.3 Hz, 2 N-CH_2); 1.16 (6H, t, J = 7.3 Hz, 2 CH_3); UV λ_{max} (EtOH) nm (ϵ): 320 (740); FABMS (NBA as matrix): m/z $[M+H]^+$ 352.2; HR-FABMS exact mass calcd for $C_{23}H_{30}NO_2$ $[M+H]^+$ 352.2277, found 352.2274.

5,5′-diallyl-3-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol (2b)

According to procedure A, reaction of magnolol (0.53 g, 2.0 mmol), pyrrolidine (0.18 mL, 2.0 mmol) and a formaldehyde solution (0.30 mL) in ethanol (25 mL) after 5 h, aqueous workup, and chromatography on silica gel afforded the title compound (0.48 g, 69%) as a pale yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13–7.10 (3H, m, Ar-H), 7.04 (1H, s Ar-H), 6.90 (1H, s, Ar-H), 6.05–5.95 (2H, m, 2 CH═), 5.13–5.03 (4H, m, 2 ═CH_2), 4.01 (2H, s, Ar-CH_2-N), 3.38 (2H, d, J = 6.5 Hz, Ar-CH_2), 3.34 (d, J = 6.8 Hz, 2H, Ar-CH_2), 2.86 (4H, s br, 2 N-CH_2); 1.90 (4H, s br, CH_2CH_2); UV λ_{max} (EtOH) nm (ϵ): 322 (5495); FABMS (NBA as matrix): m/z $[M+H]^+$ 350.2; HR-FABMS exact mass calcd for $C_{23}H_{28}NO_2$ $[M+H]^+$ 350.2208, found 350.2206.

5,5′-diallyl-3-(piperidin-1-ylmethyl)-biphenyl-2,2′-diol (2c)

According to procedure A, reaction of magnolol (0.53 g, 2.0 mmol), pyrrolidine (0.20 mL, 2.0 mmol) and a formaldehyde solution (0.30 mL) in ethanol (25 mL) after 5 h, aqueous workup, and chromatography on silica gel afforded the title compound (0.54 g, 74%) as a pale yellow solid: mp 78–80°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18–7.10 (3H, m, Ar-H), 7.06 (1H, s Ar-H), 6.90 (1H, s, Ar-H), 6.05–5.95 (2H, m, 2 CH═), 5.15–5.05 (4H, m, 2 ═CH_2), 3.86 (2H, s, Ar-CH_2-N), 3.38 (2H, d, J = 6.5 Hz, Ar-CH_2), 3.33 (2H, d, J = 6.4 Hz, Ar-CH_2), 2.86 (4H, s br, 2 N-CH_2), 1.80–1.65 (6H, s br, $CH_2CH_2CH_2$); UV λ_{max} (EtOH) nm (ϵ): 306 (5010); FABMS (NBA as matrix): m/z $[M+H]^+$ 364.2; HR-FABMS exact mass calcd for $C_{24}H_{30}NO_2$ $[M+H]^+$ 364.2365, found 364.2372.

5,5′-diallyl-3-(4-pyrrolidin-1-yl-piperidin-1-ylmethyl)-biphenyl-2,2′-diol (2d)

According to procedure A, reaction of magnolol (0.8 g, 3.0 mmol), 4-(1-pyrrolidyl)piperidine (0.46 mL, 3.0 mmol) and a formaldehyde solution (0.45 mL) in ethanol (25 mL) after 5 h, aqueous workup, and chromatography on silica gel afforded the title compound (0.62 g, 48%) as a pale yellow solid: mp 69–71°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15–7.10 (4H, m, Ar-H), 7.02 (1H, s Ar-H), 6.80 (1H, s, Ar-H), 6.05–5.90 (2H, m, 2 CH═), 5.15–5.05 (4H, m, 2 ═CH_2), 3.80 (2H, s, Ar-CH_2-N), 3.40–3.32 (4H, m, 2 Ar-CH_2), 3.15–3.00 (1H, m, N-CH), 2.64 (4H, s br, 2 N-CH_2); 2.28 (4H, s br, N-CH_2), 2.00–1.65 (8H, m, 2 CH_2CH_2); UV λ_{max} (EtOH) nm (ϵ): 300 (4073); FABMS (NBA as matrix): m/z $[M+H]^+$ 433.3; HR-FABMS exact mass calcd for $C_{28}H_{36}N_2O_2$ $[M+H]^+$ 433.2517, found 333.2523.

