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# Synthesis and Antitumor Activity of 3-[2-(4-Hydroxy-Phenyl)-Ethyl]-Benzo[*d*] Isoxazole-4,6-Diol

**Li Wang**

The First Affliated Hospital of Xi'an Medical University, Xi'an,. China
Email: 394004312@qq.com

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## Abstract

A new phloretin derivative 1 3-[2-(4-hydroxy-phenyl)-ethyl]-benzo[d] isoxazole-4,6-diol (yield 63%) was synthesized from phloretin by carbonyl nucleophilic addition condensation reaction. Its structure was characterized by 1H NMR, 13C NMR and HR-MS. The phloretin, compound 1, resveratrol and acetylated resveratrol were determined by comparing them with paclitaxel. Anti-tumor activity of alcohol on SPC-A1, EC109, A549, MCF-7 and MDA-MB-231 cell lines. Compound 1 showed better antitumor activity than docetaxel against A549 tumor cells.

## Keywords

3-[2-(4-Hydroxy-Phenyl)-Ethyl]-Benzo[*d*] Isoxazole-4,6-Diol, Synthesis, Antitumor Activity

## 1. Introduction

Phloretin (**Figure 1**), a dihydrochalcone in flavonoids, is a natural non-steroidal estrogen with a C6-C3-C6 skeleton (two benzene rings are linked by a chain containing three carbons). Phloretin is mainly distributed in the peel, rhizome and root bark of apples, pears, strawberries and other juicy fruits. The natural active substance is obtained by various extraction methods [1]. Phloretin, as a new natural skin whitening agent, has many biological and pharmacological activities, such as moisturizing and antioxidant effects [2]. Recently a large number of studies have reported that it has an antitumor effect [3] [4].

Phloridzin is the glucoside of phloretin (**Figure 1**). Since its discovery in the nineteenth century, the research results show that phloridzin and its derivatives have biological activities such as antitumor, anti-oxidation and hypoglycaemic

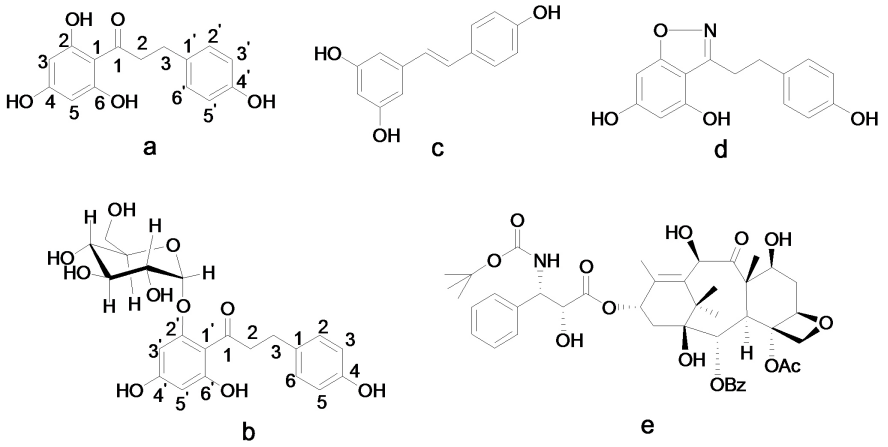



**Figure 1**. Structure of molecules: (a) Phloretin (b) Phloridzin (c) Resveratrol (d) Compound 1 (e) Docetaxel.

activity, and have been widely used in the field of medicine and biofilm research [5]. Sodium phloretin disulfonate was synthesized by Shin *et al.* [6]. The results showed that the compound could protect keratin from ultraviolet B (UVB) damage and inhibit the release of inflammatory mediators such as IL-6 and prostaglandin E2 induced by UVB. Anna *et al.* [7] synthesized a derivative of phloridzin. Its antioxidant activity is similar to that of phloridzin but more stable.

Resveratrol (**Figure 1**) is a polyphenolic compound with a structure similar to that of phloretin. Resveratrol has the functions of anti-cancer, anti-oxidation, anti-bacterial, anti-inflammatory and preventing food allergies. It can be widely used in medicine, health products, food, cosmetics and other fields. Acetylated resveratrol was synthesized by acylation of resveratrol. It was confirmed that acetylated resveratrol as a prodrug increased resveratrol accumulation in the lung [8]. Acetylated resveratrol could alleviate acute lung injury caused by seawater drowning [9] by increasing the expression of connexin 43.

