Lignans from the Fruits of *Melia toosendan* and Their Agonistic Activities on Melatonin Receptor MT_1

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Key words
- *Melia toosendan*
- Meliaceae
- lignans
- MT_1 receptor
- agonistic activity

Abstract

Investigation on the fruits of *Melia toosendan* afforded seven new lignans (**1**–**7**), along with seventeen known compounds (**8**–**24**). The structures of the new compounds, involving four neo-lignans (**1**–**4**), two sesquilignans (**5**–**6**), and a norlignan (**7**), were elucidated based on extensive spectroscopic analyses (high-resolution electrospray ionization mass spectra, ultraviolet, infrared, one-dimensional and two-dimensional nuclear magnetic resonance). Compound **24** exhibited activity on melatonin receptor type 1 with an agonistic rate of 57.77% at 1.02 mM according to the assay on HEK293 cell lines *in vitro*.

Supporting information available online at http://www.thieme-connect.de/products

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Introduction

The fruit of *Melia toosendan* Sieb. et Zucc. (Meliaceae), well-known as "Chuan-Lian-Zi" in Chinese, is an important traditional Chinese medicine (TCM) for the treatment of malaria, sting, stomachache, acute or chronic inflammations, and parasitosis [1–3], and possesses the property of "soothing liver-qi stagnation", indicating the neuroprotective potency according to TCM theory [4]. Pharmacological studies have demonstrated that toosendanin is an active component of "Chuan-Lian-Zi" as a selective presynaptic blocker, effective antibotulismic agent, and proliferation inhibitor of various human cancer cells [1,5]. In addition, "Chuan-Lian-Zi" is widely used as a botanical pesticide in agriculture due to the presence of limonoid-type triterpenoids [3,6].

Previous research has mainly focused on the limonoid-type triterpenoids of *M. toosendan*, from which more than 50 cases have been isolated [3,7,8]. However, there have been only a few investigations of lignans, another main component of "Chuan-Lian-Zi". Lignans as secondary metabolites derived from the shikimic acid pathway are widely distributed in plants and possess antitumor, antimitotic, antiviral, enzyme inhibitory, piscicidal, and antifungal activities [9–11]. Especially, it has been reported that lignans have neuroprotective activity *in vitro* and *in vivo* [12–15].

Melatonin receptor type 1 (MT_1), a high-affinity G-protein-coupled receptor widely distributed in the brain, retina, cardiovascular system, immune system, reproductive system, kidneys, pancreas, and liver, is a very important target in the treatment of most melatonin-related nervous system diseases, including sleep disorders, depression, and anxiety syndromes [16–18]. Mediation on the activation of MT_1 is an effective therapy for these psychiatric problems, based on which several agonistic drugs (circadin, ramelteon, agomelatine, etc.) have been developed successfully, along with many potential candidates undergoing clinical trials [18].

In order to clarify the neuroprotective components of the entitled plant, the bioassay-guided investigation on the EtOAc part of the fruit of *M. toosendan* led to the isolation of seven new lignans (**1**–**7**; **Fig. 1**) and seventeen known ones (**8**–**24; Fig. 1S,** Supporting Information). Among them, compounds **8**–**23** were isolated from the fruits of *M. toosendan* for the first time. Their structural elucidation and biological evaluation are reported in this paper.

Results and Discussion

Compound **1** showed a quasi-molecular ion peak at *m/z* 425.1585 $[M + Na]^+$ in the positive HRESIMS, indicating a molecular formula of $C_{22}H_{26}O_7$

