

# Sulfated modification and cytotoxicity of *Portulaca oleracea* L. polysaccharides

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**Abstract** A water-soluble polysaccharide (POP1) was isolated from *Portulaca oleracea* L. Four sulfated derivatives of POP1 (POP1-s1, POP1-s2, POP1-s3 and POP1-s4) were prepared by chlorosulfonic acid method with *N,N*-Dicyclohexylcarbodiimide (DCC) as a dehydration-condensation agent. FT-IR spectra and  $^{13}\text{C}$  NMR spectra indicated the sulfated groups had been introduced at the C-6 and C-2 positions of POP1. Sulfated derivatives had different degree of substitution (DS) ranging from 1.01 to 1.81, and different weight-average molecular mass (Mw) ranging from 41.4 to 48.5 KDa. Sulfated derivatives except POP1-s5 inhibited the growth of HepG2 cells and Hela cells *in vitro* significantly, which indicated that sulfated modification could enhance cytotoxicity of POP1 on tumor cells. Flow cytometric studies revealed that sulfated derivatives could mediate the cell-cycle arrest of Hela cells in the S phase.

**Keywords** *Portulaca oleracea* L. · Sulfated derivatives · Cytotoxicity

## Introduction

Polysaccharides from plants are safe, biocompatible and biodegradable, which have many kinds of biological activi-

ties, such as enhancing immunity, anti-inflammatory, anti-oxidation, anti-cancer and prevention of acquired immune deficiency syndrome (AIDS) [8, 24, 15, 27]. Biological activities of polysaccharides depend on their molecular structure, including sugar unit, glycosidic bond of the main chain, type and polymerization degree of the branch, flexibility and configuration of the chain [9]. Therefore, molecular modification and structure improvement of polysaccharides are a hot topic in recent years. Many studies have demonstrated that biological activities of polysaccharides are increased by molecular modification [3, 7, 8, 21].

Since the inhibitory activity of a sulfated polysaccharide against the human immunodeficiency virus (HIV) was first reported in 1987, sulfated polysaccharide had become the focus of considerable research [6]. They were a kind of polysaccharides with sulfated groups replacing their hydroxyl groups, including natural sulfated polysaccharides extracted from plants or derivatives synthesized from natural polysaccharides [12, 13]. Sulfated polysaccharides could not only enhance the water solubility but also change the chain conformation, resulting in the alteration of their biological activities [2, 10, 11]. Wang extracted a linear (1–3)-, (1–4)-linked  $\beta$ -glucan (OBG) from the oat bran and synthesized a sulfated derivative OBGS with 36.5% sulfate. OBGS had potent activity against human immunodeficiency virus (HIV-1) [17]. Yang extracted a native polysaccharide from the mycelium of a marine filamentous fungus *Phoma herbarum* YS 4108 (YCP) and synthesized its two sulfated derivatives YCP-S1 and YCP-S2. The sulfated YCP was more promising than YCP in protecting erythrocytes against oxidative damage hemolysis [23]. Two water-soluble derivatives, sulfated and carboxymethylated polysaccharides were prepared by Wang using derivatization of water-insoluble polysaccharides (GL-IV-I) from the fruit body of *Ganoderma lucidum*. The sulfated and carboxymethylated groups

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in the polysaccharides played an important part in enhancing their antitumor activities [18, 19]. Some polysaccharides had no antiviral activity, but their sulfated derivatives exhibited antiviral activity [14]. The activities of sulfated polysaccharides depended on their structural parameters, including the degree of substitution (DS) [16], the weight-average molecular mass (Mw) [25], the position of sulfation [1], and glycosidic branching [26]. Recently, sulfated modification by chlorosulfonic acid–pyridine method was evaluated as the most popular method. However, polysaccharides would be depolymerized severely in the strong acid circumstance due to the long reaction time, and even lose the original biological activities.

*Portulaca oleracea* L., which was situated in the moist areas was a green annual herb of the Portulacaceae family, and was known as “Long-lived grass” in China [4, 5]. Polysaccharide from *Portulaca oleracea* L. (POP) was the main effective ingredient with weak biological activities, such as enhancing immunity, anti-oxidation and anti-cancer.

In the present study, we extracted POP and synthesized its sulfated derivatives (POPS), and hoped that sulfated modification could improve its biological activities. Here, POP was modified by chlorosulfonic acid–pyridine method, and *N,N*-dicyclohexylcarbodiimide (DCC) was added as a dehydration-condensation agent in the reaction system for increasing the reaction rate and hence decreasing the depolymerization of polysaccharides. The effects of chain conformation and sulfated groups on the cytotoxicity of POP on tumor cells were discussed *in vitro*.

## Material and methods

### Materials and reagents

*Portulaca oleracea* L. was collected from the mountain area in Weinan city, Shaanxi Province, China. The crude POP from *Portulaca oleracea* L. was obtained from Shaanxi Lixin biotechnology Co., Ltd.

