Triterpenoid Saponins from the Seeds of *Celosia argentea* and Their Anti-inflammatory and Antitumor Activities

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Three new triterpenoid saponins, named celosin E (1), celosin F (2) and celosin G (3), together with a known compound cristatain (4), were isolated from the seeds of *Celosia argentea* L. (Amaranthaceae). All the isolated compounds were obtained for the first time from this plant. The structures of new compounds were characterized on the basis of extensive NMR experiments and mass spectrometry data. The antitumor and anti-inflammatory activities of the four compounds were tested *in vitro*.

Key words Amaranthaceae; *Celosia argentea*; triterpenoid saponin; antitumor; anti-inflammatory

Celosia L., one category of Amaranthaceae, is widely distributed in Africa, America, the subtropical zone and temperate zone of Asia.[1] There are three species in China. They are *Celosia argentea* L., *Celosia cristata* L. and *Celosia taitoensis* Hayata. We have been studying for the seeds of two species *C. argentea* and *C. cristata* in our laboratory. Semen celosiae, the seeds of *C. argentea*, has been traditionally used for treatment of hepatitis, caligo corneae, hypertention and sarcoptidosis.[2] The crude EtOH extract of Semen celosiae possesses antipyretic, antispasmodic, anticancer, diuretic, anti-inflammatory and antibacterial activities.[3] However, there wasn't any report about the seeds of *C. cristata*. In our laboratory research for bioactive compounds from Semen celosiae and the seeds of *C. cristata*, β-sitosterol, palmitic