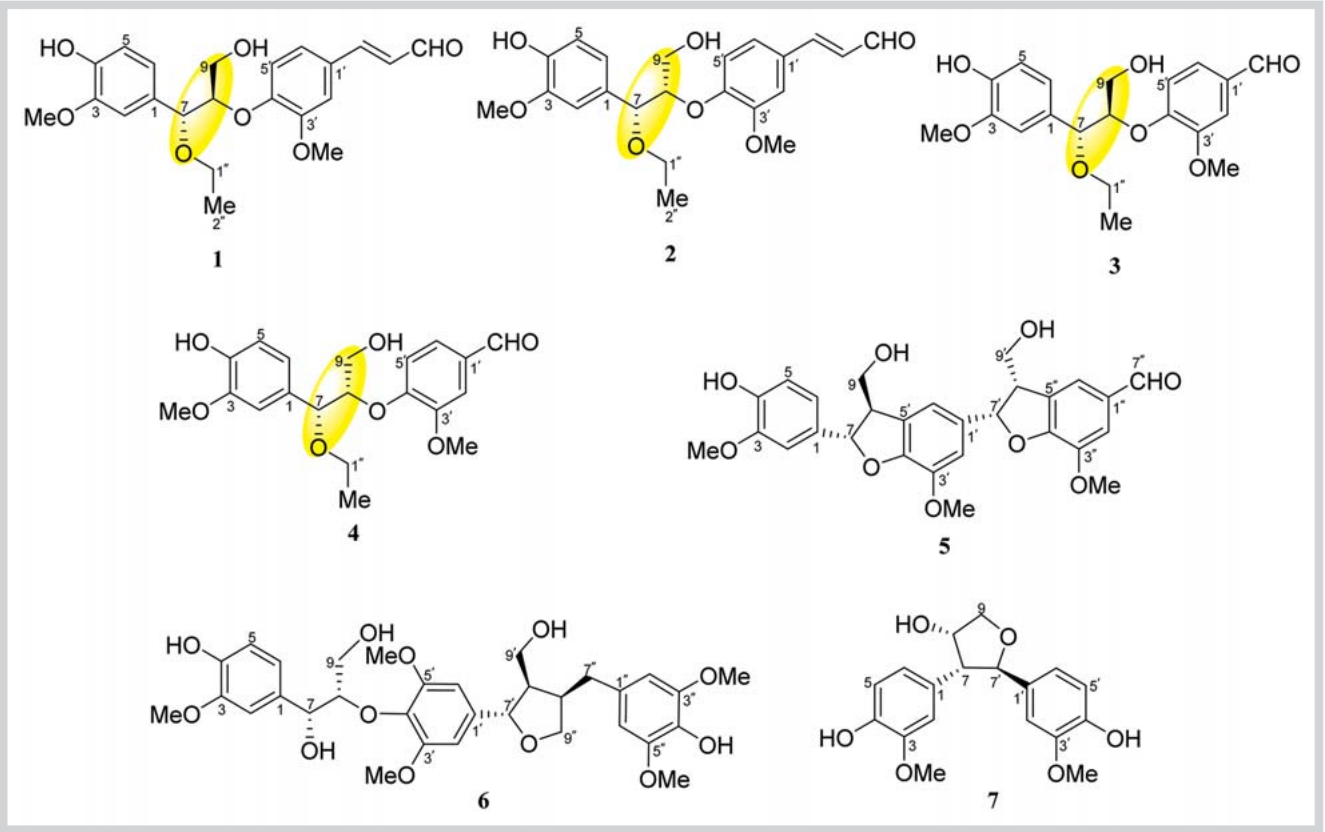

Fig. 1 The structures of compounds **1**–**7**. (Color figure available online only.)

with ten degrees of unsaturation. Its IR spectrum suggested the groups of hydroxyl (3428 cm^{-1}), *α,β*-unsaturated carbonyl (1666 cm^{-1}), aromatic rings (1597, 1511, and 1460 cm^{-1}), and ether bonds (1273, 1130, and 1033 cm^{-1}). The ^{1}H NMR spectrum showed an ethoxy group at *δ* 3.41 (1H, dq, *J* = 9.5, 7.2 Hz), 3.36 (1H, dq, *J* = 9.5, 7.2 Hz), and 1.14 (3H, t, *J* = 7.2 Hz), two methoxy groups at *δ* 3.80 (3H, s) and 3.77 (3H, s), and six aromatic protons in two ABX spin systems, *δ* 6.94 (1H, d, *J* = 1.8 Hz), 6.69 (1H, d, *J* = 8.1 Hz), and 6.80 (1H, dd, *J* = 8.1, 1.8 Hz), and *δ* 7.18 (1H, d, *J* = 1.9 Hz), 6.95 (1H, d, *J* = 8.4 Hz), and 7.12 (1H, dd, *J* = 8.4, 1.9 Hz), suggesting the presence of two 1,3,4-trisubstituted phenyl rings. The signals at *δ* 9.56 (1H, d, *J* = 7.8 Hz), 7.54 (1H, d, *J* = 15.8 Hz), and 6.64 (1H, dd, *J* = 15.8, 7.8 Hz) were owed to a conjugated formyl group with a *trans*-form double bond. Its ^{13}C NMR (DEPT) spectrum was similar with that of *threo*-guaiacylglycerol-*β*-coniferyl aldehyde ether (**15**) [19] except for the additional ethoxy group and the obvious downfield shift of C-7 from δ_C 73.4 to 82.5, suggesting that **1** was the 7-*O*-ethyl derivative of **15**. This deduction was supported by the correlations of δ_H 4.46 (H-7)/δ_C 65.6 (C-1″) and δ_H 3.41, 3.36 (H-1″)/δ_C 82.5 (C-7) in the HMBC spectrum (**Fig. 2**). Previous reports revealed that compounds with a *threo*-guaiacylglycerol unit could be distinguished from its *erythro* isomer with the chemical shifts of C-8 and the $^3J_{H,H}$ coupling constants of the H-7/H-8 protons. The chemical shifts of C-8 in the *threo*-form were slightly lower than its *erythro* isomer, and the $J_{7,8}$ value in the *threo*-form was larger than the *erythro*-form [20–24]. Thus, the *threo*-configuration of the C-7/C-8 system was indicated by the $J_{7,8}$ value of 7.1 Hz, and compound **1** was assigned as *threo*-guaiacylethoxyglycerol-*β*-*O*-4′-coniferyl aldehyde ether.

Compound **2** had a molecular formula of $C_{22}H_{26}O_7$ based on the positive HRESIMS at *m/z* 425.1589 $[M + Na]^+$. It was proposed to be the isomer of **1** due to their similar ^{1}H NMR and ^{13}C NMR (DEPT) spectral data. Detailed analyses of its ^{1}H-^{1}H COSY and HMBC spectra verified that compound **2** possessed the same planar structure with **1**. The upfield shift of H-9 from *δ* 3.86 (1H, dd, *J* = 9.6, 3.7 Hz, H-9a) and 3.84 (1H, dd, *J* = 9.6, 5.7 Hz, H-9b) in compound **1** to *δ* 3.67 (1H, dd, *J* = 10.4, 3.1 Hz, H-9a) and 3.49 (1H, dd, *J* = 10.4, 4.3 Hz, H-9b) in **2**, along with the upfield shift of C-8 from δ_C 85.3 in **1** to 84.6 in **2**, suggested the changes of the stereochemistry. In contrast to the $J_{7,8}$ value of 7.1 Hz in **1**, the obvious small value of 4.2 Hz suggested the *erythro*-configuration [20–24]. Therefore, compound **2** was characterized as *erythro*-guaiacylethoxyglycerol-*β*-*O*-4′-coniferyl aldehyde ether.

