Supporting information

Lignans from the Fruits of *Melia toosendan* and their Agonistic Activities on Melatonin Receptor MT₁

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Extraction and isolation

The air-dried and powdered fruit of *Melia toosendan* (6 kg) was extracted with 90% EtOH at room temperature three times, each for 24 h. All the extracts were combined and condensed *in vacuo* and partitioned between EtOAc and H₂O to afford EtOAc part 125 g.

The EtOAc part was subjected to silica gel column chromatography (SiCC, 200—300 mesh, 900 g, 14 × 65 cm) eluted with H₂O-MeOH-CHCl₃ (0:0:100, 0:5:95, 0:10:90, 2:20:80, v/v/v, each 15 L) to afford Frs. 1-5 based on the TLC characteristics.

According to our *in vitro* bioassay (**Table S1**), Fr. 3 (71.5 g) was further divided into six subfractions (Frs. 3-1~3-6) by SiCC (900 g, 14 × 65 cm) using Me₂CO-petroleum ether (PE, 30:70, 40:60) as the eluent. Fr. 3-1 (11.1 g) was fractionated by MCI CHP 20P gel CC (100 g, 2.54 × 50 cm) eluted with MeOH-H₂O (20:80, 40:60, 60:40, 80:20, 100:0) to get Frs. 3-1-1~3-1-5, correspondingly. The subfraction Fr. 3-1-3 (4.2 g) was subjected to SiCC (50 g, 4.0 × 45 cm) eluted with Me₂CO-PE (20:80) and SiCC (10 g, 2.0 × 45 cm) with Me₂CO-CHCl₃ (5:95), and further purified by semipreparative HPLC (Zorbax-C₁₈, 5.0 μm, 9.4 × 250 mm, UV detection at λ_max = 210, 254 nm) using MeOH-H₂O (18:82, flow rate 3 mL/min) to obtain compound 7 (6 mg, t_R = 26 min, purity > 98% detected by HPLC). Fr. 3-2 (4.5 g) with higher activity on MT₁ than other fractions was partitioned by SiCC (120 g, 6.0 × 45 cm) eluted with EtOAc-PE (50:50) to get Fr. 3-2-1~3-2-5. Fr. 3-2-2 (1.3 g) was subjected to SiCC (40 g, 4.0 × 45 cm) eluted with MeOH-CHCl₃ (1:100), resulting in five subfractions, Frs. 3-2-2-1~3-2-2-5. Fr. 3-2-2-1 (30 mg) was purified by semipreparative HPLC with
MeOH-H$_2$O (65:35, flow rate 2.5 mL/min) as a mobile phase to afford compounds 3 (5 mg, $t_R = 36$ min, purity > 97%) and 4 (5 mg, $t_R = 38$ min, purity > 96%). Compound 24 (910 mg, purity > 95%, determined by HPLC) was obtained from Fr. 3-2-2-2 (1 g) by Sephadex LH-20 CC (54 g, 1.5 × 132 cm) eluted with MeOH-CHCl$_3$ (50:50). Fr. 3-2-2-3 (35 mg) was purified by semipreparative HPLC with MeOH-H$_2$O (60:40, flow rate 3 mL/min) to obtain compounds 1 (7 mg, $t_R = 31$ min, purity > 96%) and 2 (8 mg, $t_R = 32.5$ min, purity > 96%). Fr. 3-3 (10.2 g) was firstly fractionated by MCI CHP 20P gel CC (100 g, 2.54 × 50 cm) eluted with MeOH-H$_2$O (20:80→100:0) to get Frs. 3-3-1~3-3-5, and Fr. 3-3-4 (7.7 g) was further divided into five subfractions (Frs. 3-3-4-1~3-3-4-5) by SiCC (200 g, 6.0 × 45 cm) using MeOH-CHCl$_3$ (2:98) as the eluent. Fr. 3-3-4-1 (0.1 g) was rechromatographed on Sephadex LH-20 CC (31 g, 1.3 × 120 cm) with MeOH–CHCl$_3$ (50:50) and then purified by semipreparative HPLC with MeOH-H$_2$O (38:62, flow rate 3 mL/min) to yield compound 22 (30 mg, $t_R = 21$ min, purity > 97%). Fr. 3-3-4-3 (0.5 g) was purified by SiCC (20 g, 2.0 × 45 cm) eluted with Me$_2$CO-CHCl$_3$ (20:80) and then semipreparative HPLC using an MeOH-H$_2$O (18:82, flow rate 2.5 mL/min) system to obtain compounds 17 (12 mg, $t_R = 19.5$ min, purity > 95%) and 18 (10 mg, $t_R = 21$ min, purity > 96%). Fr. 3-3-4-4 (20 mg) was subjected to semipreparative HPLC eluted with MeOH-H$_2$O (37:63, flow rate 3 mL/min) to obtain compound 20 (12 mg, $t_R = 18$ min, purity > 97%). Fraction Fr. 3-4 (3.9 g) was partitioned by MCI CHP 20P gel CC (100 g, 2.54 × 50 cm) eluted with MeOH-H$_2$O (20:80→100:0) to get Frs. 3-4-1~3-4-5. Fr. 3-4-1 (0.6 g) was further subjected to SiCC (30 g, 4.0 × 45 cm) eluted with MeOH-CHCl$_3$ (3:97) and
semipreparative HPLC with MeOH-H$_2$O (30:70, flow rate 2 mL/min) to obtain compounds 15 (21 mg, $t_R = 21$ min, purity > 96%) and 16 (30 mg, $t_R = 23$ min, purity > 97%). Semipreparative HPLC with MeOH-H$_2$O (23:77, flow rate 3 mL/min) was employed on fraction Fr. 3-4-2 (0.1 g) to give the compound 23 (27 mg, $t_R = 19$ min, purity > 97%). Fr. 3-5 (2.7 g) was divided into five fractions (Frs. 3-5-1~3-5-5) by MCI CHP 20P gel CC (100 g, 2.54 × 50 cm) eluted with MeOH-H$_2$O (20:80→100:0). Fr. 3-5-2 (0.8 g) was further partitioned by SiCC (20 g, 2.0 × 45 cm) using EtOAc-PE (30:70) to obtain subfractions Frs. 3-5-2-1~3-5-2-5. Fr. 3-5-2-1 (20 mg) was purified by semipreparative HPLC with MeOH-H$_2$O (38:62, flow rate 3 mL/min) to yield compound 13 (10 mg, $t_R = 18$ min, purity > 95%) and Fr. 3-5-2-5 (0.7 g) was chromatographed on SiCC (30 g, 4.0 × 45 cm) with Me$_2$CO-CHCl$_3$ (20:80) as the eluent, resulting in Frs. 3-5-2-5-1~3-5-2-5-3. Fr. 3-5-2-5-1 (45 mg) was then purified on semipreparative HPLC eluted with MeOH-H$_2$O (35:65, flow rate 3 mL/min) to obtain compound 9 (30 mg, $t_R = 17$ min, purity > 96%). HPLC with MeCN-H$_2$O (28:72, flow rate 3 mL/min) was applied to Fr. 3-5-2-5-2 (20 mg) to gain compound 10 (11 mg, $t_R = 16$ min, purity > 98%). Fr. 3-5-2-5-3 (51 mg) was further separated using semipreparative HPLC eluted with MeCN-H$_2$O (28:72, flow rate 3 mL/min) to yield compounds 5 (7 mg, $t_R = 19$ min, purity > 97%) and 14 (9 mg, $t_R = 23$ min, purity > 96%). Fr. 3-6 (7.8 g) was partitioned by MCI CHP 20P gel CC (100 g, 2.54 × 50 cm) eluted with MeOH-H$_2$O (20:80→100:0) to get Frs. 3-6-1~3-6-5. Fr. 3-6-1 (0.4 g) was successively separated with Sephadex LH-20 CC (31 g, 1.3 × 120 cm) using MeOH-CHCl$_3$ (50:50), SiCC (10 g, 0.8 × 30 cm) eluted with Me$_2$CO-CHCl$_3$ (40:60),
and semipreparative HPLC eluted with MeCN-H$_2$O (28:72, flow rate 3 mL/min) to yield compounds 6 (4 mg, $t_R = 17.5$ min, purity $> 97\%$) and 8 (9 mg, $t_R = 20$ min, purity $> 98\%$).

