Supplementary data

1. Monosaccharide composition analysis

1) Method

Monosaccharide composition was analyzed by PMP-HPLC method. In brief, the sample (1-2 mg) was completely hydrolyzed with 2 M trifluoroacetic acid (TFA) at 110 ℃ for 4 h. The hydrolysate was dried under vacuum, and then derivatized with 50 μL 1-phenyl-3-methyl-5-pyrazolone (PMP) solution (0.5 M in methanol) and 50 μL of 0.6 M NaOH at 70 ℃ for 100 min. The reaction was stopped by neutralization with 100 μL of 0.3 M HCl and 900 μL distilled water, followed by extraction with chloroform (1 mL, 3 times). The extract solution was applied to HPLC analysis with a Fortis C18 column (5 μm, 4.6 × 250 mm) on an Agilent 1200 instrument with a G1362A Binpump and a G1314F UV detector.

2) Result

The monosaccharide composition of S1 was shown in Table S1. Glucose (40.3%), Glucuronic acid (31.6%) and Galactose (17.2%) were major components, while Manose (1.7%), Galacturonic acid (3.2%), Xylose (0.9%), Arabinose (3.3%) and Fucose (2.0%) are minor residues in S1.

2. Primers used for qPCR

Real-time quantitative PCR primer sequences were shown in Table S2.

Table S1 The monosaccharide composition of S1

monosaccharide	Man	GlcA	GalA	Glc	Gal	Xyl	Ara	Fuc
(%)	1.7	31.6	3.2	40.3	17.2	0.9	3.3	2.0

Table S2 Real-time quantitative PCR primer sequences

Primers	Forward sequence	Reverse sequence
CREB	AGCAGCCACTCAGCCGGGTA	ACGTCTCCAGAGGCAGCTTGAA
Runx2	CCGGAATGCCTCTGCTGTTATGA	ACTGAGGCGGTCAGAGAACAAACT
EGFR	TACCATGCAGAAGGAGGCAA	CAGACATCACTCTGGTGGGT
NF-κB	AGCAAATAGACGAGCTCCGA	TCGGTAAAGCTGAGTTTGCG
SP1	TGGCAGCAGTACCAATGGC	CCAGGTAGTCCTGTCAGAACTT
SP3	GCGACAGGTGATTTGGCTTCT	TACTGCCCACTTGAAGTAGCA
FOXN2	AGAGAGCTGAAACCCCAGGAG	GCTGACTCACTGTCCACTAGAG
p300	GCCAGTTCCTTCCTCAGACT	GCTTGAGACACTGGAGCTTG
FGFR2	GGTGGCTGAAAAACGGGAAG	AGATGGGACCACACTTTCCATA
FGFR1	CCCGTAGCTCCATATTGGACA	TTTGCCATTTTTCAACCAGCG
VEGFR	GGCCCAATAATCAGAGTGGCA	CCAGTGTCATTTCCGATCACTTT
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA

3. Molecular weight analysis

1) Method

The molecular weight distribution of samples was determined by high performance gel permeation chromatography (HPGPC) on an Agilent 1260 series HPLC system equipped with a refractive index detector and two series-connect columns of KS-804 and KS-802 (8.0 mm × 300 mm; Shodex). The columns were calibrated by pullulans standards (180 Da, 667 Da, 6,000 Da, 11,300 Da, 21,700 Da, 48,800 Da, 113,000 Da, 210,000 Da, 393,000 Da, 805,000 Da; PL2090-0100, Varian). The sample (2.0 mg/mL, 20 μL) was eluted by 0.1 M NaNO3 with a flow rate of 0.6 mL/min at 40 ℃.

2) Result

The molecular distribution of S1 was shown in Fig. S1. The average molecular weight (MW) of the first peak at 21.8 min (I) was 100 kDa. The average MW of peaks at 25.5 min (II) and 26.9 min (III) was 12 kDa and 1740 Da, respectively. The peak at 29.3 min (IV) was solvent peak (sodium nitrate). Several peaks appeared after 30 min were components of small molecules.

4. Cell cytotoxicity assays

1) Method

For CCK-8 assay, BxPC-3 cells (3000 cells/well) were seeded into 96-well plates in triplicate and treated with different concentration of S1 for 72 h. 10 μL CCK-8 Solution added into each well and incubated with cells for 4 h and the absorbance was assessed at 450 nm using a microplate reader. For LDH assay, BxPC-3 cells (3000 cells/well) were seeded into 96-well plates in triplicate and treated with different concentration of S1 for 72 h. The supernatant was removed and 150 μl LDH releasing reagent was addede in each well. After incubated with cells for 1 h, 120 μl supernatant was transfered to a new 96-well plate and 60 μl LDH work detection was added in each well. After incubated with 30 min in dark at room temperature, then the absorbance was assessed at 490 nm using a microplate reader.

