Ann Microbiol (2015) 65:1303–1311
DOI 10.1007/s13213-014-0968-y

ORIGINAL ARTICLE

Biotransformation of geniposide by *Synechocystis* sp. PCC 6803 into genipin and its inhibitory effects on BEL-7402, *Escherichia coli*, and cyanobacteria

Zhi Wang · Lu Zhang · Xiao-Dong Cui · Tai-Sen Hao · Chun-Mei Wang · Xiang-Xiang Gu · Yong-Gang Liu · Jun-Gui Dai · Agaru Bao

Received: 23 April 2014 / Accepted: 26 August 2014 / Published online: 17 September 2014

Abstract *Synechocystis* sp. PCC 6803, a freshwater cyanobacterium that is widely distributed in nature, was chosen for the transformation of geniposide extracted from the Chinese traditional medicine plant *Gardenia jasminoides*. After a period of 25 days, two converted products were obtained according to the TLC and HPLC results. NMR was used to identify the products. One was genipin (A), an iridoid compound that possesses various pharmacological effects, and its in vitro anti-tumor and anti-bacterial activity were measured. This compound showed a concentration-dependent and time-dependent inhibitory effect against BEL-7402. The IC_{50} of genipin after incubation with BEL-7402 for 72 h was 16.0 μg mL^{-1}. The diameter of the inhibition zone of genipin at a concentration of 10 μg mL^{-1} on a culture of *Escherichia coli* was 24.2 mm. This manuscript provides the first demonstration of the strong in vitro anti-bacterial activity of genipin. Geniposide also showed inhibitory ability on the growth of BEL-7402 and bacterial cells, but its effects were weaker than that of genipin. In addition, the cytotoxicity of genipin against *Synechocystis* sp. PCC 6803 or another bloom-forming cyanobacterium, *Microcystis* sp. PCC 7806, was evaluated. Genipin exhibited a high level of cytotoxicity against *Synechocystis* sp. PCC 6803 and *Microcystis* sp. PCC 7806 with IC_{50} values of 1.33 μg mL^{-1} and 66.27 μg mL^{-1}, respectively. In conclusion, the results indicate *Synechocystis* sp. PCC 6803 as a potential biocatalyst for the transformation of geniposide into genipin, which not only has valuable pharmacological activities (determined in this study and by other research groups), but also shows potential for water-bloom control (based on the results of assays on two cyanobacteria reported herein).

Keywords Genipin · *Synechocystis* sp. PCC 6803 · Biotransformation · Cytotoxicity · *Microcystis* sp. PCC 7806

Z. Wang · L. Zhang · X.-D. Cui · T.-S. Hao · C.-M. Wang (✉) · X.-X. Gu · Y.-G. Liu · A. Bao
Department of Biological Pharmaceutics, School of Materia Medica, Beijing University of Chinese Medicine, Beijing, China 100102
e-mail: wchunmei@126.com

J.-G. Dai
Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
e-mail: jgdai@imm.ac.cn

Introduction

Geniposide is one of the major iridoid glucosides in Zhizi, the fruit of gardenia (*Gardenia jasminoides* Ellis), which has long been used as a traditional Chinese medicine for the treatment of liver and gall bladder disorders and inflammatory conditions (Kim and Kim 2007). Geniposide has been proven to possess hepatoprotective and anti-inflammatory activities by many research groups (Ma et al. 2011; Fu et al. 2012; Wang et al. 2012; Wang et al. 2013; Zhang et al. 2013; Deng et al. 2013). However, previous studies have demonstrated that derivatives of geniposide exert more desirable activities than geniposide itself. For instance, penta-acetyl geniposide, an acetylated derivative of geniposide, is more potent at inhibiting aflatoxin B_1-induced unscheduled DNA synthesis in rat primary hepatocytes (Tseng et al. 1994). In contrast, geniposidic acid showed greater inhibitory activity than geniposide against implanted tumor growth when administered given alone or in combination with X-irradiation (Hsu et al. 1997). Another interesting finding was that the pharmacological activities of geniposide described above are not