5,5′-diallyl-3-(morpholin-4-ylmethyl)-biphenyl-2,2′-diol (2e)

According to procedure A, reaction of magnolol (0.4 g, 1.5 mmol), morpholine (0.13 mL, 1.5 mmol) and a formaldehyde solution (0.13 mL) in ethanol (20 mL) after 5 h, aqueous workup, and chromatography on silica gel afforded the title compound (0.46 g, 84%) as a pale yellow solid: mp 102–104°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–6.84 (1H, s, Ar-H), 6.05–5.96 (2H, m, 2 CH═), 5.15–5.05 (4H, m, 2 ═CH_2), 3.81 (2H, s, Ar-CH_2-N), 3.80–3.70 (4H, s br, 2 O-CH_2), 3.39 (2H, d, J = 6.4 Hz, Ar-CH_2), 3.35 (2H, d, J = 6.5 Hz, Ar-CH_2), 2.64 (4H, s br, 2 N-CH_2); UV λ_{max} (EtOH) nm (ϵ): 296 (6606); FABMS (NBA as matrix): m/z $[M+H]^+$ 366.2; HR-FABMS exact mass calcd for $C_{23}H_{28}NO_3$ $[M+H]^+$ 366.2069, found 366.2074.

5,5′-diallyl-3,3′-bis-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol (3a)

According to procedure B, reaction of magnolol (0.27 g, 1.0 mmol), pyrrolidine (0.18 mL, 2.0 mmol) and a formaldehyde solution (0.30 mL) in ethanol (20 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.26 g, 60%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (2H, s, Ar-H), 6.88 (2H, s, Ar-H), 6.05–5.90 (2H, m, 2 CH═), 5.13–5.03 (4H, m, 2 ═CH_2), 3.94 (4H, s, 2 Ar-CH_2-N), 3.39–3.30 (4H, m, 2 Ar-CH_2), 2.68 (8H, s br, 4 N-CH_2); 1.84 (8H, s br, 2 CH_2CH_2); UV λ_{max} (EtOH) nm (ϵ): 324 (3090); FABMS (NBA as matrix): m/z $[M+H]^+$ 433.2; HR-FABMS exact mass calcd for $C_{28}H_{37}N_2O_2$ $[M+H]^+$ 433.2855, found 433.2851.

5,5′-diallyl-3,3′-bis-(piperidin-1-ylmethyl)-bipheny-2,2′-diol (3b)

According to procedure B, reaction of magnolol (0.53 g, 2.0 mmol), pyrrolidine (0.40 mL, 2.0 mmol) and a formaldehyde solution (0.60 mL) in ethanol (25 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.42 g, 46%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (2H, s, Ar-H), 6.82 (2H, s Ar-H), 6.05–5.90 (2H, m, 2 CH═), 5.15–5.05 (4H, m, 2 ═CH_2), 3.77 (4H, s br, 2 Ar-CH_2-N), 3.38–3.30 (4H,

m, 2 Ar-CH_2), 2.56 (8H, s br, 4 N-CH_2), 1.70–1.45 (12H, s br, 2 $CH_2CH_2CH_2$); UV λ_{max} (EtOH) nm (ϵ): 300 (776); FABMS (NBA as matrix): m/z $[M+H]^+$ 364.2; HR-FABMS exact mass calcd for $C_{24}H_{30}NO_2$ $[M+H]^+$ 364.2365, found 364.2372.

