Introduction
1-β-D-Arabinofuranosylcytosine (cytarabine, Ara-C) is an efficient anticancer agent for acute myelogenous leukemia, but with short plasma half-life and rapid deamination to its inactive metabolite. However, its usefulness is limited by its short plasma half-life and rapid deamination to its inactive metabolite, 1-β-D-arabinofuranosyluracil (Ara-U) by cytidine deaminase. Cytidine deaminase is a ubiquitous enzyme found in high concentrations in the intestine, liver, spleen, and kidneys. When administered by iv, systemic elimination is biphasic with an initial plasma half-life ($t_{1/2a}$) of 7–20 min and a terminal half-life ($t_{1/2b}$) of 2–3 h. Cytarabine derivatives and conjugates have been designed with the intention of increasing its stability and lipophilicity as well as avoiding rate-limiting cytarabine phosphorylation while maintaining its biological activity. For example, ancitabine (cyclocytidine), enocitabine and fostabine have been used clinically in Japan. N4-peptidyl-Ara-C, like L-valyl-Ara-C, was reported to increase cellular uptake of cytarabine through the involvement of various transporters. Relatively high activity of cytarabine conjugates was achieved through the coupling of amino groups of cytarabine with carboxyl groups of polysaccharide molecules (e.g., polygalacturonic acid (PGA) and carboxymethylated yeast beta-D-glucan (CMG)); the in vitro antileukemic activity of these conjugates is needed in the future.

Keywords: cytarabine; cholic acid; liver; antitumor drug
conjugates was comparable or higher than the activity of non-conjugated/free cytarabine combined with a polysaccharide (Figure 2).

The 5'-hydroxyl group is essential for the biological activity of cytarabine because the activation of cytarabine to triphosphate occurs at this position; thus, if the 5'-hydroxyl group is blocked by conjugation, it would prevent its phosphorylation until free cytarabine is liberated, which would result in changed pharmacokinetic parameters. The attachment of a lipidic structure to cytarabine is usually achieved through the remains of phosphates as in Ara-CDP (arabinosylcytosine diphosphate), which is a normal cytarabine metabolite preceding activation to its active metabolite Ara-CTP. Steroid-cytarabine conjugates are small molecular conjugates that deserved attention. For example, conjugating cytarabine with prednisolone or prednisone by forming a phosphodiester bond at the 5' position has produced increased activities in L1210 leukemia models [12].

Individual bile acids are endogenous markers of liver transport and synthesis function. The enterohepatic circulation of bile acid makes it an attractive tool in designing pharmacological hybrid molecules and prodrugs with the view of improving intestinal absorption, increasing the metabolic stability of pharmaceuticals, specifically targeting drugs to organs involved in enterohepatic circulation, and sustaining therapeutically reasonable systemic concentrations of active agents [13–19].

In light of these characteristics of bile acids, cholic acid was chosen to modify cytarabine to improve its in vivo function and to achieve improved liver-targeting, which would make it possible to use cytarabine for treating liver cancer. Herein, the synthesis of several cholic acid-cytarabine conjugates is described with different linkers. In vitro stability, portacaval samples and in vivo distribution were tested to investigate the metabolic process. Furthermore, the antitumor activities of the conjugates were also tested.

Materials and methods
Chemistry
Conjugate 6 consisted of cholic acid linked to cytarabine with a direct amide bond from cholic acid to cytarabine via ethyl chloroformate [20]. Cholic acid was linked to cytarabine with a direct ester bond in conjugate 7 and was synthesized via general conditions using dicyclohexylcarbodiimide and 4-dimethylaminopyridine (Scheme 1).

Conjugates 12 and 16 were designed to have a β-Alanine as the linker between cholic acid and cytarabine. The N-terminal of β-Alanine was attached to cholic acid with an amide bond, whereas the carboxyl group was linked to cytarabine via an ester bond or an amide bond to form 12 and 16, respectively. As shown in Scheme 2, commercially available β-Alanine was directly converted to an ester by thionyl chloride and EtOH. The resulting ester was coupled to cholic acid through isobutyl chloroformate to yield 10. After the hydrolysis of 10 by NaOH, compound 12 was then synthesized in similar manner as 7 using the corresponding acid, 11. Cytarabine was first coupled to a Boc-protected β-Alanine to obtain 14. After deprotection by HCl in EtOH, the resulting compound, 15, was reacted with cholic acid to produce 16.

