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SYNTHESIS AND ANTITUMOR ACTIVITIES OF RESVERATROL DERIVATIVES ON CERVICAL CANCER HELA CELLS

Lu Jin, Yu-Jie Ren,* and Cheng Du

*Using 2-thiophenecarbonyl chloride and 2-furoyl chloride to modify the structure of the natural product resveratrol, we synthesized five novel resveratrol derivatives. The target compounds were evaluated for their antitumor activities against cervical cancer HeLa cells by the MTT method. The results indicated that the compound **3a** displayed the best antitumor activities, which is higher than the value of resveratrol, and its inhibition ratio was 95.1% against HeLa cells at the concentration of 75 μmol/L.*

Keywords: resveratrol derivatives, antitumor activities, HeLa cells.

Resveratrol (**1**) is a natural nonflavanoid polyphenolic compound. It has various beneficial properties such as antitumor [1], anti-oxidation [2], anti-inflammatory [3], anti-aging [4], and cardiovascular activity [5]. Many studies have shown that resveratrol derivatives have pharmacological activities similar to resveratrol, and some compounds display better activities, selectivities, and stabilities than resveratrol. Cervical cancer is the most common female genital tract malignancy, and it is the third most common malignant tumor next to breast cancer and colorectal cancer in the global women. A study has reported that resveratrol and its derivatives can inhibit the proliferation of HeLa cells, and partial derivatives have better or equivalent inhibitory activities than resveratrol against human cervical carcinoma HeLa cells [6]. The purpose of this study was to seek resveratrol derivatives that have stronger activity, selectivity, and stability against cervical cancer.

Currently one important way of developing new drugs is to use active natural products as lead compounds and make modifications in their structures in order to obtain high activity and low toxicity. Because esterification is the most common modifying method in prodrug design, ester prodrugs are used to protect the charged groups in water-soluble drugs such as carboxylic acid and phosphoric acid, thereby increasing the fatsolubility of the original drug and enhancing the permeability of the passive film. After the ester prodrugs enter the body, the esterase in the blood, liver, and other tissues hydrolyze the ester bond to release the original function. It has been reported that approximately 49% of listed prodrugs owe their activity to enzymatic hydrolysis [7].

Several studies have reported when heterocyclic groups are introduced into the resveratrol molecule, the antitumor activities of their derivatives were stronger than resveratrol *in vitro* [8, 9], which is because resveratrol and other stilbene compounds can suppress and even reverse the cancer [10]. We researched the stilbene structural skeleton of the resveratrol molecule, which has antitumor activities, and mainly modified the phenolic hydroxyl groups on the benzene ring; meanwhile, 2-furoic acid is an antibiotic that can have a bactericidal effect in the breeding season of cells; 2-thiophenecarboxylic acid is an important intermediate that is used to synthetize the anti-cancer drug Raltitrexed. By introducing 2-thenoyl and 2-furoyl heterocyclic structures into the resveratrol molecule, five novel resveratrol derivatives were synthesized (Scheme 1). The target compounds were evaluated for their antitumor activities against cervical cancer HeLa cells *in vitro*. Finally, we expected that these new compounds can have a synergistic effect, so that a candidate compound with better anti-cervical cancer activity can be found.

School of Chemical and Environmental Engineering, Shanghai Institute of Technology, 201418, Shanghai, P. R. China, e-mail: clab@sit.edu.cn. Published in *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2015, pp. 563–565. Original article submitted October 24, 2013.