A novel phloretin derivative 1 (yield 63%) was synthesized from phloretin by nucleophilic addition condensation of carbonyl group. Its structure was characterized by 1H NMR, 13C NMR and HR-MS. Docetaxel as the positive control antitumor drug, the antitumor activities of phloretin, compound 1, resveratrol and acetylated resveratrol against five cancer cell lines SPC-A1, EC109, A549, MCF-7 and MDA-MB-231 were determined.

## 2. Experiment Section

### 2.1. Cell Line, Instruments and Reagents

SPC-A1 (human lung cancer cells), EC109 (human esophageal cancer cells), A549 (human non-small cell lung cancer cells), MDA-MB-231 (human breast cancer cells) and MCF-7 (human breast cancer cells) were provided by Tangdu Hospital of Air Force Medical University. Bruker DKX300 nuclear magnetic resonance instrument (DMSO-d6 as solvent, TMS as internal standard), Waters ACQUITY TQD-series quadrupole liquid chromatography-mass spectrometry,

enzyme-linked immunosorbent. Serum-free DMEM medium, fetal bovine serum, tetramethylazole blue (MTT), dimethyl sulfoxide (DMSO) were purchased from Sigma Company. All the other reagents used are commercially pure and have not been purified before use.

## 2.2. Synthesis

1.00 g (3.65 mmol) phloretin and 0.50 g (7.30 mmol) hydroxylamine hydrochloride were dissolved in 30 mL absolute ethanol. 10 mL potassium hydroxide ethanol solution with 20% mass fraction was added drop by drop under ice bath. Catalytic amount of glacial acetic acid was added and the mixture was heated to reflux after restoring to room temperature. TLC was used to monitor the reaction. the reaction solution was concentrated, and column chromatography separation and purification are used, eluent is petroleum ether to ethyl acetate which proportion is equal to 2:1 and 0.63 g light jujube red solid compound 1 was obtained with a yield of 63%. Melt point: 127.4˚C. HR-MS m/z: 272.07 $[M + H]^+$. $^1H$ NMR (DMSO-$d_6$, 300 MHz) δ: 11.01 (s, 1H), 10.62 (s, 1H), 9.17 (s, 1H), 7.04 (d, 2H, J = 2.0 Hz), 6.67 (d, 2H, J = 1.5 Hz), 6.34 (s, 1H), 6.22 (s, 1H), 3.04 (t, 2H), 2.93 (t, 2H). $^{13}C$ NMR (DMSO-$d_6$, 75 MHz): 165.7，161.5, 156.9, 155.5, 153.27, 131.1, 129.1, 115.1, 104.0, 97.7, 86.4, 32.6, 28.1.

## 2.3. Solution Preparation

Phloretin, docetaxel, compound 1, resveratrol and acetylated resveratrol were dissolved in DMSO to prepare mother liquor. The mother liquor was stored at 4˚C. The final concentration was 10 μg/mL, 20 μg/mL, 30 μg/mL, 40 μg/mL, 50 μg/mL, 60 μg/mL, 70 μg/mL, 80 μg/mL, 90 μg/mL. docetaxel was used as positive control drug.

## 2.4. Cell Culture

After cell resuscitation, the cells were cultured in 25 cm 2 culture flask with DMEM (10% fetal bovine serum, 33 mg/L penicillin and 100 mg/L streptomycin). The incubator conditions were 5% $CO_2$, 37 C, saturated humidity, and liquid exchange every two days. The experiment was carried out when the cell reached logarithmic growth stage and the fusion degree reached 80%.

## 2.5. MTT Assay for Cell Proliferation

The experimental cells were treated with different concentrations of drugs. After 48 hours of culture, MTT reagent was added to each pore (concentration was 5 mg/mL), incubated at 37 C for 4 hours, the supernatant was discarded, DMSO was added to each pore for 150 mL, and the crystal was fully dissolved. The absorption value of each pore at 570 nm wavelength was detected, and the mean value was taken to calculate the growth inhibition rate. The inhibition rate and drug concentration were plotted, and the dose response curve was obtained. The $IC_{50}$ (half inhibition concentration) value (μg/mL or μmol/L) was calculated.

$$\text{Growth inhibition rate}(\text{IR}) = (\text{control group absorbance-experimental group absorbance})/\text{experimental group absorbance} \times 100\%.$$

### 2.6. Antitumor Activity of Compound 1

The cytotoxicity of phloretin, compound 1, resveratrol and acetylated resveratrol to SPC-A1, EC109, A549, MDA-MB-231 and MCF-7 cancer cell lines was determined by MTT method. $IC_{50}$ (μmol/L) was calculated three times in parallel with docetaxel as control. The results are shown in Table 1.

