Four New Lignans and IL-2 Inhibitors from Magnoliae Flos

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Four new lignans, a furufuran lignan medioresinol B (10) and three tetrahydrofuran lignans kobusinol C (16), 7′-methoxy magnostellin A (21), and magnostellin D (23), along with 19 known lignans, were isolated from the flower buds of Magnolia biondii PAMP. The structures of the isolates were elucidated using spectroscopic analysis, mainly one- and two-dimensional NMR, high resolution-MS, and circular dichroism techniques as well as Mosher’s esterification method. The anti-allergic effects of the isolated compounds were evaluated by analyzing the inhibition of interleukin-2 (IL-2) expression in Jurkat T-cells. Compounds 11–14 reduced IL-2 expression in a dose-dependent manner.

Key words Magnolia biondii PAMP; Magnoliaceae; lignan; anti-allergy; interleukin-2

Allergy, also known as hypersensitivity, is an inappropriate immune response to a harmless substance, called an allergen, which leads to a characteristic set of symptoms that can be mild to potentially life threatening. 1) Allergic diseases such as asthma, rhinitis, and eczema are increasing in prevalence and they contribute considerably to public health care costs. Mast cell-derived interleukin-2 (IL-2) is an essential cytokine for the generation and maintenance of Treg cells, which antagonize immune responses. 2,3) T-cells play a crucial role in the pathogenesis of cell-mediated autoimmune diseases and chronic inflammatory diseases. 4) When T-cells are activated, IL-2 is secreted that stimulates their proliferation and differentiation into effector cells, leading to allergies.

Flos Magnoliae (also known as “Shin-yi”) is flower bud from Magnolia biondii PAMP. (Magnoliaceae) that is widely distributed in Korea, China, and Japan. The dried flower buds of M. biondii have historically been used for the symptomatic management of allergic rhinitis, sinusitis, and headache in Chinese medicine. 5) Previous phytochemical investigations of this species revealed that it is a valuable natural source of essential oils, 6,7) lignans, 8,9) neolignans, 10) sesquiterpenes, 11) cholesterol acyl transferase (ACAT) inhibitory activity, 12) anti-inflammatory activity, 13) anti-allergic activity, 14) pro-apoptotic activity, 15) and vasorelaxant effects. 16) In recent research, lignans from M. biondii were demonstrated to possess anti-allergic activity in vitro. 17)

Thus, we investigated the bioactive constituents of the flower buds of M. biondii PAMP. The column chromatographic purification of the chloroform-soluble fraction led to the isolation of four new lignans, medioresinol B (10), kobusinol C (16), 7′-methoxy magnostellin A (21), and magnostellin D (23), along with 19 known ones (1–9, 11–15, and 17–20). The structures of these compounds were determined using one and two-dimensional (1D- and 2D-)NMR, high resolution (HR)-MS, and circular dichroism (CD) techniques as well as Mosher’s esterification method. In addition, these isolates were screened for their ability to inhibit IL-2 expression in activated Jurkat T-cells. This paper describes the isolation and structural elucidation of the four new compounds (10, 16, 21, and 23) as well as their anti-allergic activities by analyzing the inhibition of IL-2.

Results and Discussion

The methanol extract of the flower buds of Magnolia biondii PAMP. was partitioned successively with chloroform, ethyl acetate (EtOAc), and water. Repeated column chromatographic purification of the chloroform-soluble fraction afforded four new lignans, a furufuran lignan (10) and three tetrahydrofuran lignans (16, 21, and 23), along with 19 known lignan derivatives (1–9, 11–15, and 17–20) (Fig. 1).

