New Adducts of Iriflophene and Flavonoids Isolated from *Sedum aizoon* L. with Potential Antitumor Activity

Mingxiao Li, Zheyuan Qi, Yimeng Hao, Chongning Lv, Lingyun Jia, Jing Wang and Jincai Lu*

Department of Medicinal Plants, school of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China; mingxiaoli0928@126.com (M.L.); sevensc@126.com (Z.Q.); haoyimeng@me.com (Y.H.); lcnmi@outlook.com (C.L.); jialingyun2003@126.com (L.J.); wangjingyk@126.com (J.W.)

* Correspondence: jincailu@126.com; Tel.: +86-024-2398-6500

Received: 15 October 2017; Accepted: 24 October 2017; Published: 2 November 2017

Abstract: Four new special compounds with character of an iriflophene unit and a flavonoid unit connecting via a furan ring were isolated from the roots of *Sedum aizoon* L. Their corresponding structures were elucidated on the basis of spectroscopic analysis. The in vitro anti-proliferative activities against BXPC-3, A549, and MCF-7 tumor cell lines were evaluated. Compounds 3 and 4 exhibited moderate cytotoxic activities with IC\textsubscript{50} ranging from 24.84 to 37.22 μmol L\textsuperscript{−1}, which was capable for further drug exploration.

Keywords: *Sedum aizoon* L.; iriflophene; flavonoids; antitumor

1. Introduction

*Sedum aizoon* L., is an endemic plant, named ‘jingtiansanqi’ in folk medicine. It is distributed in Japan, North Korea, Mongolia, and China. The whole plant is used as a traditional medicine to treat traumatism, hemorrhage, palpitation, and neurasthenia [1–3]. Previously, the phytochemical constituents of *Sedum aizoon* L. have been extensively reported but only restricted to the aerial part [4–9]. So far, no investigation has been reported regarding the chemical constituents and biological activities of the underground part. In order to find new biologically active compounds, we extracted the roots of *Sedum aizoon* L. and four new special flavonoids were obtained and identified with character of an iriflophene unit and a flavonoid unit connecting via a furan ring (Figure 1). These rare dimers were discovered for the first time. The anti-proliferative activities in vitro against BXPC-3, A549, and MCF-7 tumor cell lines were evaluated by MTT assay.

![Compound 1](image1.png)

![Compound 2](image2.png)

Figure 1. Cont.
2. Results and Discussion

The ethanol extract of Sedum aizoon L. was concentrated and stored at room temperature to yield a crude extract with sediment separated out at the bottom. The sediment was presumed to have low polarity because it dissolved in ethanol but separated out during concentration. Spectroscopic analysis of purified compounds led to the structures of Compounds 1–4. The $^1$H-NMR and $^{13}$C-NMR, IR, UV, HRESIMS, DEPT, HSQC, HMBC, and CD of Compounds 1–4 are available as supplementary materials.

Compound 1, a yellow powder, showed quasi-molecular ions at $m/z$ 561.1005 [M + H]$^+$ (calcd. for C$_{29}$H$_{27}$O$_{12}$, 561.1028) in HRESIMS spectrum. A broad and intense IR absorption band centered at 3412 cm$^{-1}$ confirmed the presence of hydroxyl groups while an intense band with a shoulder at 1633 cm$^{-1}$ showed the presence of conjugated carbonyl functionalities [10]. The $^1$H NMR spectrum for Compound 1 in dry DMSO-$d_6$ displayed an isolated proton at $\delta_H$ 3.60 (3H, s, OCH$_3$) corresponding to the characteristic of methoxyl. Two singlets at $\delta_H$ 5.86 (1H, s, H-6) and $\delta_H$ 5.77 (1H, s, H-8) indicated a disubstituted ring of a flavonoid while three groups of aromatic at $\delta_H$ 6.61 (1H, $dd$, $J = 8.3$, 1.9 Hz, H-6$'$), $\delta_H$ 6.66 (1H, $d$, $J = 8.3$ Hz, H-5$'$), and 6.76 (1H, $d$, $J = 1.9$ Hz, H-2$'$) assigned to a trisubstituted phenyl moiety. Two pairs of aromatic proton at $\delta_H$ 7.72 (2H, $d$, $J = 8.7$ Hz) and $\delta_H$ 6.85 (2H, $d$, $J = 8.7$ Hz) suggested the presence of E ring in iriflophene unit, while a single proton singlet at $\delta_H$ 6.03 (1H, s, H-14) was associated with the single hydrogen on the penta-substituted benzene ring.