Papain was from Beijing Huamei Biotechnology Co., Ltd. (China). DCC was purchased from Nanjing Chemlin Chemical Industry Co., Ltd. (China). Chlorosulfonic acid (CSA), pyridine and *N,N*-dimethylformamide (DMF) were analytical grade and obtained from Gansu Yinguang Chemical Industry Co., Ltd. (Baiyin, China). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was from Sigma Co. (France). SephadexG-100 was from Pharmacia Co. (Sweden).

### Purification of POP

The crude POP (10 g) was purified as follows: The protein was removed by Sevage method, combined with

papain (300 U/mL). After centrifugation, the supernatant was dialyzed against distilled water (2 days) and then separated through SephadexG-100 column. Three kinds of purified POP (named by POP1, POP2 and POP3) were obtained.

The contents of POP1, POP2 and POP3 were measured by Vitriol–Phenol with anhydrous glucose as standard control [22], and their purities were up to 94%. Their yields were 42.3%, 4.5%, 6.2%, respectively. POP1 was only used for further experiment, and the molecular mass of POP1 was 55.8 KDa.

### Sulfated modification of POP1

#### Preparation of sulfated reagent

CSA was dripped in anhydrous pyridine, under agitating and cooling in ice water bath [18, 19]. The process was lasted for 20 min.

#### Sulfation reaction

POP1 (200 mg) was suspended in anhydrous DMF (20 mL) at room temperature with stirring for 30 min, and sulfated reagent was added dropwise. Then 50 mg DCC was added to ensure appropriate modification of polysaccharide. The mixture was stirred for 1 h at 40°C. Later, the mixture was cooled to room temperature and the pH value was adjusted to 7–8 by 2.5 mol/L NaOH solution. The mixture was precipitated with ethanol, washed, dissolved in water, and was dialyzed (molecular weight cutoff 8–14 kDa) against distilled water for 48 h to remove pyridine, DCC, salt and potential degradation products. Four sulfated POP1-s (POP1-s1 to POP1-s4) with different DS were collected after lyophilizing.

POP1-s5 was obtained by chlorosulfonic acid–pyridine method without DCC in the reaction.

### Characterization of sulfated POP1

The homogeneity and molecular weight of sulfated derivatives were determined by high-performance gel-filtration chromatography (HPGFC) on a Waters 2695 instrument equipped with three WATO columns and a Waters 2414 Refractive Index Detector (RID). 1 mg/ml sample solution (POP1-s solution) was injected in each run, using 0.05 mol/L Na<sub>2</sub>SO<sub>4</sub> as the mobile phase. The analysis was performed at 25°C and a flow rate of 0.7 ml/min. The HPGFC system was calibrated with T-series Dextran standards (T-5, T-25, T-50, T-150 and T-270).

The Sulfur contents (S%) of the sulfated derivatives were determined by an elemental analyzer (Vario EL, Elementar Co., Germany), and DS, which referred to the

**Table 1** Characterization of the sulfated POP1

POP1-s <sup>a</sup>	CSA:PD <sup>b</sup>	Time (h)	Mw (kDa)	Elemental analysis (%)			DS
				C	H	S	
POP1-s1	1:5	1.0	48.5	19.75	3.76	12.19	1.01
POP1-s2	1:4	1.0	44.7	17.79	3.64	14.07	1.30
POP1-s3	1:3	1.0	45.2	17.26	3.58	15.69	1.59
POP1-s4	1:2	1.0	41.4	16.19	3.82	16.71	1.81
POP1-s5	1:5	2.5	31.3	15.05	3.21	16.02	1.66

<sup>a</sup> Sulfation of polysaccharide from *Portulaca oleracea*.L

<sup>b</sup> The ratio of chlorosulfonic acid to pyridine in sulfated reagent

average number of sulfated residues on each monosaccharide residue was calculated :

$$DS = (162 \times S\%)/(32 - 102 \times S\%)$$

FT-IR spectra was recorded on a FLS920 FT-IR spectrophotometer (Edinburgh, England). <sup>13</sup>C NMR spectra of 40 mg/ml solution in D<sub>2</sub>O was recorded at 40°C with a Bruker Avance 600 MHz spectrometer (Germany), and the chemical shifts were expressed in ppm relative to the resonance of internal standard 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

#### Cell lines and culture

The human hepatoma cell line (HepG2) and uterine cervix carcinoma cell line (Hela) were provided by the Biology Preservation Center in Shanghai Institute of Materia Medica and maintained with RPMI 1640 medium containing 10% fetal bovine serum and 100 ng/ml, each of penicillin and streptomycin at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>.