directly induced by this agent but rather through intestinal bacteria transformation products, one of which is genipin (Akao et al. 1994). Geniposide is hydrolyzed from a glucose molecule into genipin, which exhibits greater anti-inflammatory and hepatic-protective activities than the former (Koo et al. 2006; Kang et al. 2012; Khanal et al. 2012). Taken together, geniposide may act as a potent lead compound, at least in some aspects, and stronger or novel bioactivities can be attained after structural modifications. Among the various approaches that can be used to achieve the structural modification of natural products, biotransformation through the enzyme systems of organisms is widely accepted because it is well in agreement with the concept of "green chemistry" (Rather et al. 2012).

Synechocystis sp. PCC 6803, a type of unicellular cyanobacteria, which is a class of photoautotrophic microorganisms that have shown capabilities as biocatalysts for the transformation of various organic compounds (Faramarzi et al. 2008; Ghasemi et al. 2011), is widely distributed in freshwater. Its model organism status as a member of the class of cyanobacteria and its rapid growth and abundant enzyme systems indicate its potential to be used as a biocatalyst. Moreover, it recently showed capability for the biotransformation of inorganic arsenic (As) (Yin et al. 2011). However, its ability to transform organic compounds has not yet been evaluated. In this study, *Synechocystis* sp. PCC 6803 was used for the transformation of geniposide. After this transformation, the product was subjected to pharmacological activity, anti-tumor cell activity, and bacteriostasis tests. Given that the color of the algal suspension changed interestingly during the transformation, we then assessed the cytotoxicity of the converted product against two photosynthetic cyanobacteria.

Materials and Methods

Extraction, separation, and purification of geniposide from gardenia

The dried fruit of *Gardenia jasminoides* Ellis (Rubiaceae) was purchased from Beijing Tong Ren Tang Group Co., Ltd. (Beijing, China). A mass of 200 g of dry fruit of *Gardenia jasminoides* Ellis was powdered and reflux-extracted with 1,000 mL of boiling 50 % ethanol for 2 h. The extract was then collected, and 1,000 mL of 50 % ethanol was added to continue the reflux extraction for 1.5 h. This procedure was repeated twice before the product was concentrated under vacuum. The crude extract of geniposide was obtained using D 101 macroporous resins according to the method described by Lu et al. (2002). Then, 2 g of the dry crude extract was dissolved in 200 mL of distilled water before being loaded into a separatory funnel. The same volume of ethyl acetate was then added as an extraction solvent, and the water-soluble portion was collected and loaded into the separatory funnel for two extraction steps. The three ethyl acetate-soluble portions were mixed together and concentrated in a rotary evaporator at 55 °C. The concentrated solution was maintained at 4 °C overnight to precipitate geniposide. Pure geniposide was obtained by recrystallization. The purity of crystalline geniposide was determined to be 96 % by HPLC.

Biotransformation

Wild-type *Synechocystis* sp. PCC 6803 was kindly provided by the researcher Huang Fang (Institute of Botany, the Chinese academy of sciences, China) and was aseptically transferred to BG-11 liquid medium for shake culture. The geniposide obtained as described above was dissolved in sterile water to a concentration of 10 μg mL^{-1}. When the OD_{665} nm of the liquid cultures of *Synechocystis* sp. PCC 6803 was approximately 0.4, 1 mL of the above-mentioned geniposide solution was added into 100-mL liquid cultures. The cultures were incubated on a rotary shaker operated at 110 rpm under 5000-lx continuous white light at 30 °C for 25 days. Two control experiments were performed under the same culture conditions in the absence of either *Synechocystis* sp. PCC 6803 or substrate.

