Eight new furan derivatives, irpexins A‒H (1‒8), two new polyketides, irpexins I and J (9 and 10), together with nine known compounds were isolated from the fermentation of *Irpex lacteus*. The structures and absolute configurations were elucidated on the basis of extensive spectroscopic methods and Mosher ester reaction. All compounds show no cytotoxicity to human MCF-7 and Hela cancer cell lines at the concentration of 10 μM.

**Keywords** *Irpex lacteus* · Furan derivatives · Polyketides

**1 Introduction**

Fungi are important sources of natural products for the discovery of drugs and agricultural chemicals [1‒5], which have attracted great attention from pharmaceutical chemists and pharmacologists. *Irpex lacteus* is a pathogenic wood-decaying fungus belonging to the family Polyporaceae [6]. Its crude polysaccharide fraction has long been used as a traditional Chinese medicine for the treatment of chronic glomerulonephritis in clinic [7]. Chemists have researched the fermentation products of this fungus and the chemical constituents of fruiting bodies. In 1981, Hayashi et al. isolated three new nematicidal metabolites including two furan derivatives from their lactose liquid medium [8]. Our previous chemical investigations on the liquid fermentation of this fungus reported new carbon skeletons irlactins A–D that featured a 6/6 backbone [9], as well as a series of tremulane sesquiterpenoids [10, 11]. Later, studies on the fruiting bodies reported a series of triterpenes, irpekmins A–E, which inhibit the production of NO by RAW264.7 macrophages induced by LPS, and a new skeletal triterpenoid irpexolidal with a 6/5/6/5/6/5-fused polycyclic skeletal system [12, 13]. Recently, Duan et al. isolated four new bioactive metabolites from the endophytic fungus *Irpex lacteus* DR10-1, including one tremulane sesquiterpenoid irpexlacte A and three new furan derivatives irpexlactes B‒D [14]. In the current study, eight new furan derivatives, irpexins A‒H (1‒8), two new polyketides, irpexins I and J (9 and 10), together with nine known compounds were obtained from cultures of *I. lacteus*.
in rice medium. Their structures have been established by extensive spectroscopic methods, as well as modified Mosher’s method. All new compounds were evaluated for their cytotoxic activities against two human MCF-7 and Hela cell lines.

2 Results and Discussion

Compound 1 was isolated as a colorless oil. Its molecular formula C10H14O3 was determined on the basis of HR-ESI–MS, corresponding to four degrees of unsaturation. In the 1H NMR spectrum (Table 1), one methyl group at δH 0.96 (3H, t, J = 7.5 Hz, H-1), one aldehyde proton at δH 9.50 (1H, s, H-1), and two olefinic protons at δH 7.19 (1H, d, J = 3.5 Hz, H-3) and 6.28 (1H, d, J = 3.5 Hz, H-4) were readily identified. The 13C NMR and DEPT data (Table 2) revealed ten carbon resonances ascribable for one CH3, three CH2, four CH, and two C. Of them, signals at δC 151.8 (s, C-2), 124.1 (d, C-3), 108.9 (d, C-4), 163.8 (s, C-5) were suggested to establish a furan moiety conjugated to an aldehyde group at δC 177.0 (d, C-1). These data were very similar to these of irpexlacte C [14].

The main difference was that the position of the hydroxyl group at C-7 in irpexlacte C was moved to C-8 in 1. The δC 151.8 (s, C-2), 124.1 (d, C-3), 108.9 (d, C-4), 163.8 (s, C-5) were suggested to establish a furan moiety conjugated to an aldehyde group at δC 177.0 (d, C-1). These data were very similar to these of irpexlacte C [14].