The $^{13}$C NMR data showed 27 resonances, two of which had double intensities indicative of carbons on para-disubstituted aromatic rings. The DEPT (135 spectrum) data confirmed the presence of methane carbons at $\delta_C$ 132.06 (C-19, 23), 119.05 (C-6$'$), 114.83 (C-20, 22), 114.61 (C-5$'$), 111.62 (C-2$'$), 97.66 (C-14), 96.85 (C-6), 95.23 (C-8), and a methoxyl at $\delta_C$ 55.61 (OCH$_3$-3). The presence of seven oxygenated aromatic carbons was inferred from the carbon resonances at $\delta_C$ 168.50 (C-7), 163.29 (C-5), 162.05 (C-21), 160.69 (C-15), 160.45 (C-9), 159.62 (C-17) and 157.70 (C-13); and the carbon resonances $\delta_C$ 147.63 (C-4$'$) and $\delta_C$ 146.72 (C-3$'$) indicated the presence of two oxygenated ortho-carbons [10]. A saturated quaternary carbon with an oxygen atom at $\delta_C$ 79.89 (C-3) was readily characterized, while the dioxygenated carbon at $\delta_C$ 117.01(C-2) was identified by comparison with the chemical shifts of dihydroflavonol moieties from the daphnodorins isolated from Daphne odora Thunb [11–13].

The linkage between carbons and hydrogen was characterized by the HSQC while the HMOC data effectively positioned the hydroxyl groups and all non-protonated carbons. The hydrogen at $\delta_H$ 5.86 (H-6) showed correlations to the carbons at $\delta_C$ 168.7 (C-7), 163.3 (C-5), 98.1 (C-10), 95.2 (C-8), while the one at $\delta_H$ 5.77 (H-8) exhibited correlations to the carbons at $\delta_C$ 191.3 (C-4), 168.5 (C-7), 160.5 (C-9), 98.1 (C-10), and 96.9 (C-6) which confirmed the structure of ring A as a common disubstituted ring of a flavonoid. The structure of ring C was confirmed as a trisubstituted phenyl connected to the C-2 by a series correlations of the hydrogen at $\delta_H$ 6.61 (H-6$'$) to the carbons at $\delta_C$ 147.6 (C-4$'$), 117.0 (C-2), 111.6 (C-2$'$); the hydrogen at $\delta_H$ 6.66 (H-5$'$) to the carbons at $\delta_C$ 146.7 (C-3$'$), 124.8 (C-1$'$) and the one at $\delta_H$ 6.76 (H-2$'$) to the carbons at $\delta_C$ 147.6 (C-4$'$), 124.8 (C-1$'$), 119.1 (C-6$'$), and 117.0 (C-2). The single hydrogen at $\delta_H$ 6.03 (H-14) showed correlations to the carbons

![Figure 1. Chemical structures of 1–4.](image-url)
at $\delta_C$ 191.3 (C-11), 106.3 (C-16), 103.2 (C-12), and 79.9 (C-3) which showed the penta-substituted benzene ring D was connected to the feature structure C-2 and C-3. The hydrogen at $\delta_H$ 7.72 (H-19, 23) displayed correlations to the carbons at $\delta_C$ 191.3 (C-11) and 162.1 (C-21); and $\delta_H$ 6.85 (H-20, 22) exhibited correlations to the carbons at $\delta_C$ 162.1 (C-21) and 129.7 (C-18) which corroborated the linkage of ring E as a disubstituted ring connected to the ring D via a carbonyl [14–17]. The $\delta_H$ 6.03 (H-14) showed no correlation to the carbons at $\delta_C$ 159.62 which excluded the possibility that C-11 connected to C-14 (Figure 2).

Thus, the structure of Compound 1 was assigned as 1,3,8,10,10b-penta-hydroxy-5a-(4-hydroxy-3-methoxyphenyl)-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one, an iriflophenone unit and a quercetin unit connecting via a furan ring [18,19].