Compound **3** was determined to be $C_{20}H_{24}O_7$ on the basis of the positive HRESIMS at *m/z* 399.1435 $[M + Na]^+$. Its ^{1}H- and ^{13}C-NMR data (**Tables 1** and **3**) were similar with those of compound **1**, except for the absence of two vinylic carbons and the upfield shift of the aldehyde group from δ_C 196.1 in **1** to 192.9 in **3**, which suggested the aldehyde group was directly linked to C-1′. This deduction was confirmed by HMBC correlations (**Fig. 2**) from δ_H 9.73 (H-7′) to δ_C 111.5 (C-2′) and 127.2 (C-6′). The *threo*-configuration was indicated by the $J_{7,8}$ value of 7.1 Hz. Thus, compound **3** was elucidated as *threo*-guaiacylethoxyglycerol-*β*-*O*-4′-guaiacyl aldehyde ether.

Compound **4** had a quasi-molecular ion peak at *m/z* 399.1405 $[M + Na]^+$ (calcd. for 399.1414) in HRESIMS, suggesting a molecular formula of $C_{20}H_{24}O_7$ with nine degrees of unsaturation. Comparing the ^{1}H NMR and ^{13}C NMR (DEPT) spectral data with those of compound **3**, the upfield shift of H-9 from *δ* 3.89 (1H, dd, *J* = 11.4,

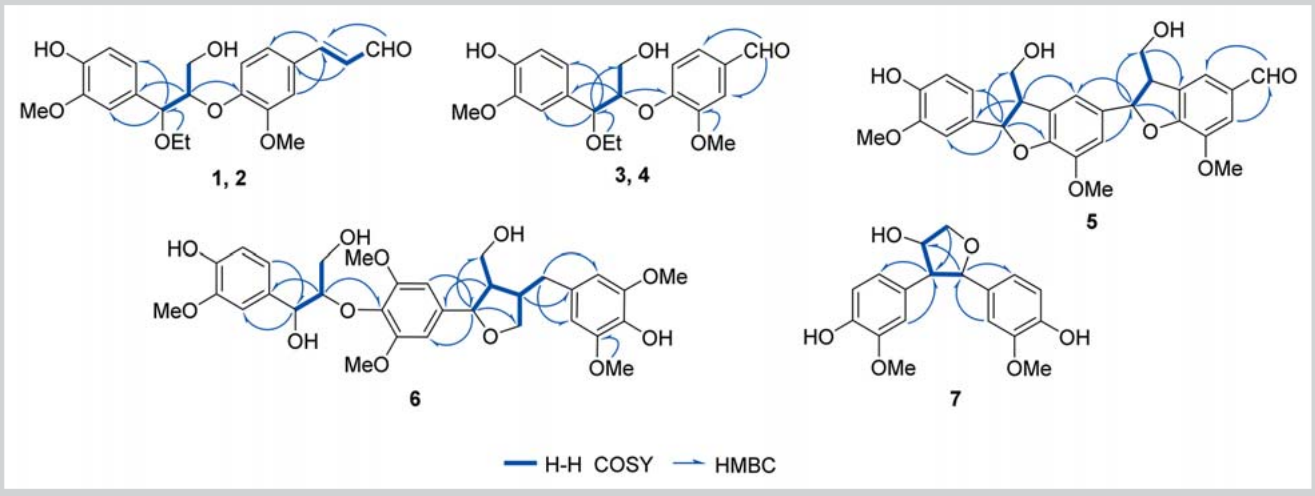

Fig. 2 The key ^{1}H-^{1}H COSY and HMBC correlations of compounds **1**–**7**. (Color figure available online only.)

Table 1 ^{1}H-NMR (600 MHz) data for compounds **1**–**4** in CD_3OD (δ in ppm, J in Hz).

No.	1	2	3	4
2	6.94 d (1.8)	6.96 d (1.8)	6.94 d (1.8)	6.96 d (1.7)
5	6.69 d (8.1)	6.76 d (8.1)	6.67 d (8.0)	6.75 d (8.0)
6	6.80 dd (8.1, 1.8)	6.80 dd (8.1, 1.8)	6.80 dd (8.0, 1.8)	6.81 dd (8.0, 1.7)
7	4.46 d (7.1)	4.52 d (4.2)	4.46 d (7.1)	4.53 d (4.5)
8	4.54 ddd (7.1, 5.7, 3.7)	4.51 ddd (4.3, 4.2, 3.1)	4.64 ddd (7.1, 5.7, 3.6)	4.62 ddd (6.2, 4.5, 3.5)
9a	3.86 dd (9.6, 3.7)	3.67 dd (10.4, 3.1)	3.89 dd (11.4, 3.6)	3.68 dd (12.0, 3.5)
9b	3.84 dd (9.6, 5.7)	3.49 dd (10.4, 4.3)	3.85 dd (11.4, 5.7)	3.53 dd (12.0, 6.2)
2′	7.18 d (1.9)	7.28 d (1.9)	7.35 d (1.9)	7.45 d (1.8)
5′	6.95 d (8.4)	7.08 d (8.4)	7.07 d (8.3)	7.19 d (8.1)
6′	7.12 dd (8.4, 1.9)	7.19 dd (8.4, 1.9)	7.38 dd (8.3, 1.9)	7.46 dd (8.1, 1.8)
7′	7.54 d (15.8)	7.59 d (15.8)	9.73 s	9.78 s
8′	6.64 dd (15.8, 7.8)	6.68 dd (15.8, 7.8)		
9′	9.56 d (7.8)	9.59 d (7.8)		
1″a	3.41 dq (9.5, 7.2)	3.42 dq (9.5, 7.2)	3.41 dq (9.5, 7.2)	3.41 dq (9.5, 7.2)
1″b	3.36 dq (9.5, 7.2)	3.36 dq (9.5, 7.2)	3.36 dq (9.5, 7.2)	3.35 dq (9.5, 7.2)
2″	1.14 t (7.2)	1.08 t (7.2)	1.14 t (7.2)	1.06 t (7.2)
3-OMe	3.77 s	3.82 s	3.77 s	3.81 s
3′-OMe	3.80 s	3.90 s	3.81 s	3.92 s