Therefore, a planar structure of 1 was figured out as shown in Fig. 1. The absolute configuration of 1 was determined by the Mosher reaction. Compound 1 reacted with R-Mosher reagent and S-Mosher reagent to obtain compounds 1a and 1b (for their 1H NMR spectra, see Figs. S8 and S9 in the Supporting Information), respectively. In the 1H NMR spectrum, the chemical shifts of δH 2.76–2.71 (2H, m, H-6) and δH 2.07–2.03 (2H, m, H-7) in 1a were higher than δH 2.63–2.56 (2H, m, H-6) and δH 2.02–1.96 (2H, m, H-7) in 1b, while the chemical shifts of δH 1.67–1.65 (2H, m, H-9) and δH 0.85–0.83 (3H, m, Me-10) were lower than δH 1.74–1.73 (2H, m, H-9) and δH 0.95–0.92 (3H, m, Me-10) in 1b. Due to the shielding effect of phenyl, this result suggested that –C9–C10– in 1a was on the same side as phenyl in R-Mosher, and –C9–C10– in 1b is not on the same side as phenyl in S-Mosher corresponding to A-type and B-type in Fig. 2, respectively. At this point, the absolute configuration of C-8 was determined as R. Compound 1 was, therefore, established as irpexin A, as depicted.

Compound 2 was isolated as a colorless oil. Its molecular formula C10H12O3 was determined on the basis of HR-ESI–MS, corresponding to five degrees of unsaturation. The 13C NMR and DEPT data of 2 were very similar to those of irpexlacte C [14], except for one double bond in 2. In 1H–1H COSY spectrum, the correlation of δH 6.84 (1H, m, H-9) with δH 2.21 (1H, m, H-8) and δH 5.06 (2H, m, H-10) revealed a double bond was formed between C-9 and C-10 in 2. The optical rotation of 2 ([α]S = –38.0), contrary to that of 1 ([α]R = +44.8) and those analogues reported in the literature [15], indicated that the absolute configuration of the C-6 in the side chain was S. Compound 2 was, therefore, established as irpexin B, as depicted.

Compound 3 was isolated as a colorless oil. Its molecular formula C10H19O3 was determined on the basis of HR-ESI–MS, corresponding to three degrees of unsaturation. The 13C NMR and DEPT data (Table 2) of 3 were very similar to these of irpexlacte C [14], except that the aldehyde unit was replaced by a hydroxymethyl (δC 57.5) in 3, as supported by the HMBC correlations from δH 4.59 (1H, s, H-1) to δC 153.3 (s, C-2) and 108.4 (d, C-3). The optical rotation of 3 ([α]S = –16.1) suggested C-6 to be S form. Compound 3 was, therefore, established as irpexin C, as depicted.

Compound 4 was isolated as a colorless oil. Its molecular formula C12H20O4 was determined on the basis of HR-ESI–MS, corresponding to three degrees of unsaturation. The 13C NMR and DEPT data of 4 were very similar to those of 1 (Table 2), except that the aldehyde unit in 1 was replaced by a dimethoxymethyl group as supported by the HMBC correlations from δH 5.38 (1H, s, H-1) to δC 149.0 (s, C-2), 109.1 (d, C-3) and two OCH3 (δC 52.8). The optical rotation of 4 ([α]S = +37.5), related to that of 1, revealed the absolute configuration of C-8 to be R. Compound 4 was, therefore, established as irpexin D, as depicted.

Compound 5 was isolated as a colorless oil. Its molecular formula C12H18O3 was determined on the basis of HR-ESI–MS, corresponding to three degrees of unsaturation. The 13C NMR and DEPT data of 5 were very similar to those of 1 (Table 2), except for two additional carbon resonances linked to a carbonyl carbon forming a propionyl group, replacing the formyl group in 1. It was supported by the 1H–1H COSY correlation of δH 1.20 (3H, t, J = 7.4 Hz, Me-1) with δH 2.80 (2H, m, H-2), together with the HMBC correlations from δH 7.11 (1H, d, H-5), H-1, and H-2 to δC 189.7 (s, C-3) and 151.2 (s, C-4). The optical rotation data ([α]S = +14.6) indicated C-10 to be R form. Compound 5 was, therefore, established as irpexin E, as depicted.

