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Research Article

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Synthesis and biological evaluation of 10-hydroxycamptothecin derivative on different tumor cells *in vitro* and *in vivo*

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ABSTRACT

10-hydroxycamptothecin (10-HCPT) is a camptothecin analogue with a powerful cytotoxic effect for cancer therapies. Increasing attention has been paid on the synthesis and pharmacological activities of 10-HCPT and its derivatives. In the study, the design, synthesis and biological evaluation of 10-HCPT derivative was researched. The newly synthesized 10-HCPT derivative was characterized by ^{1}H-NMR and the anticancer activity was also investigated by MTT assay and in vivo test. MTT results suggested that the new compound has better biological effect than 10-HCPT on four tumor cell lines including K562, HT-29, HepG2 and S180. The new compound also showed good inhibition on S180 ascites model.

Keywords: 10-HCPT, Synthesis, Antitumor activity, MTT, S180

INTRODUCTION

Cancer is inevitably one of the most studied but yet unsolved non- communicable human diseases [1], and it is predicted to continue to become the leading cause of death within the coming years [2]. Nowadays the world has more than 10 million cancer patients, and the death rate is high [3].

10-Hydroxycamptothecin (10-HCPT) as a minor camptothecin analogue was isolated from a native Chinese tree Camptotheca acuminate Decne. 10-HCPT shows a significant cytotoxic effect for cancer therapies [4-6] and is a broad spectrum anticancer agent for treatment of gastric carcinoma, hepatoma, leukemia and tumor of head and neck in clinical practice [7, 8].

Although 10-HCPT showed well anticancer effect, the application is restricted by its low aqueous solubility, *in vitro* and *in vivo* instability, especially for the short half-life about 10 min[9]. Therefore, synthesis of new 10-HCPT derivatives with high solubility and low toxicity is urgent. The existing 10-hydroxy is an important substitute for 10-HCPT compared to camptothecin and some compounds are also derived from 10-HCPT by modifying the position 10 such as the famous antitumor agent irinotecan [10]. Thus we designed and synthesized the 10-HCPT derivatives by substituting the position 10 in the prospect of better antitumor drug. Through *in vitro* tests, one 10-HCPT derivative (compound **2**) showed potent cytotoxicity. In the present study, the synthesis process and the *in vivo* and *in vitro* antitumor activity of the 10-HCPT derivative were present.

EXPERIMENTAL SECTION

2.1 Materials and Measurements

10-Hydroxycamptothecin and hydroxyl camptothecin injection with the brand name of Knowshine were purchased from Knowshine (Shanghai) Pharmachemicals Inc. (Shanghai, China). L-Alanine methyl ester hydrochloride hydrochloride was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. All reagents and solvents used in this study were analytical grade. The reaction temperature control used the oil bath temperature modulator. Thin layer chromatography (TLC) with silica gel 60 GF254 E.Merckprecoated plates (0.25 mm) was visualized using UV. 0.1 for flash chromatography on silica gel (particle size 100-200 mesh). ^{1}H-NMR spectra were recorded on Bruker AM-400 NMR spectrometers in deuterated chloroform and deuterated DMSO. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane as internal standard.

2.2. Animals and cells

Adult Kunming mice (18-22 g) were obtained from the Aoyide Lab Supplies company (Tianjin, China). The animals were housed in standard cages with white wood chips for bedding, and given free access to food and drinking water, under controlled temperature, humidity and photoperiod. Mice sarcoma 180 (S180) cells were purchased from the Instituteof Biochemistry and Cell Biology, Shanghai Institutes for BiologicalSciences, Chinese Academy of Sciences (Shanghai, China).

2.3 Chemistry

The synthesis route of the 10-HCPT derivative was showed in Fig.1.