Compound 10 was obtained as a colorless oil. Its HR-electron ionization (EI)-MS indicated a molecular ion peak at m/z 388.1522 [M]+ (Calcld for C23H12O5 388.1522), compatible with the molecular formula C23H12O5. The IR spectra of 10 revealed characteristic absorption bands of typical hydroxyls (3324 cm−1) and benzene ring (2305, 1697, and 1423 cm−1) moieties, respectively. The 1H-NMR spectrum of 10 showed proton signals of two methane protons at δH 3.09 (2H, m, H-8 and H-8′), two oxygenated methyl protons at δH 4.26 (2H, ddd, J=18.2, 9.1, 6.7Hz, H-9a and H-9b) and 3.90 (2H, m, H-9b and H-9b′), two benzyl oxymethine protons at δH 4.71 (1H, d, J=5.2Hz, H-7) and 4.72 (1H, J=5.7Hz, H-7′), which suggested a furufuran moiety, together with a 1,3,4-trisubstituted benzene ring at δH 6.89 (1H, d, J=5.1Hz, H-2), 6.88 (1H, d, J=8.0Hz, H-5), and 6.82 (1H, dd, J=8.0, 1.5Hz, H-6), and 1′,3′,4′,5′-tetrasubstituted benzene ring at δH 6.57 (1H, d, J=1.7Hz, H-2′) and 6.51 (1H, d, J=1.7Hz, H-6′) (Table 1). They correlated to the carbons resonating at δC 54.3 (C-8 and 8′), 71.9, 72.1 (C-9 and 9′), 85.9, 86.0 (C-7 and 7′), 108.7 (C-2), 114.4 (C-5), 119.2 (C-6), 105.6 (C-2′), and 101.8 (C-6′), respectively in the heteronuclear multiple-quantum coherence (HMQC) spectrum. In heteronuclear multiple-bond connectivity (HMBC) spectrum, the connection of a furufuran moiety
and benzene rings was observed by the correlation between $\delta H 4.71$ (H-7) and $\delta C 108.7$ (C-2)/$\delta C 119.2$ (C-6) and between $\delta H 4.72$ (H-7') and $\delta C 105.6$ (C-2')/$\delta C 101.8$ (C-6'). The correlations between proton/carbon signals at $\delta H 3.91$ (3H, s, 3-OCH$_3$)/$\delta C 146.9$ (C-3), $\delta H 3.89$ (3H, s, 4'-OCH$_3$)/$\delta C 135.0$ (C-4'), and $\delta H 3.88$ (3H, s, 5'-OCH$_3$)/$\delta C 152.7$ (C-5') were observed in the HMBC spectrum, thus the positions of three methoxyl groups at C-3, C-4', and C-5' were confirmed in compound 10. In the HMBC spectrum, the hydroxyl proton at $\delta H 5.77$ (1H, br s, 3'-OH) showed a correlation with the aromatic quaternary carbons at $\delta C 149.5$ (C-3') and 135.0 (C-4'), and an aromatic methine carbon at $\delta C 105.6$ (C-2'), which confirmed the position of the hydroxyl group at C-3'. Another HMBC correlation between the hydroxyl proton at $\delta H 5.59$ (1H, br s, 4-OH) with an aromatic quaternary carbon at $\delta C 145.4$ (C-4), and an aromatic methine carbon at $\delta C 114.4$ (C-5), was observed, suggest-

![Fig. 1. The Structure of Isolated Compounds 1-23 from the Flower Buds of M. biondii](image)

| Position | 10$^{a}$ | 16$^{a}$ | 21$^{b}$ | 23$^{c}$ |
|----------|---------|---------|---------|---------|
| 2        | 6.89, d (1.5) | 6.57, s | —       | 6.94, d$^{d}$ |
| 5        | 6.89, d (8.0) | —       | 6.94, d (8.2) | 6.84, d (8.5) |
| 6        | 6.82, dd (8.0, 1.5) | 6.57, s | 6.87, d (8.2) | 6.69, m$^{m}$ |
| 7        | 4.71, d (5.2)$^{e}$ | 4.96, d (6.6) | 4.59, d (6.4) | 2.55, dd (10.6, 13.5) |
| 8        | 3.09, m$^{t}$ | 2.28, m | 2.04, dd (6.4, 6.9) | 2.73, sept (6.4, 10.6, 12.5) |
| 9        | 4.26, ddd (18.2, 9.1, 6.7)$^{t}$ | 3.92, d (5.1) | 1.09, d (6.9) | 3.75, dd (6.4, 8.5) |
| 10       | 3.90, m$^{t}$ | 4.03, dd (11.2, 3.9) | —       | 4.05, dd (6.4, 8.5) |
| 2'       | 6.57, d (1.7) | 6.90, d (1.5) | 6.94, d$^{d}$ | 6.87, m$^{m}$ |
| 5'       | —       | 6.85, d (8.8) | 6.98, d (7.9) | 6.88, m$^{m}$ |
| 6'       | 6.51, d (1.7) | 6.90, dd (1.5, 8.8) | 6.94, d (7.9) | 6.80, dd (1.6, 8.1) |
| 7'       | 4.72, d (5.7)$^{e}$ | 5.11, d (3.6) | 4.33, d (6.4) | 4.79, d (6.7) |
| 8'       | 3.09, m$^{t}$ | 2.79, dt (3.6, 8.5) | 2.72, dt (6.4, 7.4) | 2.41, dt (6.4, 6.7) |
| 9'       | 4.26, ddd (18.2, 9.1, 6.7)$^{t}$ | 4.09, dd (8.0, 8.5) | 4.11, dd (7.4, 8.3) | 3.78, dd (6.4, 10.2) |
| 10'      | 9.1,6,7,9$^{t}$ 3.90, m$^{t}$ | 4.16, dd (8.0, 8.5) | 4.18, dd (8.2, 8.3) | 3.92, dd (3.3, 10.2) |
| 3-OCH$_3$ | 3.91, s | 3.85, s$^{s}$ | 3.86, s$^{s}$ | 3.92, s |
| 4-OH     | 5.59, brs | —       | 3.86, s | 5.52, brs |
| 4-OCH$_3$ | —       | 3.83, s | 3.86, s | —       |
| 5-OCH$_3$ | —       | 3.85, s$^{s}$ | —       | —       |
| 3'-OCH$_3$ | 5.77, brs | —       | —       | —       |
| 3'-OCH$_3$ | 3.89, s | 3.86, s$^{s}$ | 3.87, s | —       |
| 5'-OCH$_3$ | 3.88, s | —       | 3.86, s$^{s}$ | 5.60, brs |
| 4'-OCH$_3$ | 5.60, brs | 3.88, s | —       | —       |
| 7'-OCH$_3$ | —       | 3.22, s | —       | —       |