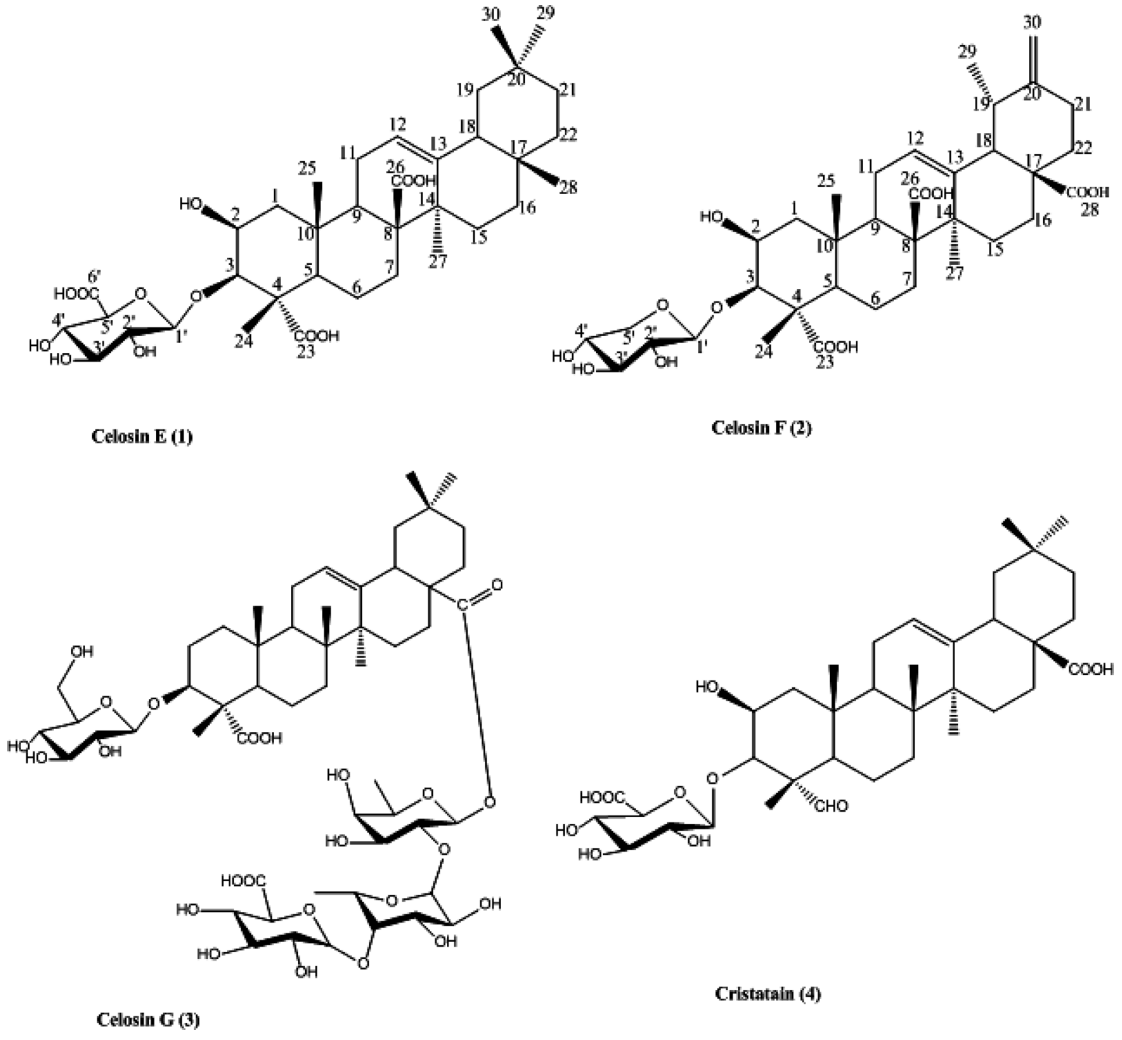

Fig. 1. Structures of Compounds **1**—**4**

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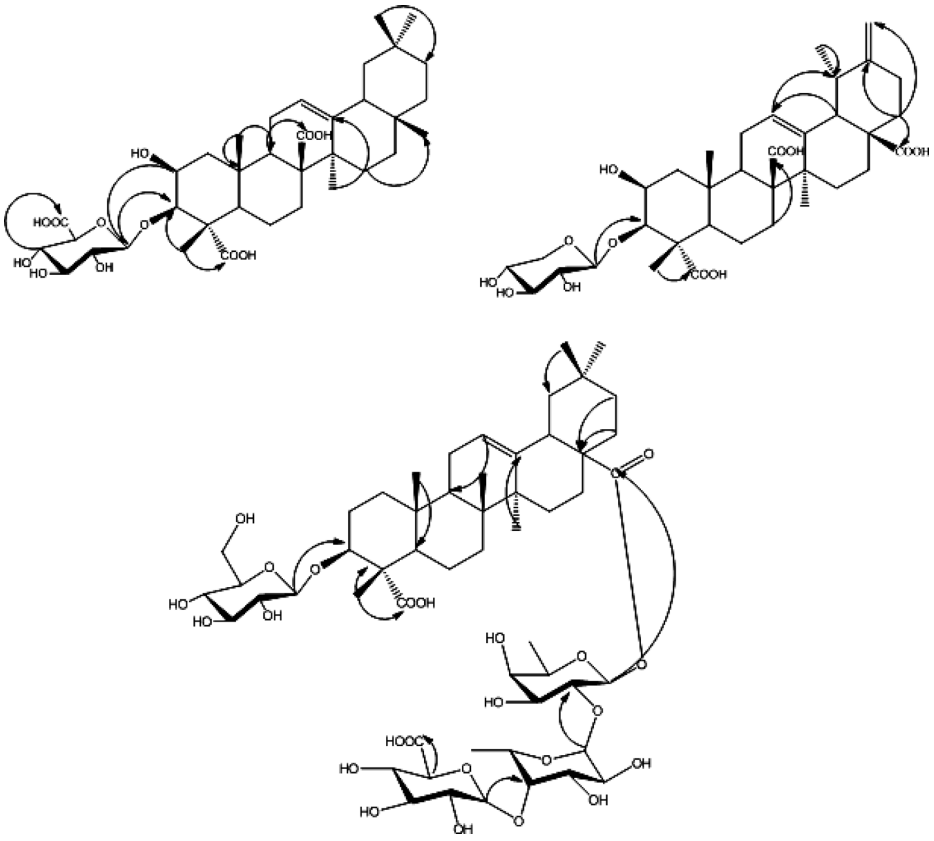

Fig. 2. The Main HMBC Correlations of Compounds **1**—**3**

acid, stigmasterol, daucosterol, oleanolic acid and four saponins named celosin A, celosin B, celosin C and celosin D were isolated from Semen celosiae and it was demonstrated that the saponins' hepatoprotective effect was significant[4—6]; meanwhile, 4-hydroxyphenethyl alcohol, kaempferol, quercetin, β-sitosterol, 2-hydroxyoctadecanoic acid, stigmasterol and a hepatoprotective saponin named cristatain were separated from the seeds of *C. cristata*.[7,8] In this paper, the isolation and characterization of three new and one known triterpenoid saponins (Fig. 1) from Semen celosiae and their antitumor and anti-inflammatory activities *in vitro* are reported.