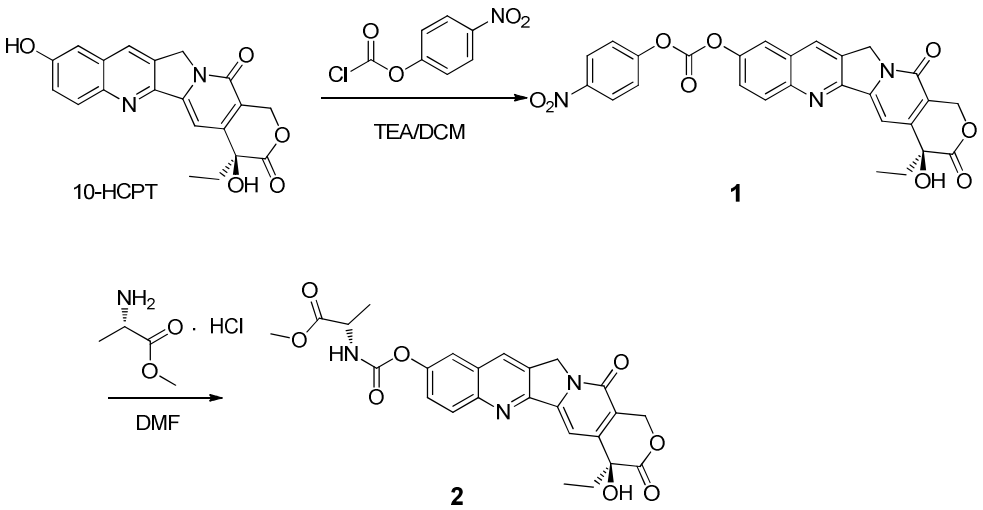

Fig .1 Synthesis route of 10-HCPT derivatives

2.3.1 Synthesis of (S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H- pyrano[3',4':6,7]indolizino[1,2-b] quinolin-9-yl (4-nitrophenyl) carbonate (compound 1)

Fetch 10-HCPT 5.00 g (13.72 mmoL) was dissolved in 500 mL of anhydrous dichloromethane, and it was added triethylamine 19.20 mL (137.20 mmoL) under ice-bath, than was added p-nitro phenyl chloroformate 11.06 g (54.89 mmoL), with stirring under ice-cooling 15 min, the reaction at room temperature for 9 h. After the reaction was completed by TLC, washed with 300 mL water three times, the organic phase was collected, dried over anhydrous sodium sulfate, dichloromethane: methanol = 200:1,200 mesh silica gel column chromatography to give 10-(4-nitro-phenyl carbonate) - camptothecin 4.65 g, yield 64%.

2.3.2 Synthesis of S)-methyl2-(((((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano [3',4': 6,7]i-ndolizino[1,2-b]quinolin-9-yl)oxy)carbonyl)amino)propanoate (compound 2)

Fetch compound **1** 0.50 g (0.94 mmoL) was dissolved in 3 mL of anhydrous DMF, and it was added L-alanine methyl ester hydrochloride under the ice-bath 0.26 g (1.89 mmol) and anhydrous potassium carbonate 0.26 g (1.89 mmol), with stirring under ice-cooling 2 h. After completion of the reaction by TLC, the reaction mixture was added 15 mL of water and extracted with 3 times 25 mL of dichloromethane, and the combined organic phases, the organic phase was washed with saturated brine, dried over anhydrous sodium sulfate, dichloromethane: methanol = 175:1,200 mesh silica gel column chromatography to give 10 - [(L-alanine) carbamate] - camptothecin 0.22 g, yield is 48%.

2.4 MTT Assay

Four tumor cells (K562, HT-29, HepG2 and S180) were seeded in 96-well plates at a density of 5×10^4 cells/mL (100 μL of medium per well). After overnight incubation, cells were synchronbized by changing to a medium free of fetal calf serum, After another 24 h, serial concentrations of the compounds (0.5 μL) were added and further incubated for 48 h (final concentrations of each compound: 1 nM, 10 nM, 0.1 μM, 1 μM and 10 μM). The culture plates were incubated for 4 h after adding 20 μL MTT. The supernatant was then removed and 100 μL DMSO was added into each well with samples being shaken for further 10 min. The control group was cells without any addition and was referred to as 100% of viable cells. The optical density (OD) was measured at 492 nm (or 570 nm) and 630 nm. Cell viability was calculated by OD value according to the corresponding formula and a graph is plotted of cell viability (y-axis) against drug concentration (x-axis). The experiment was carried out three times independently. The IC_{50} concentration represents the concentration resulting in a 50% decrease in cell growth after 2 days incubation.

2.5 *In vivo* mice Tumors Model

S180 cells suspension (1×10^7 cell/mL) were given into mice by intraperitoneal injection after fasting 24 h. Forty Kunming mice (half male and female) were divided into five groups including control group, 10-HCPT (1 mg/kg) and compound **2** (1, 3 and 5 mg/kg). Control group was given normal saline every day. All animals were treated for 26 days by intraperitoneal injection. During the experiment, the body weight of the mice was weighted every day. Finally, all the living animls were sacrificed. The animal study was approved by the Institutional Aninmal Care and Use Committee of China, and institutional guidelines for animal welfare and experimental conduct were followed.