3.6 Hz, H-9a) and 3.85 (1H, dd, J = 11.4, 5.7 Hz, H-9b) in **3** to δ 3.68 (1H, dd, J = 12.0, 3.5 Hz, H-9a) and 3.53 (1H, dd, J = 12.0, 6.2 Hz, H-9b) in **4**, along with the upfield shifts of C-7 (from 82.5 in **3** to 81.6 in **4**) and C-8 (from 85.1 in **3** to 84.3 in **4**), suggested that compound **4** should be the stereoisomer of **3**. The *erythro*-configuration was concluded by the $J_{7,8}$ value of 4.5 Hz. Hence, compound **4** was elucidated as *erythro*-guaiacylethoxy glycerol-*β*-*O*-4′-guaiacyl aldehyde ether.

Compound **5** had a molecular formula of $C_{28}H_{28}O_9$ with 15 degrees of unsaturation based on its $[M + H]^+$ ion peak at *m/z* 509.1778 (calcd. for 509.1806) in HRESIMS. The functional groups of hydroxyl (3442 cm^{-1}), aromatic rings (1516, 1493, and 1452 cm^{-1}), and ether bonds (1276, 1136, and 1032 cm^{-1}) were deduced from the IR spectrum. The ^{1}H NMR spectrum showed three methoxyls at δ 3.95, 3.87, and 3.84 (each 3H, s), one 1,3,4-trisubstituted phenyl ring at δ 6.97 (1H, d, J = 1.8 Hz, H-2), 6.84 (1H, d, J = 8.1 Hz, H-5), and 6.78 (1H, dd, J = 8.1, 1.8 Hz, H-6), and two 1,3,4,5-substituted phenyl rings at δ 7.55, 7.48, 6.96, and 6.94 (each 1H). Three methoxy groups, eleven quaternary carbons, twelve methines, and two methylenes observed in the ^{13}C NMR (DEPT) spectrum indicated a sesquilignan skeleton. The proton resonances at δ_H 7.55 (H-6″), 7.48 (H-2″), 6.96 (H-6′), 6.94 (H-2′), 5.73 (H-7), and 5.55 (H-7′), together with the carbon resonances at δ_C 154.1 (C-4″), 148.2 (C-4′), 129.7 (C-5′), 129.2 (C-5″), 89.3 (C-7), 87.9 (C-7′), 53.8 (C-8), and 53.0 (C-8′), suggested the presence of two dihydrobenzofuran moieties. The correlations from H-2′ and H-6′ to C-7′, and from H-7′ to C-2′ and C-6′ in HMBC (**Fig. 2**) indicated the two dihydrobenzofuran moieties were linked by the $C_{1'}$-$C_{7'}$ bond. The linkage of a guaiacyl moiety with C-7 was further confirmed by the correlations from H-2 and H-6 to C-7, together with the correlations from H-7 to C-2 and C-6 in the HMBC spectrum. Compared with the coupling constant (6.9 Hz) of $J_{7',8'}$ in rosalaevin A (**8**) [25], the *trans*-orientation of H-7 and H-8 was evident from the $J_{7,8}$ value of 6.9 Hz, while the *cis*-configuration of H-7′ and H-8′ was verified by the $J_{7',8'}$ value of 4.7 Hz. Thus, the structure of **5** was finally determined as *trans*-2-guaiacyl-3-hydroxymethyl-5-(*cis*-3′-hydroxymethyl-5′-formyl-7′-methoxybenzofuranyl)-7-methoxybenzofuran.