Fr. 4 (14 g) was divided into five fractions (Frs. 4-1~4-5) by MCI CHP 20P gel CC (100 g, 2.54 × 50 cm) eluted with MeOH-H$_2$O (20:80→100:0). Fr. 4-2 (1.3 g) was separated with SiCC (40 g, 4.0 × 45 cm) using MeOH-EtOAc (5:95). Fr. 4-2-4 (50 mg) was separated by Sephadex LH-20 CC (31 g, 1.3 × 120 cm), eluting with MeOH-CHCl$_3$ (50:50) to afford compound 19 (13 mg, purity $> 95\%$).

Fr. 5 (7.8 g) was subjected to MCI CHP 20P gel CC (100 g, 2.54 × 50 cm), eluting with MeOH-H$_2$O (20:80→100:0), resulting in five fractions (Frs. 5-1~5-5). Fr. 5-2 (4.4 g) was separated by SiCC (100 g, 6.0 × 45 cm) eluted with Me$_2$CO-CHCl$_3$ (20:80) to obtain Frs. 5-2-1~5-2-5. Fr. 5-2-1 (0.1 g) was further purified by SiCC (10 g, 0.8 × 45 cm) with Me$_2$CO-PE (30:70) to afford compound 12 (13 mg, purity $> 95\%$). Fr. 5-2-3 (37 mg) was purified by semipreparative HPLC eluting with MeCN-H$_2$O (28:72, flow rate 3 mL/min) to get compounds 11 (5 mg, $t_R = 18$ min, purity $> 97\%$) and 21 (3 mg, $t_R = 15$ min, purity $> 95\%$).