#### Growth inhibition assay

The inhibition effects of sulfated derivatives on the HepG2 cells and Hela cells were evaluated *in vitro* using MTT assay. HepG2 cells and Hela cells (5 × 10<sup>3</sup> cells/well) were incubated for 12 h before adding the compounds. Cells were exposed and were treated with varied concentration of sulfated derivatives for 48 h at 37°C. After drug exposure, the culture medium was removed and 100 μl of MTT reagent (diluted in culture medium, 0.5 mg/ml) was added. Following incubation for 4 h, the MTT/medium was removed and DMSO (100 μl) was added to dissolve the formazan crystals. Absorbance of the colored solution was measured on a microplate photometer (PerkinElmer Victor3) using a test wavelength of 570 nm. Results were evaluated by comparing the absorbance of the wells containing

compound treated cells with the absorbance of wells containing 0.1% DMSO alone (solvent control). Conventionally, cell viability was determined for each assay including blank wells that did not contain cells. All experiments were performed in triplicate. To validate the induction of apoptosis by identifying morphological features, phase-contrast microscopy was used to show the dose-dependent manner in cell viability in treatment of HepG2 cells or Hela cells with sulfated derivatives.

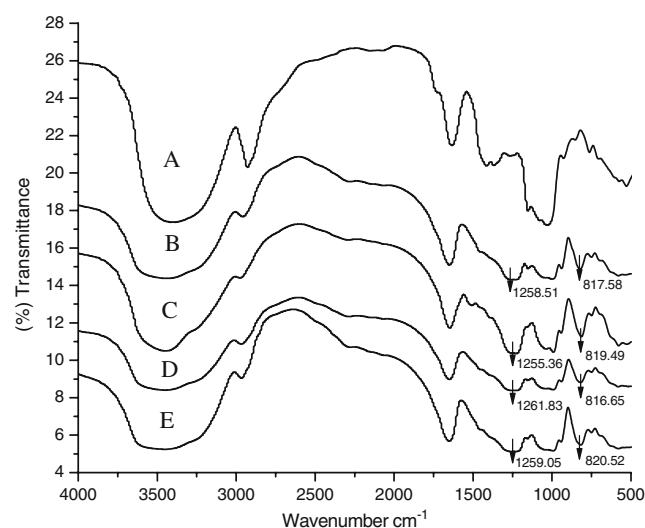
The inhibition rate (IR) was calculated according to the formula below:

#### Growth inhibition rate (%)

$$= (1 - A_{\text{drug-blank}}/A_{\text{control-blank}} \times 100)$$

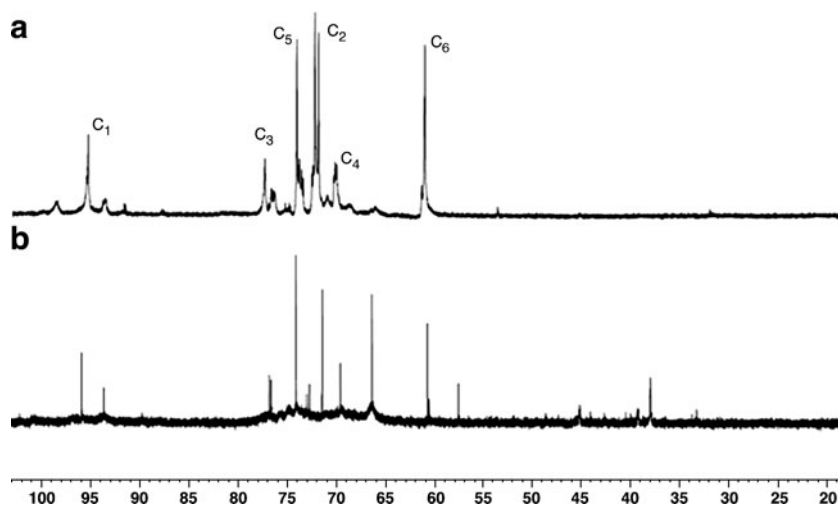
#### Cell-cycle analysis

The effects of sulfated derivatives on Hela cell-cycle were assessed by flow cytometry. The cells (5 × 10<sup>3</sup> cells/well) were



**Fig. 1** FT-IR spectra of POP1 and its sulfated derivatives. (A) POP1, (B) POP1-s1, (C) POP1-s2, (D) POP1-s3, (E) POP1-s4

**Fig. 2**  $^{13}\text{C}$  NMR (600 MHz) spectra of POP1 and its sulfated derivatives. (A) POP1; (B) POP1-s1



incubated on a 6-well plate with POP1-s3 for 48 h. After the incubation, the cells were washed with phosphate-buffered saline (PBS) twice, fixed in 70% cold ethanol, and stored at  $-4^{\circ}\text{C}$  overnight. Prior to the analysis, the fixed cells were washed with PBS twice and were stained with 200  $\mu\text{l}$  of 1.12 mg/ml propidium iodide (PI) (Bender MedSystems Inc., Burlingame, CA). The stained cells were transferred to flow tubes by passing through nylon mesh with a pore size of 40  $\mu\text{m}$ . Flow cytometric analysis was performed on a flow cytometer (EPICS XL, U.S. COULTER).

