Compounds Isolated From the Fruits of *Xanthium strumarium*, Including a New Neo-Lignan, and Their Anticancer Effects

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Abstract
A new neo-lignan, (7′,8′)-4′,5′,9′-trihydroxy-4,6-dimethoxy-5,8′-oxyneolign-7-en-9-yl (1), along with 5 known compounds (2-6), were isolated from the fruits of *Xanthium strumarium*. Their structures were elucidated by extensive spectroscopic methods. All the isolates were evaluated for in vitro cytotoxicities against the human cancer lines HepG2, A549, HCT-116, and SGC-7901. Compounds 1 and 3 showed potent antiproliferative effects against A549 cancer cells with half-maximal inhibitory concentration (IC₅₀) values of 11.2 and 8.3 µM, respectively. In addition, compound 3 exhibited moderate cytotoxicity to SGC-7901 cancer cells, with an IC₅₀ value of 12.9 µM.

Keywords
*Xanthium strumarium*, Compositae, neo-lignan, cytotoxicity, A549

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*Xanthium strumarium* L. (family Compositae), commonly known as “Cang-Er-Zi” in China, is widely distributed in most regions of China. Its fruits are included in the Chinese Pharmacopoeia for treating nasosinusitis, headache caused by cold, pruritus, and rheumatic arthralgia. Recent studies have shown that *X. strumarium* contains several classes of compounds, including lignans, sesquiterpene lactones, phenolic acids, ent-kauranoid glycosides, and flavonoids. Some of these compounds exhibited bioactivity, including antidiabetic, anti-inflammatory, diuretic, anthelmintic, antifungal, and anticancer. As part of our ongoing program toward the discovery of novel bioactive constituents, the ethyl acetate (EtOAc) fraction of *X. strumarium* fruits was investigated; a new neo-lignan, together with 5 known compounds (2-6), were isolated. Herein, we report the isolation, structural elucidation, and cytotoxic activities of these compounds.

Results and Discussion
Compound 1 was obtained as a yellow amorphous powder. The high-resolution electron ionization mass spectrometry (HREIMS) showed a major peak at *m/z* 390.1289 [M]+, ascribable to the molecular formula C₂₀H₂₂O₈ (Supplemental Figure S5). The ¹H nuclear magnetic resonance (NMR) spectrum (Supplemental Figure S1; Table 1) of 1 displayed a characteristic aldehyde signal at δ fourteen 9.62 (1H, d, J = 1.5 Hz, H-6′, 2 olefinic signals at δ fourteen 7.61 (d, J = 16.0 Hz, H-7) and 6.74 (dd, J = 8.0, 16.0 Hz, H-8), an ABX type aromatic ring at δ fourteen 7.01 (1H, d, J = 1.5 Hz, H-6′), 7.07 (1H, d, J = 8.0 Hz, H-3′), 6.77 (1H, d, J = 8.0, 1.5 Hz, H-8′), a tetra-substituted aromatic ring at δ fourteen 7.02 (2H, s, H-1/3), and 2 methoxy groups at δ fourteen 3.89 (6H, s). The ¹³C NMR spectrum (Supplemental Figure S2; Table 1) of 1 showed 22 carbons, including 12 aromatic carbons, an aldehyde carbon at δ fourteen 196.1, 2 olefinic carbons at δ fourteen 155.2 (C-7) and 129.2 (C-8), and 2 methoxy carbons at δ fourteen 57.0. The neo-lignan nature of 1 was supported by a comparison of its NMR spectroscopic data with those of *three*-7E-4′,9′-dihydroxy-3,5,3′-trimethoxy-4,8′-oxyneolign-7-en (compound A). Comparing the NMR data of 1 with those of compound A established that 1 differs from compound A by the absence of a methoxy group in 1. The heteronuclear multiple bond correlations of δ fourteen 3.89 with δ fourteen 154.8 (C-4/6), 107.4 (C-1/3), and of δ fourteen 7.61/6.74 with δ fourteen 131.7 (C-2) established the structure of 1 (Supplemental Figure S3, Figure 1). The relative configuration of H-7′ and H-8′ was determined as trans from the coupling constant *J*₇,₈g = 6.5 Hz. Unfortunately, the nuclear
Overhauser effect spectroscopy spectrum was missing because of some external factors. The absolute configuration was assigned as 7S, 8R from the negative Cotton effect at 229 nm in the circular dichroism spectrum (Supplemental Figure S4) and the study of similar systems. Therefore, compound 1 was assigned as (7′S,8′R)-4′,5′,9′-trihydroxy-4,6-dimethoxy-5,8-oxynelign-7-en-9-ol.