Compound 6 was isolated as a colorless oil. Its molecular formula C10H16O3 was determined on the basis of HR-ESI–MS data. The 1H and 13C NMR spectra were very close to those of 5 (Tables 1 and 2), except for the hydroxyl group at C-10 (δC 72.3) in 5 moved to the C-11 (δC 67.7) in 6, as verified by the 1H–1H COSY correlation of δH 3.84 (1H, m, H-11) with δH 1.51 (2H, m, H-10) and δH 1.21 (3H, d, J = 6.2 Hz, Me-12). The calculated optical rotation data ([α]S = –10.7) matched well with the experimental data.
Table 1 \(^1\)H NMR (600 MHz) Data of 1–10 (\(\delta\) in ppm and \(J\) in Hz)

| No | 1 \(^a\) | 2 \(^a\) | 3 \(^a\) | 4 \(^a\) | 5 \(^a\) | 6 \(^a\) | 7 \(^a\) | 8 \(^b\) | 9 \(^a\) | 10 \(^a\) |
|----|---------|---------|---------|---------|--------|--------|--------|--------|--------|--------|
| 1  | 9.50, s | 9.59, s | 4.59, s | 5.38, s | 1.20, t (7.4) | 1.20, t (7.5) | 1.47, d (7.0) | 1.20, d (6.1) | 0.98, t (7.6) |
| 2  | 2.80, m | 2.80, q (7.5) | 4.83, q (7.0) | 3.96, m | 6.22, d (5.7) | 2.21, q (7.6) |
| 3  | 7.19, d (3.5) | 7.22, d (3.5) | 6.24, d (3.1) | 6.31, d (3.1) | 7.11, d (3.5) | 7.10, d (3.5) | 7.23, d (3.5) | 6.25, d (3.1) | 2.69, m |
| 4  | 6.28, d (3.5) | 6.49, d (3.5) | 6.18, d (3.1) | 5.98, d (3.1) | 6.18, d (3.5) | 6.17, d (3.5) | 6.25, d (3.5) | 6.01, d (3.1) | 2.27, m |
| 5  | 7.11, d (3.5) | 7.10, d (3.5) | 6.25, d (3.1) | 2.69, m | 2.55, m | 2.37, m |
| 6a | 2.93, m | 4.82, dd (8.1, 5.1) | 4.65, t (6.9) | 2.80, m 2.71, m | 6.18, d (3.5) | 6.17, d (3.5) | 6.25, d (3.5) | 6.01, d (3.1) | 2.27, m |
| 6b | 2.83, m | 1.91, m 1.77, m | 1.98, m | 1.86, m | 1.84, m 1.71, m |
| 7a | 1.91, m 1.77, m | 1.98, m | 1.86, m | 1.84, m 1.71, m |
| 7b | 1.91, m 1.77, m | 1.98, m | 1.86, m | 1.84, m 1.71, m |
| 8a | 3.56, m | 2.21, m | 1.43, m 1.31, m | 3.55, m | 2.90, m 2.81, m | 2.73, t (7.6) | 3.03, t (7.3) | 2.62, t (7.3) | 2.44, m |
| 8b | 2.81, m | 1.36, m | 1.53, m 1.47, m | 1.89, m 1.77, m | 1.83, m 1.74, m | 2.84, t (7.3) | 1.86, m | 1.06, t (7.3) | 1.72, m 1.63, m |
| 9a | 1.51, m | 6.84, m | 1.36, m | 1.53, m 1.47, m | 1.89, m 1.77, m | 1.83, m 1.74, m | 2.84, t (7.3) | 1.86, m | 1.06, t (7.3) |
| 9b | 1.72, m 1.63, m | 1.51, m | 1.51, m | 1.36, m | 1.89, m 1.77, m | 1.83, m 1.74, m | 2.84, t (7.3) | 1.86, m | 1.06, t (7.3) | 1.72, m 1.63, m |
| 10 | 0.96, t (7.5) | 5.06, m | 0.91, t (7.3) | 0.95, t (7.5) | 3.57, m | 1.51, m | 2.52, t (7.2) | 3.56, m |
| 11a | 1.53, m 1.49, m | 3.84, m | 2.46, q (7.3) | 1.21, d (6.2) | 1.08, t (7.3) | 2.10, s | 3.23, s | 3.20, s |
| 11b | 1.55, m 1.50, m | 0.96, t (7.4) | 1.21, d (6.2) | 1.08, t (7.3) | 2.10, s | 3.23, s | 3.20, s |
| OCH\(_3\) | 3.35, s | 3.35, s |
| OCH\(_3\) | 3.35, s |