## Results and discussion

### Characterization of sulfated derivatives

The molecular masses of sulfated derivatives with different DS were investigated (Table 1). It showed that the molecular masses of sulfated derivatives increased from 31.3 to 48.5 kDa, and DS increased from 1.01 to 1.81. It implied that hydroxyl groups were substituted efficiently by sulfated groups on the polysaccharide.

After sulfated modification, the molecular mass of polysaccharide had not been decreased obviously except

POP1-s5. It might be the reason of adding DCC in synthesis reaction, and making the reaction achieve dynamic equilibrium fast, which decreased the depolymerization of polysaccharide. DCC worked as a dehydration agent to afford dicyclohexylurea in the reaction.

The FT-IR spectra of the native POP1 and sulfated derivatives were shown in Fig. 1. Compared with POP1, two characteristic absorption bands appeared in the FT-IR spectra of sulfated derivatives, one at near  $1258\text{ cm}^{-1}$  describing an asymmetrical S=O stretching vibration and the other at near  $815\text{ cm}^{-1}$  representing a symmetrical C–O–S vibration. They indicated that POP1-s1, POP1-s2, POP1-s3 and POP1-s4 were successfully sulfated.

The sulfated position on the polysaccharide was usually determined by  $^{13}\text{C}$  NMR spectrum. The  $^{13}\text{C}$  NMR spectra for the native POP1 and its derivative (POP1-s1) were shown in Fig. 2. The  $^{13}\text{C}$  NMR spectrum of the native POP1 exhibited six signals around 96.4, 78.2, 74.9, 73.1, 70.9, and 60.8 ppm, attributed to C-1, C-3, C-5, C-2, C-4, and C-6, respectively. Compared with the native POP1, there were several new signals caused by sulfated groups in POP1-s1. The peak at 66.8 was assigned to the signal of C-6s; the peak at 72.7 was assigned to the signal of C-2s. The peak at 61.6 was weakened, which indicated that C-6 had been substituted by the sulfated group, but C-2 had

**Table 2** Growth inhibition of POP1 and its sulfated derivatives against HepG2 cells *in vitro*

Group	Result	$\text{C}^{\text{b}}$ ( $\mu\text{g}/\text{ml}$ )				
		100	200	500	1000	2000
POP1	IR <sup>a</sup> (%)	$-27.2 \pm 2.4$	$-27.8 \pm 1.7$	$-24.3 \pm 2.3$	$-27.9 \pm 1.9$	$-8.9 \pm 1.3$
POP1-s1	IR (%)	$-15.2 \pm 0.9$	$-4.1 \pm 1.8$	$5.3 \pm 3.6$	$10.0 \pm 0.8$	$24.1 \pm 2.1$
POP1-s2	IR (%)	$6.2 \pm 1.7$	$5.4 \pm 2.3$	$10.2 \pm 1.2$	$19.7 \pm 2.9$	$28.0 \pm 3.1$
POP1-s3	IR (%)	$17.6 \pm 2.2$	$32.0 \pm 1.9$	$35.4 \pm 2.2$	$44.8 \pm 2.4$	$51.3 \pm 1.3$
POP1-s4	IR (%)	$3.0 \pm 1.4$	$7.4 \pm 1.2$	$8.2 \pm 2.6$	$13.8 \pm 1.2$	$39.7 \pm 0.8$
POP1-s5	IR (%)	$-1.2 \pm 0.7$	$3.9 \pm 1.3$	$6.8 \pm 1.6$	$19.4 \pm 1.8$	$27.5 \pm 1.6$

<sup>a</sup> IR, growth inhibition rate

<sup>b</sup> C, concentration of POP1 and its sulfated derivatives

**Table 3** Growth inhibition of POP1 and its sulfated derivatives against Hela cells *in vitro*

Group	Result	C <sup>b</sup> (μg/ml)				
		100	200	500	1000	2000
POP1	IR <sup>a</sup> (%)	-1.8±1.2	-2.1±0.9	6.3±2.3	23.5±1.2	21.5±2.1
POP1-s1	IR (%)	18.4±3.1	31.6±1.3	41.3±2.8	43.7±1.8	48.2±1.9
POP1-s2	IR (%)	15.6±1.7	22.5±1.7	44.3±1.9	53.4±3.2	67.7±2.8
POP1-s3	IR (%)	9.4±1.5	13.4±2.2	39.2±2.2	52.6±2.6	62.5±1.1
POP1-s4	IR (%)	3.5±0.7	6.3±1.4	27.2±2.6	30.1±2.0	35.2±1.8
POP1-s5	IR (%)	4.2±1.2	14.8±1.1	24.9±1.8	27.2±2.0	35.4±1.9

<sup>a</sup> IR, growth inhibition rate

<sup>b</sup> C, concentration of POP1 and its sulfated derivatives

been partially substituted. Because their strong peaks existed in the NMR spectra, the peak at 72.7 for C-2s was much weaker than that of C-6s at 66.8. We could conclude that the C-6 position was more active than the C-2 position due to the steric hindrance. Furthermore, a new peak appeared at 93.9, and the native C-1 peak at 96.4 became weaker in NMR spectra of POP1-s1. It was known that the signal of C-1 splitted if hydroxyl group on C-2 was functionalized. It could be explained by the fact that C-2 had been substituted, which could influence the adjacent C-1 to split into two peaks. New peaks at 65–75 ppm meant sulfation of other positions occurred besides C-6 and C-2. As the consequence of a heterogeneous reaction, sulfated groups were distributed unevenly.

