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Synthesis and biological evaluation of hydroxycinnamic acid hydrazide derivatives as inducer of caspase-3

Zheng-Rong Wu [a], Jian Liu [b], Jian-Ying Li [a], Li-Fang Zheng [a], Yang Li [a], Xing Wang [a], Qing-Jian Xie [c], Ai-Xia Wang [a], Ying-Hui Li [a], Rong-Hui Liu [c], Hong-Yu Li [a, c, *]

[a] School of Pharmaceutics, Lanzhou University, 222 Tian Shui South Road, Lanzhou 730000, People's Republic of China
[b] The First Hospital of Lanzhou University, Lanzhou 730000, People's Republic of China
[c] Institute of Microbiology, School of Life Sciences, Lanzhou University, 222 Tian Shui South Road, Lanzhou 730000, People's Republic of China

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ABSTRACT

In order to generate compounds with superior antitumor activity and reduced toxicity, twelve new hydroxycinnamic acid hydrazide derivatives were synthesized and evaluated for their antiproliferative activities against two cancer cell lines (H1299 lung carcinoma cells and MCF-7 breast cancer cells), and compared to two normal counterparts (NL-20 lung epithelial cells and H184B5F5/M10 breast cells) by MTT method. The results demonstrated that some of these compounds possessed good antiproliferative activity against the two cancer cell lines. Among them, compound **2c** was active against the growth of H1299 lung carcinoma cells with IC_{50} values of 1.50 μM, which was more active than the positive topotecan (IC_{50} = 4.18 μM). Simultaneously, it showed lower cytotoxic effects on normal NL-20 lung epithelial cells (IC_{50} > 10 μM). Mechanism studies indicated that it induced cell cycle arrest at G2/M phase followed by activation of caspase-3, and consequently caused the cell death. Further studies on the structure optimization are ongoing.

1. Introduction

Cancer is one of the major causes of death worldwide and chemotherapy is a major therapeutic approach for the treatment, the purpose of the chemotherapy is to kill the tumor cells as these cells are more susceptible to the actions of these drugs because of their growth at a much faster rate than healthy cells [1–3]. Over the past few years, research efforts to improve chemotherapy have led to an improvement in patient survival but there is still a need for improvement, the effectiveness of the treatment is directly related to the treatment's ability to target and to kill the cancer cells while affecting as few healthy cells as possible, but at present, most of standard chemotherapy agents lack selectivity toward cancerous cells cause significant damage to rapidly proliferating normal cells [4–6]. In searching for molecules that selectively induce apoptosis in cancer cells, a compound to be medicinally useful it is critical that this apoptotic induction be selective for cancerous versus non-cancerous cells [7].

Natural products have generated significant attention as potential chemotherapeutic agents because many of them are free from harmful adverse effects [8]. Hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic and sinapic acid) (Fig. 1) are widely distributed in the plant kingdom in the form of esters, amides and glycosides, and are reported as cellular antioxidants, anti-inflammatory agents, or inhibitors of enzymes involved in cell proliferation [9,10]. Particularly, the derivatives is considered to be promising anticancer drug due to its efficient induction of proliferation arrest and apoptosis in a variety of tumor cells [11–13]. A number of studies indicated that most of their pharmacological properties are considered to be due to their antioxidant action [14,15].

Based upon the above results and in our continuing efforts to find new compounds with potent activities and low toxicity from natural products. Considering the high antioxidant activity and good water solubility when actively transplanted into mammalian tissue of hydroxycinnamic acids. In this work we described the synthesis of a series of hydroxycinnamic acid hydrazide derivatives and evaluated for their antiproliferative activities against two cancer cell lines (H1299 lung carcinoma cells and MCF-7 breast cancer cells), and compared to two normal counterparts (NL-20 lung epithelial cells and H184B5F5/M10 breast cells) by MTT method. Simultaneously, we also assessed the antiproliferative

* Corresponding author. Institute of Microbiology, School of Life Sciences, Lanzhou University, 222 Tian Shui South Road, Lanzhou 730000, People's Republic of China
E-mail address: lihy@lzu.edu.cn (H.-Y. Li).

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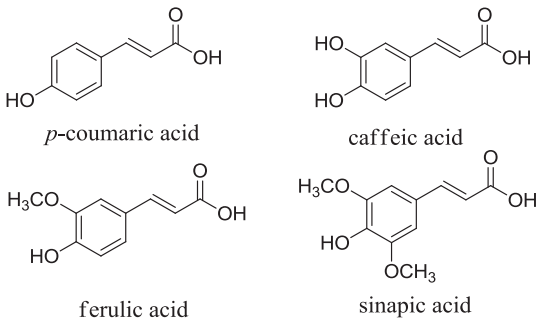

Fig. 1. Structural formulas of Hydroxycinnamic acids.

activity of steric and electronic parameters on the aromatic ring of cinnamic acids group. On the other hand, the most active compound was selected for further mechanism studies, including its effect on cell cycle progression, apoptosis, and induction to caspase-3.

2. Chemistry

The synthesis of hydroxycinnamic acid hydrazide derivatives was done according to our previously reported [16]. Compounds were purified by silica gel column chromatography using a gradient mixture of petroleum ether-ethyl acetate. The yields were between 52 and 84% and all the synthesized compounds were characterized by IR, ^{1}H and ^{13}C NMR spectroscopy, and HRMS mass spectrometry.

The IR spectra showed the carbonyl peaks at 1639–1677 cm^{-1}, and the NH and OH stretching vibrations at 3004–3396 cm^{-1}. The ^{1}H NMR spectra displayed the hydrazide (NH) protons as singlets between 9.92 and 10.51 ppm. The ^{13}C NMR spectra showed C=O signals at 161.5–165.9 ppm. All of the compounds showed mass spectra with molecular ions characteristic of their chemical structure. The elemental analyses were performed on an Elemental Vario-EL elemental analyzer and within 0.2% of theoretical values.