The formation of the amide and ester bonds was determined by 1H-NMR. The chemical shift, δ, of hydrogen on
Scheme 1. Reagents and conditions: (a) ethyl chloroformate, triethylamine, N,N-dimethylformamide, -15 °C to RT; (b) dicyclohexylcarbodiimide, 4-dimethylaminopyridine, N,N-dimethylformamide.

Scheme 2. Reagents and conditions: (a) thionyl chloride, EtOH; (b) 5, isobutyl chloroformate, triethylamine, N,N-dimethylformamide, -15 °C to RT; (c) 10% NaOH, EtOH; (d) 1, dicyclohexylcarbodiimide, 4-dimethylaminopyridine, N,N-dimethylformamide; (e) ethyl chloroformate, triethylamine, N,N-dimethylformamide, -15 °C to RT; (f) HCl in EtOH; (g) 5, ethyl chloroformate, triethylamine, N,N-dimethylformamide, -15 °C to RT.
the 5-, 6-, and 5'carbon of cytarabine was 6.0, 7.78, and 3.85, respectively. When N4 was coupled with the carboxyl group of cholic acid (compounds 6 and 7), δ of 5- and 6-hydrogen moved to 7.4 and 8.2, respectively; when the 5'-OH was coupled with the carboxyl group of cholic acid, δ of 5'-H shifted to 4.2 (Figure 3). Similar changes were observed in compounds 12 and 14.

In vitro stability
Taking into account the possibility of the oral administration of the conjugates, the stability of all the newly synthesized compounds was tested in simulated gastric and intestinal fluids, mouse blood and liver homogenate. After incubation with the four matrixes, samples at different time points were subjected to HPLC to determine the extent of metabolism. Simulated gastric and intestinal fluids were prepared according to the Chinese Pharmacopoeia. Mouse blood was collected, and heparin was added; the solution was then incubated at 37°C. Mouse liver homogenate was prepared using a buffer containing 50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 10% glycerol, 1% Tween 20, 1.5 mmol/L MgCl2, 10 mmol/L Na3PO4, 100 mmol/L NaF, and 2 mmol/L Na3VO4.

Cytarabine, 6, 7, 12, and 16 were weighed (2 mg each), dissolved in 50 μL DMSO and added to an 800 μL matrix solution described above. The mixtures were incubated at 37°C, and the samples were obtained at different time points (gastric and intestinal fluid: 0, 2, 5, and 24 h; blood and liver homogenate: 0, 1, 3, 5, and 24 h). All target compounds were analyzed using an HPLC system (HP1100) with UV-detection at 214 nm. The separation was performed using a ZORBAX SB-C18 (5 μm, 4.6 mm×150 mm) with a 5 mmol/L ammonium acetate solution/methanol gradient at a flow rate of 1.0 mL/min (column temperature was set to 35°C).

Portacaval sampling
It has been reported that cytarabine deaminates rapidly in the liver[4]. To determine the forms of the conjugates after intestinal absorption and before reaching liver, portacaval blood samples were investigated by LC/MS after oral administration. Four male Sprague-Dawley rats, weighing 200 to 230 g, were orally administered conjugates 6, 7, 12, and 16 separately (each compound was equivalent to 50 mg/kg cytarabine). The compounds administered were prepared by dissolving the compounds in propanediol and diluting them with the same volume of 0.5% carboxyl methyl cellulose (CMC) to 35 mmol/L. The rats were anaesthetized by barbital and the abdominal cavity was opened. The portacaval vein was intubated and blood samples were collected at 0.25, 0.5, and 1 h. All of the samples were examined by LC-MS/MS. The analysis was performed using a LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA), an Atlantis analytical HILIC Silica column (2.1 mm×100 mm, 5 μm) from Waters (made in Ireland); a mixture of methanol, 5 mmol/L ammonium acetate solution, water and acetonitrile containing 0.1% acetic acid was used as the mobile phase [Mobile phases A and B consisted of methanol and 5 mmol/L ammonium acetate; Mobile phases C and D consisted of water and acetonitrile containing 0.1% acetic acid, respectively. Gradient chromatographic separation using mobile phases A, B, C, and D was as follows: 0 min (2% A, 5% B, and 93% D), 2.1 min (2% A, 5% B, and 93% D), 2.5 min (2% A, 5% B, 60% D, and 33% C), 3.0 min (2% A, 5% B, 60% D, and 33% C), 3.5 min (2% A, 5% B, and 93% D) and finished at 6.5 min at a constant flow rate of 0.3 mL/min]. The MS/MS reactions selected to monitor cytarabine and Acyclovir (internal standard) were the transitions from m/z 244 → m/z 112 and m/z 226 → m/z 152 with collision energies of 32% and 30% of the instrumental maximum for MS2 experiments, respectively.