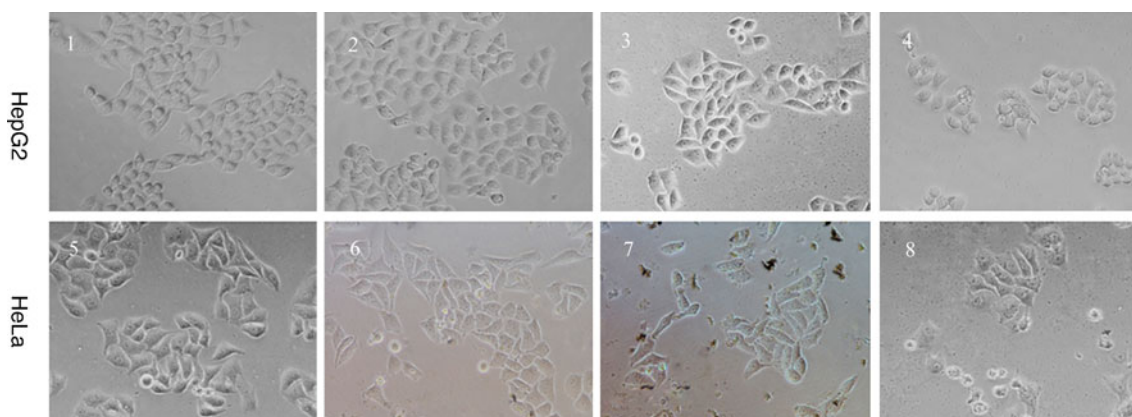
#### Growth inhibition of sulfated derivatives on HepG2 cells

It was reported that sulfated polysaccharides might lead to antitumor activity [3, 7, 20]. In the present study, the growth inhibitory effects of POP1 and the five sulfated derivatives against HepG2 cells *in vitro* were first examined in Table 2. The native POP1 was determined to have no cytotoxicity against HepG2 cells, but all sulfated derivatives showed cytotoxicity. In the concentration range from 100 μg/ml to

2,000 μg/ml, POP1-s3 and POP1-s4 inhibited significantly the growth of HepG2 cells. Furthermore, POP1-s3 exhibited significantly higher inhibition ratios than other fractions at all concentrations. Especially, at the concentration of 2,000 μg/ml, the inhibition activity of POP1-s3 was the highest with an inhibition ratio of 51.3%±1.3%. This suggested that sulfated modification could improve cytotoxicity of POP1 on tumor cells. However, POP1-s5 exhibited lower inhibition activity from 100 μg/ml to 2,000 μg/ml.