\(^a\)Measured in CDCl\(_3\)

\(^b\)Measured in methanol-d\(_4\)
(\([\alpha]\) = −14.3) for 6 suggested C-10 to be R form (see Supporting Information). Compound 6 was, therefore, established as irpexin I, as depicted.

Compound 7 was isolated as a colorless oil. Its molecular formula C_{12}H_{16}O_{4} was determined on the basis of the positive high resolution (HR) ESI-MS at m/z 225.11211 [M + H]^{+} (calcd for C_{12}H_{17}O_{4}^{+}, 225.11214), corresponding to five degrees of unsaturation. The $^{13}$C and DEPT spectra displayed 12 carbon resonances which were closely related to that of 5 (Table 2), except that the signals at $\delta_C$ 31.3 (t, C-2) and $\delta_C$ 72.3 (d, C-10) in 5 were replaced by signals of $\delta_C$ 69.3 (d, C-2) and $\delta_C$ 209.0 (s, C-10) in 7, respectively. The $^1$H-$^1$H COSY correlation of $\delta_H$ 5.47 (3H, d, J = 7.0 Hz, Me-1) with $\delta_H$ 4.83 (1H, q, J = 7.0 Hz, H-2) suggested that C-2 in 5 was substituted by a hydroxyl group. In addition, the $^1$H-$^1$H COSY correlation of $\delta_H$ 3.03 (2H, t, J = 7.3 Hz, H-8) with $\delta_H$ 2.84 (2H, t, J = 7.3 Hz, H-9), and $\delta_H$ 2.46 (2H, q, J = 7.3 Hz, H-11) with $\delta_H$ 1.08 (3H, t, J = 7.3 Hz, Me-12), as well as the HMBC correlation from H-12 to C-10 and $\delta_C$ 35.9 (t, C-11) suggested that C-10 in 5 was substituted by a ketone group. The optical rotation (\([\alpha]\) = −14.0), comparing with those analogues reported in the literature [16],

| No | 1$^a$ | 2$^a$ | 3$^a$ | 4$^a$ | 5$^a$ | 6$^a$ | 7$^a$ | 8$^a$ | 9$^a$ | 10$^a$ |
|----|------|------|------|------|------|------|------|------|------|-------|
| 1  | 177.0, d | 177.5, d | 57.5, t | 98.0, d | 8.4, q | 8.4, q | 22.3, q | 17.9, q | 169.7, s | 13.3, q |
| 2  | 151.8, s | 152.0, s | 153.3, s | 149.0, s | 31.3, t | 31.3, t | 69.3, d | 81.4, d | 124.7, d | 16.3, t |
| 3  | 124.1, d | 123.1, d | 108.4, d | 109.1, d | 189.7, s | 189.7, s | 190.0, s | 68.2, d | 153.6, d | 142.0, s |
| 4  | 108.9, d | 108.6, d | 106.5, d | 105.4, d | 151.2, s | 151.2, s | 148.6, s | 150.5, s | 110.4, s | 210.0, s |
| 5  | 163.8, s | 163.4, s | 157.0, s | 156.1, s | 118.6, d | 118.5, d | 120.9, d | 109.8, d | 35.7, t | 34.3, t |
| 6  | 24.7, t | 67.4, d | 67.8, d | 24.3, t | 108.1, d | 108.0, d | 109.1, d | 105.5, d | 30.9, t | 29.0, t |
| 7  | 34.5, t | 34.6, t | 55.1, t | 35.0, t | 161.4, s | 161.3, s | 161.2, s | 155.3, s | 210.0, s | 173.2, s |
| 8  | 72.2, d | 29.4, t | 27.7, t | 72.5, d | 24.7, t | 28.2, t | 22.4, t | 26.7, t | 36.0, t | 27.3, t |
| 9  | 30.3, t | 137.3, s | 22.4, t | 30.2, t | 34.7, t | 23.9, t | 39.5, t | 21.8, t | 7.8, q | 34.5, t |
| 10 | 9.9, q | 115.7, t | 14.0, q | 9.9, q | 72.3, d | 38.5, t | 209.0, s | 41.8, t | 72.9, d | 30.3, t |
| 11 | 30.3, t | 67.7, d | 35.9, t | 210.0, s | 30.3, t |
| 12 | 9.9, q | 23.6, q | 7.7, q | 28.5, q | 9.8, q |
| OCH$_3$ | 52.8, q |
| OCH$_3$ | 52.8, q |