Coupling constants ($J$) are in Hz; $^1$H-NMR measured at 500 MHz in a) CDCl$_3$ and b) CD$_3$OD; c) Overlapped.
ing one hydroxyl group was located at C-4 (Fig. 2). All the spectral data of 10 including the 1D- and 2D-NMR spectra were similar to those of (7R,8R,7′S,8′R)-3′,4-dihydroxy-3,4′,5′-trimethoxy-7,9,7′,9-diepoxylignane (M4), except the absolute configuration. The nuclear Overhauser effect spectroscopy (NOESY) spectrum showed cross-peaks of H-7 (H-7′) with H-9a (H-9′a) and H-8 (H-8′) with H-9b (H-9′b) which indicated that the protons were on the same orientations, respectively (Fig. 3). The CD spectrum of M4 showed the Cotton effects at 226 nm (Δε +0.72) and 291 nm (Δε −0.20) which suggested a stereoisomer of 10. The CD spectrum of 10 was compared with that of 8 {[(7S,8R,7′S,8′R)-configuration]. Those of 10 showed two positive Cotton effects at 210 nm (Δε+2.5) and 280 nm (Δε+0.8), which were identical to those of 8 (medioresinol). On the basis of the spectral data, the structure of 10 was determined to be (7S,8R,7′S,8′R)-3′,4-dihydroxy-3,4′,5′-trimethoxy-7,9,9-diepoxylignane and named as medioresinol B.

Compound 16 was isolated as a colorless oil. The molecular formula of 16 was deduced to be C23H30O8 from the HR-El-MS spectra, which showed an ion peak at m/z 434.1941 [M]+ (Calcd for C23H30O8, 434.1941). The IR spectra of 16 showed absorption bands typical of hydroxyl (3329 cm−1) and benzene ring (2375, 1748, and 1423 cm−1) moieties. The 1H-NMR spectrum of 16 displayed proton signals for five methoxyl protons at δH 3.85 (3- and 5-OCH3), 3.83 (4-OCH3), 3.89 (3′-OCH3), and 3.88 (4′-OCH3), a symmetric 1,3,4,5-tetrasubstituted proton at δH 6.57 (2H, s, 2- and 6-H), and 1′,3′,4′-trisubstituted aromatic moiety at δH 6.90 (2H, dd, J=8.8, 1.5 Hz, H-2′ and H-6′) and 6.85 (1H, d, J=8.8 Hz, H-5′), together with oxygenated aliphatic proton signals at δH 5.11 (1H, d, J=3.6 Hz, H-7′), 4.96 (1H, d, J=6.6 Hz, H-7), 4.16 (1H, dd, J=8.0, 8.5 Hz, H-9′a), 4.09 (1H, dd, J=8.0, 8.5 Hz, H-9′b), 4.03 (1H, dd, J=3.9, 11.2 Hz, H-9a), and 3.92 (1H, d, J=5.1 Hz, H-9b), two aliphatic methine protons at δH 2.28 (1H, m, H-8) and 2.79 (1H, dt, J=3.6, 8.5 Hz, H-8′),
suggesting the presence of a tetrahydrofuranoid lignan skeleton, compared with 7S,8R,7'S,8'R-3,4,3',4'-tetramethoxy-9,7'-dihydroxy-8,8'7,O,9'-lignan (15) (Table 1). In the correlation spectroscopy (COSY) spectrum, a correlation of H-8 with H-7/H-9, H-8' with H-7'/H-9', and H-8 with H-8' was observed, which showed the connection of two propanoyl groups, a tetrahydrofuran moiety (Fig. 2). The HMBC experiments indicated the correlation of δH 5.11 (H-7) with δC 136.0 (C-1′)/109.2 (C-2′)/118.0 (C-6′) and δH 4.96 (H-7) with δC 138.3 (C-1)/102.7 (C-2′, C-6), suggesting that the veratryl and 3,4,5-trimethoxylphenyl groups were linked to C-8′ and C-7′. The positions of five methoxyl groups at C-3, C-4, C-5, C-7′, and C-8′ were demonstrated by the HMBC correlations between protons/carbon signals at δH 3.85 (6H, s)/δC 153.5 (C-3, C-5), δH 3.83 (3H, s)/δC 137.4 (C-4), δH 3.89 (3H, s)/δC 149.3 (C-3′), and δH 3.88 (3H, s)/δC 148.7 (C-4′) (Fig. 2).