No.	5	6	7
2	6.97 d (1.8)	6.97 d (1.8)	6.94 d (1.8)
5	6.84 d (8.1)	6.77 d (8.1)	6.66 d (8.1)
6	6.78 dd (8.1, 1.8)	6.73 dd (8.1, 1.8)	6.75 dd (8.1, 1.8)
7	5.73 d (6.9)	4.90 d (3.6)	3.16 dd (10.8, 4.4)
8	3.68 ddd (6.9, 5.1, 3.6)	4.22 ddd (5.6, 3.8, 3.6)	4.45 ddd (4.4, 4.2, 0.5)
9a	3.91 dd (9.4, 3.6)	3.89 dd (12.2, 5.6)	4.42 dd (9.4, 4.2)
9b	3.89 dd (9.4, 5.1)	3.56 dd (12.2, 3.8)	3.99 dd (9.4, 0.5)
2′	6.94 d (1.3)	6.65 d (1.9)	6.82 d (1.8)
5′			6.71 d (8.1)
6′	6.96 d (1.3)	6.65 d (1.9)	6.70 dd (8.1, 1.8)
7′	5.55 d (4.7)	4.83 d (6.9)	5.13 d (10.8)
8′	3.52 ddd (5.2, 4.7, 3.4)	2.35 m	
9′a	3.82 dd (12.9, 3.4)	3.86 dd (10.8, 5.5)	
9′b	3.79 dd (12.9, 5.2)	3.68 dd (10.8, 3.2)	
2″	7.48 d (1.6)	6.50 brs	
6″	7.55 d (1.6)	6.50 brs	
7″a	9.81 s	2.92 dd (13.4, 5.9)	
7″b		2.50 dd (13.4, 10.4)	
8″		2.71 m	
9″a		4.01 dd (9.6, 2.6)	
9″b		3.76 dd (9.6, 6.4)	
3-OMe	3.87 s	3.82 s	3.82 s
3′-OMe	3.95 s	3.81 s	3.75 s
3″-OMe	3.84 s	3.81 s	
5′-OMe		3.81 s	
5″-OMe		3.81 s	

Table 2 ^{1}H-NMR (600 MHz) data for compounds **5**–**7** in CD_3OD (δ in ppm, J in Hz).

Table 3 ^{13}C-NMR (150 MHz) data for compounds **1**–**7** in CD_3OD (δ_C values).

No.	1	2	3	4	5	6	7
1	131.4 s	131.3 s	131.6 s	131.6 s	134.3 s	133.8 s	133.8 s
2	112.0 d	112.5 d	111.9 d	112.4 d	110.5 d	111.3 d	114.7 d
3	149.1 s	148.8 s	149.1 s	148.8 s	144.2 s	148.7 s	148.9 s
4	147.5 s	147.4 s	147.6 s	147.4 s	146.2 s	146.8 s	146.7 s
5	116.0 d	115.6 d	116.0 d	115.6 d	114.4 d	115.7 d	115.9 d
6	121.4 d	122.0 d	121.4 d	122.0 d	114.8 d	120.6 d	123.9 d
7	82.5 d	81.7 d	82.5 d	81.6 d	89.3 d	74.0 d	60.2 d
8	85.3 d	84.6 d	85.1 d	84.3 d	53.8 d	87.4 d	76.0 d
9	62.4 t	62.5 t	62.3 t	62.5 t	63.3 t	61.6 t	77.1 t
1′	129.1 s	129.1 s	131.3 s	131.1 s	133.0 s	141.0 s	128.6 s
2′	112.7 d	112.7 d	111.5 d	111.4 d	109.1 d	104.0 d	111.0 d
3′	151.7 s	151.7 s	151.7 s	151.7 s	144.9 s	154.4 s	148.8 s
4′	153.6 s	152.8 s	156.3 s	155.5 s	148.2 s	134.8 s	147.3 s
5′	117.2 d	117.1 d	116.0 d	115.8 d	129.7 s	154.4 s	116.0 d
6′	124.5 d	124.3 d	127.2 d	127.0 d	118.3 d	104.0 d	120.5 d
7′	155.6 d	155.5 d	192.9 d	192.9 d	87.9 d	84.0 d	84.8 d
8′	127.6 d	127.6 d			53.0 d	54.2 d	
9′	196.1 d	196.1 d			63.1 t	60.6 t	
1″	65.6 t	65.3 t	65.6 t	65.3 t	131.4 s	132.8 s	
2″	15.6 q	15.6 q	15.5 q	15.6 q	112.5 d	106.8 d	
3″					147.7 s	149.3 s	
4″					154.1 s	135.8 s	
5″					129.2 s	149.3 s	
6″					120.9 d	106.8 d	
7″					191.3 d	34.1 t	
8″						43.8 d	
9″						73.7 t	
3-OMe	56.3 q	56.3 q	56.3 q	56.3 q	55.3 q	56.3 q	56.5 q
3′-OMe	56.6 q	56.6 q	56.5 q	56.4 q	55.4 q	56.7 q	56.3 q
5′-OMe						56.7 q	
3″-OMe					55.0 q	56.6 q	
5″-OMe						56.6 q	

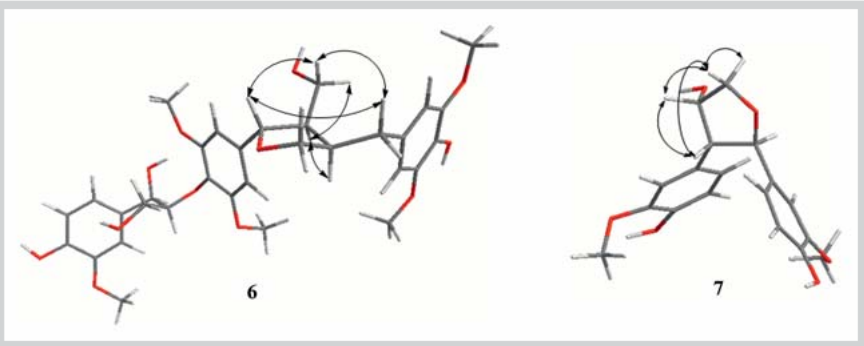

Fig. 3 The key correlations in ROESY experiments of compounds **6**–**7**. (Color figure available online only.)