$^a$ Measured in CDCl$_3$

$^b$ Measured in methanol-$d_4$

Fig. 1 Chemical structures of compounds 1–19

([\alpha] = −14.3) for 6 suggested C-10 to be R form (see Supporting Information). Compound 6 was, therefore, established as irpexin I, as depicted.

Compound 7 was isolated as a colorless oil. Its molecular formula C$_{12}$H$_{16}$O$_{4}$ was determined on the basis of the positive high resolution (HR) ESI-MS at m/z 225.11211 [M + H]$^+$ (calcd for C$_{12}$H$_{17}$O$_{4}^+$, 225.11214), corresponding to five degrees of unsaturation. The $^{13}$C and DEPT spectra displayed 12 carbon resonances which were closely related to that of 5 (Table 2), except that the signals at $\delta_C$ 31.3 (t, C-2) and $\delta_C$ 72.3 (d, C-10) in 5 were replaced by signals of $\delta_C$ 69.3 (d, C-2) and $\delta_C$ 209.0 (s, C-10) in 7, respectively. The $^1$H-$^1$H COSY correlation of $\delta_H$ 1.47 (3H, d, J = 7.0 Hz, Me-1) with $\delta_H$ 4.83 (1H, q, J = 7.0 Hz, H-2) suggested that C-2 in 5 was substituted by a hydroxyl group. In addition, the $^1$H-$^1$H COSY correlation of $\delta_H$ 3.03 (2H, t, J = 7.3 Hz, H-8) with $\delta_H$ 2.84 (2H, t, J = 7.3 Hz, H-9), and $\delta_H$ 2.46 (2H, q, J = 7.3 Hz, H-11) with $\delta_H$ 1.08 (3H, t, J = 7.3 Hz, Me-12), as well as the HMBC correlation from H-12 to C-10 and $\delta_C$ 35.9 (t, C-11) suggested that C-10 in 5 was substituted by a ketone group. The optical rotation ([\alpha] = −14.0), comparing with those analogues reported in the literature [16],
indicated C-2 to be S form. Therefore, the structure of 7 was established as irpexin G, as shown.

Compound 8 was isolated as a colorless oil. Its molecular formula C_{12}H_{14}O_{4} was determined on the basis of HR-ESI-MS. The \(^{13}\)C NMR and DEPT data of 8 were very similar to those of 7 (Table 2), except that the hydroxy at C-2 was replaced by a methoxy in 8, as supported by a HMBC correlation from δ\(_{H} 3.23\) (3H, s, OCH\(_{3}\)) to δ\(_{C} 81.4\) (d, C-2). Detailed analysis of 2D NMR data suggested that other parts of 8 were the same to those of 7. Therefore, compound 8 was established as irpexin K, as depicted.