In vivo distribution
Male Kunming mice, weighing 18 to 20 g, were administered cytarabine (50 mg/kg), compound 6 or 16 orally, which was equivalent to 50 mg/kg cytarabine. Blood and liver samples were collected at different time points. All of the samples were examined by LC/MS.

The liver target index was calculated by the following formula:

\[
\text{Liver target index} = \frac{\text{Concentration in liver}}{\text{Concentration in blood}}
\]
Liver target index (LTI) = \frac{\text{AUC}_{\text{in liver}}}{\text{AUC}_{\text{in plasma}}}

Normal, non-fasted male Kuming mice were administrated ig with a dose of 50 mg/kg of cytarabine, 130 mg/kg of 6 and 145 mg/kg of 16, respectively. At specific times after administration, mice were anesthetized, and blood was drawn. The liver was removed, rapidly frozen on crushed ice and then stored at -20 °C. The livers were slit, added to methanol with THU (final concentration of 0.1 mmol/L) and homogenized. The centrifugation was carried out at 3500 revolutions per minute and 4 °C for 5 min. A 100 μL aliquot of supernatant was added to 25 μL of the internal standard (Acyclovir) and 100 μL of acetonitrile and mixed by vortexing for 1 min. After centrifugation, the supernatant was transferred to a glass tube and evaporated until dry using an evaporator at 40 °C under a stream of nitrogen. The dried extract was then reconstituted in 100 μL of the mobile phase and a 20 μL aliquot was injected into the LC-MS/MS system for quantitative analysis.

Blood was added to THU (final concentration of 0.1 mmol/L) and chilled on crushed ice. The plasma was then separated by centrifugation (11 000 revolutions per minute, 4 °C), frozen and stored at -20 °C. The mouse plasma samples were prepared using the protein precipitation technique. A 100 μL aliquot of an acetonitrile solution containing 25 μL of plasma, and mixed by vortexing for 1 min. After centrifugation, the supernatant was transferred to a glass tube and evaporated at 40 °C under a stream of nitrogen. The dried extract was then reconstituted in 100 μL of the mobile phase and a 20 μL aliquot was injected into the LC-MS/MS system for quantitative analysis.

The pharmacokinetics results of cytarabine, 6, and 16. The concentration of the compounds in the plasma, after the administration of cytarabine, reached their peaks at 0.5 h, and only trace amounts were left in the blood after 4 h. The C_{max} was 1.14 μg/mL. The C_{max}, T_{max} and AUC_{in} of the livers were all lower than those of the plasma; additionally, the elimination rate in liver was found to be much faster (Figure 5A). The released cytarabine of conjugate 6 was observed in the plasma samples but not in the liver. The C_{max} of the conjugate in the plasma and liver were 0.091 and 3.25 μg/mL respectively, and 0.188 μg/mL for released cytarabine. Released cytarabine was detected in the sample of conjugate 7, which had cytarabine directly linking to cholic acid via an ester bond, was completely converted into cytarabine in mouse blood within one hour.

The conjugated forms of 6, 12, and 16 were observed in portal caval samples, among which 16 had the most obvious MS characteristic peak. A small amount of cytarabine was detected at one hour only after the administration of conjugate 12. Neither the conjugate nor released cytarabine was observed when conjugate 7 was administered. The results showed that either conjugate 7 was not absorbed by the intestine or was metabolized to an unknown form (Figure 4).