Compound 2 was obtained as a yellowish amorphous solid. The molecular formula was determined to be C$_{28}$H$_{18}$O$_{11}$ from HRESIMS which showed a quasi-molecular ion peak at $m/z$: 531.0907 [M + H]$^+$ (calcd. for C$_{28}$H$_{18}$O$_{11}$, 531.0922). Its IR, $^1$H NMR, and $^{13}$C NMR spectrum was alike with Compound 1, revealed a similar structure. However, the observation of four pairs of aromatic proton of $^1$H at $\delta_H$ 7.70 (2H, $d$, $J = 8.4$ Hz, H-19, 23), 7.06 (2H, $d$, $J = 8.4$ Hz, H-20, 22), 6.85 (2H, $d$, $J = 8.4$ Hz, H-20, 22), 6.67 (2H, $d$, $J = 8.4$ Hz, H-21′ 5′) as well as $^{13}$C at $\delta_C$ 132.54 (C-19, 23), 114.93 (C-3′ 5′), 128.57 (C-2′ 6′), 115.36 (C-20, 22) suggested that there were two disubstituted rings instead of a trisubstituted phenyl moiety as such structures would show carbon proton between $\delta_C$ 144-148. According to the analysis of HSQC and HMBC, the structure of Compound 2 was elucidated as 1,3,8,10,10b-penta-hydroxy-9-(4-hydroxybenzoyl)-5a-(4-hydroxyphenyl) -5a,10b-dihydro-11H-benzofurochromen-11-one, an iriflophenone unit and a kaempferol unit connecting via a furan ring [20].

Compound 3 was isolated as a yellowish amorphous solid. The HRESIMS showed a quasi-molecular ion peak at $m/z$: 547.0858 [M + H]$^+$ (calcd. for C$_{28}$H$_{19}$O$_{12}$, 547.0871), corresponding to a molecular formula of C$_{28}$H$_{19}$O$_{12}$. Its IR, $^1$H NMR, and $^{13}$C NMR spectrum was similar to Compound 1 but without the signal of methoxyl, which suggested that Compound 3 has the same frameworks as 1 and the methoxy should turn into a hydroxyl. The HSQC and HMBC data supported the postulate. From the above information, the structure of Compound 3 was assigned as 5a-(3,4-dihydroxyphenyl)-1,3,8,10,10b-penta-hydroxy-9-(4-hydroxybenzoyl)-5a,10b-dihydro-11H-benzofurochromen-11-one, an iriflophenone unit, and a quercetin unit connecting via a furan ring [21].

Compound 4 was obtained as a yellowish amorphous solid. The molecular formula was determined to be C$_{30}$H$_{22}$O$_{12}$ from HRESIMS which showed a quasi-molecular ion peak at $m/z$: 575.1162 [M + H]$^+$ (calcd. for C$_{30}$H$_{23}$O$_{12}$, 575.1184). Its IR, $^1$H NMR, and $^{13}$C NMR spectrum was similar to Compound 1. Another methoxyl at $\delta_H$ 3.77 suggested that a hydroxyl should
be replaced by methoxyl. The HMBC correlation from 7-OCH$_3$ ($\delta_H$ 3.77) to C-7 ($\delta_C$ 167.88) located the methoxy group at C-7. Therefore, the structure of Compound 4 was elucidated as 1,8,10,10b-tetrahydroxy-5a-(4-hydroxy-3-methoxyphenyl)-9-(4-hydroxybenzoyl)-3-methoxy-5a,10b-dihydro-11H-benzofuro[2,3-b]chromen-11-one, an iriflophene unit and a rhamnazin unit connecting via a furan ring [22].

The determination of the absolute configuration of C-2 and C-3 in Compounds 1–4 was established by circular dichroic (CD) spectra (Figure 3). The CD spectra showed a negative cotton effect similar to that of daphnodorin F and H at 275 and 321 nm. Therefore, the absolute configuration of C-2 and C-3 was assigned as 2$^S$, 3$^R$ [23,24].

Subsequently, the isolated Compounds 1–4 were evaluated for in vitro cytotoxicity against BXCP-3, MCF-7, and A549 tumor cell lines. The result revealed that Compounds 3 and 4 exhibited moderate cytotoxic activities to all three cell lines with IC$_{50}$ ranging from 24.84 to 37.22 µmol L$^{-1}$, as shown in Table 1. With those distinct frameworks and promising activities, Compounds 3 and 4 can be considered as potential lead compounds for the further structural modification and biological evaluation.