All the spectral data of 16 including 1D- and 2D-NMR spectra were similar with those of (7R,8S,7'S,8'R)-3,4,3',4'-pentamethoxy-9,7'-dihydroxy-8,8',7,O,9'-lignan,20 except the absolute configuration. The NOESY spectrum showed cross-peaks of H-8′ with H-8, H-9 with H-8′, and H-9′ with H-8′, which indicated that the protons were on the same orientation (Fig. 3). Mosher’s esterification method was performed to determine the absolute configuration at C-7′. Analysis of 1H-NMR chemical shift differences between δH and δH′ showed the assignment of the absolute configurations of C-7′, C-3, C-7′, and C-8′ as R, S, R, and S, respectively (Fig. 4). Thus, compound 16 was assigned as (7R,8S,7'S,8'R)-3,4,3',4'-pentamethoxy-9,7'-dihydroxy-8,8',7,O,9'-lignan and named as kobusolin C.

Compound 21 was obtained as a colorless oil. The molecular formula of 21 was determined to be C39H40O4 according to HR-ESI-MS, which showed a molecular ion peak at m/z 402.2042 [M]+ (Calcd for C39H40O4: 402.2042). The 1H-NMR spectrum of 21 indicated proton signals of two methoxyl groups at δH 3.86 (12H, s, 3,4,3',4'-OCH3) and 3.22 (3H, s, 7'-OCH3), 20 methyl groups at δH 1.09 (9H, d, J = 6.9Hz, H-9, two trisubstituted benzene rings at δH 6.89 (1H, d, J = 7.9Hz, H-5'), 6.94 (2H, d, J = 7.9Hz, H-2,5), 2H, d, J = 8.2Hz, H-2',6'), and 6.87 (1H, d, J = 8.2Hz, H-6), oxygenated aliphatic protons at δH 4.59 (1H, d, J = 6.4Hz, H-7), 4.33 (1H, d, J = 6.4Hz, H-7′), 4.18 (1H, dd, J = 8.2, 8.3Hz, H-9′a), and 4.11 (1H, dd, J = 7.4, 8.3Hz, H-9′b) together with two aliphatic methane protons at δH 2.72 (1H, dt, J = 6.4, 7.4Hz, H-8′) and 2.04 (1H, dd, J = 6.4, 6.9Hz, H-8), suggesting the presence of a tetrahydrofuran moiety (Table 1). They correlated to the carbons resonating at δC 56.5 (3,4,3',4'-OCH3), 56.2 (7'-OCH3), 13.1 (C-9), 113.0 (C-5′), 112.0 (C-2′), 121.1 (C-6′), 110.8 (C-2), 112.9 (C-5), 119.6 (C-6), 89.3 (C-7), 83.5 (C-7′), 70.4 (C-9′), 49.5 (C-8′), and 45.2 (C-8), respectively in the HMOC spectrum. The 1H- and 13C-NMR spectra of 21 were similar to those of magnostellin A (20).21 However, compound 21 has a methoxyl group at C-7′ instead of the hydroxyl group seen in 20. This was demonstrated by HMBC correlation of δH 3.22 (3H, s) with δC 83.5 (C-7′) (Fig. 2). The NOESY spectrum showed cross-peaks of H-8 with H-8′, H-9 with H-7′, and H-9′ with H-9′, which indicated that the protons were on the same orientations, respectively (Fig. 3). The CD spectrum of 21 [(ε)20 +42.7 (c 0.1, MeOH)] exhibited two positive Cotton effects at 210nm (Δε+2.8) and 280nm (Δε+0.7) which were similar to those of the known compound 20 [(ε)20 +68.0 (c 0.75, CHCl3)].22 On the basis of spectral evidence, the structure of 21 was elucidated as (7S,8S,7'S,8'R)-3,4,3',4'-pentamethoxy-8,8',7,O,9'-lignan and named as 7'-methoxy magnostellin A.