Table 2 showed in vivo pharmacokinetics results of cytarabine, 6, and 16. The concentration of the compounds in the plasma, after the administration of cytarabine, reached their peaks at 0.5 h, and only trace amounts were left in the blood after 4 h. The C_{max} was 1.14 μg/mL. The C_{max}, T_{max} and AUC_{in} of the livers were all lower than those of the plasma; additionally, the elimination rate in liver was found to be much faster (Figure 5A). The released cytarabine of conjugate 6 was observed in the plasma samples but not in the liver. The C_{max} of the conjugate in the plasma and liver were 0.091 and 3.25 μg/mL respectively, and 0.188 μg/mL for released cytarabine in plasma (Figure 5B). Conjugate 16 also released cytarabine in vivo. The C_{max} of the conjugate in plasma and liver were 0.826 and 14.868 μg/mL, whereas released cytarabine levels in plasma and liver were 0.097 and 0.455 μg/mL (Figure 5C), respectively. Although 12 was present in the portal caval samples, only a small amount of the conjugate form could be detected in the liver, and it was not found in plasma. Released cytarabine was not detected in the liver or plasma. Thus, data on conjugate 12 were not provided. According to the calculation of AUC_{in}, the liver targeting index was 0.17 for cytarabine, 34.9 for 6, and 16.3 for 16.

Both conjugates 6 and 16 showed good liver targeting compared to cytarabine. The LTI of 6 reached 34.9, which was 200 times more than cytarabine. The LTI of 16 also reached 16.3, and the C_{max} was 14.868 μg/mL, which demonstrated good absorption. The stability of the amide bond prevented the conjugates from degradation through oral absorption,
whereas *in vivo*, the bond could be metabolized, which made it an appropriate link. The $C_{\text{max}}$ of 16 was approximately 4 times larger than conjugate 6, which showed that using β-Alanine as the linker might increase cytarabine’s absorption.

The antitumor activities of conjugates 6, 16, and 12 were tested on HL60 cells (Table 3). All three compounds showed potent antitumor activities on cytarabine-sensitive HL60 cells, which indicated that conjugating cytarabine to cholic acid allowed its antitumor activities to be retained.

In conclusion, a new series of cholic acid-cytarabine conjugates with antitumor activities have been described. Some of them exhibited excellent targeting of the liver and good absorption compared to cytarabine alone. Further studies will...
continue by developing more conjugates with different linkers. Biological evaluations will be conducted on conjugates 6 and 16 in the near future.

**Experimental**

The reagents were purchased from Alfa-Aesar (Karlsruhe, Germany), Acros (Geel, Belgium) and Shanghai Chemical Reagent Company (Shanghai, China) and were used without further purification. Analytical thin-layer chromatography was performed on HSGF 254 (150–200 μm thickness; Yantai Huiyou Company, Yantai, Shandong, China). The IR spectra were recorded on a Nicolet Magna 750 FTIR spectrometer, and the $^1$H NMR (300 MHz or 400 MHz) spectra were recorded on a Varian Mercury-300 or a 400 High Performance Digital FT-NMR using tetramethylsilane as an internal standard. The $^{13}$C NMR (100 MHz) spectra were determined using a Varian Mercury-400 High Performance Digital FT-NMR. HPLC analysis was performed using a Gilson HPLC system with UV detection at 214 and 254 nm. UV scanning was performed on an Agilent HP1100. LC-MS spectra were obtained using a LCQ Deca XP ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). Accurate mass measurements were carried out on a Q-TOF ultima Globe mass spectrometer (Micromass, Manchester, UK).

(4R)-N-((3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-y)-2-oxo-1,2-dihydropyrimidin-4-yl)-4-((3R,5S,7R,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethyhexadecahydro-1H-cyclopenta[alphenanthren-17-y]pentanoate (6)

Cholic acid (1.16 g, 3 mmol) and triethylamine (TEA, 0.39 mL, 3 mmol) were dissolved in dry N,N-dimethylformamide (DMF, 20 mL). The mixture was cooled to -15 °C, and ethyl chloroformate (0.31 mL, 3 mmol) was then added. After being stirred for 20 min, cytarabine (0.69 mg, 3 mmol) in 15 mL DMF was added the mixture, dropwise. The reaction mixture was stirred at -15 °C for 1 h and at RT overnight. The mixture was filtered, and the filtrate was concentrated. The residue was subjected to silica gel chromatography (dichloromethane/methanol, 10:1 to 4:1) to yield 6 as a white solid (1.34 g, 75%).