Table 1. Cytotoxicity activities of Compounds 1–4 from Sedum aizoon L.

| Compound | IC$_{50}$ (µmol L$^{-1}$) |
|----------|-------------------------|
|          | BXPC-3  | MCF-7  | A549  |
| 1        | >100    | >100   | >100  |
| 2        | >100    | >100   | >100  |
| 3        | 24.84   | 35.89  | 37.20 |
| 4        | 31.22   | 33.90  | 26.11 |
| 5-FU     | 15.81   | 17.36  | 2.96  |

5-FU: positive control.

3. Materials and Methods

3.1. General Procedures

Optical rotations were determined on an Anton Paar MCP-200 polarimeter (Anton Paar, Graz, Austria) in MeOH at 20 °C. UV spectra were obtained on a UV-1700 visible spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded using a Bruker IFS-55 IR spectrometer with KBr
disks. NMR experiments were performed on a Bruker 400 MHz 600 MHz AV III HD spectrometers (Bruker Biospin, Rheinstetten, Germany). HR-ESI-MS carried out on an Agilent Technologies 6540 UHD accurate mass Q-TOF MS apparatus (Agilent, Santa Clara, CA, USA). ECD spectra were recorded on a BioLogic ECD spectrometer (BioLogic, Pariset, France). Column chromatography (CC) was performed with silica gel (100–200 mesh, 200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China). Silica GF254 (10–40 µm; Qingdao Marine Chemical, Inc., Qingdao, China) and Silica G (10–40 µm; Qingdao Marine Chemical, Inc., Qingdao, China) were used for TLC. Spots were observed by UV light or by spraying with 10% H2SO4-EtOH followed by heating. Preparative HPLC was performed on a Shimadzu 20A system with a YMC-pack (ODS-A, 20 × 250 mm, 5 µm) running with a flow rate of 3.5 mL min⁻¹.

3.2. Plant Material

The roots of *S. aizoon* L. were collected at Heze, Shandong Province, China, in August 2016 and identified by Prof. Jincai Lu (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China). A voucher specimen (No.20160930) was deposited in the Herbarium of Shenyang Pharmaceutical University.

3.3. Extraction and Isolation

The dry roots of *S. aizoon* (17 kg) was extracted with 70% ethanol (136 L × 3 times) and filtered. The filtrate was concentrated under reduced pressure and stored at room temperature to yield a crude extract (20 L) with sediment separated out at the bottom. The crude extract was then centrifuged to separate sediment. The sediment (220 g) was chromatographed on silica gel column, eluting with CH2Cl2/CH3OH (1.0, 50:1, 35:1, 20:1, 10:1, 1:1) to obtain six fractions (fraction 1–6). The fraction 2 (6.0 g) of CH2Cl2/CH3OH (50:1) continued silica gel column elution with petroleum ether-EtOAc (4:1, 2:1, 1:1). The fraction 2-2(1.0 g) of petroleum ether-EtOAc (2:1) was further purified by preparative HPLC (YM-pack ODS-A, 20 × 250 mm, 5 µm, 60% MeOH in H2O) to afford Compound 1 (8 mg). The fraction 4 (5.0 g) of CH2Cl2/CH3OH (20:1) was chromatographed on silica gel column, eluting with CH2Cl2/CH3OH (30:1, 10:1, 1:1). Fraction 4-2 (1.0 g) continued silica gel column elution with CH2Cl2/CH3OH (15:1) and further purified by preparative HPLC (YM-pack ODS-A, 20 × 250 mm, 5 µm, 60% MeOH in H2O) to afford Compound 2 (8 mg). The fraction 6 was subjected to silica gel column, eluting with CH2Cl2/CH3OH (20 × 250 mm, 5 µm, 60% MeOH in H2O) to obtain Compound 3 (20 mg). The fraction 6 was subjected to silica gel column, eluting with CH2Cl2/CH3OH (30:1, 10:1, 1:1), and fraction 6–2 (800 mg) continued silica gel column elution with CH2Cl2/CH3OH (25:1) to yield Compound 4 (13 mg).

3.4. Compound Characterization

**Compound 1**: Yellowish amorphous solid (MeOH); [α]D20 +13.06 (c 0.1033, MeOH); UV λmax(MeOH): 300(3.98) nm, 342(3.80) nm; IR(KBr) νmax/cm⁻¹ 3412, 1633, 1515, 1428, 1284, 1170, 1048, 1023. 1H and 13C NMR data see Table 2; HRESIMS m/z: 561.1005 [M + H]+ (calcd. for C20H12O12, 561.1028).

