Arylsulfonamides From the Roots and Rhizomes of *Tupistra chinensis* Baker

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Abstract

One new arylsulfonamide (1) and a novel natural product (2) were isolated from the roots and rhizomes of *Tupistra chinensis* Baker. Their structures were characterized by physicochemical properties and spectroscopic methods, as 2-[2-[[1,1′-biphenyl]-4-ylsulfonylamino]-benzoylamino]-benzoic acid methyl ester (1), 2-[[1,1′-biphenyl]-4-ylsulfonylamino]-benzoic acid (2), respectively. Additionally, the cytotoxic activity of 1-2 was evaluated on human HCT116, HT29, A549, and H1299 tumor cell lines in vitro, respectively. Whereas, the result showed that these compounds displayed weak cytotoxicity in the human cancer cell lines.

Keywords

Arylsulfonamide, *Tupistra chinensis* Baker, Cytotoxicity

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*Tupistra chinensis* Baker, a herbaceous perennial plant, is mostly distributed in China and India. It is a species in the *Tupistra* genus of Liliaceae family, which is medicinally known as “Kai-Kou-Jian” in the Qinba Mountains of Shaanxi Province in China,¹ and the roots and rhizomes are commonly used as one of the most important ingredients in many medical prescriptions for the treatment of throat irritation, rheumatic diseases, and snake-bite.² According to the results of modern pharmacological experiments, the extracts of this species possessed significant antitumor activity.³,⁴

Previous investigations of bioactive constituents from the roots and rhizomes of *T. chinensis* by our research group had the presence of steroidal saponins.⁵-⁸ In order to explore more bioactive lead compounds from the medicinal herbs in the Qinba mountains of China,⁹,¹² the chemical constituents and pharmacological studies of *T. chinensis* were studied, and 1 new arylsulfonamide 2-[2-[[1,1′-biphenyl]-4-ylsulfonylamino]-benzoylamino]-benzoic acid methyl ester (1) (Figure 1) and an

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aryl sulfonamide 2-[[1,1'-biphenyl]-4-ylsulfonyl]amino]-benzoic acid (2) firstly reported in plants. Natural products that contain sulfonamide groups in their structure were known, but they were rare. Sulfonamides acted on a range of molecular targets, including the \( \alpha \)-carbonic anhydrase, which was a valid target for the development of novel antiglaucoma, antitumor, antibesity, or anticonvulsant drugs. In this article, we reported the isolation and structure elucidation of 2 sulfonamides and their cytotoxic evaluation against HCT116, HT29, A549, and H1299 tumor cells.