Extraction, separation, and purification of converted products

After incubation for 25 days, the algal suspension was placed in a -80 °C ultra-low temperature refrigerator and subjected to freezing treatment for 8 h. The frozen mixture was then thawed in a 40 °C water bath for 15 min, then freezing and thawing steps were repeated four times. The sample obtained after the last thawing step was mixed with ethyl acetate at a ratio of 1:1 (*v*:*v*), and the mixture was loaded into a separatory funnel for extraction. The extraction procedure was the same as described above and was repeated three times, and the sample was then placed in a rotary evaporator at 45 °C. The dried products were then transferred to a dry place for further analysis. The control samples were subjected to the same treatment.

Structural identification of the converted products

The dry products were dissolved in methanol and analyzed by thin-layer chromatography (TLC) for preliminary confirmation. TLC was performed on pre-coated silica-gel G_{254} plates (Qingdao Haiyang Chemical Group Company, Shandong, China). The silica-gel plates were activated in an oven at 110 °C for 20 min before spotting. A chloroform:methanol (5:1, *v*/*v*) mixture was used as the developing solvent. Visualization of TLC plates was performed under an ultraviolet lamp at 254 nm.

The methanol-soluble samples were filtered through a 0.22-μm organic cellulose ester microporous membrane before being analyzed by high-performance liquid chromatography (HPLC). HPLC analysis was conducted with a Waters 1525 unit connected to a Breeze workstation (Waters Corporation, MA, USA). A 2489 UV/Visible detector (Waters Corporation, MA, USA) and a Grace Apollo C_{18} Column (250 mm× 4.6 mm, 5 μm) (Alltech, CA, USA) were also used. The whole pipeline and column were rinsed with methanol for 20 min and then with mobile phase for 1 h prior to detection. The compounds were eluted with a methanol:water mixture at a ratio of 35:65 (*v*/*v*) at a definite flow rate of 0.8 mL min^{-1} over a period of 40 min. The column temperature was 30 °C, and the samples were detected at 238 nm. The sample injection volume was 10 μL. The biotransformation rates were calculated through the peak area normalization method.

The pure converted products were attained via preparative high-performance liquid chromatography, which was performed using the same procedure as ordinary HPLC with the exception that the sample injection volume was 20 μL each time, and the samples were then subjected to nuclear magnetic resonance (NMR) analysis. The NMR analysis was performed on a Bruker AVANCE 600 nuclear magnetic resonance spectrometer (Bruker Corporation, Switzerland) with the following parameters: PROBHD=5 mm PABBO BB-, Pulprong = ZG30, TD=65536 K, SOLVENT = MeOD, NS=16, DS=2, TE=300 K, SWH=12335.5 Hz, FIDRES=0.188 Hz, AQ= 2.66 s, RG=181, DW=40.5 μs, and DE=6.5 μs.

Elucidation of the enrichment site of the converted products

To elucidate the enrichment site of the converted products, cultures of *Synechocystis* sp. PCC 6803 incubated for 25 days were transferred to a 50-mL centrifuge tube and centrifuged at 5,000 rpm and 4 °C for 10 min. After the supernatant was removed, fresh BG-11 liquid medium was added to the precipitate, and the mixture was maintained in a -80 °C ultra-low temperature refrigerator and subjected to freezing treatment for 8 h. The frozen mixture was then thawed in a 40 °C water bath. The algal cells were fully broken after three freezing and thawing cycles. The supernatant and broken algal cells in the precipitate were loaded into a separatory funnel for three rounds of extraction. The dry products were attained after rotary evaporation and were then subjected to HPLC analysis as described above. The enrichment site was determined through the peak area normalization method.