Along with this new neo-lignan, 5 known compounds (2-6) were obtained from the fruits of X. strumarium. The known compounds were identified based on NMR spectroscopic data as 5-O-caffeylshikimic acid (2), (3R,4R,7R)-7-(3,4-methylenedioxybenzyl)-4-(4-hydroxy-3-methoxyphenyl)-1,5- dioxaspiro[2.4]heptene (3), solariciresinol (4), rel-(2α,3β)-7-O-methylcedrusin (5), and rel-(7R,8R,7′R,8′R)-manglisin E (6) (Figure 2).

The isolated compounds 1-6 were evaluated for their anti-proliferative activities in vitro against 4 human cancer cell lines (HepG2, A549, HCT-116, and SGC-7901); doxorubicin was used as the positive control (Table 2). The data suggested that all isolates possess different levels of cytotoxic activities on the 4 cancer cells. Compound 3 showed significant cytotoxic activity against A549 cancer cells, with a half-maximal inhibitory concentration (IC50) value of 8.3 µM. In addition, compound 1 also showed moderate cytotoxic activity against A549 cancer cells with an IC50 value of 11.2 µM.

In conclusion, we obtained 6 compounds, including 1 new neo-lignan, from the fruits of X. strumarium, and determined

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Table 1. Nuclear Magnetic Resonance Spectroscopic Data of Compound 1 in Deuterated Methanol (1H: 500 MHz, 13C: 125 MHz).

| No. | δC | δH  |
|-----|----|-----|
| 1   | 107.4 | 7.02 s |
| 2   | 131.7 |     |
| 3   | 107.4 | 7.02 s |
| 4   | 154.8 |     |
| 5   | 140.5 |     |
| 6   | 154.8 |     |
| 7   | 155.2 | 7.61 d (16.0) |
| 8   | 129.2 | 6.74 dd (8.0, 16.0) |
| 9   | 196.1 | 9.62 d (8.0) |
| 1′  | 133.6 |     |
| 2′  | 121.0 | 6.87 dd (8.0, 1.5) |
| 3′  | 116.0 | 6.77 d (8.0) |
| 4′  | 148.9 |     |
| 5′  | 147.3 |     |
| 6′  | 111.9 | 7.01 d (1.5) |
| 7′  | 74.6  | 5.01 d (6.5) |
| 8′  | 89.0  | 4.24 m |
| 9′  | 62.1  | 3.79 dd (4.5, 12.0) |
| 4/6-OCH3 | 57.0 | 3.89 s |

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Figure 1. Key heteronuclear multiple bond correlations of compound 1.

Figure 2. The structure of compounds 1-6.
Table 2. Cytotoxicities of Compounds 1-6 on Cancer Cell Lines.

| Compounds | IC_{50} µM |
|-----------|-----------|
|           | HepG2 | A549 | HCT-116 | SGC-7901 |
| 1         | 17.8  | 11.2 | 32.5    | 27.2     |
| 2         | 24.2  | 41.8 | >50     | 41.1     |
| 3         | 14.7  | 8.3  | 17.5    | 12.9     |
| 4         | 25.8  | 38.9 | 45.7    | >50      |
| 5         | >50   | 37.6 | 45.3    | >50      |
| 6         | 32.5  | 41.8 | >50     | >50      |
| Doxorubicin\(^a\) | 1.9  | 1.5  | 2.3     | 1.1      |

Abbreviation: IC_{50}, half-maximal inhibitory concentration. IC_{50} values were calculated from regression lines using 5 different concentrations with triple determinations. 

\(^a\)Positive control.

their cytotoxic activities on HepG2, A549, HCT-116, and SGC-7901 cancer cell lines. Among these isolates, compound 3 showed relatively significant cytotoxic activity on A549 cancer cells, and this might provide a basis for treating lung cancer.