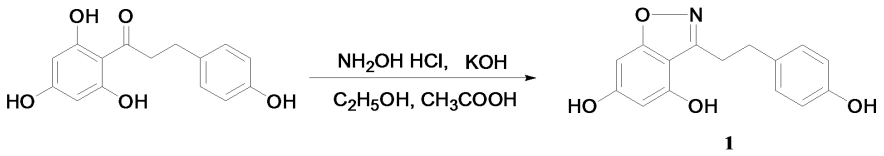
## 3. Results and Discussion

The main functional groups in the structure of phloretin are the phenolic hydroxyl group and carbonyl group. The oxime was synthesized by nucleophilic addition and condensation of hydroxylamine and carbonyl. The phloretin, hydroxylamine hydrochloride, potassium hydroxide, anhydrous ethanol and catalytic amount of glacial acetic acid were heated and refluxed, and the product was purified to obtain a light jujube red solid as shown in Scheme 1. By analyzing the structure of compound 1, the possible mechanism of its formation was found as follows: 1) Hydroxylamine first reacted with carbonyl group in phloretin to form oxime by nucleophilic addition and condensation. 2) A new heterocyclic isoxazole structure (Scheme 2) was formed by dehydration reaction with the nearest phenolic hydroxyl group. The principle of this method is similar to that reported in the literature [10]. The difference is that this method uses anhydrous reaction conditions, but the results are similar.

Table 1. $IC_{50}$(μM) of compound 1 for five cell lines.

| Compound | $IC_{50}$(MM) | | | | |
|---|---|---|---|---|---|
| | MDA-MB-231 | A549 | SPC-A1 | EC109 | MCF-7 |
| Phloretin | 30.54 ± 1.95 | 133 > 100 | 147 > 100 | 148 > 100 | 78.64 ± 0.86 |
| Compound 1 | 18.02 ± 0.21 | 53.00 ± 0.89* | 126 > 100 | 105 > 100 | 43.51 ± 1.06 |
| Resveratrol | 44.968 ± 0.77 | 35.82 ± 1.01 | 123 > 100 | 55.06 ± 1.67 | 141 > 100 |
| Acetylated resveratrol | 47.370 ± 2.32 | 93.13 ± 0.87 | 119 > 100 | 105 > 100 | 26.92 ± 0.99 |
| Docetaxel | 1.00 ± 1.04 | 70.30 ± 1.06* | 149 > 100 | 56.44 ± 1.62 | 1.46 ± 1.52 |

*: For A549 cells, compared with compound 1 IC50 (μM), t = −21.65, $P^* < 0.05$, there were statistical differences.

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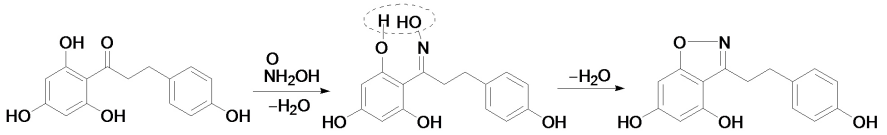
Scheme 1. The synthesis of compound **1**.




Scheme 2. A formation mechanism of compound 1.

The cytotoxicity data of compound 1 against SPC-A1, EC109, A549, MDA-MB-231 and MCF-7 cell lines showed that compound 1 exhibited certain antitumor activity. Compound 1 has stronger antitumor activity than phloretin on five cancer cell lines. For the A549 cell line, compound 1 with the value of $IC_{50}$ (53 μM) has a statistical difference in t = −21.65, $P < 0.05$ in contrast to docetaxel with the value of $IC_{50}$ (70.3 μM); therefore compound 1 has better antitumor activity than docetaxel on A549 cells. Phloretin, resveratrol and acetylated resveratrol all exhibit certain antitumor activity, but their activity is weaker than compound 1, compound 1 is a potential antitumor substance.

## 4. Conclusion

In conclusion, the novel synthetic route of compound 1 provides a new process for the synthesis of these compounds, and its excellent antitumor activity provides a basis for the study of phloretin derivatives.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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