Compound 9 was isolated as a colorless oil. Its molecular formula C_{10}H_{14}O_{4} was determined on the basis of HR-ESI-MS at m/z 219.15361 [M + H\(^{+}\)] (calcd for C_{10}H_{14}O_{4}Na\(^{+}\), 221.07843), corresponding to four degrees of unsaturation. In the \(^1\)H NMR spectrum (Table 1), two methyl group at δ\(_{H} 0.98\) (t, J = 7.6 Hz, Me-9) and from δ\(_{H} 2.52\) (1H, m, H-8a) and 2.60 (1H, m, H-8b), as well as at δ\(_{H} 5.7\) (J = 7.6 Hz, H-2) to δ\(_{H} 3.56\) (1H, m, H-10) and 3.20 (3H, s, OCH\(_{3}\)) to δ\(_{H} 3.23\) (3H, s, OCH\(_{3}\)) to δ\(_{C} 81.4\) (d, C-2). Other known compounds were identified by comparison of their NMR spectroscopic data with the literature, namely irpxelacte B (11) [14], irpxelacte C (12) [14], irpxelacte D (13) [14], 1-(5-(hydroxymethyl)-2-furanyl)-pentanone (14) [18], 5-(3-oxopentyl)-2-furancarboxaldehyde (15) [19], (1R,2R)-1-(5-(pent-4-4-en-1-yl)furan-2-yl)propane-1,2-diol (16) [20], (1R,2R)-1-(5-pentylfuran-2-yl)propane-1,2-diol (17) [20], 2,5,8-decanetione (21) [19], 2-butyl-3-hydroxy-2-cyclopenten-1-one (19) [22].

All new compounds were evaluated for their cytotoxicity against human MCF-7 and Hela cancer cell lines. However,
no compounds showed cytotoxicity against two cell lines at the concentration of 10 μM.

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were measured with a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Tenor 27 spectrophotometer using KBr pellets. 1D and 2D spectra were run on a Bruker Avance III 600 MHz spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra were recorded on an Agilent 6200 Q-TOF MS system. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People’s Republic of China), RP-18 gel (20–45 μm, Fuji Siliesia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Medium Pressure Liquid Chromatography (MPLC) was performed on a Biotage SP1 equipment, and columns packed with RP-18 gel. Preparative High Performance Liquid Chromatography (prep-HPLC) was performed on an Agilent 1260 liquid chromatography system equipped with Zorbax SB-C18 columns (5 μm, 9.4 mm × 150 mm or 21.2 mm × 150 mm) and a DAD detector. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd. Qingdao), and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 in EtOH.

3.2 Fungal Material

Irpex lacteus was collected from the Wangtianshu Scenic Area, Xishuangbanna, Yunnan Province in July 2014, and authenticated by Prof. Yu-Cheng Dai of Beijing Forestry University. A voucher specimen of I. lacteus was deposited at the Higher Fungi Chemistry Group of School of Pharmaceutical Sciences, South-Central University for Nationalities (No. HFG 201407-SCUN201804.2).

3.3 Fermentation Condition

This strain was cultured on PDA medium for 8 days, and then was cut into small pieces to incubate on solid rice medium. The rice medium was inoculated in 500-mL Erlenmeyer flasks, each containing 100 g of rice medium and 100 mL of water. Flask cultures were put into a high pressure sterilizing pot and sterilized at 121 °C for 15 min. Then, the strain of I. lacteus was cultured in the rice medium, and two hundred 500-mL Erlenmeyer flasks were dark incubated fixedly at 25 °C for 30 days.

3.4 Extraction and Isolation

The culture of I. lacteus in rice medium (20 kg) was extracted five times with Me2CO to give a crude extract, which was partitioned into water and EtOAc layers. The EtOAc layer (72 g) was subjected to CC over silica gel (80–100 mesh) eluted with a solvent system of CHCl3/MeOH (from 1:0 to 0:1) to obtain nine fractions (A–I).