5,5′-diallyl-3,3′-bis-(morphorin-4-ylmethyl)-biphenyl-2,2′-diol (3c)

According to procedure B, reaction of magnolol (0.2 g, 0.75 mmol), morpholine (0.13 mL, 1.5 mmol) and a formaldehyde solution (0.20 mL) in ethanol (15 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.19 g, 55%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (2H, s, Ar-H), 6.85 (2H, s, Ar-H), 6.05–5.92 (2H, m, 2 CH=), 5.15–5.05 (4H, m, 2 $=CH_2$), 3.75 (4H, s, 2 Ar-CH_2-N), 3.70 (8H, s br, 4 O-CH_2), 3.35 (4H, d, J = 6.3 Hz, 2 Ar-CH_2), 2.70–2.45 (8H, m, 4 N-CH_2); UV λ_{max} (EtOH) nm (ϵ): 296 (4570); FABMS (NBA as matrix): m/z $[M+H]^+$ 465.2; HR-FABMS exact mass calcd for $C_{28}H_{37}N_2O_4$ $[M+H]^+$ 465.2753, found 465.2743.

5,5′-diallyl-3-diethylaminomethyl-3′-(morphorin-4-ylmethyl)-biphenyl-2,2′-diol (4a)

According to procedure B, reaction of 3-substituted magnolol **2e** (0.46 g, 1.3 mmol), diethylamine (0.13 mL, 1.3 mmol) and a formaldehyde solution (0.20 mL) in ethanol (20 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.20 g, 35%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (2H, s, Ar-H), 6.84 (2H, s, Ar-H), 6.05–5.95 (2H, m, 2 CH=), 5.15–5.05 (4H, m, 2 $=CH_2$), 3.78 (2H, s, Ar-CH_2-N), 3.74 (2H, s, Ar-CH_2-N), 3.70 (4H, s br, 2 O-CH_2), 3.35 (4H, s br, 2 Ar-CH_2), 2.80–2.65 (4H, m, 2 N-CH_2), 2.60 (4H, s br, 2 N-CH_2), 1.15 (6H, s br, 2 CH_3); UV λ_{max} (EtOH) nm (ϵ): 300 (5495); FABMS (NBA as matrix): m/z $[M+H]^+$ 451.2; HR-FABMS exact mass calcd for $C_{28}H_{39}N_2O_3$ $[M+H]^+$ 451.3014, found 451.3022.

5,5′-diallyl-3-(piperidin-1-ylmethyl)-3′-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol (4b)

According to procedure B, reaction of 3-substituted magnolol **2c** (0.35 g, 1.0 mmol), pyrrolidine (0.09 mL, 1.0 mmol) and formaldehyde solution (0.15 mL) in ethanol (20 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.35 g, 49%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (2H, s, Ar-H), 6.85 (2H, s, Ar-H), 6.05–5.95 (2H, m, 2 CH=), 5.15–5.00 4H, m, 2 $=CH_2$), 4.01 (2H, s, Ar-CH_2-N), 3.78 (2H, s, Ar-CH_2-N), 3.38–3.30 (4H, m, 2 Ar-CH_2), 2.83–2.60 (8H, m, 4 N-CH_2), 1.88–1.50 (10H, m, 5 CH_2CH_2); UV λ_{max} (EtOH) nm (ϵ): 302 (5128); FABMS (NBA as matrix): m/z $[M+H]^+$ 447.3; HR-FABMS exact mass calcd for $C_{29}H_{38}N_2O_2$ $[M+H]^+$ 447.2702, found 447.2709.

5,5′-diallyl-3-(morphorin-4-ylmethyl)-3′-(pyrrolidin-1-ylmethyl)-biphenyl-2,2′-diol (4c)

According to procedure B, reaction of 3-substituted magnolol **2e** (0.46 g, 1.3 mmol), pyrrolidine (0.18 mL, 2.0 mmol) and a formaldehyde solution (0.20 mL) in ethanol (20 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.35 g, 61%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (2H, s, Ar-H), 6.84 (2H, s, Ar-H), 6.05–5.95 (2H, m, 2 CH=), 5.15–5.00 (4H, m, 2 $=CH_2$), 3.98 (2H, s, Ar-CH_2-N), 3.76 (2H, s, Ar-CH_2-N), 3.75–3.65 (4H, m, 2 O-CH_2), 3.37–3.30 (4H, m, 2 Ar-CH_2), 2.77 (4H, s br, 2 N-CH_2), 2.60 (4H, s br, 2 N-CH_2), 1.87 (4H, t, J = 6.2 Hz, CH_2CH_2); UV λ_{max} (EtOH) nm (ϵ): 306 (4570); FABMS (NBA as matrix): m/z $[M+H]^+$ 449.2; HR-FABMS exact mass calcd for $C_{28}H_{37}N_2O_3$ $[M+H]^+$ 449.2804, found 449.2807.