**Compound 2**: Yellowish amorphous solid (MeOH); [α]D20 +11.58 (c 0.0944, MeOH); UV λmax(MeOH): 301(3.55) nm, 345(3.57) nm; IR(KBr) νmax/cm⁻¹ 1632, 1512, 1439, 1274, 1170, 1044, 1023. 1H and 13C NMR data see Table 2; HRESIMS m/z: 531.0907 [M + H]+ (calcd. for C25H15O11, 531.0922).

**Compound 3**: Yellowish amorphous solid (MeOH); [α]D20 +18.39 (c 0.1142, MeOH); UV λmax(MeOH): 300(4.28) nm, 343(4.26) nm; IR(KBr) νmax/cm⁻¹ 3396, 1632, 1510, 1403, 1272, 1169, 1022. 1H and 13C NMR data see Table 3; HRESIMS m/z: 547.0858 [M + H]+ (calcd. for C28H15O12, 547.0871).

**Compound 4**: Yellowish amorphous solid (MeOH); [α]D20 -10.69 (c 0.0842, MeOH); UV λmax(MeOH): 301(3.10) nm, 340(2.98) nm; IR(KBr) νmax/cm⁻¹ 3405, 1632, 1514, 1282, 1129, 1047, 1023. 1H and 13C NMR data see Table 3; HRESIMS m/z: 575.1162 [M + H]+ (calcd. for C30H23O12, 575.1184).
Table 2. $^1$H NMR and $^{13}$C NMR data of Compounds 1 and 2 in DMSO-$d_6$.

| Position | 1 $^a$ | 2 $^b$ |
|----------|--------|--------|
|          | $\delta$C | $\delta$H | $\delta$C | $\delta$H |
| 4        | 191.25 | 191.64 | 191.25 | 191.64 |
| 11       | 191.02 | 191.64 | 191.02 | 191.64 |
| 7        | 168.50 | 168.26 | 168.50 | 168.26 |
| 5        | 163.29 | 163.75 | 163.29 | 163.75 |
| 21       | 162.05 | 162.57 | 162.05 | 162.57 |
| 15       | 160.69 | 161.15 | 160.69 | 161.15 |
| 9        | 160.45 | 160.75 | 160.45 | 160.75 |
| 17       | 159.62 | 160.07 | 159.62 | 160.07 |
| 13       | 157.7  | 158.10 | 157.7  | 158.10 |
| 4'       | 147.63 | 158.77 | 147.63 | 158.77 |
| 3'       | 146.72 | 114.93 | 146.72 | 114.93 |
| 19, 23   | 132.06 | $7.72 \, (d, J = 8.7 \, Hz, 2H)$ | 132.54 | $7.70 \, (d, J = 8.4 \, Hz, 2H)$ |
| 18       | 129.70 | 130.00 | 129.70 | 130.00 |
| 1'       | 124.81 | 124.67 | 124.81 | 124.67 |
| 6'       | 119.05 | 6.61 $\, (dd, J = 8.3, 1.9 \, Hz, 1H)$ | 128.57 | 7.06 $\, (d, J = 8.4 \, Hz, 2H)$ |
| 2        | 117.01 | 117.76 | 117.01 | 117.76 |
| 20, 22   | 114.84 | 6.85 $\, (d, J = 8.6 \, Hz, 2H)$ | 115.36 | 6.85 $\, (d, J = 8.4 \, Hz, 2H)$ |
| 5'       | 114.61 | 6.66 $\, (d, J = 8.3 \, Hz, 1H)$ | 114.93 | 6.67 $\, (d, J = 8.4 \, Hz, 2H)$ |
| 2'       | 111.62 | 6.76 $\, (d, J = 1.9 \, Hz, 1H)$ | 128.57 | |
| 19, 23   | 106.31 | 106.84 | 106.31 | 106.84 |
| 18       | 103.23 | 104.00 | 103.23 | 104.00 |
| 10       | 98.08  | 98.76  | 98.08  | 98.76  |
| 14       | 97.66  | 6.03 $\, (s, 1H)$ | 97.96  | 6.04 $\, (s, 1H)$ |
| 6        | 96.85  | 5.86 $\, (s, 1H)$ | 97.10  | 5.90 $\, (d, J = 2.0 \, Hz, 1H)$ |
| 8        | 95.23  | 5.77 $\, (s, 1H)$ | 95.28  | 5.80 $\, (d, J = 2.0 \, Hz, 1H)$ |
| 3        | 79.89  | 80.32  | 79.89  | 80.32  |
| 3'-OCH$_3$ | 55.61 | 3.60 $\, (s, 3H)$ | 55.61 | 3.60 $\, (s, 3H)$ |

$^a$ $^{13}$C 150 Hz, $^b$ $^{13}$C 100 Hz.