2) Result

As the CCK-8 assay result showed (Fig. S2-A), the cell cytotoxicity on BxPC-3 cell of S1 could reach to 90.2% at the concentration of 1.0 mg/mL. As the LDH assay result showed (Fig. S2-B), the cell cytotoxicity on BxPC-3 cell of S1 could reach to 59.2% at the concentration of 1.0 mg/mL.

5. The growth factor receptor and transcription factor mRNA levels analysis

1) Method

RNA was extracted using TRIzol reagent (Invitrogen, USA), cDNA was generated from 5 μg total RNA using M-MLV reverse transcriptase (TaKaRa, Japan). PCR was performed using an ABI system with PCR reagents (TaKaRa, Japan) according to manufacturer's instructions.

2) Result

As shown in Fig. S3, the expression of epidermal growth factor receptor (EGFR) and cyclic AMP (cAMP) response element binding protein (CREB) were significantly inhibited on mRNA level when treated with S1.

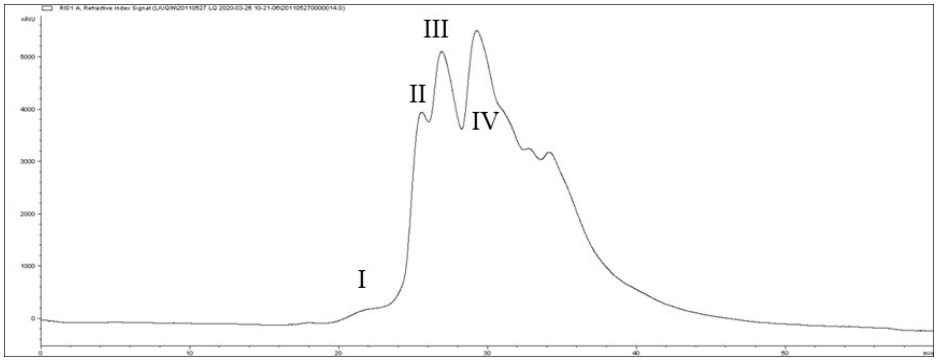

Figure S1. The HPGPC spectrum of S1

The molecular weight distribution of samples was determined by high performance gel permeation chromatography (HPGPC) on an Agilent 1260 series HPLC system equippd with a refractive index detector and two series-connect columns of KS-804 and KS-802 (8.0 mm × 300 mm; Shodex). The columns were calibrated by pullulans standards (180 Da, 667 Da, 6,000 Da, 11,300 Da, 21,700 Da, 48,800 Da, 113,000 Da, 210,000 Da, 393,000 Da, 805,000 Da; PL2090-0100, Varian). The sample (2.0 mg/mL, 20 μL) was eluted by 0.1 M NaNO3 with a flow rate of 0.6 mL/min at 40 °C.

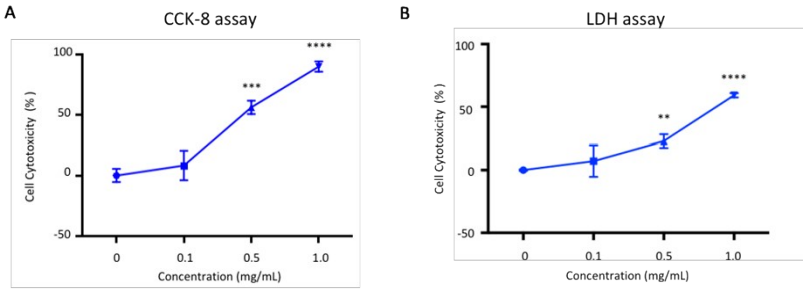

Figure S2. The cell cytotoxicity assay result using CCK-8 kit and LDH kit

A: The cells were treated with indicated concentration of S1 for 72h. Cell cytotoxicity of these cells was analysed by CCK-8 method. B: The cells were treated with indicated concentration of S1 for 72h. Cell cytotoxicity of these cells was analysed by LDH method. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

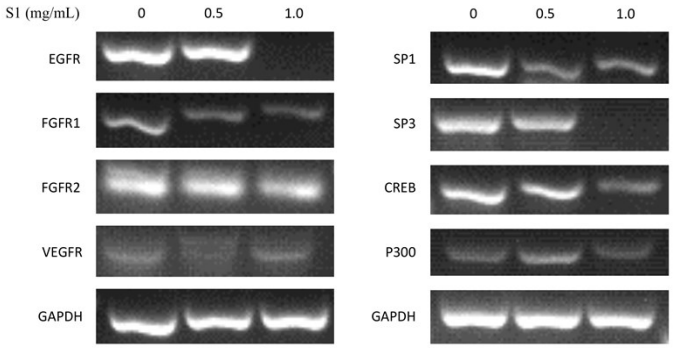

Figure S3. S1 decreased the expression of EGFR and CREB on mRNA level in BxPC-3 cell

Growth factor receptor and transcription factor mRNA levels were detected after treatment with S1 at the indicated concentration using PCR.