Compound **6** had a molecular formula of $C_{32}H_{40}O_{12}$ with 13 degrees of unsaturation determined by its quasi-molecular ion peak at *m/z* 639.2400 $[M + Na]^+$ (calcd. for 639.2412) in the positive HRESIMS. The sesquilignan structure was evident from the existence of one 1,3,4-trisubstituted aromatic ring at δ 6.97 (1H, d, J = 1.8 Hz, H-2), 6.77 (1H, d, J = 8.1 Hz, H-5), and 6.73 (1H, dd, J = 8.1, 1.8 Hz, H-6), and two 1,3,4,5-substituted phenyl rings (δ 6.65 and 6.50, each 2H) in the ^{1}H NMR spectrum (**Table 2**). Comparing the ^{1}H and ^{13}C NMR (DEPT) data with those of rosalaevins A (**8**), the presence of an additional methoxyl at C-5″ in **6** was confirmed by the downfield shift of C-5″ from δ_C 114.4 in **8** to 149.3 in **6**, and the correlation of δ_H 3.81 to δ_C 149.3 in the HMBC spectrum (**Fig. 2**). The $J_{7,8}$ (3.6 Hz) and $J_{7',8'}$ (6.9 Hz) values indicated the 7,8-*erythro* configuration and 7′,8′-*trans* orentation [25]. Furthermore, the absence of a ROESY correlation of H-7′/H-8″ together with the correlations of H-7′/H-8′/H-9′, H-7′/H-7″ (δ 2.50), H-8′/H-8″ and H-7″/H-9′ (δ 3.86) (**Fig. 3**) indicated the *trans*-orentation of H-7′ and H-8″. Hence, the structure of **6** was deduced as *erythro*-guaiacylglycerol-*β*-*O*-4′-(+)-5,5′-dimethoxylariciresinol ether.

Compound **7** was obtained as pale yellow oil, and had a molecular formula of $C_{18}H_{20}O_6$ by the negative HRESIMS at *m/z* 331.1157 $[M - H]^-$, requiring nine degrees of unsaturation. The hydroxy (3494 and 3444 cm^{-1}), aromatic (1609, 1517 and 1456 cm^{-1}), and ether bond (1274, 1233, 1124, and 1034 cm^{-1}) groups were revealed in the IR spectrum. In the ^{1}H NMR spectrum, signals of two 1,3,4-trisubstituted aromatic groups at δ 6.94 (1H, d, J = 1.8 Hz, H-2), 6.66 (1H, d, J = 8.1 Hz, H-5) and 6.75 (1H, dd, J = 8.1, 1.8 Hz, H-6), δ 6.82 (1H, d, J = 1.8 Hz, H-2′), 6.71 (1H, d, J = 8.1 Hz, H-5′) and 6.70 (1H, dd, J = 8.1, 1.8 Hz, H-6′), together with signals of a substituted epoxybutyl unit, were unambiguously designated by ^{1}H-^{1}H COSY and HSQC experiments (**Fig. 2**). The correlations from H-2 and H-6 to C-7, from H-7 to C-2 and C-6, from H-2′ and H-6′ to C-7′, and from H-7′ to C-2′ and C-6′ in the HMBC experiment suggested that two 4-hydroxy-3-methoxybenzyl units were located at C-7 and C-7′, respectively. Therefore, the planar structure of **7** was established as norlignan from the ^{1}H and ^{13}C NMR data (**Tables 2** and **3**). The 7,8-*cis* and 7,7′-*trans* orentation were indicated by the $J_{7,8}$ (4.4 Hz) and $J_{7,7'}$ (10.8 Hz) values, which were confirmed by the ROESY experiment (**Fig. 3**). Finally, compound **7** was elucidated as (2*R**,3*R**,4*S**)-2,3-diguaiacyl-4-hydroxyl tetrahydrofuran.

The known lignans were identified as rosalaevins A (**8**) [25], ficusesquilignans A (**9**) [26], buddlenol E (**10**) [27], 5′-demethoxybuddlenol E (**11**) [28], leptolepisol D (**12**) [24], lyoniside (**13**) [29], picrasmalignan A (**14**) [30], *threo*-guaiacylglycerol-*β*-coniferyl aldehyde ether (**15**) [19], *erythro*-guaiacylglycerol-*β*-coniferyl aldehyde ether (**16**) [19], *threo*-guaiacylglycerol-*β*-coniferyl ether (**17**) [31], *erythro*-guaiacylglycerol-*β*-coniferyl ether (**18**) [31], *erythro*-dihydroxydehydrodiconifery alcohol (**19**) [20], vladinol D (**20**) [32], (−)-secoisolariciresinol (**21**) [33], ficusal (**22**) [26], (2*S*)-3,3-diguaiacyl-1,2-propanediol (**23**) [34], and (+)-pinoresinol (**24**) [19] by comparing their spectroscopic data with the literature.

Seven new compounds (**1**–**7**) and the abundant known (+)-pinoresinol (**24**) were evaluated for agonistic activity on human melatonin receptor MT_1 on HEK293 cell lines *in vitro*. As shown in **Table 4**, (+)-pinoresinol (**24**), the major lignan, exhibited the strongest agonistic effect among the tested compounds with an agonistic rate of 57.77% at the concentration of 1.02 mM. Compounds **7** and **2** possessed moderate agonistic activities on MT_1 with agonistic rates of 37.55% and 21.16% at about 1.00 mM, respectively. Compounds **1** and **5** showed weak agonistic activities on MT_1, while no obvious effects for compounds **3, 4**, and **6** were detected at the tested concentration.