Results and Discussion

Compound **1** was obtained as a white amorphous powder, and its molecular formula, $C_{36}H_{54}O_{12}$, was deduced from the high resolution-electrospray ionization-mass spectra (HR-ESI-MS): *m/z* 679.3675 $[M+H]^+$ (Calcd 679.3694), indicating the presence of ten unsaturation degrees. There were 36 carbon signals in ^{13}C-NMR, among which 30 carbon signals were assigned to the aglycone, and 6 carbon signals to the saccharide moiety. The ^{1}H-NMR of **1** indicated that the aglycone contained six methyl protons at δ 0.65, 0.72, 0.76, 0.96, 1.12, and 1.38 (each 3H, s), and one olefinic proton at δ 5.11. Correspondingly, six methyl carbons at δ 18.0, 33.9, 24.4, 26.9, 17.4, and 14.3 and two olefinic carbons at δ 123.6 and 145.5 exhibited in the ^{13}C-NMR spectrum, respectively. Additionally, three carbonyl carbons at δ 175.5, 181.8, and 182.2 exhibited in the ^{13}C-NMR spectrum, respectively. The presence of the 2-OH group was indicated by a signal at δ 70.9 in the downfield region of the ^{13}C-NMR spectrum.[8] In heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 2), the methyl proton at δ 1.38 (H-24) showed a long-range correlation with the carbonyl carbon at δ 182.2 (C-23), a methine proton at δ 2.76 (H-9) correlation with the carbonyl carbon at δ 181.8 (C-26) and a methine proton at δ 4.27 (H-4′) correlation with the carbonyl carbon at δ 175.5 (C-6′).

On acid hydrolysis with 2 M trifluoroacetic acid (TFA), **1** afforded sugar moieties that identified as D-glucuronopyranose (GlcA) based on the GC-MS analysis of the aldononitrile peracetate and the data of the literature.[9] The saccharide of **1** was confirmed by the presence of an anomeric proton signal at δ 4.44 (d, J=7.5 Hz) [with d, J=7.5 Hz, indicating its β configuration] in the ^{1}H-NMR spectrum and assigned to D-GlcA. The ^{1}H–^{1}H correlation spectroscopy (COSY) experiment allowed the sequential assignment of most of the resonances for the sugar ring, staring from the easily distinguished signals due to anomeric proton. The assignment of their corresponding carbons was made by heteronuclear single quantum coherence (HSQC) spectrum. The sugar was attached to C-3 of the oleanane residue, which was deduced from the HMBC correlations between H-1 (δ 4.44) of GlcA unit and C-3 (δ 87.0). The relative stereochemistry of **1** was established with aid of a nuclear Overhauser effect spectroscopy (NOESY) experiment. Thus, **1** was elucidated as 2-hydroxy-12,13-ene-23,26-dicarboxy-3-*O*-[β-D-glucuronopyranosyl]-oleanene and named celosin E.

Compound **2** was obtained as a white amorphous powder, and its molecular formula, $C_{35}H_{50}O_{12}$, was deduced from the HR-ESI-MS: *m/z* 661.3255 $[M-H]^-$ (Calcd 661.3224), indicating the presence of eleven unsaturation degrees. There

Table 1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) Data for Compounds **1**, **2** and **3** (**1** and **2** in CD_3OD; **3** in C_5D_5N. δ in ppm and J in Hz)