Evaluation of in vitro anti-tumor activity of the converted product

One of the converted products was assessed to determine its cytotoxic activity against the human hepatocellular carcinoma cell line (BEL-7402) using the MTT assay method. BEL-7402 cells were cultivated in aseptic RPMI-1640 medium with 10 % (*v*/*v*) fetal bovine serum in 5 % CO_2 at 37 °C. Cells at the logarithmic phase were used for the MTT assay. The procedure was performed according to Verma et al. (2010) with slight modifications. Briefly, the cell density was adjusted to 2.0×10^4 cells mL^{-1}, and 100 μL of the cell suspension was seeded into 96-well plates and cultivated for 24 h before being incubated with the converted product at different concentrations for 12 to 72 h. Then, 10 μL of the MTT solution (5 mg mL^{-1}) was added, and the OD was read at 490 nm after the mixture was incubated for 4 h. The cell inhibitory rate was calculated using the following formula: inhibitory rate (IR) $\left(1-\frac{OD \text{ value of group with cnverted product}}{OD \text{ value of group without cnverted product}}\right)\times 100$. The IC_{50} values of the converted product at different incubating times were also calculated. The cellular IR of geniposide was also evaluated for comparison purposes.

Inhibitory effect of the converted product on the growth of *E. coli*

The disk diffusion method was used to estimate the inhibitory effects of genipin and geniposide against *E. coli* DH-5α cells, which were maintained in solid LB medium at our laboratory. A colony was transferred into 5 mL of liquid LB medium and cultivated on a rotary shaker operated at 170 rpm and 37 °C for 14 h in the dark before the assay. Solid LB plates and different concentrations of the aseptically converted product and geniposide were also prepared. Bacteria liquid was then streaked out on the surface of solid LB plates. Pieces of filter paper with a diameter of 1 cm soaked with the converted product or geniposide were tiled on the surface of solid LB plates containing *E. coli* DH-5α cells. Filter papers soaked with DMSO were used as the control. All of the plates were incubated at 37 °C for 48 h in the dark, and the inhibition zones were observed.

Cytotoxicity of the converted product on two photosynthetic cyanobacteria

Microcystis sp. PCC 7806 cells were obtained from the Institute of Hydrobiology of the Chinese Academy of Sciences (Wuhan, China) and grown in BG-11 medium. For the assay of the cytotoxicity against *Synechocystis* sp. PCC 6803, 3.5-mL cell suspensions of *Synechocystis* sp. PCC 6803 (OD_{665} of 0.1) with different concentrations of the converted product or geniposide was added into a 12-well plate and incubated on a rotary shaker operated at 110 rpm under 5000-lx continuous white light at 30 °C for 48 h. For the assay of cytotoxicity against *Microcystis* sp. PCC 7806, 0.5-mL cell suspensions of *Microcystis* sp. PCC 7806 (OD_{650} of 0.3) with different concentrations of the converted product or geniposide were added into a 24-well plate and incubated on a rotary shaker operated at