Pharmacological studies demonstrated that serotonin (5-hydroxytryptamine, 5-HT) receptors, especially the subtypes of 5-HT_{1A} and 5-HT_{2C}, were closely involved in the pathogenesis and treatment of psychiatric problems such as depression and anxiety disorders [35, 36]. Consequently, these compounds were further assayed for the agitating activities on 5-HT_{1A} and 5-HT_{2C} receptors on HEK293 cell lines *in vitro*. However, no meaningful agonistic activities were observed at the concentration of about 1 mM (**Table 4**).

Different from the previous research, this investigation was focused on the lignans and resulted in seven new together with seventeen known ones. A bioassay on HEK293 cell lines *in vitro* revealed that (+)-pinoresinol (**24**) exhibited a moderate activity against MT_1 mediation. This study may provide another view for understanding the active components of "Chuan-Lian-Zi".

Materials and Methods

General

Melting points (m.p.) were measured by an SGW®X-4B melting point apparatus. UV spectra were recorded on a UV-2401A spectrophotometer with methanol (MeOH) as the solvent. Optical rotations were obtained on a Jasco model 1020 polarimeter in MeOH solution. IR spectra were collected on a Bio-Rad FTS-135 spectrometer with KBr pellets. HRESIMS data were acquired on a liquid chromatography-mass spectrometry-ion trap-time-of-flight (LCMS-IT-TOF). 1D and 2D NMR experiments were carried out using Advance III-600 NMR spectrometers with tetramethylsilane (TMS) as an internal standard. Semipreparative HPLC was performed on a Newstyle™ (pump: NP-7000 serials, detector:

Compounds	Test concentration (mM)	Agonistic rate[d] (%)		
		MT_1	$5\text{-}HT_{1A}$	$5\text{-}HT_{2C}$
1	0.91	4.28	0.64	6.94
2	0.99	21.16	5.07	9.80
3	0.97	ND[e]	ND	5.08
4	0.95	ND	3.49	11.39
5	1.05	8.04	ND	10.28
6	0.92	ND	7.73	5.25
7	0.90	37.55	ND	7.89
24	1.02	57.77	14.88	7.40

Table 4 Agonistic activities of compounds **1**–**7** and **24** on MT_1[a], $5\text{-}HT_{1A}$[b], and $5\text{-}HT_{2C}$[c] receptors.

[a] The positive control was melatonin (EC_{50} 0.54 nM); [b] the positive control was 5-hydroxytryptamine (EC_{50} 43.28 nM); [c] the positive control was 5-hydroxytryptamine (EC_{50} 0.94 nM); [d] the agonistic rate was the percentage versus the control (normalized to 100%) and was the average of two independent tests; [e] no agonistic activity was detected

NU-3000 serials) with a reversed-phase (RP) C_{18} column (9.4 × 250 mm, 5 µm). Silica gel (200–300 mesh), MCI gel CHP-20P, and Sephadex LH-20 were used for column chromatography (CC). Thin-layer chromatography (TLC) was conducted on silica gel GF_{254} plates and the spots were visualized under UV light or by spraying with 10% H_2SO_4 in 95% ethanol (EtOH) followed by heating. Melatonin (purity > 99%) and serotonin hydrochloride (purity > 99%) were purchased from Alfa Aesar as positive controls.

Plant material

The fruit of *M. toosendan* Sieb. et Zucc. was purchased from Jvhuacun traditional Chinese medicine market, Kunming, Yunnan Province of China, in July 2012, and identified by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS). A voucher specimen (No. 2012–07–18) was deposited in the Laboratory of Antivirus and Natural Medicinal Chemistry, KIB, CAS.

Extraction and isolation

The air-dried and powdered fruit of *M. 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 H_2O to afford EtOAc part 125 g.

The EtOAc part was subjected to silica gel column chromatograph (SiCC, 900 g, 14 × 65 cm) eluted with H_2O-MeOH-$CHCl_3$ (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 1S,** Supporting Information), Fr. 3 (71.5 g) was further separated by SiCC to obtain Frs. 3–1 ~ 3–6. Fr. 3–1 (11.1 g) was fractionated by MCI CHP-20P gel CC to get Frs. 3–1–1 ~ 3–1–5. Fr. 3–1–3 was subjected to repeated SiCC and further purified by semipreparative HPLC (Zorbax-C_{18}, 5.0 µm, 9.4 × 250 mm, UV detection at λ_{max} = 210, 254 nm) using MeOH-H_2O (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) was partitioned by repeated SiCC to obtain Frs. 3–2–2–1 ~ 3–2–2–5. Fr. 3–2–2–1 was purified by semipreparative HPLC with MeOH-H_2O (65 : 35, flow rate 2.5 mL/min) to afford compounds **3** (5 mg, t_R = 36 min, purity > 97%) and **4** (5 mg, t_R = 38 min, purity > 96%). Fr. 3–2–2–2 was purified by Sephadex LH-20 CC to get compound **24** (910 mg, purity > 95%). Fr. 3–2–2–3 was purified by semipreparative HPLC with MeOH-H_2O (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 subjected to MCI CHP-20P gel CC, SiCC, and Sephadex LH-20 CC, respectively, and finally purified by semipreparative HPLC to yield known compounds **17** (12 mg), **18** (10 mg), and **22** (30 mg). Fr. 3–4 (3.9 g) was successively partitioned by MCI CHP-20P gel CC, SiCC, and semipreparative HPLC to obtain known compounds **15** (21 mg), **16** (30 mg), and **23** (27 mg). Fr. 3–5 (2.7 g) was repeatedly isolated to yield compounds **9** (30 mg), **10** (11 mg), **13** (10 mg), and Fr. 3–5–2–5–3 (51 mg), which was further separated by semipreparative HPLC eluted with MeCN-H_2O (28 : 72, flow rate 3 mL/min) to yield compounds **5** (7 mg, t_R = 19 min, purity > 97%) and **14** (9 mg). Fr. 3–6 (7.8 g) was partitioned by repeated CC and finally purified by semipreparative HPLC eluted with MeCN-H_2O (28 : 72, flow rate 3 mL/min) to yield compounds **6** (4 mg, t_R = 17.5 min, purity > 97%) and **8** (9 mg). Fr. 4 (14 g) was repeatedly isolated to afford compound **19** (13 mg) and Fr. 5 (7.8 g) was partitioned to obtain compounds **11** (5 mg), **12** (13 mg), and **21** (3 mg), respectively (the detailed isolation procedure is provided in Supporting Information). The purities of all known compounds were > 95% as determined by HPLC.