**Figure 5.** A) Plasma and liver concentrations following oral administration of Ara-C (50 mg/kg). B) Plasma and liver concentrations following oral administration of 6 (130 mg/kg). C) Plasma and liver concentrations following oral administration of 16 (145 mg/kg). Mean±SD. n=3.

| Compounds | IC_{50} (μmol/L) |
|-----------|------------------|
| Cytarabine| 0.19±0.13        |
| 6         | 2.09±1.23        |
| 12        | 0.99±0.65        |
| 16        | 2.96±2.03        |

Table 3. Antitumor activities on HL60 cells after 72 h. Mean±SD. n=3.
A white solid was formed, filtered and then evaporated. Ether was added dropwise. The reaction mixture was stirred at -15 °C for 3 h, β-alanine dissolved. The mixture was stirred at rt overnight and then poured into ice water and acidified with 1 mol/L HCl, which was extracted with ethyl acetate 3 times. The organic layer was combined and washed with 0.5 mol/L acetate and washed with water 4 times (water/ethyl acetate, 1:2). The organic layer was combined and washed with brine, dried (Na2SO4) and then filtered. The reaction mixture was stirred at rt overnight. The mixture was filtered, and the filtrate was concentrated. The residue was dissolved in ethyl acetate (1.7 mL, 15 mmol) and added to a solution of 10% NaOH (30 mL). The reaction mixture was stirred at rt overnight and then poured into ice water. The mixture was filtered and subjected to silica gel chromatography (dichloromethane/methanol, 20:1) to yield 16 as a white solid 3.2 g (87%).

**Ethyl 3-((4R)-4-((3R,5S,7R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamido)propanoate (10)**

Thionyl chloride (20 mL) was added dropwise to anhydrous ethanol (80 mL), and the temperature did not exceed 25 °C. β-Alanine (9 g, 0.1 mol) was then added. After stirring at 50 °C for 3 h, β-Alanine dissolved. The mixture was stirred at RT overnight. The solvent was evaporated and ether was added to the residue. A white solid was formed, filtered and then dried (Na2SO4) to yield 16 g (100%). Cholonic acid (5.57 g, 13.6 mol) and triethylamine (TEA, 1.92 mL, 15 mmol) were dissolved in dry DMF (45 mL). The mixture was cooled to -15 °C, and isobutyl chloroformate (1.78 mL, 15 mmol) was dissolved in dry DMF (45 mL). The mixture was cooled to -15 °C for 30 min and then stirred at rt. The reaction mixture was stirred at rt overnight and then poured into ice water. The mixture was filtered, and the filtrate was concentrated. The residue was dissolved in ethyl acetate and washed with water 4 times (water/ethyl acetate, 1:2). The organic layer was combined and washed with 0.5 mol/L NaOH, 0.5 mol/L HCl and brine; it was then dried and concentrated to yield 10 as a white solid 5.67 g (87%).

**1H-NMR (CD3OD, 300 MHz): δ 4.12 (m, 2H), 3.94 (brs, 1H), 3.78 (brs, 1H), 3.4 (m, 3H), 2.5 (t, J=6.6 Hz), 2.4–1.2 (m, 24H), 1.24 (t, J=7.2 Hz), 1.0 (d, 3H, J=6.4 Hz), 0.9 (s, 3H), 0.7 (s, 3H).**

**MS (ESI): m/z 333 (M+H)+.**

**Compound 10 (3.9 g, 7.7 mmol) was dissolved in ethanol (30 mL) and added to a solution of 10% NaOH (30 mL). The reaction mixture was stirred at RT overnight and then poured into ice water and acidified with 1 mol/L HCl, which was extracted with ethyl acetate 3 times. The organic layer was combined and washed with brine, dried (Na2SO4) and concentrated to yield 11 as a white solid 3.2 g (87%).**