Positon	**1**		**2**		**3**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.93 (d, 13.5)	45.1	1.18 (d, 6.5)	44.9	1.35 (s), 2.36 (d, 12.5)	44.6
2	4.27 (s)	70.9	4.18 (s)	71.3	2.11 (m)	32.5
3	4.07 (s)	87.0	3.98 (s)	86.7	4.80 (d, 3.5)	86.3
4		53.6		53.6		53.1
5	1.51 (d, 8.5)	53.3	1.48 (m)	53.3	2.15 (m)	52.8
6	1.65 (m), 0.83 (d, 14)	29.0	1.06 (s), 1.58 (m)	21.9	1.86 (m), 1.72 (m)	21.2
7	1.07 (s)	30.9	2.03 (m)	31.1	1.30 (m)	34.1
8		37.6		37.7		40.3
9	2.77 (dd, 13.5, 2.5)	42.9	2.43 (t, 13.5)	43.0	1.82 (m)	48.9
10		41.1		41.2		37.0
11	1.77 (m)	24.8	1.62 (m)	24.4	1.00 (s), 2.17 (m)	23.8
12	5.11 (s)	123.6	5.23 (s)	124.3	5.50 (s)	122.9
13		145.5		144.8		144.9
14		43.2		43.3		42.4
15	1.52 (s)	21.9	1.18 (d, 6.5)	30.7	1.99 (s)	23.5
16	1.50 (s)	47.4	1.44 (m), 1.73 (m)	39.3	2.11 (m)	32.5
17		47.7		47.9		47.2
18		49.8	2.62 (dd, 18.0, 4.0)	49.1	3.19 (dd, 14.5, 4.0)	42.3
19	1.17 (s)	35.1	2.43 (t, 13.5)	43.0	1.82 (m), 1.32 (s)	46.5
20		31.8		149.9		31.1
21	1.41 (s)	33.8	1.18 (s), 1.43 (m)	33.8	1.49 (d, 9.0)	34.4
22		34.0	2.75 (d, 11.0), 1.95 (m)	42.9	2.17 (m), 1.35 (s)	28.4
23		182.2		182.1		180.3
24	1.38 (s)	14.3	1.30 (s)	13.9	2.07 (s)	14.3
25	0.65 (s)	18.0	1.18 (s)	17.3	1.65 (s)	17.0
26		181.8		181.9	1.07 (s)	17.6
27	0.96 (s)	26.9	0.73 (s)	17.8	1.33 (s)	26.2
28	1.12 (s)	17.4		181.1		176.8
29	0.72 (s)	33.9	1.09 (s)	26.7	1.35 (s)	33.4
30	0.76 (s)	24.4	4.51 (s)	107.5	1.00 (s)	23.9
3-O-GlcA						
1	4.44 (d, 7.5)	105.5				
2	3.32 (t, 8.0)	75.3				
3	3.45 (m)	77.9				
4	4.27 (s)	70.9				
5	3.55 (m)	73.9				
6		175.5				
3-O-Xyl						
1			4.31 (d, J=7.5)	105.5		
2			3.19 (m)	75.0		
3			3.25 (m)	77.5		
4			3.39 (m)	73.5		
5			3.45 (t, 6.5)	62.9		
3-O-Glc						
1					5.28 (d, 7.5)	106.2
2					4.01 (m)	75.0
3					4.24 (m)	77.7
4					4.02 (m)	78.6
5					4.26 (m)	71.1
6					4.32 (m)	67.6
28-O-Fuc						
1					6.11 (d, 8.0)	94.9
2					4.71 (m)	74.3
3					4.24 (m)	76.7
4					4.02 (m)	73.3
5					4.77 (m)	72.5
6					1.65 (s)	17.0
Fuc-2-Rha						
1					6.47 (br s)	101.5
2					4.91 (s)	71.9
3					4.77 (m)	72.5
4					4.40 (m)	85.5
5					4.85 (d, 3.0)	70.9
6					1.77 (d, 6.0)	18.7
Rha-4-glcA						
1					5.10 (d, 7.5)	107.8
2					4.13 (d, 5.5)	76.4
3					4.01 (m)	75.0
4					4.26 (m)	71.1
5					4.13 (dd, 14.5, 5.5)	78.9
6						170.7