3. Pharmacology

All the synthesized hydroxycinnamic acid hydrazide derivatives (**1a**–**c**, **2a**–**c**, **3a**–**c** and **4a**–**c**) were evaluated *in vitro* against two cancer cell lines (H1299 lung carcinoma cells and MCF-7 breast cancer cells), and the cytotoxicity were compared to two normal counterparts (NL-20 lung epithelial cells and H184B5F5/M10 breast cells) by MTT method. The concentration that inhibited the growth of 50% of cells (IC_{50}) was determined from the linear portion of the curve by calculating the concentration of tested agent that reduced absorbance in treated cells, compared to control cells, by 50%. In addition, the most active compound was selected for further mechanism studies.

4. Results and discussion

The antiproliferative activity of the synthesized compounds are summarized in Table 1, the results demonstrated that some of these compounds possessed good antiproliferative activity against the two cancer cell lines. Among the synthesized molecules, compound **2c** exhibited the most potent antiproliferative activity against the growth of H1299 lung carcinoma cells with IC_{50} values of 1.50 μM, which was more active than the positive topotecan (IC_{50} = 4.18 μM), encouragingly it showed lower cytotoxic effects on NL-20 normal lung epithelial cells (IC_{50} > 10 μM), and therefore, was selected for further mechanism studies.

According to SARs analysis, it was observed that both the steric and the electronic parameters play major role in the activity of this series of compounds. Addition of one methoxyl group or hydroxyl group *ortho* to the hydroxyl group of the aromatic ring of acids (1 → 2 and 1 → 3), the antiproliferative activity against the two tumor cell lines (H1299 and MCF-7) were increased. But addition of a second methoxyl group, at the other *ortho* to the hydroxyl group of the aromatic ring of acids (1 → 4), the antiproliferative activity were decreased. Results presented herein suggested that 4-hydroxy-3-methoxy group on the aromatic ring of acid was the key substituent group for the higher antiproliferative activity.

Further mechanism studies indicated that compound **2c** induced cell cycle arrest at G2/M phase followed by activation of caspase-3, and then caused the cell death.

Table 1
Antiproliferative activities of compounds **1a**–**c**, **2a**–**c**, **3a**–**c** and **4a**–**c**.

Compounds	Cell lines (IC_{50}, μM)[a]			
	H1299	NL-20	MCF-7	H184B5F5/M10
1a	7.36 ± 1.72	9.75 ± 0.22	7.39 ± 1.04	9.32 ± 1.25
1b	6.64 ± 0.32	>10	6.02 ± 0.35	8.44 ± 0.83
1c	5.47 ± 0.60	8.62 ± 1.15	8.80 ± 0.16	>10
2a	6.16 ± 0.23	9.55 ± 1.38	5.22 ± 0.30	9.17 ± 1.24
2b	5.42 ± 0.36	>10	5.62 ± 0.15	>10
2c	1.50 ± 0.09	>10	4.36 ± 0.47	>10
3a	6.77 ± 1.09	8.85 ± 0.86	4.75 ± 0.08	8.12 ± 0.65
3b	5.42 ± 0.16	9.39 ± 1.27	4.86 ± 0.27	9.57 ± 1.42
3c	4.48 ± 1.40	>10	6.05 ± 1.32	8.68 ± 1.57
4a	>10	>10	>10	>10
4b	7.45 ± 1.30	9.42 ± 1.83	7.17 ± 1.06	9.45 ± 1.43
4c	8.71 ± 1.57	>10	9.32 ± 1.25	>10
Topotecan	4.18 ± 0.23	>10	3.46 ± 1.02	>10

[a] Data are the mean of three independent experiment.

The effects of **2c** on cell cycle progression were determined by FACS analysis in H1299 cells [17]. Cells in the Sub-G1 phase were considered as apoptotic cells, as shown in Fig. 2A, the percentage of apoptotic cells in the untreated control was 5.3%, whereas cells treated with **2c** had a significantly higher percentage of apoptotic cells (8.9 and 12.3%) after 12 and 24 h of treatment ($p < 0.05$). Moreover, it can also be seen in Fig. 2A, the proportion of cells were decreased in the G1 and accumulated in G2/M phase after 12 and 24 h treatment of **2c** ($p < 0.05$), while the Sub-G1 phase increased. Compared with 5.4% in untreated cultures, G2/M phase arrest was initially detectable after 12 h of treatment, there were 31.7% and 46.2% of the cells in G2/M phase after **2c** exposure for 12 h and 24 h respectively ($p < 0.01$). These results demonstrate that the treatment with 1.50 μM **2c** lead to a time-dependent accumulation of cells in the G2/M phase with a concomitant decrease in the population of G1 phase cells.

In order to compare the effects of **2c** on cell cycle changes between H1299 cells and normal NL-20 cells, the effects of **2c** on cell cycle progression were also determined in NL-20 cells *in vitro*. As shown in Fig. 2B, cells in the sub-G1 phase were considered as apoptotic cells, as expectedly, the percentage of apoptotic cells in the untreated control was 4.6%, there were similar percentages of apoptotic cells (5.2 and 4.9%) after 12 and 24 h treatment with 1.50 μM **2c** ($p > 0.05$). Thus, results presented herein suggested that treatment of NL-20 cells with **2c** did not affect the cell-cycle progression.

As above mentioned, **2c** interfered with H1299 cells proliferation by arresting the cell cycle at G2/M phase, no such current was observed in the normal NL-20 cells.