Fraction C (6 g) was separated by MPLC over RP-18 silica gel column eluted with MeOH/H2O (from 5:95 to 100:0, v/v) to give five sub-fractions (C1–C5). Fraction C1 was repeatedly separated by CC over silica gel eluted with petroleum ether/ethyl acetate (15:1) to gain 18 (4.6 mg), and then purified by prep-HPLC (CH3CN/H2O from 5:95 to 25:75 in 25 min, v/v) to give 13 (0.8 mg, tR = 21.6 min). Fraction C2 was separated by CC over Sephadex LH-20 (methanol), and then purified by prep-HPLC (CH3CN/H2O = 16:84, v/v) to give 2 (2.2 mg, tR = 20.1 min), D (23.7 mg, tR = 18.0 min) and 19 (2.0 mg, tR = 24.1 min). Fraction C3 was repeatedly separated by CC over silica gel eluted with petroleum ether/ethyl acetate (15:1), and then purified by prep-HPLC (CH3CN/H2O from 8:92 to 20:80 in 40 min, v/v) to give 7 (4.5 mg, tR = 20.8 min) and 15 (0.6 mg, tR = 17.3 min). Fraction C4 was separated by CC over Sephadex LH-20 (methanol), and then purified by prep-HPLC (MeOH/H2O from 25:75 to 55:45 in 30 min, v/v) to give 1 (24.8 mg, tR = 22.6 min) and 4 (2.5 mg, tR = 17.4 min).

Fraction D (8 g) was separated by MPLC over RP-18 silica gel column eluted with MeOH/H2O (from 5:95 to 100:0, v/v) to give eleven sub-fractions (D1–D11). Fraction D2 was purified by prep-HPLC (CH3OH/H2O = 26:74, v/v) to give 3 (2.5 mg, tR = 18.2 min) and 9 (1.0 mg, tR = 14.6 min). Fraction D3 was separated by CC over Sephadex LH-20 (methanol), and then purified by prep-HPLC (CH3CN/H2O = 21:79, v/v) to give 6 (2.4 mg, tR = 20.6 min) and 10 (5.0 mg, tR = 25.0 min), and by prep-HPLC (CH3CN/H2O = 26:73, v/v) to give 11 (14.1 mg, tR = 16.2 min) and 14 (2.4 mg, tR = 18.9 min). Fraction D4 was repeatedly separated by CC over silica gel eluted with petroleum ether/acetone (10:1), and then purified by prep-HPLC (CH3CN/H2O = 32:68, v/v) to give 7 (3.3 mg, tR = 15.4 min). Fraction D5 was separated by CC over silica gel eluted with petroleum ether/acetone (10:1), and then purified by prep-HPLC (CH3CN/H2O = 32:68, v/v) to give 16 (2.2 mg, tR = 17.5 min) and 17 (12.8 mg, tR = 19.8 min). Fraction E (7.2 g) was separated by MPLC over RP-18 eluted with MeOH/H2O (from 5:95 to 100/0, v/v) to give five subfractions (E1–E5). Fraction E4 was subjected to CC over silica gel (200–300 mesh) and Sephadex LH-20 (methanol), and then purified by prep-HPLC (CH3CN/H2O from 20:80 to 70:30 in 20 min, v/v) to give 8 (3.2 mg, tR = 8.9 min).
3.5 Spectroscopic Data of Compounds

3.5.1 Irpexin A (1)
Colorless oil; $[\alpha]_{D}^{27} + 44.8$ (c 0.5, MeOH); IR (KBr) $\nu_{\text{max}}$: 3444, 2937, 1668, 1517, 1097, 1029, 968 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI-MS: $m/z$ 183.10158 [M + H]$^+$ (calcd for C$_{10}$H$_{15}$O$_3$$^+$, 183.10157).

3.5.2 Irpexin B (2)
Colorless oil; $[\alpha]_{D}^{27} − 38.0$ (c 0.5, MeOH); IR (KBr) $\nu_{\text{max}}$: 1670, 1508 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI-MS: $m/z$ 181.08589 [M + H]$^+$ (calcd for C$_{10}$H$_{13}$O$_3$$^+$, 181.08592).

3.5.3 Irpexin C (3)
Colorless oil; $[\alpha]_{D}^{27} − 16.1$ (c 0.27, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 225 (3.31) and 275 (3.12) nm. IR (KBr) $\nu_{\text{max}}$: 3338, 2943, 2831, 1452, 1114, 1031 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI-MS: $m/z$ 207.09825 [M + Na]$^+$ (calcd for C$_{10}$H$_{16}$O$_3$Na$^+$, 207.09917).