Table 3. $^1$H NMR and $^{13}$C NMR data of Compounds 3 and 4 in DMSO-$d_6$.

| Position | 3 $^a$ | 4 $^b$ |
|----------|--------|--------|
|          | $\delta$C | $\delta$H | $\delta$C | $\delta$H |
| 4        | 192.53 | 192.89 | 192.53 | 192.89 |
| 11       | 191.55 | 191.64 | 191.55 | 191.64 |
| 7        | 167.30 | 167.88 | 167.30 | 167.88 |
| 5        | 163.66 | 163.25 | 163.66 | 163.25 |
| 21       | 162.64 | 162.52 | 162.64 | 162.52 |
| 15       | 161.36 | 161.32 | 161.36 | 161.32 |
| 9        | 160.46 | 161.10 | 160.46 | 161.10 |
| 17       | 160.02 | 160.29 | 160.02 | 160.29 |
| 13       | 157.64 | 157.50 | 157.64 | 157.50 |
| 3'       | 146.91 | 148.20 | 146.91 | 148.20 |
| 4'       | 144.87 | 147.26 | 144.87 | 147.26 |
| 19, 23   | 132.58 | $7.71 \, (d, J = 8.7 \, Hz, 2H)$ | 132.56 | $7.73 \, (d, J = 8.6 \, Hz, 2H)$ |
| 18       | 129.92 | 130.18 | 129.92 | 130.18 |
| 1'       | 124.97 | 124.85 | 124.97 | 124.85 |
| 6'       | 118.40 | 6.33 $\, (dd, J = 8.3, 2.2 \, Hz, 1H)$ | 119.36 | 6.60 $\, (dd, J = 8.6, 2.0 \, Hz, 1H)$ |
| 2        | 117.91 | 117.94 | 117.91 | 117.94 |
| 20, 22   | 115.43 | 6.87 $\, (d, J = 8.6 \, Hz, 2H)$ | 115.33 | 6.86 $\, \hspace{0.2cm} (d, J = 8.6 \, Hz, 2H)$ |
| 5'       | 115.15 | 6.67 $\, (d, J = 8.3 \, Hz, 1H)$ | 115.15 | 6.67 $\, (d, J = 8.3 \, Hz, 1H)$ |
| 2'       | 114.80 | 6.70 $\, (d, J = 8.3 \, Hz, 1H)$ | 111.91 | 6.75 $\, (d, J = 2.0 \, Hz, 1H)$ |
| 16       | 106.94 | 106.80 | 106.94 | 106.80 |
| 12       | 104.26 | 103.79 | 104.26 | 103.79 |
| 10       | 98.98  | 99.92  | 98.98  | 99.92  |
| 14       | 97.87  | 6.06 $\, (s, 1H)$ | 98.17  | 6.04 $\, (s, 1H)$ |
| 6        | 96.79  | 5.95 $\, (d, J = 2.0 \, Hz, 1H)$ | 95.81  | 6.13 $\, (d, J = 1.7 \, Hz, 1H)$ |
| 8        | 94.97  | 5.85 $\, (d, J = 2.0 \, Hz, 1H)$ | 93.75  | 6.07 $\, (d, J = 1.7 \, Hz, 1H)$ |
| 7-OCH$_3$ | 94.97 | 56.07  | 3.77 $\, (s, 3H)$ |
| 3-OCH$_3$ | 94.97 | 55.37  | 3.61 $\, (s, 3H)$ |