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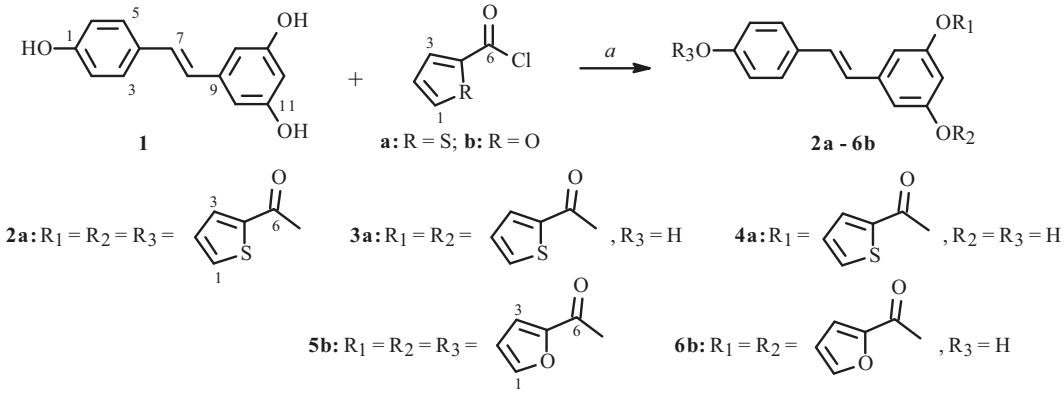
TABLE 1. Result of Antitumor Activities of the Target Compounds **2a–6b** on HeLa cells (inhibition ratio, %)

Compound	IC_{50}, μmol/L	Concentration/(ммol/L)			
		50	75	100	150
2a	–	–2.7	3.6	0	–2.0
3a	26.7	88.0	95.1	95.0	94.5
4a	–	0	0.4	4.0	17.1
5b	74.5	37.6	51.5	87.0	86.2
6b	–	–17.7	–24.5	–11.0	32.5
Resveratrol	112	15.8	30.1	43.7	71.2

–: Inhibition effect was not obvious on HeLa cells.



a. Et_3N, DCM, r.t., 4 h

Scheme 1

Resveratrol has a phenolic hydroxyl hydrogen with certain activities, so it needs a base or acid as catalyst to react with the acid chloride to form esters. With sodium hydroxide as the alkali, tetrahydrofuran, acetone, dichloromethane, and water were used as a solvent at room temperature or at the reflux temperature to explore the reaction conditions, but the results indicated that the reaction did not occur; when using triethylamine as the catalyst and an acid-binding agent, dichloromethane, as the solvent to control the temperature at 25°C, the reaction was optimum. If the reaction temperature is increased, resveratrol could be oxidized, with was not conducive to the reaction; with decreasing temperature, the reaction time would be prolonged. The effects of reaction conditions were investigated, and the most suitable conditions for the reaction are as follows: triethylamine as the catalyst and acid binding agent, and methylene chloride as the solvent at 25°C for 4 h; after purification by silica gel column chromatography, we obtained the target compounds.

In the IR spectra, compounds **3a**, **4a**, and **6b** had the typical phenolic hydroxyl O–H stretching vibration characteristic absorption peaks at 3300 cm^{-1}, and compounds **2a**, **3a**, **4a**, **5b**, and **6b** had typical C=O and C–O–C absorption peaks at 1735 to 1770 cm^{-1} and 1012–1315 cm^{-1}, which indicated that the compounds contain the ester bond. In the ^1H NMR spectrum, we observed that the reaction occurred at the phenolic hydroxyl group by comparison of the nuclear magnetic resonance data. Taking compounds **2a**, **3a**, and **4a** as examples, we see that **4a** has single peaks of two phenolic hydroxyl group at δ 9.81 and 9.62, **3a** has an peak at δ 9.90 for two phenolic hydroxyl groups at the same position, and **2a** has no active hydrogen peak within this range, indicating that the reaction took place in the phenolic hydroxyl group; from the ^{13}C NMR spectrum, it was seen that compounds **2a**, **3a**, and **4a** near δ 158.0 had the peak of the carbon atoms in the carboxylic acid ester. These spectral data characterize the structures of compounds **2a–6b**.

The results of antitumor activities of compounds on HeLa cells are shown in Table 1. The results indicated that compounds **3a** and **5b** displayed better activities against HeLa cells than resveratrol, but the other compounds **2a**, **4a**, and **6b** did not display obvious activities. Among all the compounds, **3a** displayed the best inhibition effect, with an inhibition ratio of 95.1% against HeLa cells at the concentration of 75 μmol/L, and its IC_{50} was 26.7 μmol/L, which was 4.2 times lower than that of resveratrol (IC_{50} = 112 μmol/L).