#### Growth inhibition of sulfated derivatives on Hela cells

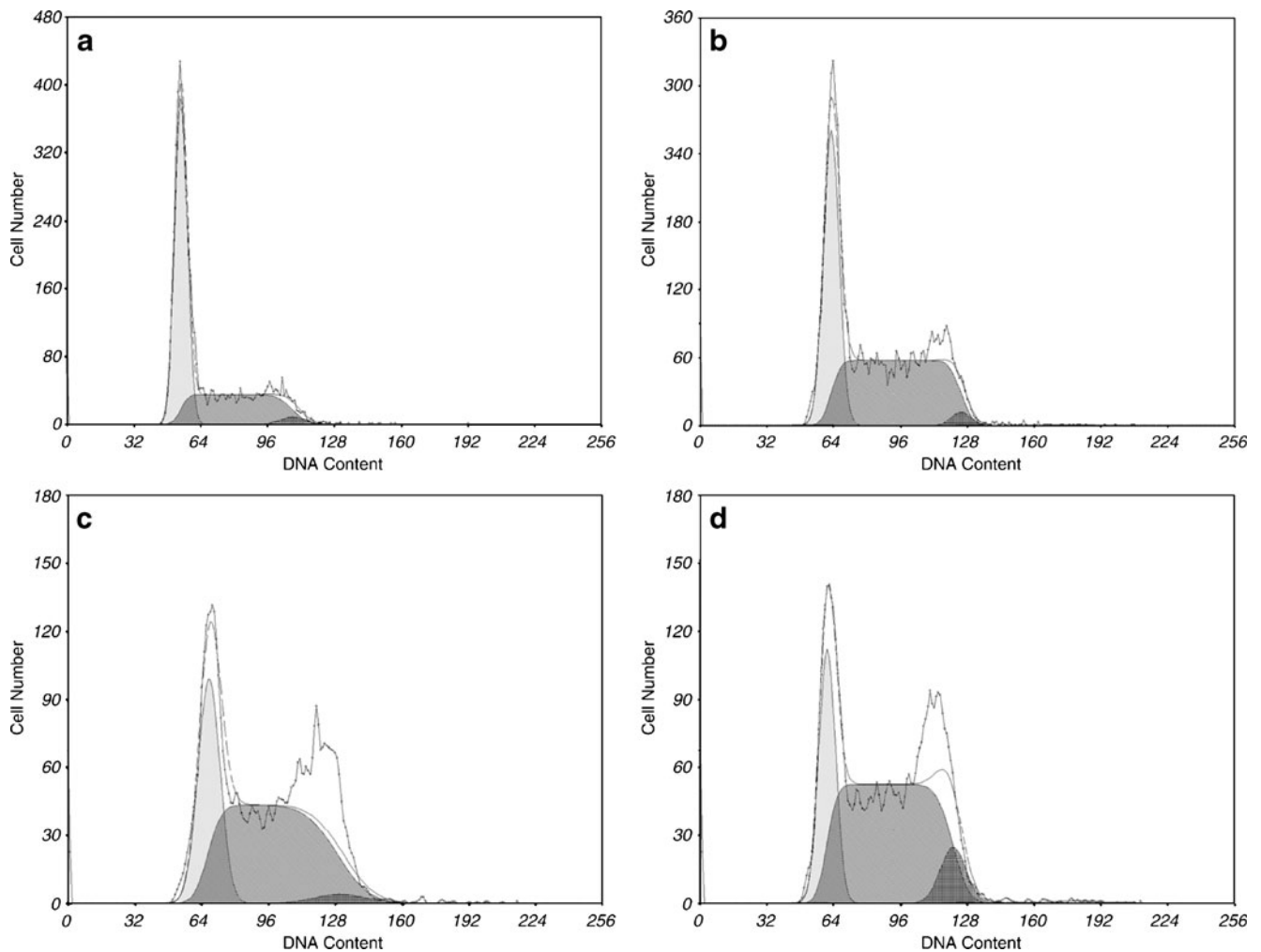
The inhibition ratios of POP1 and four fractions against Hela cells *in vitro* were summarized in Table 3. The results indicated that POP1 exhibited lower inhibition activity against Hela cells, but POP1-s1, POP1-s2 and POP1-s3 exhibited much stronger inhibition ratios against the growth of Hela cells. It indicated that the sulfated groups could contribute to direct cytotoxicity of POP1 on tumor cells *in vitro*. However, it did not seem that polysaccharide with higher sulfate content exhibited stronger cytotoxicity on tumor cells. In our experiment, POP1-s2 with DS of 1.30 showed the highest activity with inhibition ratio of 67.7%±2.8% at 2,000 μg/ml, and the effect was in a dose-dependent



**Fig. 3** Morphological characteristics of HepG2 cells and Hela cells treated with POP1-s3. HepG2 cells and Hela cells were seeded into tissue culture flasks and treated with increasing concentrations of POP1-s3. 1-4 was HepG2 cells: (1) Control, (2)–(4) Concentration of

POP1-s3 was 100, 1000 and 2,000 μg/ml, respectively. 5–8 were Hela cells: (5) Control, (6)–(8) Concentration of POP1-s3 was 100, 1000 and 2,000 μg/ml, respectively. All cells were photographed after 48 h drug treatment





**Fig. 4** Cell cycle analysis of HeLa cells treated with POP1-s3. **a** Control; **b–d** Concentration of POP1-s3 was 100, 1000 and 2,000  $\mu\text{g/ml}$ , respectively

manner at the concentration ranging from 100  $\mu\text{g/ml}$  to 2,000  $\mu\text{g/ml}$ , suggesting that a moderate DS of sulfated derivatives was necessary for a high cytotoxicity on tumor cells *in vitro*. However, POP1-s5 had been found lower inhibition activity from 100  $\mu\text{g/ml}$  to 2,000  $\mu\text{g/ml}$ . We believed that POP1-s5 might be depolymerized due to longer reaction time under strong acid conditions, and the structure of polysaccharide was damaged severely.

#### Changes about HepG2 cells and HeLa cells morphological features

Figure 3 showed that HepG2 cells and HeLa cells treated with POP1-s3 were accompanied by morphological changes. Two days after treatment with POP1-s3, cell numbers decreased visibly with POP1-s3 concentration increasing from 100  $\mu\text{g/ml}$  to 2,000  $\mu\text{g/ml}$ . Moreover, the distinctive morphological features of cells included detachment and cell shrinkage. After cells were killed, cytoplasm

came out. We believed that the death of HepG2 cells and HeLa cells induced by POP1-s3 were very obvious *in vitro*.