Compound 23 was also obtained as a colorless oil. The HR-ESI-MS showed an ion peak at m/z 360.1573 [M]+ (Calcd for C20H25O4: 360.1573), which corresponds to the molecular formula C20H25O4. The IR spectrum of 23 indicated the absorption bands typical of hydroxyl (3324 cm⁻¹) and benzene ring (1558 and 1423 cm⁻¹) moieties. The 1H-NMR spectrum possessed signal protons of two methoxyl groups at δH 3.92 (3-OCH3) and 3.87 (3'-OCH3), two trisubstituted benzene rings at δH 6.87 (1H, m, H-5'), 6.88 (1H, m, H-2'), 6.84 (1H, d, J = 8.5Hz, H-5), 6.80 (1H, dd, J = 1.6, 8.1Hz, H-6), and 6.69 (2H, m, H-2, 6), oxygenated aliphatic protons at δH 4.79 (1H, d, J = 6.7Hz, H-7′), 4.05 (1H, dd, J = 6.4, 8.5Hz, H-9′a), 3.92 (1H, dd, J = 3.3, 10.2Hz, H-9′a), 3.78 (1H, dd, J = 6.4, 10.2Hz, H-9′b), and 3.75 (1H, dd, J = 6.4, 8.5Hz, H-9b) together with two aliphatic methane protons at δH 2.73 (1H, sept, J = 6.4, 10.6, 12.5Hz, H-8) and 2.41 (1H, dt, J = 6.4, 6.7Hz, H-8′), which revealed the existence of a tetrahydrofuran moiety, and two aliphatic methylene protons at δH 2.91 (1H, dd, J = 5.2, 13.5Hz, H-7a) and 2.55 (1H, dd, J = 10.6, 13.5Hz, H-7b) (Table 1). The 13C and distortionless enhancement by polarization transfer (DEPT) NMR spectra showed 20 signal carbons including six quaternary carbons at δC 146.8, 146.7, 145.2, 144.2, 134.9, and 133.4, nine methine carbons at δC 121.4, 118.9, 114.6, 114.3, 111.4, 108.5, 83.0, 52.3, and 42.6, two methylene carbons at δC 73.1, 61.1, and 33.5, and two methyl carbons at δC 56.1 and 56.1. These spectroscopic data were similar to those of reported compound 3-(α,4-dihydroxy-3-methoxybenzyl)-4-(4-hydroxy-3-methoxybenzyl)tetrahydrofuran,23 however the absolute configuration of 23 has not been determined before. The relative configuration of 23 was determined by the coupling constant value and NOESY correlation. The relative configurations of C-7′ and C-8′ were determined as erythro by comparison with a reported compound, tanegool (J7,8,erythro=7.4Hz),23 wherein

![Fig. 4](image-url)
the C-7′ and C-8′ moieties are similar to those of 23. The coupling constant between H-7′ and H-8′ is 6.7 Hz, which is smaller than the value of threo form (J_{7′-8′} = 9.7 Hz) of the known compound (7S,8R,7′S,8′R)-3,4,3′,4′-tetramethoxy-9,7′-dihydroxy-8,8′,7′,O,9′-ligan (15).24) Furthermore, the NOESY spectrum showed cross-peaks of H-7′ with H-7_{axial} and H-9′ which indicated that the protons were on the same orientation (Fig. 3). Mosher’s esterification method was performed to determine the absolute configuration at C-7′. Analysis of 1H-NMR chemical shift differences between δS and δR allowed the assignment of the absolute configurations of C-7′, C-8′, and C-8 as R, R, and S, respectively (Fig. 4). Based on above evidences, the structure of compound 23 was determined to be (8S,7′R,8′R)-3,3′-dimethoxy-4,4′,7′-trihydroxy-8,8′,9′,O,9′-ligan and named as magnostellin D.

Additionally, the chemical structures of 19 known compounds were confirmed by comparison with previous literature data to be: (+)-fargesin (1),25) (+)-kobusin (2),25) (+)-aschantin (3),25) (+)-eudesmin (4),25) (+)-epimagnolin (5),25) (+)-magnolin (6),25) (+)-syringaresinol (7),26) (+)-medioresinol (8),27) (+)-pinoresinol (9),28) (+)-veraguensin (11),29) (+)-galgravin (12),30) nectandrin A (13),31) futokadsurin C (14),32) 7S,8R,7′S,8′R-3,4,3′-tetramethoxy-9,7′-dihydroxy-8,8′,7′,O,9′-ligan (15),33) 7R,8S,7′S,8′R-3,4,3′-4′-tetramethoxy-9,7′-dihydroxy-8,8′,7′,O,9′-ligan (17),33) 7R,8S,7′S,8′R-3,4,3′,4′-pentamethoxy-9,7′-dihydroxy-8,8′,7′,O,9′-ligan (18),20) kobusin B (19),30) magnostellin A (20),32) and (+)-larciresinol dimethyl ether (22).33) Compounds 7, 8, 13–15, 17, and 18 were isolated from the flower buds of M. biondii for the first time. In this study, all isolated compounds were tested to inhibit IL-2 production in Jurkat T-cells, which were stimulated with phorbol 12-myristate 13-acetate (PMA, 200 nM) and A23187 (1 µM). The furofuran lignin, yangambin was reported to have an anti-inflammatory effect in a previous study.36) Among the tested furofuran lignans (1–10), compounds 2 and 3 exhibited inhibitory activities against IL-2, which suggested that the benzodioxole moiety of furofuran lignans may have a positive effect on the inhibition of IL-2. Among the tested tetrahydrofuran lignans (11–23), compounds 11–14 showed significant inhibition of IL-2 (Fig. 5). Particularly, compounds 12, 13, and 14 bearing the position of methyl group located on opposite sides (8S,8′R) in their structures, showed stronger inhibitory effects than 11 which has a methyl group on the same side (8S,8′S). Thus, we tested the dose-dependent inhibition of IL-2 of these compounds. As shown in Fig. 6, compounds 12–14 demonstrated the significant inhibition of IL-2, in a dose-dependent manner. As shown in Fig. 6B, optimal inhibition was observed at the concentrations ranging from 25 to 50 µM of compounds 12–14. Our data implied that the orientation of the methyl group attached to tetrahydrofuran moiety may influence the inhibitory effects of the compounds on IL-2 production in activated Jurkat T-cells. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay indicated that none of the lignans showed cytotoxicity in activated Jurkat T-cells at concentrations effective for the inhibition of IL-2 production (data not shown). Therefore, these lignans might be valuable candidates as anti-allergic agents developed from natural sources. Further...
studies are required to elucidate the relationship between the chemical structures of the active compounds 12–14 and their various inhibitory mechanisms on IL-2 expression.