were 35 carbon signals in ^{13}C-NMR, among which 30 carbons were assigned to the aglycone, and 5 carbons to the saccharide moiety. The ^{1}H-NMR of **2** indicated that the aglycone contained four methyl protons at δ 0.72 (H-27), 1.09 (H-29), 1.18 (H-25) and 1.29 (H-24) (each 3H, s), two olefinic protons at δ 5.23 (H-12) and 4.51 (H-30). Correspondingly, four methyl carbons were at δ 13.9 (C-24), 17.3 (C-25), 17.8 (C-27) and 26.7 (C-29), four olefinic carbons at δ 107.5 (C-30), 124.3 (C-12), 144.8 (C-13) and 149.9 (C-20). Additionally, three carbonyl carbons at δ 181.2 (C-28), 181.9 (C-26) and 182.1 (C-23) exhibited in the ^{13}C-NMR spectrum (Table 1), respectively. The presence of the 2-OH group was indicated by a signal at δ 71.3 in the downfield region of the ^{13}C-NMR spectrum. In HMBC spectrum, the methyl proton at δ 1.29 (H-24) showed long-range correlation with the carbonyl carbon at δ 182.2 (C-23), a methylene proton at δ 2.03 (H-7) was correlated with the carbonyl carbon at δ 181.9 (C-26) and a methylene proton at δ 2.74 (H-22) was correlated with the carbonyl carbon at δ 181.1 (C-28). The methylene proton at δ 2.74 (H-22) was also correlated with the carbonyl carbon at δ 149.9 (C-20) and 107.5 (C-30). It indicated that the aglycone might be one type of ursolic acid.[10] The sugar moiety of **2** were determined to be D-xylose[11] which was analyzed by GC-MS with the aldononitrile peracetate of standard sugar. A ^{1}H–^{1}H COSY experiment allowed analysis of their spin systems and assignments of their proton resonances. This was also confirmed by the monosaccharide anomeric proton signals at δ 4.31 (d, J=7.5 Hz) [with d, J=7.5 Hz, indicating its β configuration], showed correlation with the aglycone carbon at δ 86.7 (C-3) in the HMBC spectrum. The relative stereochemistry of **2** was established with aid of the NOESY experiment. Based on the above results, **2** was elucidated as 2-hydroxy-20,29-ene-23,26-dicarboxy-3-*O*-[β-D-xylopyranosyl]-ursolic acid and named celosin F.

Compound **3** was obtained as a white amorphous powder, and its molecular formula, $C_{54}H_{84}O_{24}$, was deduced from the HR-ESI-MS: *m*/*z* 1139.5248 $[M+Na]^+$ (Calcd 1139.5250), indicating the presence of thirteen unsaturation degrees. There were 54 carbon signals in ^{13}C-NMR, among which 30 carbons were assigned to the aglycone, and 24 carbons to the saccharide moiety. Most signals due to the aglycone of **3** were similar to celosin D.[6] On acid hydrolysis with 2 M TFA, **3** afforded sugar moieties that identified as D-glucose, D-fucose, L-rhamnose and D-glucuronopyranosyl in the relative proportions of 1 : 1 : 1 : 1 based on the GC-MS analysis of the aldononitrile peracetates of standard sugars. And the absolute configuration of sugars was further confirmed by consulted the literature.[11,12] This compound occurred as a C-3 and C-28 bisdesmosidic triterpene glycoside. In the NMR spectrum of compound **3**, four anomeric proton [δ_H 5.28 (d, J=7.5 Hz), 6.11 (d, J=8.0 Hz), 6.47 (br s), 5.10 (d, J=7.5 Hz)] and four anomeric carbon (δ_C 106.3, 94.9, 101.5, 107.8) signals were observed, corresponding to D-glucose, D-fucose, L-rhamnose and D-glucuronopyranosyl. In the HMBC spectrum, the main correlations from H-1 of glucose to C-3 and from H-1 of fucose to C-28 confirmed that the sugar units were located at C-3 and C-28 of the aglycon, respectively. Other correlations were observed between signals at δ_H 4.71 (H-2 of Fuc) and δ_C 101.5 (C-1 of Rha) and between δ_H 5.10 (H-1 of GlcA) and δ_C 85.5 (C-4 of Rha). The relative stereochemistry of **3** was established with aid of a NOESY experiment. Therefore, compound **3** was assigned as 23-carboxy-3-*O*-[β-D-glucopyranosyl]-28-*O*-[β-D-glucuronopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl]-oleanolic acid and named celosin G.

Compounds **1**, **2**, **3** and **4** were tested for their antitumor activities toward five human cancer cell lines (SHG44, HCT116, CEM, MDA-MB-435 and HepG2) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Table 2).