**Experimental**

**General**

Optical rotations were determined on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). Ultraviolet (UV) spectra were recorded on a Shimadzu UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Infrared (IR) spectra were obtained on a Shimadzu FT-IR-8400S spectrophotometer (Shimadzu Corporation, Kyoto, Japan), and NMR spectra on a Bruker Avance III 500 MHz spectrometer (Bruker, Karlsruhe, Germany). Mass spectra were obtained on an Applied Biosystems Mariner 5140 spectrometer (Applied Biosystems, Florida, USA), and HPLC was carried out using a Shimadzu LC-6AD instrument with a SPD-20A detector (Shimadzu, Kyoto, Japan) with a YMC-Pack ODS-A (250 × 20 mm, 5 µm) column (YMC Co., Ltd., Kyoto, Japan).

**Plant Material**

The fruits of *X. strumarium* were collected in Yichang, Hubei province, People’s Republic of China, and authenticated by Professor Hua Li (College of Pharmacy, Guangzhou Medical University). A voucher specimen of the plant (No. 20180524) was deposited at the College of Pharmacy, Guangzhou Medical University, Guangzhou, People’s Republic of China.

**Extraction and Isolation**

The dried and powdered fruits of *X. strumarium* (10.0 kg) were extracted with 75% aqueous ethanol (EtOH) to give an extract (1.4 kg). This was suspended in water and partitioned with light petroleum, EtOAc, and n-butanol, respectively. The EtOAc fraction (123.4 g) was fractionated by silica gel column chromatography by eluting with a gradient of dichloromethane (CH2Cl2)-methanol (MeOH) (from 200:1 to 0:1) to afford 12 fractions (Fr.1-Fr.12). Fr.6 (15.6 g) was divided into 8 parts (Fr.6.1-Fr.6.8) on a MCI gel CHP 20P column eluting with a gradient of aqueous MeOH (35:65-100%). Fr.6.3 (3.7 g) was subjected to RP-18 column chromatography, eluting with MeOH-water (H2O) (from 20:80 to 100%) to afford 10 fractions (Fr.6.3.1-Fr.6.3.10). Fr.6.3.4 (700.3 mg) was applied to a C18 reversed-phase HPLC column and eluted with a gradient of 45%-65% MeOH in H2O at a flow rate of 3.0 mL/min over 90 minutes. This resulted in the isolation of compound 1 (2.7 mg, t_R = 38.8 minutes), compound 2 (5.3 mg, t_R = 45.8 minutes), compound 3 (8.8 mg, t_R = 50.3 minutes), compound 3 (3.2 mg, t_R = 58.5 minutes), compound 4 (5.3 mg, t_R = 63.5 minutes), and compound 6 (3.2 mg, t_R = 71.0 minutes)

\(\text{(7′S,8′R)-4′,5′,9′-trihydroxy-4,6-dimethoxy-5,8′-oxyneolignan-7-en-9-yl (1). A yellow amorphous powder; } [\alpha]_{\text{D}}^{25} +7.3 \ (c 0.1, \text{MeOH); UV (MeOH) } \lambda_{\text{max}} \text{ (log } \varepsilon) : 289 (3.31), 218 (3.53) \text{ nm; IR (potassium bromide disc) } \nu_{\text{max}} \text{ 3487, 2916, 2824, 1615, 1602, 1517, 1459 cm}^{-1}; ^1H (500 MHz) \text{ and } ^13C \text{ NMR (125 MHz) spectral data in deuterated methanol, see Table 1; } \text{HREIMS: } m/z \ 390.1289 \text{ [M]^+ (calked for } C_{20}H_{22}O_{8}, 390.1351).} \)

**Cytotoxicity Assay**

The cytotoxic activities of compounds 1-6 were evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded in 96-well plates (5 × 10^4 cells/well) and allowed to adhere for 24 hours at 37 °C. Compounds 1-6 and the positive control were dissolved in dimethyl sulfoxide (DMSO) and diluted with the medium to the different tested concentrations (100 µM, 50 µM, 25 µM, 12.5 µM, 6.25µM, 3.125µM). Then, cells were treated with the test compounds and the positive control. After 48 hours, 20 µL of MTT solution (5 mg/mL, Solarbio, Beijing, China) was added to each well. After 5 hours, the produced formazan crystals were solubilized with DMSO, and the absorbance of each well was measured at 570 nm with a microplate reader (Thermo Fisher Scientific Inc.).

**Statement of Human and Animal Rights**

All of the experimental procedures involving animals were conducted in accordance with the Institutional Animal Care guidelines of First People’s Hospital of Foshan, China, and approved by the Administration Committee of Experimental Animal, Guangdong Province, China.

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