Experimental

General Experimental Procedures  The optical rotations were measured using a Jasco P-1020 polarimeter. The IR spectra were recorded using a Bruker IFS-66/S Fourier transform (FT)-IR spectrometer. The CD spectra were measured using a Jasco J-810 spectropolarimeter. The UV spectra were recorded using an Agilent 8453 UV-visible spectrophotometer. The NMR spectra were recorded using a Bruker 500 NMR spectrometer using TMS as the internal standard. The HR-MS spectra were recorded on a JEOL JMS-700 mass spectrometer under EI conditions in the Korea Basic Science Institute (Daegu). Silica gel 60 (Merck, 230–400 mesh) and reversed phase (RP)-C18 silica gel (Merck, 75 mesh) were used for column chromatography (CC), TLC was performed using Merck precoated silica gel F254 plates and plates and RP-18 F254. Plates. HPLC was performed using a Waters 600 Controller system with a UV detector and a YMC Pak ODS-A column (20×250mm, 5 μm particle size, YMC Co., Ltd., Japan), and HPLC solvents were from Burdick & Jackson, U.S.A.

Plant Material  The flower buds of M. biondii were purchased from Kyungdong traditional market in Seoul (February 2015) and identified by Professor Byung Sun Min at Catholic University of Daegu. It has been deposited at pharmacognosy laboratories in the College of Pharmacy, Kyungpook National University, Korea.

Extraction and Isolation  The dried flower buds of M. biondii (9.0 kg) were extracted three times refluxing with MeOH (3×4L) at 60°C. The filtered MeOH-soluble part was concentrated in vacuo to obtain brown residue (1.2 kg) which was suspended in H2O and partitioned with organic solvents, then afford extracts of chloroform (591.7 g), EtOAc (28.2 g), and aqeous extract (580.1 g), successfully. Chloroform-soluble extract was chromatographed on a silica gel vacuum liquid chromatography (VLC) (63–200 μm particle size, Merck) and eluting with a gradient mixture of methylene chloride (MC):hexane (3:7→1:0) and EtOAc:MC (1:9→1:19) to afford nine fractions (MB1-1 to MB9-9). Fraction MB5-9 (53.0 g) was separated by silica gel CC using a gradient elution of MC:hexane (0:1→1:4) to afford two fractions (MB5-1 and MB5-2). From fraction MB5-1 (8.0 g) continuously was subjected on silica gel CC with a gradient elution of ACN:hexane (1:4→0:1) and acetonitrile (ACN):MC (1:9→1:19) to afford eighteen fractions (MB51-1 to MB51-18). Fraction MB51-4 (60.5 mg) was purified by HPLC using acetonitrile eluting with a gradient mixture of ACN:MC (1:99→1:19) to afford 1 compound (37.2 mg). Fraction MB51-15 (34.4 mg) was purified by HPLC using an acidic elution of 60% ACN–H2O to afford compounds 11 (12.9 mg, tR=50.3 min) and 12 (3.6 mg; tR=52.8 min). Fraction MB5-2 (42.0 g) was fractionated on a silica gel VLC eluting with gradient mixture of acetone:hexane (1:9→2:3) to give seven fractions (MB52-1 to MB52-7). Fraction MB52-5 (940.8 mg) was subjected to RP-18 CC with a mixture of MeOH:H2O (1:1→3:2) to afford compound 2 (629.4 mg). Fraction MB52-6 (36.2 g) was chromatographed on silica gel column eluting with acetone (1:9→3:7) to gain to four fractions (MB526-1 to MB526-4). Fraction MB526-1 (201.5 mg) was purified by HPLC using an isocratic elution of 70% MeOH–H2O to afford 4 (62.1 mg, tR=19.58 min) and 5 (92.7 mg, tR=21.17 min). Compounds 3 (371.1 mg) and 13 (2.5 mg) were obtained from fraction MB526-2 (632.3 mg) by RP-18 CC eluting with MeOH:H2O (1:1→3:2). Fraction MB9-9 (176.5 g) was fractionated on a silica gel VLC eluting with gradient mixture of acetone:hexane (1:9→2:3) to gain six fractions (MB9-1 to MB9-6). Fraction MB9-2 (58.0 g) was subjected on to RP-18 CC with a mixture of MeOH–H2O (1:1→3:2) to obtain 18 (351.5 mg). Fraction MB9-4 (7.8 g) was fractionated to eight fractions (MB94-1 to MB94-8) by silica gel CC eluting with acetone:hexane (1:9→2:3). Fraction MB94-5 (2.6 g) chromatographed on RP-18 column eluting with a mixture of MeOH–H2O (2:3→1:1) to yield 20 (36.7 mg) and eight fractions (MB945-1 to MB945-8). Fraction MB95-9 (8.3 g) was subjected on to silica gel CC eluted by MeOH:MC (1:99→1:19) to afford several compounds (MB95-1 to MB95-9). Fraction MB95-5 (60.3 g) fractionated to nine fractions (MB95-1 to MB95-9). Fraction MB95-5 (8.3 g) was subjected on to silica gel CC eluted by MeOH:MC (1:99→1:19) to afford 7 (37.4 mg) and nine fractions (MB95-1 to MB95-9). Fraction MB95-5 (178.6 mg) was purified by using HPLC using MeOH:H2O (2:3→7:10) to give 8 (9.7 mg, tR=33.8 min) and fraction MB95-2 (21.4 mg) was isolated by using HPLC using MeOH:H2O (2:3→4:1) to afford 12 (12.4 mg, tR=25.5 min). Fraction MB95-6 (2.4 g) was subjected to RP-18 CC eluting with MeOH:H2O (2:3→1:1) to afford 19 (580.1 mg) and five fractions (MB9556-1 to MB9556-5). MB9556-2 (64.9 mg) was purified by HPLC using MeOH–H2O (1:1→3:2) to give compounds 15 (20.1 mg, tR=25.8 min), 16 (2.3 mg, tR=27.9 min), 17 (5.3 mg, tR=35.3 min), and 18 (13.5 mg, tR=37.6 min).