RESULTS AND DISCUSSION

3.1 Characteristics of 10-HCPT Derivatives by ^{1}H NMR

3.1.1(S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl (4-nitrophenyl) carbonate by ^{1}H NMR (compound 1)

^{1}H-NMR (d6-DMSO, 400 MHz): δ/ppm 0.879-0.916 (m, 3H), 1.851-1.921(m, 2H), 5.317 (s, 2H), 5.442 (s, 2H), 6.539 (s, 1H), 7.371 (s, 1H), 7.776 (d, 2H), 7.963-7.993 (m, 1H), 8.21 (d, 1H), 8.401 (d, 2H), 8.739 (s, 1H).

3.1.2(S)-methyl2-(((((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)carbonyl)amino)propanoate by ^{1}H NMR (compound 2)

^{1}H-NMR (d6-DMSO, 400 MHz): δ/ppm 0.889 (t, 3H), 1.395(d, 3H), 1.841-1.929 (m, 2H), 3.695 (s, 3H), 4.068-4.107 (m, 1H), 4.216-4.253 (m, 1H), 5.296 (s, 1H), 5.431 (s, 2H), 6.521 (s, 1H), 7.343 (s, 1H), 7.625-7.654 (m, 1H), 7.904 (d, 1H), 8.183 (d, 1H), 8.498 (d, 1H), 8.679 (s, 1H).