The four compounds were also detected for their ability to inhibit nitric oxide (NO) production by LPS-induced RAW 264.7 cells. The inorganic free radical NO, synthesized by a family of enzymes termed NO-synthase (NOS), acts as a host defense mechanism by damaging pathogenic DNA and also acts as a regulatory molecule with homeostatic activities.[13] However, excess production of NO, due to the reaction with superoxide in biological systems, gives rise to various diseases such as inflammation, carcinogenesis, and atherosclerosis.[14] Therefore, down-regulation of NO production may be of therapeutic benefit in various diseases induced by pathological levels of NO. In the present study, it was found that the four compounds had inhibiting nitric oxide production activity compared with the positive control indomethacin. The IC_{50} values was showed in Table 3. From these data, it can be concluded that the sequence of anti-inflammatory activities of these compounds was as follows: compound **4**>compound **1**>compound **3**>compound **2**>indomethacin (reference). Cell viability was also determined by application of the MTT method in order to evaluate whether inhibition of NO production was due to the cytotoxicity of these tested compounds. It was found that none of the concentrations used in the experiment was cytotoxic (cell viability >85%). The experiment results supported the following ideas: 1. It seemed that the structure of the aglycone es-

Table 2. Cytotoxic Activity of Compounds **1**—**4** against Five Human Cancer Cell Lines

Sample	IC_{50} (μg/ml)				
	SHG44	HCT116	CEM	MDA-MB-435	HepG2
1	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	>100
3	>100	>100	>100	>100	>100
4[b]	23.71±2.96	26.72±4.11	31.62±2.66	27.63±2.93	28.35±2.32
DOX[a]	0.117±0.024	0.176±0.015	0.00134±0.00013	0.0717±0.0162	0.116±0.025

a) Hydroxydaunomycin hydrochloride (DOX) as positive control. *b*) The data represent the mean plus S.D. of three independent experiments in which each compound concentration was tested in two replicate wells.

Table 3. IC_{50} Values of Compounds **1**—**4** and Indomethacin with Different Concentration

	1	2	3	4	Indomethacin
IC_{50} value (μmol/ml)	0.158	0.384	0.278	0.047	0.371

LPS: Negative control; indomethacin: positive control.

pecially the carbonyl group at C-23 and the hydroxyl group at C-2 played an important role in terms of anti-inflammatory activity against RAW 264.7 cells; 2. The anti-inflammatory activities of oleanene-type saponins were more available than ursanene-type ones. However, more extensive studies are required before a clear structure-activity relationship can be reached.

Experimental

General Experimental Procedures The IR spectra were obtained on a Bruker Vector 22 spectrometer with KBr pellets. ESI-MS and HR-ESI-MS were measured on Micromass Q-TOF and the Agilent 6538 Q-TOF mass spectrometer, respectively. The melting points were measured on a Yanaco micromelting point apparatus without correction. Gas chromatography analysis was done on an HP-5892 II with FID detector, and an HP-20M (Carbowx 20M) capillary column (25 m×0.32 mm×0.3 μm) was used. Column chromatography separations were carried out on D101 macroporous resin (Chemical Factory of Nankai University, Tianjin, P. R. China), Octadecyl silane (ODS) (50 mesh, AA12S50, YMC). The ^{1}H-NMR, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and HMBC spectra were recorded on a Bruker DMX-500 NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The preparative high-speed counter-current chromatography instrument (Model TBE-300B, Shanghai Tauto Biological Co., China), the constant-flow pump (Model TBP5002 Shanghai Tauto Biological Company, China), Model HX-1050 constant-temperature controller (Beijing Detianyou Technology, Beijing, China), evaporative light scattering detection (SEDEX 85 ELSD, France).

Plant Material The seeds (Semen celosiae) were collected in Bozhou, Anhui province of China in October 2004, and authenticated by Prof. Mei-li Guo, School of Pharmacy, Second Military Medical University (Shanghai, China). A voucher specimen (SMMU 04063) was deposited at the herbarium of Second Military Medical University of China.