threo-Guaiacylethoxyglycerol-β-O-4′-coniferyl aldehyde ether (**1**): pale yellow amorphous powder, m. p. 59–61 °C; $[\alpha]_D^{20}$: − 3.93 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 337 (4.21) nm; IR (KBr) ν_{max} 3428, 1666, 1597, 1511, 1460, 1273, 1130, 1033, 867, 816 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see **Tables 1** and **3**; (+)HRESIMS *m/z* 425.1585 $[M + Na]^+$ (calcd. for $C_{22}H_{26}O_7Na$, 425.1571).

erythro-Guaiacylethoxyglycerol-β-O-4′-coniferyl aldehyde ether (**2**): pale yellow amorphous powder, m. p. 43–45 °C; $[\alpha]_D^{20}$: − 6.13 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 337 (3.89) nm; IR (KBr) ν_{max} 3441, 1661, 1597, 1511, 1454, 1272, 1130, 1032, 875, 819 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see **Tables 1** and **3**; (+)HRESIMS *m/z* 425.1589 $[M + Na]^+$ (calcd. for $C_{22}H_{26}O_7Na$, 425.1571).

threo-Guaiacylethoxyglycerol-β-O-4′-guaiacyl aldehyde ether (**3**): pale yellow oil; $[\alpha]_D^{20}$: − 4.52 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 309 (3.50), 279 (3.67), 228 (3.85) nm; IR (KBr) ν_{max} 3449, 1673, 1594, 1510, 1463, 1273, 1130, 1033, 867, 814 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see **Tables 1** and **3**; (+)HRESIMS *m/z* 399.1435 $[M + Na]^+$ (calcd. for $C_{20}H_{24}O_7Na$, 399.1414).

erythro-Guaiacylethoxyglycerol-β-O-4′-guaiacyl aldehyde ether (**4**): pale yellow oil; $[\alpha]_D^{20}$: − 9.07 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 309 (3.57), 279 (3.71), 228 (3.88) nm; IR (KBr) ν_{max} 3450, 1677, 1596, 1508, 1466, 1271, 1136, 1031, 868, 815 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see **Tables 1** and **3**; (+)HRESIMS *m/z* 399.1405 $[M + Na]^+$ (calcd. for $C_{20}H_{24}O_7Na$, 399.1414).

trans-2-Guaiacyl-3-hydroxymethyl-5-(cis-3′-hydroxymethyl-5′-formyl-7′-methoxybenzofuranyl)-7-methoxybenzofuran (**5**): pale yellow amorphous powder, m. p. 67–69 °C; $[\alpha]_D^{20}$: − 10.38 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε): 378 (2.36), 288 (4.10), 235 (4.33) nm; IR (KBr) ν_{max} 3442, 1631, 1516, 1493, 1452, 1276, 1136, 1032 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see ▶ **Tables 2** and **3**; (+)HRESIMS *m/z* 509.1778 $[M + H]^+$ (calcd. for $C_{28}H_{29}O_9$, 509.1806).

erythro-Guaiacylglycerol-β-O-4′-(+)-5,5′-dimethoxylariciresinol ether (**6**): pale yellow amorphous powder, m. p. 92–94 °C; $[\alpha]_D^{20}$: − 9.00 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 378 (2.74), 279 (3.73) nm; IR (KBr) ν_{max} 3441, 3433, 1620, 1517, 1462, 1274, 1121, 1035, 877, 857 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see ▶ **Tables 2** and **3**; (+)HRESIMS *m/z* 639.2400 $[M + Na]^+$ (calcd. for $C_{32}H_{40}O_{12}Na$, 639.2412).

(2R,3R*,4S*)-2,3-Diguaiacyl-4-hydroxyl tetrahydrofuran* (**7**): pale yellow oil; $[\alpha]_D^{20}$: − 5.25 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 281 (3.88), 228 (4.26) nm; IR (KBr) ν_{max} 3494, 3444, 1609, 1517, 1456, 1274, 1233, 1124, 1034, 874, 817 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data see ▶ **Tables 2** and **3**; (−)HRESIMS *m/z* 331.1157 $[M - H]^-$ (calcd. for $C_{18}H_{19}O_6$, 331.1187).

Agonistic activity assay

The agonistic rates of the test compounds were performed according to our previous report [37].

Supporting information

1D and 2D NMR spectra as well as HRESIMS spectra of the seven new compounds, detailed extraction and isolation, and the procedure of the bioassay are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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