#### Cell-cycle analysis of HeLa cells by POP1-s3

According to cytotoxicity studies described, a cell cycle analysis was performed for POP1-s3 to determine whether these sulfated derivatives influenced the progression of cell

**Table 4** Cell cycle perturbations induced by POP1-s3

Group	concentration ( $\mu\text{g/ml}$ )	Cell cycle phase (%)		
		G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M
POP1-s3	100	39.89%	57.94%	2.16%
	1000	31.79%	65.26%	2.95%
	2000	24.22%	67.55%	8.23%
Control	0	59.54%	37.98%	2.48%

cycle of the HeLa cells. As shown in Fig. 4 and Table 4, control cells were 59.54%, 37.98% and 2.48% on the cell-cycle phases (G<sub>0</sub>/G<sub>1</sub>, S, and G<sub>2</sub>/M), respectively. After treatment with POP1-s3 for 48 h at the concentration ranging from 100 µg/ml to 2,000 µg/ml, an accumulation of S phase was observed. The percentage of HeLa cells in the S phase increased obviously at the ranging from 57.94% to 67.55%, which indicated apoptotic phenomenon, as well as led to the S phase arrest. These results suggested that sulfated derivatives could inhibit the growth of HeLa cells through cell-cycle arrest in the S phase and induce the apoptosis.

## Conclusions

Four sulfated derivatives of POP1 from *Portulaca oleracea* L. were prepared by chlorosulfonic acid method using DCC as a dehydration-condensation agent. FT-IR spectra and <sup>13</sup>C NMR spectra indicated that the sulfation reaction had actually occurred. Depending on the reaction conditions, sulfated derivatives showed different DS ranging from 1.01 to 1.81, and different Mw ranging from 41.4 to 48.5 KDa. It implied that hydroxyl groups were substituted efficiently by sulfated groups on the polysaccharide with a little degradation.

Sulfated derivatives exhibited obvious inhibition effects on HepG2 cells and HeLa cells *in vitro*, compared with POP1-s5. Flow cytometric studies revealed that treatment of sulfated derivatives with HeLa cells could mediate the cell-cycle arrest in the S phase. It was determined that the sulfation of the native POP1 could improve its cytotoxicity on tumor cells. We think sulfated derivatives of POP1 will have an assistant role with anticancer agent in the treatment of cancer in the future.