In order to find good candidate compounds with anti-cancer activity, the phenolic hydroxyl group of resveratrol on the benzene ring was modified, and five novel resveratrol derivatives were synthesized; their structures were confirmed by IR,

^{1}H NMR, ^{13}C NMR, and HR-MS; when using triethylamine as the catalyst and acid-binding agent, and dichloromethane as the solvent to control the temperature at 25°C, the reaction was optimum. The results of antitumor activities of compounds on HeLa cells indicated that compounds **3a** and **5b** displayed better antitumor activities against HeLa cells than resveratrol. Compound **3a** displayed the best antitumor activities with an IC_{50} value of 26.7 μmol/L, which was higher than the value of resveratrol (IC_{50} = 112 μmol/L), and its inhibition ratio was 95.1% against HeLa cells at the concentration of 75 μmol/L.

EXPERIMENTAL

All chemicals and reagents were purchased from commercial sources and were used without further purification. All reactions were monitored by TLC (silica gel plates; Merck 60 F254). Column chromatography (CC): silica gel (Merck, 300–400 mesh). Mp: WPS-2A apparatus, uncorrected. ^{1}H and ^{13}C NMR Spectra: Bruker-Avance 500 (500 MHz) in DMSO-d_6, at 500 and 125 MHz, respectively, δ relative to Me_4Si as internal standard, in ppm, J in Hz. IR spectra: Nicolet-IR-6700 spectrometer (KBr); ν in cm^{-1}. MS: Shimadzu 2020 spectrometer. HR-MS: SolariX-70FT-MS Bruker spectrometer.

Measurement of the Antitumor Activities on Cervical Cancer HeLa Cells. The antitumor activities of the target compounds on human cervical carcinoma cells (HeLa cells) *in vitro* were measured by the MTT assay, we collected the cells in the log phase and adjusted the concentration of the cell suspension, then added the cell suspension (100 μL) to each well and adjusted the density of the cells under test to 1000–10000 holes by decking (the edge holes were filled with sterile PBS). The cells were cultured at 37°C for 24 h in a 5% CO_2 incubator until the cell monolayers covered the bottom of the hole (96-well flat-bottom plates).Then we added gradient concentration drugs in five gradients, 100 μL per well, and cultured the cells for 24 h in a 5% CO_2 incubator at 37°C. Then we observed them with an inverted microscope, discarded the supernatant, added 20 μL MTT solution (5 mg/mL, namely 0.5% MTT) to each well, continued to culture the cells for 4 h, discarded the supernatant, added 150 μL DMSO to each hole, and oscillated the cells for 10 min on a low-speed shaker until the crystals were fully dissolved. Finally, the absorbance value of each well was measured by an automatic multimicroplate reader at OD570 nm and 630 nm.

General Methods for the Preparation of the Compounds 2a–6b. A solution of 2-thiophenecarboxylic acid or 2-furoic acid (2.8 g, 22.0 mmol) in thionyl chloride (5.2 g, 44.0 mmol) was stirred and refluxed for 3 h. Then the excess solvents were removed under reduced pressure to give 2-thiophenecarbonyl chloride (**a**) or 2-furoyl chloride (**b**), which were then dissolved in dichloromethane (5.0 mL). The resulting solution was slowly added to a mixture of resveratrol (1.0 g, 4.4 mmol) and triethylamine (4.0 mL) in dichloromethane (20.0 mL) at 25°C. Then the mixture was stirred for 4 h; after completion of the reaction as monitored by thin-layer chromatography (TLC) and vaporizing the dichloromethane, the residue was extracted with ethyl acetate and washed with brine. The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to give a brown crude product .The residue was purified by flash chromatography (silica gel, ethyl acetate–petroleum ether, 8:1–1:1) to afford compounds **2a–6b** as a white solid.

(*E*)-5-(4-((Thiophene-2-carbonyl)oxy)styryl)-1,3-phenylenebis(thiophene-2-carboxylate) (2a). Yield 30%, mp 104–106°C. IR (ν, cm^{-1}): 1724.7, 1251.2, 1058.6. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.14 (2H, d, J = 4.9), 8.11 (1H, d, J = 4.9), 8.08 (2H, d, J = 2.7), 8.05 (1H, d, J = 2.6), 7.70 (2H, d, J = 8.5), 7.54 (2H, s), 7.48 (1H, d, J = 16.4), 7.34 (6H, s), 7.27 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 160.5, 160.3, 151.5, 150.5, 140.2, 136.1, 135.8, 135.7, 135.1, 132.3, 132.0, 130.3, 129.3, 129.2, 128.3, 127.4, 122.8, 118.1, 115.6. ESI-MS *m/z* 559.0 $[M + H]^+$; HR-MS calcd for $C_{29}H_{18}O_6S_3$, 559.0338.