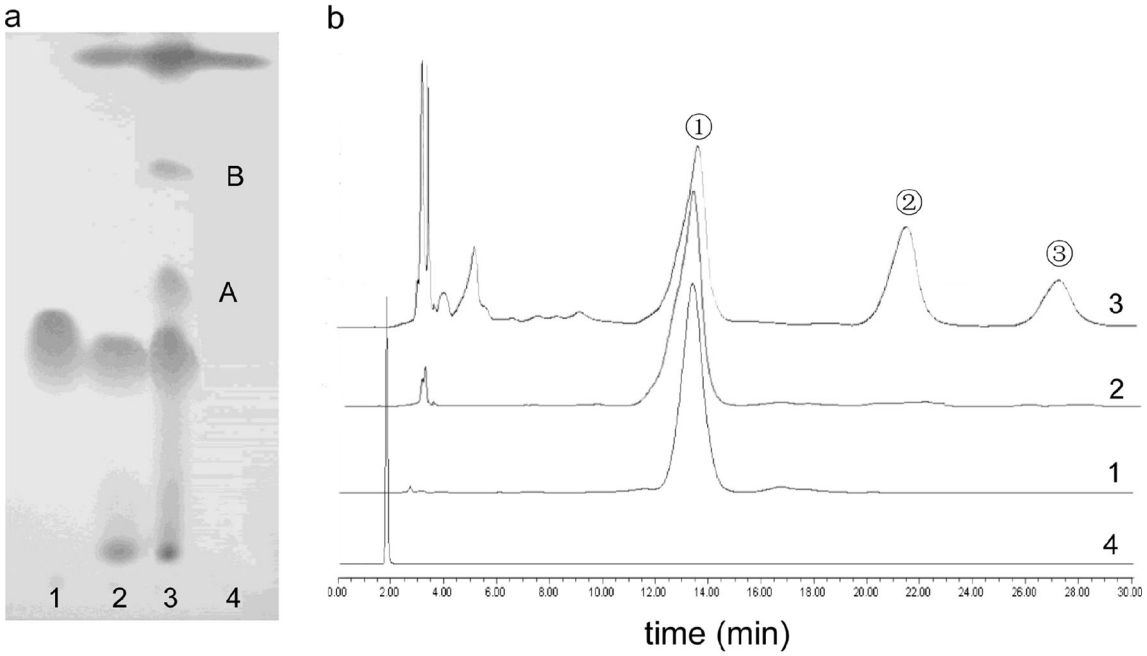

Fig. 1 Structural identification of the converted product. **a**) TLC analysis of the geniposide transformation by *Synechocystis* sp. PCC 6803. TLC analysis was performed after 25 days of incubation. **b**) HPLC chromatograms of the biotransformation products of geniposide by *Synechocystis* sp. PCC 6803. The compounds were eluted with a methanol:water mixture at a ratio of 35:65 (*v*/*v*) as the mobile phase at a definite flow rate of 0.8 mL min^{-1}. The profiles were recorded at 238 nm. All of the groups with the exception of group **1** were incubated in BG-11 liquid medium for 25 days and received the same treatment before HPLC analysis. **1,** geniposide standard; **2,** geniposide in BG-11 liquid medium in the absence of *Synechocystis* sp. PCC 6803; **3**, geniposide and its converted products transformed by *Synechocystis* sp. PCC 6803; **4**, *Synechocystis* sp. PCC 6803 in BG-11 liquid medium in the absence of geniposide; **A** and **B,** two converted products with different polarities. ①, geniposide; ②, product A; ③, product B

140 rpm under 5000-lx continuous white light at 28 °C for 72 h. The subsequent procedures of the two assays were the same as that used in the normal MTT assay described above.

Results

Structural identification of product A

The converted products obtained from the algal culture incubated for 25 days were attained. To facilitate the extraction and purification procedure of the converted products and save cost, the distribution of the converted products was determined by HPLC analysis. The results showed that the majority of the two converted products were found in the supernatant of the algal suspension and very few was found in the cell sediments (the percentages of products A and B in the supernatant were 93.99 % and 92.96 %, respectively), which suggested that the products were first synthesized in the algal cells and then transferred out from *Synechocystis* sp. PCC 6803 cells. The dry products obtained were preliminarily analyzed via TLC and HPLC. The results were shown in Fig. 1a and b. Two additional spots with R_f values of 0.5 and 0.7 were obtained in comparison with the control groups, and these

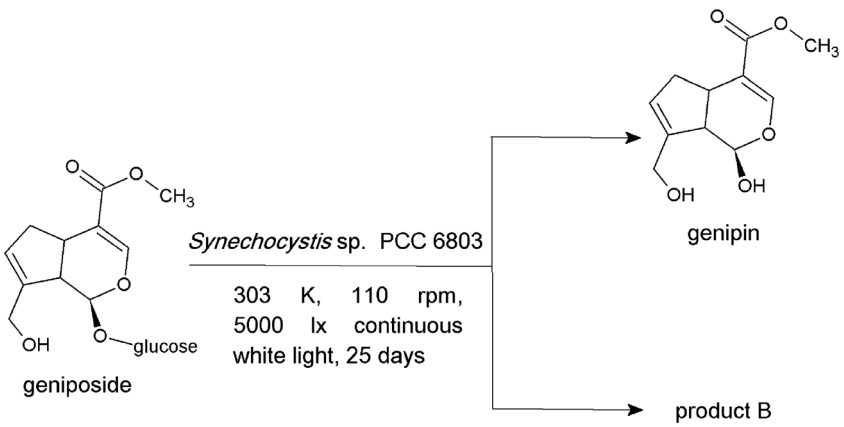

Fig. 2 The likely biotransformation process of geniposide by *Synechocystis* sp. PCC 6803. One of the two products was identified as genipin, whereas the structure of product B is unknown