Compound 1 was obtained as a white amorphous powder and soluble in chloroform. Its molecular formula was determined as C\(_{27}\)H\(_{22}\)N\(_2\)O\(_5\)S from the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) (Supplemental Figure S2) at \( m/z \) 485.1192 [M − H]\(^-\) (calcd for C\(_{27}\)H\(_{21}\)N\(_2\)O\(_5\)S, 485.1171). In the 1H- nuclear magnetic resonance (NMR) spectrum (Supplemental Figure S3), 1 methyl proton signal was observed at \( \delta_H \) 3.75 (3H, s, Me-23), 2 single peaks with nitrogenous proton signals at 11.7 (1H, s, N- H- b) and 10.46 (1H, s, N- H- a) in the low field region. In the aromatic region, 9 protons at \( \delta_H \) 7.34 (1H, m, H-1), \( \delta_H \) 7.33 (1H, m, H-2), \( \delta_H \) 7.31 (1H, m, H-3), \( \delta_H \) 7.31 (1H, m, H-3'), \( \delta_H \) 7.31 (1H, m, H-4), \( \delta_H \) 7.30 (1H, m, H-4'), \( \delta_H \) 7.45 (1H, d, \( J = 1.8 \) Hz, H-6), \( \delta_H \) 7.43 (1H, d, \( J = 1.8 \) Hz, H-6'), \( \delta_H \) 7.45 (1H, d, \( J = 1.8 \) Hz, H-7), and \( \delta_H \) 7.43 (1H, d, \( J = 1.8 \) Hz, H-7') suggesting the presence of a biphenylyl group, along with 8 aromatic protons at \( \delta_H \) 7.77 (1H, dd, \( J = 1.0 \), \( J = 8.3 \) Hz, H-10), \( \delta_H \) 7.51 (1H, m, H-11), \( \delta_H \) 7.22 (1H, m, H-12), \( \delta_H \) 7.30 (1H, dd, \( J = 1.0 \) Hz, \( J = 8.3 \) Hz, H-13), \( \delta_H \) 8.73 (1H, dd, \( J = 1.6 \) Hz, \( J = 8.0 \) Hz, H-17), \( \delta_H \) 7.61 (1H, m, H-18), \( \delta_H \) 7.14 (1H, m, H-19), \( \delta_H \) 7.95 (1H, dd, \( J = 1.6 \), \( J = 8.0 \) Hz, H-20) assigned to 2 bis-substituted benzene rings. The \( ^{13}\)C-NMR (Supplemental Figure S4) and distortionless enhancement by polarization transfer spectrum (Table 1) of 1 displayed 27 carbon resonances, among them, 12 carbon resonances at \( \delta_C \), 128.6 (C-1), 129.0 (C-2, C-2'), 127.2 (C-3, C-3'), 139.0 (C-4), 145.4 (C-5), 127.4 (C-6, C-6'), 128.0 (C-7, C-7'), and 137.7 (C-8) were attributed to a biphenyl group. In the aromatic region, 2 benzene rings at \( \delta_C \); 138.9 (C-9), 123.8 (C-10), 133.1 (C-11), 125.1 (C-12), 127.3 (C-13), 124.4 (C-14), and 141.2 (C-16), 120.5 (C-17), 135.1 (C-18), 123.5 (C-19), 131.3 (C-20), 115.4 (C-21). The above analysis was finally verified by the 2-dimensional (2D) NMR data. The heteronuclear multiple-quantum coherence (Supplemental Figure S5) experiment allowed for the assignments of the proton and protonated carbon resonances in the NMR spectra of 1. The heteronuclear multiple bond correlation (HMBC) (Supplemental Figure S6) of H-1/ C-2 and C-3, H-3/C-1 and C-5, H-6/C-4, C-7 and C-8, H-11/C-9 and C-13, H-12/C-10 and C-14, H-13/C-9, C-11 and C-15, H-17/C-16, C-19 and C-21, H-20/C-16, C-18 and

Table 1. \( ^1\)H-NMR (400 MHz, CDCl\(_3\)) and \( ^{13}\)C-NMR (100 MHz, CDCl\(_3\)) Data of Compound 1.

| Position | \( \delta_H \) | \( \delta_C \) |
|----------|----------------|----------------|
| 1        | 7.34 (m)       | 128.6          |
| 2        | 7.33 (m)       | 129.0          |
| 2'       | 7.33 (m)       | 129.0          |
| 3        | 7.31 (m)       | 127.2          |
| 3'       | 7.31 (m)       | 127.2          |
| 4        |                | 139.0          |
| 5        |                | 145.4          |
| 6        | 7.45 (br s)    | 127.4          |
| 6'       | 7.43 (br s)    | 127.4          |
| 7        | 7.72 (br s)    | 128.0          |
| 7'       | 7.74 (br s)    | 128.0          |
| 8        |                | 137.7          |
| 9        |                | 138.9          |
| 10       | 7.77 (1H, dd, \( J = 1.0, J = 8.3 \)) | 123.8          |
| 11       | 7.51 (m)       | 133.1          |
| 12       | 7.22 (m)       | 125.1          |
| 13       | 7.30 (1H, dd, \( J = 1.0, J = 8.3 \)) | 127.3          |
| 14       |                | 124.4          |
| 15       |                | 167.2          |
| 16       |                | 141.2          |
| 17       | 8.73 (1H, dd, \( J = 1.6, J = 8.0 \)) | 120.5          |
| 18       | 7.61 (m)       | 135.1          |
| 19       | 7.14 (m)       | 123.5          |
| 20       | 7.95 (1H, dd, \( J = 1.6, J = 8.0 \)) | 131.3          |
| 21       |                | 115.4          |
| 22       |                | 169.0          |
| 23       | 3.75 (br s)    | 52.6           |