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## References

- Alban, S., Franz, G.: Gas-liquid chromatography-mass spectrometry analysis of anticoagulant active curdlan sulfates. *J. Thromb. Haemost.* **20**, 152–158 (1994)
- Chaidedgumjorn, A., Toyoda, H., Woo, E.R., Lee, K.B., Kim, Y. S., Toida, T.: Effect of (1-3)- and (1-4)-linkages of fully sulfated polysaccharides on their anticoagulant activity. *Carbohydr. Res.* **337**, 925–933 (2002)
- Du, Y.G., Gu, G.F., Hua, Y.X., Wei, G.H., Ye, X.S., Yu, G.L.: Synthesis and antitumor activities of glucan derivatives. *Tetrahedron* **60**, 6345–6351 (2004)
- Garti, N., Slavin, Y., Aserin, A.: Surface and emulsification properties of a new gum extracted from *Portulaca oleracea* L. *Food Hydrocolloids* **13**, 145–155 (1999)
- Garti, N., Aserin, A., Slavin, Y.: Competitive adsorption in O/W emulsions stabilized by the new *Portulaca oleracea* hydrocolloid and nonionic emulsifiers. *Food Hydrocolloids* **13**, 139–144 (1999)
- Ito, M., Baba, M., Sato, A., Pauwels, R., Clercq, E., Shigeta, S.: Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) *in vitro*. *Antivir. Res.* **7**, 361–367 (1987)
- Lin, Y.L., Zhang, L.N., Chen, L., Jin, Y.: Molecular mass and antitumor activities of sulfated derivatives of β-glucan from *Poria cocos* mycelia. *Int. J. Biol. Macromol.* **34**, 231–236 (2004)
- Liu, Y.H., Liu, C.H., Tan, H.N., Zhao, T., Cao, J.C., Wang, F.S.: Sulfation of a polysaccharide obtained from *Phellinus ribis* and potential biological activities of the sulfated derivatives. *Carbohydr. Polym.* **77**, 370–375 (2009)
- Lu, Y., Wang, D.Y., Hu, Y.L., Huang, X.Y., Wang, J.M.: Sulfated modification of epimedium polysaccharide and effects of the modifiers on cellular infectivity of IBDV. *Carbohydr. Polym.* **71**, 180–186 (2008)
- Qiu, H., Tang, W., Tong, X., Ding, K., Zuo, J.: Structure elucidation and sulfated derivatives preparation of two α-D-glucans from *Gastrodia elata* Bl. and their anti-dengue virus bioactivities. *Carbohydr. Res.* **342**, 2230–2236 (2007)
- Shi, B.J., Nie, X.H., Chen, L.Z., Liu, Y.L., Tao, W.Y.: Anticancer activities of a chemically sulfated polysaccharide obtained from *Grifola frondosa* and its combination with 5-fluorouracil against human gastric carcinoma cells. *Carbohydr. Polym.* **68**, 687–692 (2007)
- Talarico, L.B., Pujol, C.A., Zibetti, R.G.M., Faria, P.C.S., Noseda, M.D., Duarte, M.E.R., Damonte, E.B.: The antiviral activity of sulfated polysaccharides against dengue virus is dependent on virus serotype and host cell. *Antivir. Res.* **66**, 103–110 (2005)
- Tao, J., Hu, Q.X., Yang, J., Li, R.Y., Li, X.Y., Lu, C.P.: *In vitro* anti-HIV and HSV activity and safety rutin sulfate as a microbicide candidate. *Antivir. Res.* **75**, 227–233 (2007)
- Tian, G.Y., Li, S.T., Song, M.L., Zheng, M.S., Li, W.: Synthesis of *Achyranthes bidentata* polysaccharide sulfate and its antiviral activity. *Acta Pharmacol. Sin. Chin.* **30**(2), 107–111 (1995)
- Tommonaro, G., Segura Rodriguez, C.S., Santillana, M., Immirzi, B., De Prisco, R., Nicolaus, B., Poli, A.: Chemical composition and biotechnological properties of a polysaccharide from the peels and antioxidative content from the pulp of *Passiflora ligularis* fruits. *J. Agric. Food Chem.* **55**, 7427–7433 (2007)
- Vogl, H., Paper, D.H., Franz, G.: Preparation of a sulfated linear (1→4)-β-D-galactan with variable degrees of sulfation. *Carbohydr. Polym.* **41**, 185–190 (2000)
- Wang, S.C., Annie Bligh, S.W., Zhu, C.L., Shi, S.S., Wang, Z.T., Hu, Z.B., Crowder, J.: Sulfated β-Glucan derived from *oat bran* with potent anti-HIV activity. *J. Agric. Food Chem.* **56**, 2624–2629 (2008)
- Wang, J.G., Zhang, L.N., Yu, Y.H., Peter, C.K.: Enhancement of antitumor activities in sulfated and carboxymethylated polysaccharides of *Ganoderma lucidum*. *J. Agric. Food Chem.* **57**, 10565–10572 (2009)
- Wang, L., Li, X.X., Chen, Z.X.: Sulfated modification of the polysaccharides obtained from defatted rice bran and their antitumor activities. *Int. J. Biol. Macromol.* **44**, 211–214 (2009)
- Williams, D.L., Pretus, H.A., McNamee, R.B., Jones, E.L., Ensley, H.E., Browder, W.: Development, physicochemical characterization and preclinical efficacy evaluation of a water soluble glucan sulfate derived from *Saccharomyces cerevisiae*. *Immunopharmacology* **22**, 139–156 (1991)
- Xing, R., Liu, S., Yu, H., Guo, Z., Li, Z., Li, P.: Preparation of high-molecular weight and high-sulfate content chitosans and

- their potential antioxidant activity *in vitro*. Carbohydr. Polym. **61**, 148–154 (2005)
22. Xu, G.Y., Yan, J., Guo, X.Q., Liu, W., Li, X.G., Gou, X.J.: The betterment and apply of phenol-sulphate acid method. Food Science in Chinese **26**(8), 342–345 (2005)
23. Yang, X.B., Gao, X.D., Han, F., Tan, R.X.: Sulfation of a polysaccharide produced by a marine filamentous fungus *Phoma herbarum* YS4108 alters its antioxidant properties *in vitro*. Biochim. Biophys. Acta **1725**, 120–127 (2005)
24. Yang, X.B., Zhao, Y., Yang, Y., Ruan, Y.: Isolation and characterization of immunostimulatory polysaccharide from an herb tea, *Gynostemma pentaphyllum* Makino. J. Agric. Food Chem. **56**, 6905–6909 (2008)
25. Yanga, J.H., Dua, Y.M., Yan, W.N., Li, T.Y., Hub, L.: Sulfation of Chinese lacquer polysaccharides in different solvents. Carbohydr. Polym. **52**, 397–403 (2003)
26. Zhang, M., Zhang, L.N., Wang, Y.F., Cheungb, P.C.K.: Chain conformation of sulfated derivatives of  $\beta$ -glucan from sclerotia of *Pleurotus tuber-regium*. Carbohydr. Res. **338**, 2863–2870 (2003)
27. Zhu, J.F., Wu, M.: Characterization and free radical scavenging activity of rapeseed meal polysaccharides WPS-1 and APS-2. J. Agric. Food Chem. **57**, 812–819 (2009)