$^a$ $^{13}$C 100 Hz, $^b$ $^{13}$C 150 Hz.
3.5. Cytotoxicity Assay

The cytotoxicity assay of Compounds 1–4 was performed via the MTT method using three kinds of human cancer cell lines, including BXPC-3, MCF-7, and A549 (American Type Culture Collection, Rockville, MD, USA). BXPC-3 and MCF-3 were grown in Dulbecco’s modified Eagle medium (DMEM) while A549 was grown in 1640 medium, supplemented with 10% fetal bovine serum and cultured at a density of $6 \times 10^4$ cells mL$^{-1}$ in 96-well microtiter plate for overnight. Compounds were dissolved in DMSO at five different concentrations and subsequently added to the wells in triplicates. After incubation at 37 °C with 5% CO$_2$ for 72 h, the cells were incubated with 15 µL of MTT (5 mg mL$^{-1}$) for another 4 h. The residual liquid was removed while 150 µL DMSO was added. The absorbance was detected using a microplate reader at 492 nm. All tests and analyses were carried out in three independent assays with DMSO (final concentration of 0.1%) and 5-FU applied as the blank control and positive control, respectively. The anti-proliferative activities were expressed as the IC$_{50}$ value (50% inhibitory concentration).

4. Conclusions

In the present study, four new special adducts of iriflophene and flavonoids connecting via a furan ring were discovered. The dimers of iriflophene and flavonoids were reported for the first time, which also enriched the chemical constituents of the Crassulaceae family. Previously, only a few bioflavonoids analogues were found in Daphane odora but their biological activities were indistinctive [13]. In our research, Compounds 3 and 4 exhibited moderate cytotoxic activities against BXPC-3, A549, and MCF-7 tumor cell lines. Their activities were better than the uncombined unit maybe due to the furan ring connections. Therefore, the special flavonoids isolated from Sedum aizoon L. were meaningful as potential antitumor leading compounds in the medicine industry.

Supplementary Materials: The $^1$H-NMR and $^{13}$C-NMR, IR, UV, HRESIMS, DEPT, HSQC, HMBC, and CD of Compounds 1–4 are available as supplementary materials. Supplementary materials are available online.

Acknowledgments: This project was supported financially by National Natural Science Foundation of China (No. 81373900); Science and technology program of Benxi characteristic industrial base (No. 2013226050); and the Special Fund for TCM supported by State Administration of Traditional Chinese Medicine of China (No. 201407002).

Author Contributions: In this paper, Jincai Lu and Mingxiao Li designed the experiments; Mingxiao Li, Zheyuan Qi, and Yimeng Hao performed the experiments; Mingxiao Li, Chongning Lv, Lingyun Jia, and Jing Wang analyzed the data; Mingxiao Li wrote the paper. All authors approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Wang, J.Y.; Gao, H. Tusanqi and hepatic sinusoidal obstruction syndrome. J. Digest. Dis. 2014, 15, 105–107. [CrossRef] [PubMed]
2. Shao, H.; Chen, H.Z.; Zhu, J.S.; Ruan, B.; Zhang, Z.Q.; Lin, X.; Gan, M.F. Computed tomography findings of hepatic veno-occlusive disease caused by Sedum aizoon with histopathological correlation. Braz. J. Med. Biol. Res. 2015, 48, 1145. [CrossRef] [PubMed]
3. Jin, C.; Wei, X.; Yang, S.; Yao, L.; Gong, G. Microwave-assisted Extraction and Antioxidant Activity of Flavonoids from Sedum aizoon Leaves. Food Sci. Technol. Res. 2017, 23, 111–118. [CrossRef]
4. Kim, J.H.; Hart, H.T.; Stevens, J.F. Alkaloids of some Asian Sedum species. Phytochemistry 1996, 41, 1319–1324. [CrossRef]
5. Niu, X.F.; Liu, X.; Pan, L.; Qi, L. Oleanene triterpenes from Sedum lineare Thunb. Fitoterapia 2011, 82, 960–963. [CrossRef] [PubMed]
6. Li, W.L.; Luo, Q.Y.; Wu, L.Q. Two new prenylated isoflavones from Sedum aizoon L. Fitoterapia 2011, 82, 405–407. [CrossRef] [PubMed]
7. Li, W.L.; Luo, Q.Y.; Wu, L.Q. Two new flavonol glycosides from Sedum aizoon L. Heterocycles 2011, 83, 135. [CrossRef]
8. Li, Z.; Fang, Y.; Huang, A.; Che, L.; Guo, S.; Che, J. Chemical constituents from *Sedum aizoon* and their hemostatic activity. *Pharm. Biol.* 2014, 52, 1429–1434. [CrossRef] [PubMed]