5,5′-diallyl-3-(morphorin-4-ylmethyl)-3′-(piperidin-1-ylmethyl)-biphenyl-2,2′-diol (4d)

According to procedure B, reaction of 3-substituted magnolol **2e** (0.46 g, 1.0 mmol), piperidine (0.15 mL, 1.5 mmol) and a formaldehyde solution (0.10 mL) in ethanol (15 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.20 g, 44%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (2H, s, Ar-H), 6.85 (2H, s, Ar-H), 6.05–5.95 (2H, m, 2 CH=), 5.15–5.00 (4H, m, 2 $=CH_2$), 3.77 (4H, s, 2 Ar-CH_2-N), 3.72 (4H, s br, 2 O-CH_2), 3.35 (4H, d, J = 6.7 Hz, 2 Ar-CH_2), 2.60 (8H, s br, 4 N-CH_2), 1.70–1.45 (6H, m, $CH_2CH_2CH_2$); UV λ_{max} (EtOH) nm (ϵ): 298 (5650); FABMS (NBA as matrix): m/z $[M+H]^+$ 463.3; HR-FABMS exact mass calcd for $C_{29}H_{39}N_2O_3$ $[M+H]^+$ 463.2961, found 463.2960.

5,5′-diallyl-3-(4-benzyl)-piperazin-1-ylmethyl)-3′-(morphorin-4-ylmethyl)-biphenyl-2,2′-diol (4e)

According to procedure B, reaction of 3-substituted magnolol **2e** (0.46 g, 1.0 mmol), 4-benzylpiperazine (0.17 mL, 1.0 mmol) and a formaldehyde solution (0.10 mL) in ethanol (15 mL) after two days, aqueous workup, and chromatography on silica gel afforded the title compound (0.39 g, 60%) as a light brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (5H, s, br, Ar-H), 7.14 (2H, s, Ar-H), 6.85 (2H, s, Ar-H), 6.08–5.94 (2H, m, 2 CH=), 5.18–5.04 (4H, m, 2 $=CH_2$), 3.88–3.70 (8H, m, 2 Ar-CH_2 & O-CH_2), 3.56 (2H, s, $PhCH_2N$), 3.34 (4H, d, J = 6.8 Hz, 2 Ar-CH_2), 2.70–2.4 (12H, m, 3 N-$(CH_2)_2$); UV λ_{max} (EtOH) nm (ϵ): 296 (6920); FABMS (NBA as matrix): m/z $[M+H]^+$ 554.3; HR-FABMS exact mass calcd for $C_{35}H_{44}N_3O_3$ $[M+H]^+$ 554.3436, found 554.3413.

Pharmacological evaluation

Free radical scavenging actions

DPPH (diphenyl-*p*-picrylhydrazyl), a stable nitrogen-centred free radical, was dissolved in methanol to give a 100 μM solution. The tested compound (20 or 80 μM) was added to 1.0 mL methanolic DPPH in a cuvette. The decrease in absorption at 517 nm was correlated with the scavenging action of the tested compound. The concentration of the antioxidant that induced a change of 0.20 in absorbance during the 30-min observation time was taken as evaluation of the antioxidant activity.

Measurement of chemiluminescence

To a sample containing 180-μL heparinized whole blood in a 96-well plate was added the tested compound with gradient concentration followed by phorbol myristic acetate (PMA; 2 μg mL^{-1}) or lipopolysaccharide (LPS; 10 μg mL^{-1}) and lucigenin (1 μg mL^{-1}). After standing for 15 min in dark conditions, the final volume of each well was set to 200 μL with the addition of phosphate-buffered saline (PBS). Chemiluminescence was monitored in a luminometer (Packard LumiCount) and the peak height was recorded in mV. Two negative models, which were devoid of either lucigenin or PMA, and a positive model that contained both lucigenin and PMA were conducted as background for control.