Extraction and Isolation Dried Semen celosiae (10 kg) was ground to a coarse powder and extracted with 50% ethanol at room temperature after 24 h maceration. The extracted liquid was concentrated under reduced pressure to give an ethanol extract (3.6 kg). A portion of the ethanol extract (3.0 kg) was subjected to chromatographic separation over D101 MR (10 kg), eluted sequentially with a gradient of H_2O, 30%, 60% and 95% EtOH to give four fractions with the yields of 43.5 g, 55.8 g, 95.4 g and 15.8 g, respectively. The 30% EtOH fraction was chromatographed through ODS, eluted with a gradient of MeOH–H_2O (5 : 95→30 : 70→50 : 50, v/v) to afford three fractions. The second fraction was separated by high-speed counter-current chromatography using a solvent system of dichloromethane–methal–*n*-butanol–water (4 : 3 : 0.3 : 2, v/v/v/v) with addition of 0.5% glacial acetic acid to afford compounds **1** (15 mg), **2** (18 mg), **3** (13 mg) and **4** (10 mg).

Celosin E (**1**): White amorphous powder, mp: 235—237 °C, $[\alpha]_D^{20}$ −5.6 (c=0.02, MeOH).[15] The IR spectrum (KBr) showed absorptions at 3418, 2944, 1697, 1624, 1263, 1057 cm^{-1}. HR-ESI-MS (m/z): 679.3675 $[M+H]^+$ (Calcd 679.3694). ^{1}H- and ^{13}C-NMR: see Table 1.

Celosin F (**2**): White amorphous powder, mp: 225—227 °C, $[\alpha]_D^{20}$ −7.5 (c=0.03, MeOH). The IR spectrum (KBr) showed absorption at 3439, 2927, 1694, 1655, 1384, 1084 cm^{-1}. HR-ESI-MS (m/z): 661.3255 $[M-H]^-$ (Calcd 661.3224). ^{1}H- and ^{13}C-NMR: see Table 1.

Celosin G (**3**): White amorphous powder, mp: 239—241 °C, $[\alpha]_D^{20}$ −4.3 (c=0.03, MeOH). The IR spectrum (KBr) showed absorption at 3426, 2943, 1723, 1691, 1629, 1384, 1076 cm^{-1}. HR-ESI-MS (m/z): 1139.5248 $[M+Na]^+$ (Calcd 1139.5250). ^{1}H- and ^{13}C-NMR: see Table 1.

Cristatain (**4**): White amorphous powder, mp: 243—245 °C; $[\alpha]_D^{20}$ −7.0 (c=0.04, MeOH); The IR spectrum (KBr) showed absorption at 3403, 3322, 2937, 2855, 1781, 1696, 1640, 1025 cm^{-1}; HR-ESI-MS (m/z): 661.3591 $[M-H]^-$ (Calcd 661.3588). The ^{1}H- and ^{13}C-NMR spectral data are consistent with the literature.[8]

Process of Acid Hydrolysis[6] Each saponins (3 mg) was heated in an ampule with 5 ml of aqueous 2 M CF_3COOH at 120 °C for 2 h. The aglycone was extracted with dichloromethane, and the aqueous residue was evaporated under reduced pressure. Then 0.8 ml of pyridine and 2 mg of $NH_2OH \cdot HCl$ were added to the dry aqueous residue, and the mixtures were heated at 90 °C for 30 min. After the reaction mixtures were cooled, 0.8 ml of Ac_2O was added and the mixtures were heated at 90 °C for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS using the aldononitrile peracetates of standard sugars as reference samples.

MTT Assay[16] Standard 3-(4,5-dimethylthiazole)-2,5-diphenyltetraazolium bromide (MTT assay) procedures were used with the slight modification. Cells were placed in 96-well microassay culture plates (4×10^4 cells per well) and grown overnight at 37 °C in a 5% CO_2 incubator. Test compounds were then added to the wells to achieve six final concentrations ranging from 10^{-3} to 10^2 μg/ml. Control wells were prepared by addition of culture medium (100 μl). Wells containing culture medium without cells were used as blanks. The plates were incubated at 37 °C in a 5% CO_2 incubator for 72 h. Upon completion of the incubation, stock MTT dye solution (20 μl, 5 mg/ml) was added to each well. After 4 h incubation, buffer (100 μl) containing *N*,*N*-dimethylformamide (50%) and sodium dodecyl sulfate (20%) was added to solubilize the MTT formazan. The optical density of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The IC_{50} values were determined by plotting the percentage viability *versus* concentration on a logarithmic graph and reading off the concentration at which 50% of cells remain viable relative to the control. Each experiment was repeated three times to get the mean values. Five different tumor cell lines were the subjects of this study: SHG44 (human glioma cells), HCT116 (human colon cancer cells), CEM (human leukemia cells), MDA-MB-435 (human breast cancer cells) and HepG2 (human hepatocellular carcinoma cells).