Medioresinol B (10)  Colorless oil; [α]D +23.9 (c=0.1, MeOH); IR (ATR) cm−1: 3234, 2942, 2305, 1697, 1647, 1558, 1507, 1451, 1423, and 1024; UV λmax (MeOH) nm (logε): 209 (4.12), 230.5 (3.92), and 280.5 (3.5); 1H- and 13C-NMR data (500/125 MHz, CDCl3): see Tables 1, 2; HR-EI-MS m/z: 388.1522 [M]+ (Calcd for C21H24O7, 388.1522).

Kobusinol C (16)  Colorless sticky oil; [α]D +27.3 (c=0.1, MeOH); IR (ATR) cm−1: 3239, 2942, 2319, 1654, 1507, 1458, and 1025; UV λmax (MeOH) nm (logε): 212.2 (4.16), 232.0 (4.1), and 278.5 (3.6); 1H- and 13C-NMR data (500/125 MHz, CDCl3): see Tables 1, 2; HR-EI-MS m/z: 434.1941 [M]+ (Calcd for C23H30O6, 434.1941).

Magnostellin D (23)  Colorless sticky oil; [α]D +8.16 (c=0.1, MeOH); IR (ATR) cm−1: 3237, 2943, 1457, and 1025; UV λmax (MeOH) nm (logε): 205 (4.3), 232.0 (4.0), and 279.5 (3.6); 1H- and 13C-NMR data (500/125 MHz, CDCl3): see Tables 1, 2; HR-EI-MS m/z: 402.2042 [M]+ (Calcd for C23H29O6, 402.2042).
Table 2. 13C-NMR Data for Compounds 10, 16, 21, and 23

| Position | 10(c) | 16(c) | 21(d) | 23(d) |
|----------|-------|-------|-------|-------|
| 1        | 133.0 | 138.3 | 136.7 | 132.4 |
| 2        | 108.7 | 102.7 | 110.8 | 111.4 |
| 3        | 146.9 | 153.5 | 150.5 | 146.8 |
| 4        | 145.4 | 137.4 | 150.0 | 144.2 |
| 5        | 114.4 | 153.5 | 112.9 | 114.6 |
| 6        | 119.2 | 102.7 | 119.6 | 121.4 |
| 7        | 86.0  | 82.7  | 89.3  | 33.5  |
| 8        | 54.3  | 52.0  | 45.2  | 42.6  |
| 9        | 72.1  | 60.7  | 13.1  | 73.1  |
| 1'       | 137.6 | 136.0 | 134.6 | 134.9 |
| 2'       | 105.6 | 109.2 | 112.0 | 108.5 |
| 3'       | 149.5 | 149.3 | 150.7 | 146.7 |
| 4'       | 135.0 | 148.7 | 150.2 | 145.2 |
| 5'       | 152.7 | 111.3 | 113.0 | 114.3 |
| 6'       | 101.8 | 118.0 | 121.1 | 118.9 |
| 7'       | 85.9  | 72.1  | 83.5  | 83.0  |
| 8'       | 54.3  | 47.9  | 49.5  | 52.3  |
| 9'       | 71.9  | 68.6  | 70.4  | 61.1  |
| 3'-OCH3 | 56.1  | 56.3  | 56.3  | 56.1  |
| 4'-OCH3 | —     | 60.9  | 56.5  | —     |
| 5'-OCH3 | —     | 56.3  | —     | —     |
| 3'-OCH3 | —     | 56.1  | 56.3  | 56.1  |
| 4'-OCH3 | 61.1  | 56.1  | —     | —     |
| 7'-OCH3 | —     | —     | 56.2  | —     |