Statistical analysis

All data are presented as the mean $\pm$ s.e.m. for the number of experiments indicated in the legends. Statistical analysis was evaluated using Student's *t*-test and $P < 0.05$ was regarded as significantly different.

Results and Discussion

The natural magnolol was extracted from the dried stem bark of *Magnolia officinalis* and identified by published data (Fujita et al 1972). We first modified magnolol with aminomethylation to furnish a series of mono- and diaminomethylated derivatives of magnolol, which were readily prepared following the aromatic Mannich reaction (Lubben & Feringa 1994), from which *ortho*-aminomethylated phenols could be synthesized in mild conditions. Thus, starting from magnolol, mono- and diaminomethylated magnolols were obtained. In these reaction processes, magnolol, secondary amines (1.1 equiv.), and formaldehyde were reacted together, usually in alcohol at room temperature for 5 h, to provide mono-aminomethylated magnolols **2a**–**e** in 68–84% yields with the isolation of more polar diaminomethylated magnolols 5–10% (Figure 1). With the addition of 2 equiv. secondary amines and under reflux for 24 h, the sequential Mannich reaction in-situ was driven in the absence of acid to the symmetric diaminomethylated magnolols **3a**–**c** in 52–70% yields. Starting from mono-aminomethylated magnolols **2a**–**e** and secondary amines via an additional Mannich reaction the non-symmetric diaminomethylated magnolols **4a**–**e** were obtained in 34–62% yields.

A series of in-vitro tests were carried out to assess the possible radical scavenging activity of magnolol and its aminomethylated derivatives. To evaluate and provide direct information about the antioxidative reactivity of phenolic derivatives the scavenging diphenyl-*p*-picrylhydrazyl (DPPH) free radicals model (Mellors & Tappel 1966) was used. A decrease in absorbance of DPPH after reaction with the test compound indicates the reduction of free radical concentration. Although all of the test compounds were less potent against DPPH than the classical antioxidant probucol (Table 1), at a concentration of 80 μM most of the aminomethylated magnolols were better free radical scavengers than magnolol. Interestingly, all the pyrrolidyl-containing aminomethylated magnolols (**2b**, **3a**, **4b** and **4c**) displayed much improved bleaching effects against DPPH compared with magnolol. They shut down over 50% of DPPH at the test concentration. Further testing at 20 μM showed that **2b**, **3a**, **4b** and **4c** maintained a potency of bleaching of over 30% DPPH. It is well established that the DPPH radical scavenging of phenolic components is attributed to their hydrogen-donating ability. The *ortho*-pyrrolidyl group of **2b**, **3a**, **4b** and **4c** might play a critical role in lowering the oxygen–hydrogen bonding via sterically facilitated intramolecular hydrogen bonding with the neighbouring amino group. This effect may be one of the factors leading to enhancement of their reactivity toward the free radicals (Madsen et al 1997). Other aminomethylated magnolols derived from more bulky secondary amines or devoid of the pyrrolidyl moiety were less active under the DPPH test.

To evaluate the antioxidant activity of these pyrrolidyl-containing magnolols (**2b**, **3a** and **4c**) we used the PMA/lucigenin-dependent enhanced chemiluminescence of human whole blood (Chan et al 1996; Shen et al 1998). This method investigates the scavenging effects of tested compounds on the reactive oxygen metabolites such as hydrogen peroxide and superoxide radicals mainly derived from neutrophils. Intriguingly, the radical scavenging effect displayed by compound **4c** was approximately 2-fold more potent (60% of control) at the 1 μM level compared with magnolol (80% of control) and probucol (Figure 2). Compounds **2b** and **3a** showed antioxidative profiles similar to magnolol. These results suggested that derivative **4c** might be a selective cytoprotective agent at certain oxidative stress conditions. Most of the tested compounds showed decreased radical scavenging effects at the higher concentrations (e.g. 10 μM) in this model. From these results we assumed that, at higher concentrations, magnolol-related components that scavenge strongly the primary reactive species might also generate certain levels of cytotoxicity to the tested cells (Kim & Ryu 1999).