were marked A and B, respectively, at the corresponding sites in Fig. 1a. HPLC analysis revealed the retention times of products A and B as 21.35 min and 27.10 min, respectively, whereas the retention time of geniposide was 13.49 min. The HPLC result also showed 31.9 and 15.5 % conversion of geniposide to products A and B, respectively. These results indicated that geniposide was transformed into two different lower-polarity products in *Synechocystis* sp. PCC 6803. More detailed structural information of the products was determined by NMR. Both products A and B were subjected to NMR analysis, but product B was too hard to prepare, which resulted in an insufficient amount for NMR analysis. The NMR spectral data obtained for product A were as follows: ^{1}H-NMR (CD_3OD) δ: 7.52 (s, H-3), 5.83 (s, H-7), 4.80 (d, J=7.8 Hz, H-1), 4.29 (d, J=13.2 Hz, H-10), 4.29 (d, J=13.2 Hz, H-10), 3.71 (s, -OCH_3), 3.16 (ddd, J=9.5, 8.5, 8.5 Hz, H-5), 2.89 (H-6), 2.54 (H-9), 2.07 (H-6). ^{13}C-NMR (CD_3OD) δ: 170.0 (-CO_2-), 154.5 (C-3), 145.8 (C-8), 128.4 (C-7), 111.9 (C-4), 97.9 (C-1), 61.9 (C-l0), 51.9 (-OCH_3), 48.6 (C-9), 40.2 (C-6), 37.8 (C-5). These data were in accordance with those reported before for genipin (Yang et al. 2011), identifying product A as genipin. Accordingly, the whole biotransformation process of geniposide by *Synechocystis* sp. PCC 6803 may be as detailed in Fig. 2.

Inhibitory effect of product A (genipin) on the growth of hepatocellular carcinoma cells, ***E. coli*** and cyanobacteria

The evaluation of the in vitro anti-tumor activity of genipin reveals that a low concentration of genipin has high IR against BEL-7402. Moreover, the IR values increased with increases in the concentration of genipin (Fig. 3a) and the incubation time (Fig. 3b) until it reached a maximum value. The IC_{50} values of genipin after incubation for 12, 24, 48, and 72 h were 72.2 μg mL^{-1}, 42.9 μg mL^{-1}, 44.0 μg mL^{-1} , and 16.0 μg mL^{-1}, respectively. Geniposide was also subjected to the cytotoxic assay for comparison with genipin. The results revealed that geniposide has moderate inhibitory activity against BEL-7402 growth after 48 h of incubation, which was lower than that obtained with genipin at concentrations ranging from 20 μg mL^{-1} to 120 μg mL^{-1} (Fig. 3c). This result demonstrated that the converted product (genipin) possesses better cell proliferation inhibitory activity on BEL-7402 cells than the substrate (geniposide).

The results of the disk diffusion method indicate that both genipin (Fig. 4a) and geniposide (Fig. 4b) can markedly inhibit the growth of *E. coli* because the diameters of the inhibition zones obtained with 10 μg mL^{-1} genipin and geniposide were 24.2 mm and 18.9 mm, respectively. The diameter of the inhibition zone increased with an increase in the concentration of genipin or geniposide, but the diameters obtained with genipin were always larger than those obtained with geniposide (Fig. 4c), which showed that the converted