<math>13C-NMR measured at 125 MHz in a) CDCl₃ and b) CD₃OD; c) Overlapped; d) Assignments can be interchanged.</math>

cm⁻¹ : 3566, 2943, 1558, 1507, 1423, and 1024; UV <math>λ_{max}</math> (MeOH) nm (log ε): 210 (3.9), 230 (3.7), and 281 (3.3); <math>1H</math>- and <math>13C</math>-NMR data (500/125 MHz, CDCl₃); see Tables 1, 2; HR-ESI-MS m/z: 360.1573 [M⁺] (Calcd for C₉H₂₂O₈, 360.1573).

Preparation of (S)- and (R)-<math>α</math>-Methoxy-<math>α</math>-trifluoromethylphenyl-<math>α</math>-acytetyl (MTPA) Ester Derivatives of 16 and 23 by Mosher’s Esterification Method (S)- and (R)-MTPA esters of compounds 16 and 23 were prepared using Mosher’s esterification method performed in NMR tubes. Compounds 16 and 23 (1.0 mg) and 4-(dimethylamino)-pyridine (0.2 mg) were transferred into each NMR tube, and this mixture was dried. The (S)-MTPA ester of (S)-MTPA ester of (R)-MTPA ester of (S)-MTPA ester of (R)-MTPA esters (23b) and (R)-MTPA esters (23b) were expressed as fold changes relative to that of the untreated Jurkat T cells (ATCC TIB-152, Manassas, VA, U.S.A.) were grown in RPMI medium ( Gibco-BRL, Gaithersburg, MD, U.S.A.) supplemented with 10% fetal bovine serum (FBS), penicillin G (100 units/mL), streptomycin (100 µg/mL), and 1-glutamine (2 mM). The cells were cultured at 37°C in a humidified incubator containing 5% CO₂ and 95% air. Quantitative PCR and Real-Time PCR Jurkat T cells (5 ×10⁴) were incubated with indicated concentrations of compounds for 30 min at 37°C. Incubated cells were stimulated with PMA (200 nM) and A23187 (1 µM) for 6 h for PCR. In the case of quantitative PCR experiments, cells were harvested and total RNAs were isolated with TRIZOL reagent (JBI, Korea). Reverse transcription of the RNA was performed using RT PreMix (enzynomics, Korea). The primers and PCR conditions for each gene were used as following: human IL-2, 5'-CAC GTC TTG CAC TGG TCA C-3' and 5'-CCT TCT TGG GCA TGT AAA ACT-3' Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 5'-CGG ATG CAA CGG ATT TGG TCG TAT-3' and 5'-AGC CTT CTC CAT GGT GGT GAA GAC-3'. The amplification profile was composed of denaturation at 94°C for 30 s, annealing at 60°C for 20 s, and extension at 72°C for 40 s. The 30 cycles were preceded by denaturation at 72°C for 7 min. For real-time PCR, amplification was performed in DNA Engine OpticonI for continuous fluorescence detection system (MJ Research, Waltham, MA, U.S.A.) in a total volume of 10 μL containing 1 µL of cDNA/control and gene specific primers using SYBR Premix Ex Taq (TaKaRa, Japan). Each PCR reaction was performed using the following conditions: 94°C 30 s, 60°C 30 s, 72°C 30 s, plate read (detection of fluorescent product) for 40 cycles followed by 7 min extension at 72°C 17s. Melting curve analysis was done to characterize the double stranded DNA (dsDNA) product by slowly raising the temperature (0.2°C/s) from 65 to 95°C with fluorescence data collected at 0.2°C intervals. The levels of IL-2 mRNA normalized for GAPDH were expressed as fold changes relative to that of the untreated controls. The fold change in gene expression was calculated using the following equation: Fold change = 2⁻DACT, where DACT=(CT, Target-CT, GAPDH)/(Time 0 h - Time 0 h) represents the 1x expression of the target gene of untreated cells, which was normalized to GAPDH. All experiments were performed at least three times unless otherwise indicated.
supernatants were measured using the Duoset Human IL-2 ELISA kit (R&D Systems, Inc., Minneapolis, MN, U.S.A.) according to the manufacturer’s instructions.

**Cell Viability Assays** Jurkat T cells (3×10⁵) were seeded in a 24 well-plate and incubated with isolates (1–23) for 24h. After incubation, cells (180 µL) were added with MTT solution (20µL, 5 mg/mL). After 2h of incubation on 37°C incubator, cells were centrifuged and supernatants were taken out. One hundred fifty microliters of dimethyl sulfoxide (DMSO) were added and incubated for 15min on RT. After incubation, absorbance was detected in 590nm wavelength.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

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