CDCl\(_3\), deuterated chloroform; NMR, nuclear magnetic resonance.
C-22, N-H-a/C-10. Among them, 2 carbonyl carbon signals at $\delta_C 167.2$ (C-15) and 169.0 (C-22) showed correlations with proton signals of benzene moiety at $\delta_H 7.30$ (H-13) and 7.95 (H-20), respectively. These results reveal that the 2 carbonyl carbons, respectively, connected with benzene rings. 1H-1H correlation spectroscopy (COSY) correlations (Supplemental Figure S7) of H-1/H-2/H-3, H-6/H-7, H-6′/H-7′, H-10/H-11/H-12/H-13, H-17/H-18/H-19/H-20; comparison with a known compound of NMR and HR-ESI-MS data of [2-(naphthalene-2-sulfonylamino)-benzoylamino]benzoic acid methyl ester, showed that compound 1 had a similar structure, except the naphthalene group became the biphenyl group. In addition, the main fragmentation peaks of these 2 compounds were analyzed by HR-ESI-MS, and the following description indicates that the compound contains a sulfonamide bond. After correlation of all the protons with their directly bonded carbon partners via a heteronuclear single quantum coherence spectrum, it was possible from the HMBC and 1H-1H COSY spectrum (Figure 2) to deduce the planar structure of 1. Through a systematic literature search, compound 1 was found to be a new compound, and its structure was identified as 2-[2-[(1,1′-biphenyl)-4-sulfonyl]amino]-benzoylaminobenzoic acid methyl ester.

Compound 2 was obtained as a white amorphous powder and soluble in chloroform. Its molecular formula was determined as C$_{19}$H$_{15}$NO$_4$S. When comparing the 1H-NMR and 13C-NMR data of 2 with the reported compound, 4-[[1,1′-biphenyl]-4-sulfonyl]amino]-benzoylaminobenzoic acid, they showed almost the identical NMR features excepted for the location of the carboxyl. The only difference was that the para-position carboxyl unit became the ortho-position carboxyl unit. The structure of 2 was finally confirmed by the 2D-NMR as 2-[[1,1′-biphenyl]-4-sulfonyl]amino]-benzoic acid. Compound 2 is a novel natural product and the first time to report the spectrum data.

The compounds 1-2 were evaluated for their cytotoxic activity against HCT116, HT29, A549, and H1299 human cancer cell lines (Table 2). The compound 1 exhibited weak cytotoxicity against HT29 cell lines, with a half-maximal inhibitory concentration (IC$_{50}$) value of 89.3 µM. Compound 2 showed cytotoxicity against human cancer cell lines, HCT116 and H1299, with IC$_{50}$ values of 87.3 and 79.8 µM, respectively.

### Experimental

#### General

ESI-MS was performed on Waters Quattro Premier instrument. The HR-ESI-MS spectra were taken on an Agilent Technologies 6550 Q-TOF. D and 2D NMR spectra were recorded on a Bruker-AVANCE 400 instrument with TMS as an internal standard. The analytical high-performance liquid chromatography (HPLC) was performed on a Waters 2695 Separations Module coupled with a 2996 Photodiode Array Detector and an Accurasil C18 column (4.6 mm × 250 mm, 5 mm particles, Ameritech, America). Semipreparative HPLC was performed on a system comprising an LC-6AD pump equipped with an SPD-20A UV detector and an Ultimate XB-C18 (10 mm × 250 mm, 5 mm particles). D101 was from Sunresin New Materials Co., Ltd. (Xi’an, China). Silica gel was purchased from Qingdao Haiyang Chemical Group Corporation (Qingdao, China).

#### Plant Material

The roots and rhizomes of T. chinensis Baker were collected from Taibai region of Qinba mountains in Shaanxi Province in September 2018 and identified by Senior experimentalist Jitao Wang. A voucher specimen (herbarium no. 20180916) has been deposited in the Medicinal Plants Herbarium (MPH), Shaanxi University of Chinese Medicine, Xianyang, China.