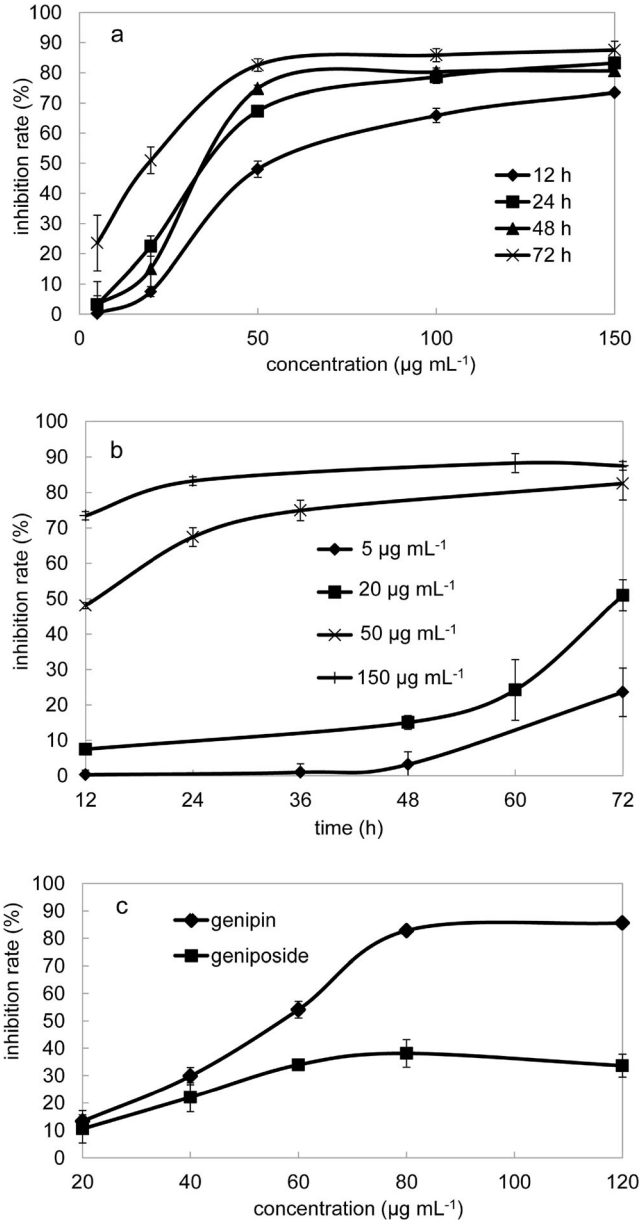

Fig. 3 Inhibition rates of genipin (**a**, **b,** and **c**) and geniposide (**c**) against the human hepatocellular carcinoma cell line (BEL-7402). A volume of 100 μL of BEL-7402 cells (2.0×10^4 /mL) was seeded in 96-well plates. After a 24-h period of incubation, different concentrations of converted product A (genipin) and geniposide were added. For the controls, RPMI-1640 medium was added instead. The cells were incubated for different incubation periods ranging from 12 to 72 h. The cells were then subjected to MTT assay, and the results are presented as the means±SD (n=6 for **a** and **b**, n=4 for **c**). **a**, cell proliferation inhibition rates against different concentrations of genipin with different incubation periods. **b,** cell proliferation inhibition rates of genipin with different incubation periods at different concentrations. **c**, comparison of the inhibitory rates of genipin and geniposide

product of geniposide exhibited better anti-bacteria activity than geniposide itself.

Geniposide can be transformed by *Synechocystis* sp. PCC 6803, but it was also slightly toxic to *Synechocystis* sp. PCC