**1H-NMR (CD3OD, 300 MHz): δ 3.95 (brs, 1H), 3.8 (brs, 1H), 3.4 (t, J=7 Hz), 2.5 (t, 2H, J=6.8 Hz), 2.4–1.05 (m, 24H), 1.0 (d, 3H, J=6.1 Hz), 0.9 (s, 3H), 0.7 (s, 3H).**

**MS (ESI): m/z 959 (2M+H)+.**

**3-((4R)-4-((3R,5S,7R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamido)propanoic acid (11)**

**3-((4R)-4-((3R,5S,7R,9S,10S,12S,13R,14S,17R)-3,7,12-trihydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanamido)propanoic acid (11)**

**Compound 10 (3.9 g, 7.7 mmol) was dissolved in ethanol (30 mL) and added to a solution of 10% NaOH (30 mL). The reaction mixture was stirred at RT overnight and then poured into ice water and acidified with 1 mol/L HCl, which was extracted with ethyl acetate 3 times. The organic layer was combined and washed with brine, dried (Na2SO4) and concentrated to yield 11 as a white solid 3.2 g (87%).**

**1H-NMR (CD3OD, 300 MHz): δ 3.95 (brs, 1H), 3.8 (brs, 1H), 3.4 (t, J=7 Hz), 2.5 (t, 2H, J=6.8 Hz), 2.4–1.05 (m, 24H), 1.0 (d, 3H, J=6.1 Hz), 0.9 (s, 3H), 0.7 (s, 3H).**

**MS (ESI): m/z 959 (2M+H)+.**

**Compound 14 (700 mg, 1.7 mmol) was dissolved in saturated HCl in ethanol (5 mL) and stirred at RT overnight. The solvent was evaporated and methanol (10 mL) was added to the residue then stirred for 1 h. The solid was filtered and washed with methanol. The white solid was dried, and the crude product 15 (400 mg) was obtained, which was used in...**
the next step without purification.

Cholic acid (408 mg, 1 mmol) and triethylamine (0.12 mL, 1 mmol) were dissolved in dry DMF (6 mL). The mixture was cooled to -15°C, and ethyl chloroformate (0.1 mL, 1 mmol) was added. After stirring for 20 min, compound 15 (351 mg, 1.1 mmol) was added to the mixture by a dropwise addition in 5 mL DMF. The reaction mixture was stirred at -15°C for 30 min and at RT overnight. The reaction mixture was filtered and concentrated. Subsequently, the residue was subjected to silica gel chromatography (dichloromethane/methanol, 20:1 to 5:1) to yield 16 as a white solid (300 mg, total yield: 82%).

mp 178–180°C.

1H-NMR (CD3OD, 300 MHz): δ 8.24 (d, 1H, J=7.8 Hz), 7.46 (d, 1H, J=7.7 Hz), 6.19 (d, 1H, J=4.1 Hz), 4.28 (m, 1H), 4.01 (d, 1H, J=6.3 Hz), 4.08 (m, 1H), 3.89 (s, 1H), 3.80 (m, 3H), 3.47 (t, 2H, J=6.4 Hz), 2.66 (t, 2H, J=6.2 Hz), 2.4-1.01 (m, 25H), 0.98 (d, 3H, J=6.3 Hz), 0.9 (s, 1H), 0.65 (s, 1H).

13C NMR (100 MHz, d6-DMSO) δ 172.9, 172.2, 162.1, 154.6, 146.7, 94.5, 87.1, 85.8, 76.2, 74.6, 71.1, 70.5, 66.4, 61.1, 46.2, 45.8, 41.6, 41.4, 36.5, 35.4, 35.2, 34.9, 34.5, 32.5, 31.8, 30.8, 30.4, 28.5, 27.4, 26.3, 22.9, 22.7, 17.2, 12.4.

MS (ESI): m/z 705 (M+H)+; HRMS calcd for C33H56N4O14Na: 727.3894 (M+Na)+; Found: 727.3898.

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Author contribution

Jing-kang SHEN and Dan-qi CHEN designed research; Dan-CHEN, Xin WANG, Lin CHEN, Jin-xue HE and Ze-hong MIAO performed research; Dan-qi CHEN and Xin WANG contributed new analytical tools and reagents; Dan-qi CHEN and Xin WANG analyzed data; and Dan-qi CHEN wrote the paper.

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