#### Extraction and Isolation

The air-dried and powdered underground parts of T. chinensis (1.5 kg) were extracted with 65% ethanol (EtOH) 3 times at 80°C. The combined EtOH extracts were evaporated to 5 L.

| Compounds | Cell lines |
|-----------|------------|
|           | HCT116     | HT29       | A549       | H1299      |        |
| b5-Fu     | 2.4 ± 1.9  | 4.3 ± 2.1  | 4.0 ± 1.6  | 3.07 ± 0.52|        |
| 1         | >100       | 89.3 ± 3.1 | >100       | >100       |        |
| 2         | 87.3 ± 2.6 | >100       | >100       | 79.8 ± 2.4 |        |

Table 2. Cytotoxicity of Compounds 1-2 Against 4 Human Cancer Cell Lines.
and applied to a resin D101 column, eluting with water (H₂O), 20% EtOH, 60% EtOH, and 95% EtOH to give 4 fractions (Fr.1-Fr.4). Fr.3 (45 g) was separated by silica gel column chromatography (CC), eluting with a gradient solvent system (chloroform [CHCl₃]-methanol [MeOH], 100:0-50:50) to yield 4 fractions (Fr.3-1-Fr.3-4). Fr.3-2 (20.3 g) was recrystallized with CHCl₃-MeOH (1:1) to obtain compound 1 (30 mg). Fr.3-2-3 (8.3 g) was chromatographed repeatedly on silica gel elution with CHCl₃-MeOH (100:0-70:30) to afford 2 fractions (Fr.3-2-3-1 and Fr.3-2-3-2). Fr.3-2-3-1 (230 mg) was purified by HPLC (flow rate: 1.5 mL/min) with MeOH-H₂O (28:72) as the mobile phase, yielding compound 2 (10.6 mg; retention time [tᵣ] = 41.5 minutes).

Cytotoxicity Assay

The cytotoxic activity assay toward the HCT116, HT29, A549, and H1299 tumor cell lines were measured by the MTT method in vitro, using 5-fluorouracil as a positive control. Briefly, 1 × 10⁴ mL cells were seeded into 96-well plates and allowed to adhere for 24 hours. Compounds 1-2 were dissolved in dimethyl sulfoxide (DMSO) and diluted with complete medium to 6 degrees of concentration (from 0.001 to 0.4 mmol/L) for inhibition rate determination. After incubation at 37°C for 4 hours, the supernatant fraction was removed before adding DMSO (100 μL) to each well. The inhibition rate and IC₅₀ were calculated (Table 2). Values are mean ± standard deviation, n = 3.

2-((1,1′-Bisphenyl)-4-Ylsulfonfylamino)-Benzoylaminobenzoic Acid Methyl Ester (1)

White amorphous powder.

IR (potassium bromide [KBr]) νₘₐₓ: 3440, 2926, 2857, 1725, 1644, 1450, 1271, 1044, 845, 765 cm⁻¹.

H₁-NMR and ¹³C-NMR: Table 1.

HR-ESI-MS m/z 485.1192 [M − H] - (calcd. for C₂₇H₂₁N₂O₅S, 485.1171).

2-((1,1′-Bisphenyl)-4-Ylsulfonfylamino)-Benzoylaminobenzoic Acid (2)

White amorphous powder.

IR (KBr) νₘₐₓ: 3438, 2932, 2844, 1716, 1622, 1455, 1298, 1277, 1157 cm⁻¹.

H₁-NMR (CDCl₃, 100 MHz): δH 7.38 (1H, m, H-10), δH 7.33 (1H, m, H-11), δH 7.59 (1H, m, H-13), δH 7.38 (1H, m, H-13), δH 7.33 (1H, m, H-12), δH 7.42 (1H, m, H-2), δH 7.41 (1H, m, H-1), δH 7.57 (1H, m, H-3), δH 7.60 (1H, m, H-3), δH 7.74 (1H, m, H-6), δH 7.73 (1H, m, H-6), δH 8.11 (1H, t, J = 1.9 Hz, H-7), δH 8.09 (1H, t, J = 1.8 Hz, H-7), δH 7.18 (1H, d, J = 3.0 Hz, H-10), δH 7.53 (1H, m, H-11), δH 7.33 (1H, m, H-12), δH 7.38 (1H, m, H-13). ¹³C-NMR (CDCl₃, 100 MHz): δC 129.1 (C-1), δC 129.3 (C-2), δC 129.3 (C-3), δC 127.7 (C-3′), δC 127.7 (C-3′), δC 139.4 (C-4), δC 147.5 (C-5), δC 127.9 (C-6′), δC 130.4 (C-7), δC 136.3 (C-8), δC 134.5 (C-9), δC 129.4 (C-10), δC 132.2 (C-11), δC 130.8 (C-12), δC 128.9 (C-13), δC 133.7 (C-14), δC 165.7 (C-15).

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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