3.5.4 Irpexin D (4)
Colorless oil; $[\alpha]_{D}^{27} + 37.5$ (c 0.5, MeOH); IR (KBr) $\nu_{\text{max}}$: 3504, 1635, 1653, 1246 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI-MS: $m/z$ 251.12544 [M + Na]$^+$ (calcd for C$_{12}$H$_{20}$O$_4$Na$^+$, 251.12538).

3.5.5 Irpexin E (5)
Colorless oil; $[\alpha]_{D}^{27} + 14.6$ (c 0.1, MeOH); IR (KBr) $\nu_{\text{max}}$: 3348, 2943, 2831, 1452, 1114, 1031 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI-MS: $m/z$ 211.13280 [M + H]$^+$ (calcd for C$_{12}$H$_{19}$O$_3$$^+$, 211.13287).

3.5.6 Irpexin F (6)
Colorless oil; $[\alpha]_{D}^{27} − 14.3$ (c 0.1, MeOH); IR (KBr) $\nu_{\text{max}}$: 3336, 2943, 2831, 1454, 1114, 1031 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI-MS: $m/z$ 221.07843 [M + Na]$^+$ (calcd for C$_{10}$H$_{14}$O$_4$Na$^+$, 221.07842).

3.5.7 Irpexin G (7)
Colorless oil; $[\alpha]_{D}^{27} − 17.9$ (c 0.2, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 210 (3.48) and 280 (2.79) nm. IR (KBr) $\nu_{\text{max}}$: 3398, 2949, 1651, 1450, 1024 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-d$_4$) and $^{13}$C NMR (150 MHz, methanol-d$_4$) spectroscopic data, see Tables 1 and 2; HR-ESI–MS: $m/z$ 239.12790 [M + H]$^+$ (calcd for C$_{13}$H$_{19}$O$_4$$^+$, 239.12779).

3.5.8 Irpexin H (8)
Colorless oil; $[\alpha]_{D}^{27} − 14.6$ (c 0.2, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 210 (3.48) and 280 (2.79) nm. IR (KBr) $\nu_{\text{max}}$: 3398, 2949, 1651, 1450, 1024 cm$^{-1}$; $^1$H NMR (600 MHz, methanol-d$_4$) and $^{13}$C NMR (150 MHz, methanol-d$_4$) spectroscopic data, see Tables 1 and 2; HR-ESI–MS: $m/z$ 239.12790 [M + H]$^+$ (calcd for C$_{13}$H$_{19}$O$_4$$^+$, 239.12779).

3.5.9 Irpexin I (9)
Colorless oil; $[\alpha]_{D}^{27} − 9.7$ (c 0.12, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\varepsilon$) 210 (3.48) and 280 (2.79) nm. IR (KBr) $\nu_{\text{max}}$: 3338, 2943, 1653, 1246 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) and $^{13}$C NMR (150 MHz, CDCl$_3$) data, see Tables 1 and 2; HR-ESI–MS: $m/z$ 197.15361 [M + H]$^+$ (calcd for C$_{12}$H$_{21}$O$_2$$^+$, 197.15361).

3.6 Mosher Reaction
Compound 1 (25 μmol) was added to a 10 mL round bottom flask, then 2.5 mL of dichloromethane and 50 μmol of pyridine were added. Finally, 50 μmol of (R) or (S) -MTPA-Cl was added dropwise, which was detected by the thin-layer chromatography. After 3 h of stirring at room temperature, the reaction was terminated, and the reaction solution was washed twice with 1 M diluted hydrochloric acid. The organic phase was concentrated and separated by HPLC to obtain compounds 1a and 1b respectively.

3.7 Cytotoxicity Assay
Two human cancer cell lines, breast cancer MCF-7 and Hela, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA),
supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO₂ at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates [23]. Briefly, 100 µL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1 × 10⁵ cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 µM in triplicates for 48 h, with taxol as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed. IC₅₀ value was calculated by Reed and Muench’s method [24].

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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