**Bioassay procedure**

The HEK293 cell line was cultured with 5% CO$_2$ at 37°C in Dubecco's modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cells were seeded in a Matrigel coated 96-well black wall/clear bottom plate at a density of $4 \times 10^4$/well, and incubated in a CO$_2$ incubator for 24 h. Then, the cells
were dyed by HDB Wash Free Fluo-8 Calcium Assay kit at 37°C in dark for 1 h, and treated with tested compounds and a positive drug, respectively. The absorption values were read with the Flex Station 3 Benchtop Multi-Mode Microplate Reader at room temperature with an excitation wavelength at 485 nm, emission wavelength at 525 nm, and emission cutoff at 515 nm. The agonistic rates of the test compounds were calculated by comparing with the positive control whose agonistic rate was normalized as 100%.
### Table S1 Agonistic activity of extract and fractions of “Chuan-Lian-Zi” on MT\textsubscript{1} receptor.

<table>
<thead>
<tr>
<th>Test component</th>
<th>Weight (mg)</th>
<th>Test concentration (μg/mL)</th>
<th>Agonistic rate(^a) (%)</th>
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<tr>
<td>EtOAc part</td>
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<td>373.33</td>
<td>19.06(^b)</td>
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<tr>
<td>Aqueous part</td>
<td>0.99</td>
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<td>EtOAc part Fr.1</td>
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<td>363.33</td>
<td>ND</td>
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<td>EtOAc part Fr.2</td>
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<td>EtOAc part Fr.3</td>
<td>1.15</td>
<td>383.33</td>
<td>16.58(^d)</td>
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<td>363.33</td>
<td>9.71(^d)</td>
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<td>EtOAc part Fr.5</td>
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<td>EtOAc part Fr.3-1</td>
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<td>340.00</td>
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<tr>
<td>EtOAc part Fr.3-2</td>
<td>0.97</td>
<td>323.33</td>
<td>36.16(^e)</td>
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<td>EtOAc part Fr.3-3</td>
<td>1.15</td>
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\(^a\)The agonistic rate was the percentage versus the control (normalized to 100%) and was the average of two independent tests; \(^b\)The positive control was melatonin (EC\textsubscript{50} 0.59 nM); \(^c\)No agonistic activity was detected; \(^d\)The positive control was melatonin (EC\textsubscript{50} 0.54 nM); \(^e\)The positive control was melatonin (EC\textsubscript{50} 0.61 nM).
Fig. S1 The structures of compounds 8-24.
$^1$H NMR spectrum of compound 1.

$^{13}$C NMR spectrum of compound 1.
$^1$H–$^1$H COSY spectrum of compound 1.

HSQC spectrum of compound 1.
HMBC spectrum of compound 1.

HRESIMS spectrum of compound 1.
$^1$H NMR spectrum of compound 2.

$^{13}$C NMR spectrum of compound 2.
$^1$H-$^1$H COSY spectrum of compound 2.

HSQC spectrum of compound 2.
HMBC spectrum of compound 2.

HRESIMS spectrum of compound 2.
$^1$H NMR spectrum of compound 3.

$^{13}$C NMR spectrum of compound 3.
$^1$H-$^1$H COSY spectrum of compound 3.

HSQC spectrum of compound 3.
HMBC spectrum of compound 3.

HRESIMS spectrum of compound 3.
$^1$H NMR spectrum of compound 4.

$^{13}$C NMR spectrum of compound 4.
$^1$H–$^1$H COSY spectrum of compound 4.

HSQC spectrum of compound 4.
HMBC spectrum of compound 4.

HRESIMS spectrum of compound 4.

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<th>Pred. m/z</th>
<th>Df. (mDa)</th>
<th>Df. (ppm)</th>
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$^1$H NMR spectrum of compound 5.

$^{13}$C NMR spectrum of compound 5.
\(^1\)H\(^1\)H COSY spectrum of compound 5.

HSQC spectrum of compound 5.
HMBC spectrum of compound 5.

HRESIMS spectrum of compound 5.
$^1$H NMR spectrum of compound 6.

$^{13}$C NMR spectrum of compound 6.
$^{1}\text{H}-^{1}\text{H}$ COSY spectrum of compound 6.

HSQC spectrum of compound 6.
HMBC spectrum of compound 6.

ROESY spectrum of compound 6.
HRESIMS spectrum of compound 6.

![HRESIMS Spectrum](image)

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$^1$H NMR spectrum of compound 7.

$^{13}$C NMR spectrum of compound 7.
$^1$H-$^1$H COSY spectrum of compound 7.

HSQC spectrum of compound 7.
HMBC spectrum of compound 7.

ROESY spectrum of compound 7.
HRESIMS spectrum of compound 7.

ECD spectrum of compound 7.