9. Xu, T.; Wang, Z.; Lei, T.; Lv, C.; Wang, J.; Lu, J. New flavonoid glycosides from *Sedum aizoon* L. *Fitoterapia* 2015, 101, 125. [CrossRef] [PubMed]

10. Canuto, K.M.; Leal, L.K.A.M.; Lopes, A.A.; Coleman, C.M.; Ferreira, D.; Silveira, E.R. Amburanains A and B from Amburana cearensis: Daphnodorin-type biflavonoids that modulate human neutrophil degranulation. *J. Braz. Chem. Soc.* 2014, 25, 639–647. [CrossRef]

11. Baba, K.; Yoshikawa, M.; Taniguchi, M.; Kozawa, M. Biflavonoids from Daphne odora. *Phytochemistry* 1995, 38, 1021–1026. [CrossRef] [PubMed]

12. Taniguchi, M.; Fujiwara, A.; Baba, K. Three flavonoids from Daphne odora. *Phytochemistry* 1997, 45, 183–188. [CrossRef]

13. Taniguchi, M.; Fujiwara, A.; Baba, K.; Wang, N.H. Two biflavonoids from Daphne acutiloba. *Phytochemistry* 1998, 49, 863–867. [CrossRef]

14. Zhou, T.; Zhang, S.W.; Liu, S.S.; Cong, H.J.; Xuan, L.J. Daphnodorin dimers from Edgeworthia chrysantha with α-glucosidase inhibitory activity. *Phytochem. Lett.* 2010, 3, 242–247. [CrossRef]

15. Luo, Q.Y.; Li, W.L.; Wu, L.Q. Acylated flavonol glycosides from *Sedum aizoon*. *Chem. Nat. Compd.* 2012, 3, 242–247. [CrossRef]

16. Yuan, H.; Bi, K.; Chang, W.; Yue, R.; Li, B.; Ye, J.; Sun, Q.; Jin, H.; Shan, L.; Zhang, W. Total synthesis of Daphnodorin A. *Tetrahedron* 2014, 70, 9084–9092. [CrossRef]

17. Jia, B.X.; Zeng, X.L.; Ren, F.X.; Jia, L.; Chen, X.Q.; Yang, J.; Liu, H.M.; Wang, Q. Baeckeins F-I, four novel C-methylated biflavonoids from the roots of Baeckea frutescens and their anti-inflammatory activities. *Food Chem.* 2014, 155, 31–37. [CrossRef] [PubMed]

18. Li, J.; Huang, D.; Chen, W.; Xi, Z.; Chen, C.; Huang, G.; Sun, L. Two new phenolic glycosides from Gnaphalium affine D. Don and their anti-complementary activity. *Molecules* 2013, 18, 7751–7760. [CrossRef] [PubMed]

19. Znati, M.; Ben, J.H.; Cazaux, S.; Souchard, J.P.; Harzallah, S.F.; Bouajila, J. Antioxidant, 5-lipoxygenase inhibitory and cytotoxic activities of compounds isolated from the Ferula lutea flowers. *Molecules* 2014, 19, 16959–16975. [CrossRef] [PubMed]

20. Elsayed, N.H.; Wojcinska, M.; Drostkarbowska, K.; Matlawska, I.; Williams, J.; Mabry, T.J. Kaempferol triosides from Silphium perfoliatum. *Phytochemistry* 2002, 60, 835. [CrossRef]

21. Lallemand, J.Y.; Duteil, M. C13 NMR Spectra of Quercetin and Rutin. *Magn. Reson. Chem.* 1977, 9, 179–180. [CrossRef]

22. Itokawa, H.; Oshida, Y.; Ikuta, A.; Inatomi, H.; Ikegami, S. Flavonol glycosides from the flowers of Cucurbita pepo. *Phytochemistry* 1981, 20, 2421–2422. [CrossRef]

23. Taniguchi, M.; Baba, K. Three biflavonoids from Daphne odora. *Phytochemistry* 1996, 42, 1447–1453. [CrossRef]

24. Li, Q.; Gao, W.; Cao, J.; Bi, X.; Chen, G.; Zhang, X.; Xia, X.; Zhao, Y. New cytotoxic compounds from flowers of Lawsonia inermis L. *Fitoterapia* 2014, 94, 148–154. [CrossRef] [PubMed]

**Sample Availability:** Not available.