Inhibition Ability against LPS-Induced NO Production and Cell Viability The inhibition ability against LPS-induced NO production and cell viability was evaluated according to the literature[17] with some modification. RAW 264.7 macrophages were seeded at 1×10^6/ml in 96-well microtiter plates. The cells were co-incubated with the isolated compounds and LPS (5 μg/ml) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernatants with Griess reagent (1% sulfanilamide/0.1% naphthylethylendiamine dihydrochloride/2.5% H_3PO_4). The absorbance at 540 nm was read using a microplate reader (POLARstar). Cell viability was determined using the mitochondrial respiration-dependent MTT reduction method. After transferring the required supernatant to another plate for the Griess assay, the remaining supernatant was aspirated from the 96-well plates, and 100 μl of fresh medium containing 2 mg/ml of MTT was added to each well. The cells were incubated at 37 °C in humidified air with 5% CO_2. After incubating for 4 h, the medium was removed and the violet crystals of formazan in viable cells were dissolved in dimethyl sulfoxide (DMSO). Absorbance at 570 nm was measured using a microplate reader.

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References

1) Chinese Academy of Sciences in China Plant Volunteer Editors Committee, "Flora Reipublicae Popularis Sinicae," 1st ed., Vol. 25, Science Press, Beijing, 1979, p. 200.
2) Institute of Materia Medica Chinese Academy of Medical Sciences, "Chi Traditional Chinese Medicine," 2nd ed., Vol. 3, People's Health Publishing House, Beijing, 1984, p. 441.
3) Hase K., Kadota S., Basnet P., *Planta Med.*, **63**, 216—219 (1997).
4) Xue Q., Guo M. L., Zhang G., *Pharm. Care Res*., **6**, 345—347 (2006).
5) Xue Q., Guo M. L., Sun Z. L., Wang Y., Zhang G., Wang X. K., *Nat. Prod. Res.*, 2009, doi:10.1080/14786410902833948
6) Sun Z. L., Wang Y., Li Y. X., Guo M. L., *Fitoterapia*, **81**, 375—380 (2010).
7) C2hi X., Guo M. L., Song H., Chen Y. D., *J. Jilin Agric. Univ.*, **32**, 657—660 (2010).
8) Wang Y., Lou Z. Y., Wu Q. B., Guo M. L., *Fitoterapia*, **81**, 1246—1252 (2010).
9) Wanas A. S., Matsunami K., Otsuka H., Desoukey S. Y., Fouad M. A., Kamel M. S., *Chem. Pharm. Bull.*, **58**, 1596—1601 (2010).
10) Reynolds W. F., McLean S., Poplawski J., Enriquez R. G., Escobar L.

I., Leon I., *Tetrahedron*, **42**, 3419—3428 (1986).
11) Inoue M., Ohtani K., Kasai R., Okukubo M., Andriantsiferana M., Yamasaki K., Koike T., *Phytochemistry*, **70**, 1195—1202 (2009).
12) Luo J. G., Ma L., Kong L. Y., *Bioor. Med. Chem.*, **16**, 2912—2920 (2008).
13) Kuo P. C., Schroeder R. A., *Ann. Surg.*, **221**, 220—226 (1995).
14) Pacher P., Joseph S., Beckman J. S., Liaudet L., *Physiol. Rev.*, **87**, 315—424 (2007).
15) Chen Q., Luo J. G., Kong L. Y., *Chem. Pharm. Bull.*, **58** 412—414 (2010).
16) Liu Y. J., Zeng C. H., Liang Z. H., Yao J. H., Huang H. L., Li Z. Z., Wu F. H., *Eur. J. Med. Chem.*, **45**, 3087—3095 (2010).
17) Zheng C. J., Huang B. K., Han T., Zhang Q. Y., Zhang H., Rahman K., Qin L. P., *J. Nat. Prod.*, **72**, 1627—1630 (2009).