Accordingly, the inhibition of lipopolysaccharide-stimulated macrophage activation of whole blood was performed to evaluate antioxidizing actions of magnolol derivatives against the nitric oxide-related free radicals (Chan et al 1996). Although they all were marginally more potent than probucol in this model, the derivatives in the range 0.01–10 μM showed moderate scavenging effects similar to those of magnolol (Figure 3).

In summary, we have found that 2-pyrrolidylmethyl

Table 1 Scavenging effects of magnolol derivatives on DPPH.

Compound (concn)	% of DPPH bleaching[a]	
	(80 μM)	(20 μM)
Control (MeOH)	0.0	
Magnolol	18.76±2.00	20.71±2.04
Probucol	79.16±8.38	37.53±3.61
2a	36.02±3.81	19.42±1.35
2b	63.79±6.75	37.28±2.66
2c	22.11±2.55	18.70±1.41
2d	18.60±1.97	17.38±1.73
2e	11.80±1.42	10.33±1.08
3a	64.32±6.81	37.62±3.11
3b	16.21±1.72	20.18±2.02
3c	13.63±1.44	15.05±1.72
4a	34.00±3.60	26.35±3.12
4b	49.34±5.22	32.84±4.01
4c	64.92±6.87	38.72±4.26
4d	7.65±0.81	11.08±1.38
4e	11.60±0.67	10.40±1.26

[a]n = 4, % of DPPH bleaching = [(absorbance of control (MeOH) − absorbance of tested compound) × 100] /absorbance of control (MeOH).

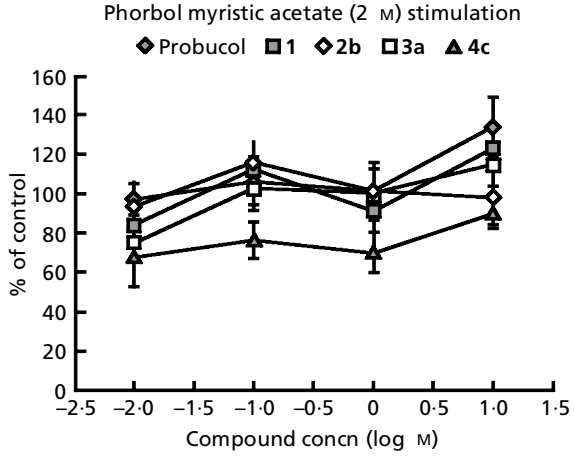

Figure 2 Scavenging effects of magnolol derivatives on phorbol myristic acetate-stimulated oxygen-derived metabolites. The values shown are the mean±s.e.m. from six experiments.

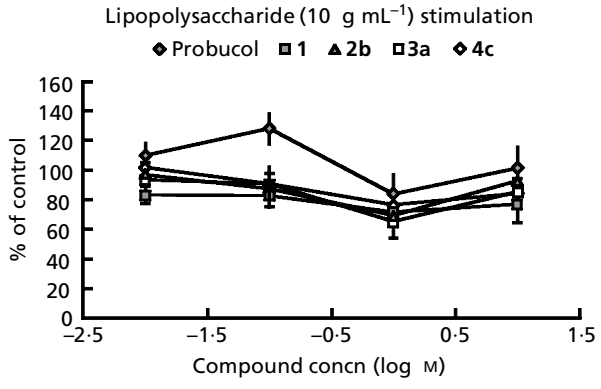

Figure 3 Scavenging effects of magnolol derivatives on lipopolysaccharide-stimulated oxygen-derived free radicals. Data are the mean±s.e.m. from six experiments.

derivatives of magnolol (**2b**, **3a** and **4c**) displayed improved scavenging effects on the stable free radical DPPH. Compound **4c** was found also to be a potent scavenger of reactive oxygen metabolites in human blood as compared with magnolol. Recent investigations proved that magnolol could prevent ischaemia/reperfusion injury by inhibiting PMA-activated neutrophil adhesion (Shen et al 1998). This could account for its blocking the accumulation of reactive oxygen-derived species in whole cells. Compound **4c** should be chosen for further evaluation on certain peroxidative damage due to its promising results from this study.

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