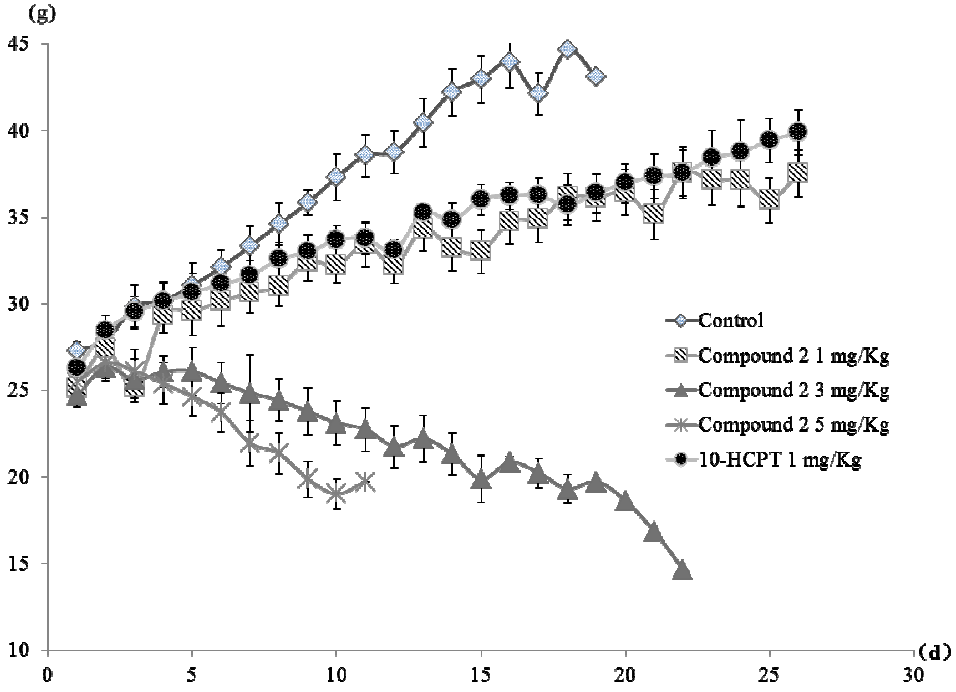

Fig.2: Male mice weight survival time

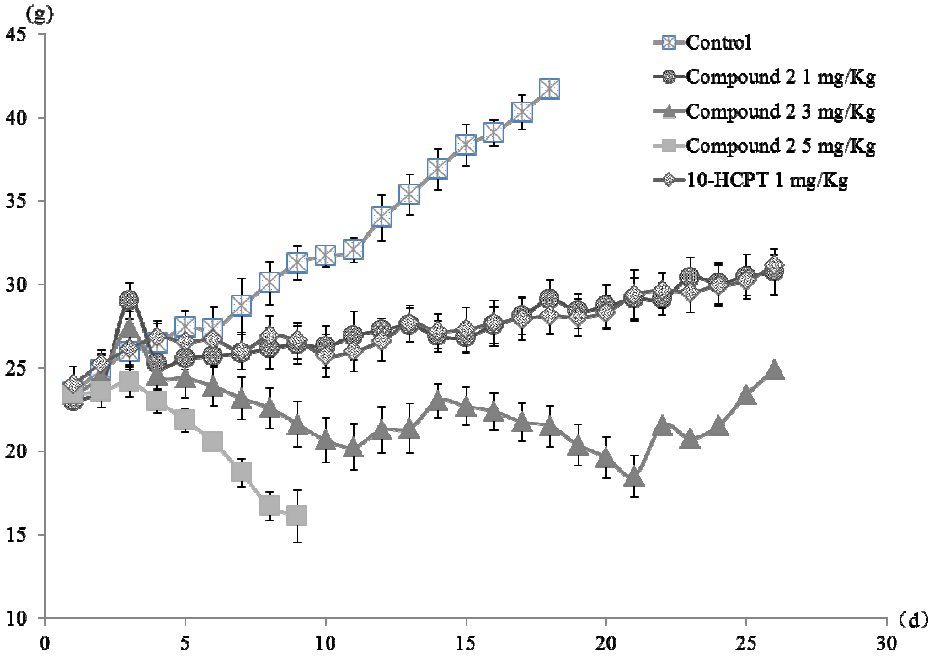

Fig.3: Female mice weight survival time

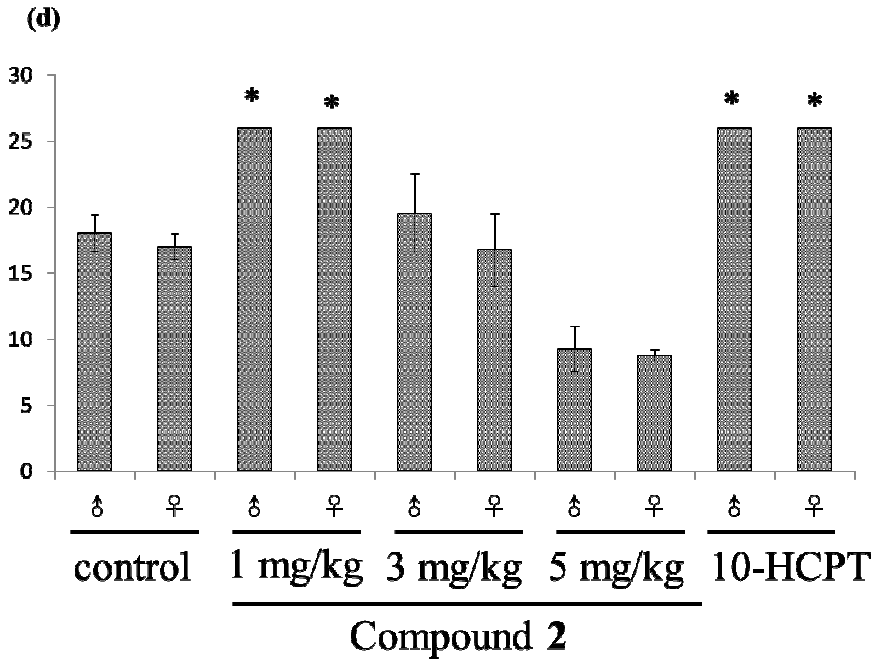

Fig. 4: Mice survival time chart * means $p<0.05$ (*vs* control)

3.2 Anti-proliferation effects of compound 2 against cancer cells

Compound **2** exhibited a different growth inhibitory activity against S180, K562, HT-29 and HepG2 cells. As shown in the Table 1, compound **2** gave stronger cytotoxicity on K562 and HT-29 than 10-HCPT.

Table 1 Inhibition activity in different cancer cells (IC_{50}, μM)

	S180	K562	HepG2	HT-29
10-HCPT	0.06	0.14	0.33	0.23
Compound **2**	0.12	0.04	0.33	0.13

3.3 Effect of compound 2 *in vivo*

Male and female mice were calculated separately in this test. Fig.**2** and Fig.**3** showed that 10-HCPT and compound **2**

(1 mg/kg) showed good inhibitory effect on S180 tumor model compared to control group from the slowly increasing weight. At the doses of 3 and 5 mg/kg, compound **2** sharply reduced the body weight due to the cumulative toxicity.

In the experiment, as shown in Fig.4, 10-HCPT group survived 26 days, compound **2** exhibited 9.25 days (♂) and 8.75 days (♀) at 5 mg/kg, 19 days (♂) and 16.75 days (♀) at 3 mg/kg, and 26 days (♂&♀) at 1 mg/kg, while the control group only survived 18 days (♂) and 17 days (♀). By calculating, both of the compound **2** and 10-HCPT gave about 50% of the life extension rate compared with the control group.

In conclusion, a new 10-HCPT derivative was designed and synthesized in the study. The antitumor activity of this compound was also investigated by MTT assay and *in vivo* test. According to the results, compound **2** shows well anticancer effect on S180, K562, HT-29 and HepG2, and has stronger cytotoxicity than 10-HCPT on K562 and HT-29. At the same time, compound **2** exhibites good inhibition on S180 ascites model. Further biological study and mechanism of the new derivative are ongoing in our laboratory.

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