(*E*)-5-(4-Hydroxystyryl)-1,3-phenylenebis(thiophene-2-carboxylate) (3a). Yield 26%, mp 148.4–149.6°C. IR (ν, cm^{-1}): 3389.2, 1717.8, 1251.2, 1065.7. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.90 (1H, s), 8.11 (2H, d, J = 4.8), 8.04 (2H, s), 7.70 (2H, d, J = 8.5), 7.36–7.25 (6H, m), 7.02 (1H, s), 6.93 (1H, s), 6.59 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 159.0, 158.3, 151.9, 139.7, 135.6, 135.3, 132.4, 132.1, 128.7, 128.5, 128.2, 117.6, 116.2, 112.1. ESI-MS *m/z* 449.0 $[M + H]^+$; HR-MS calcd for $C_{24}H_{16}O_5S_2$, 449.0512.

(*E*)-3-Hydroxy-5-(4-hydroxystyryl)phenylthiophene-2-carboxylate (4a). Yield 10%, mp 207.6–208.1°C. IR (ν, cm^{-1}): 3384.5, 1704.1, 1251.2, 1058.6. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.81 (1H, s), 9.62 (1H, s), 8.10 (1H, s), 8.02 (1H, t, J = 6.7), 7.43 (2H, d, J = 8.5), 7.35–7.30 (1H, m), 7.13 (1H, d, J = 16.3), 6.95 (2H, d, J = 16.7), 6.85 (1H, s), 6.77 (2H, d, J = 8.5), 6.53 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 158.9, 157.9, 151.8, 140.3, 135.6, 129.9, 129.2, 128.5, 128.3, 124.8, 116.0, 111.5, 110.2, 108.1. ESI-MS *m/z* 339.0 $[M + H]^+$; HR-MS calcd for $C_{19}H_{14}O_4S$, 339.0686.

(*E*)-5-(4-((Furan-2-carbonyl)oxy)styryl)-1,3-phenylenebis(furan-2-carboxylate) (5b). Yield 25%, mp 149.2–150.3°C. IR (ν, cm^{-1}): 1735.8, 1289.8, 1085.7 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 8.14 (2H, s), 8.11 (1H, s), 7.70 (2H, d, J = 8.1), 7.63 (2H, d, J = 2.8), 7.59 (1H, d, J = 2.7), 7.53 (2H, s), 7.47 (1H, d, J = 16.4), 7.34–7.27 (3H, m), 7.24 (1H, s), 6.83 (2H, s), 6.81 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 156.8, 156.5, 151.1, 150.1, 149.3, 149.1, 143.4, 143.3, 140.2, 135.1, 130.4, 128.3, 122.7, 121.0, 120.7, 118.1, 113.3, 113.2. ESI-MS *m/z* 512.1 $[M + H]^+$; HR-MS calcd for $C_{29}H_{18}O_9$, 511.1024.

(*E*)-5-(4-Hydroxystyryl)-1,3-phenylenebis(furan-2-carboxylate) (6b). Yield 17%, mp 106.2–107.8°C. IR (ν, cm^{-1}): 3441.2, 1725.7, 1289.6, 1075.7. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 9.90 (1H, s), 8.13 (2H, d, J = 9.6), 7.69 (2H, d, J = 8.2), 7.58 (2H, d, J = 3.5), 7.29 (2H, d, J = 7.8), 7.24 (2H, d, J = 24.6), 6.97 (2H, d, J = 33.8), 6.81 (2H, s), 6.59 (1H, s). ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm): 162.8, 158.9, 151.6, 149.3, 139.7, 135.3, 128.9, 128.7, 128.5, 128.2, 122.6, 120.7, 116.1, 112.1. ESI-MS *m/z* 417.1 $[M + H]^+$; HR-MS calcd for $C_{24}H_{16}O_7$, 417.0969.

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