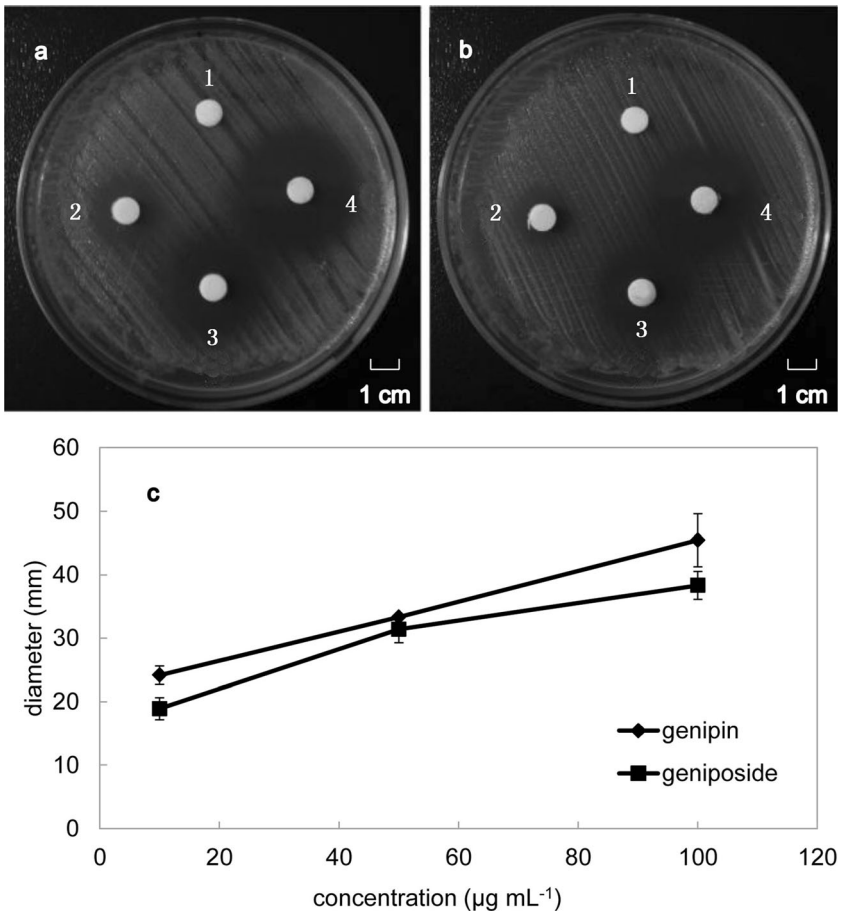

Fig. 4 Inhibitory effects of genipin (**a**, **c**) and geniposide (**b**, **c**) against *E. coli* DH-5α cells. The disk diffusion method was used as described in "materials and methods". **a** and **b,** inhibition zones obtained with different concentrations of genipin (**a**) and geniposide (**b**); **c**, diameters of the inhibition zones of genipin and geniposide at different concentrations. 2, 3, and 4 in a and b represent the following different concentrations of genipin or geniposide: 10 μg mL^{-1}, 50 μg mL^{-1}, and 100 μg mL^{-1}, respectively. 1 represents DMSO, which was used as the control

6803 (Fig. 5a). In addition, one of its products, genipin, presented high IRs against both *Synechocystis* sp. PCC 6803 (Fig. 5a) and another photosynthetic cyanobacterium, *Microcystis* sp. PCC 7806 (Fig. 5b), at a very low concentration with IC_{50} values of 1.33 μg mL^{-1} and 66.27 μg mL^{-1}, respectively. Similar to the assay performed on BEL-7402 cells, the IRs described herein were also concentration-dependent and reached maximal values when the concentrations of genipin were sufficiently high. The activities of the other product were not analyzed due to the insufficient amount of the product obtained.

Discussion

Synechocystis sp. PCC 6803 has been widely used as a model organism for the study of photosynthesis. Herein, its novel capability to transform an organic compound, geniposide, into genipin was confirmed for the first time. Given that geniposide is reported to be transformed into genipin through a reaction catalyzed by β-glucosidase (Li et al. 2014), *Synechocystis* sp. PCC 6803 may catalyze the hydrolysis of geniposide in a similar manner as β-glucosidase. We can also further infer that it likely has the ability to transform glycosides (Quan et al. 2012) and of transglucosylation (Rather et al. 2012), similarly to β-glucosidase.

Synechocystis sp. PCC 6803 has been used for the biotransformation of inorganic arsenic (As), as shown by Yin et al. 2011. In their report, treatment of these cells with sodium arsenite [As(III)] resulted in considerable oxidation activity and slight methylation activity. Approximately 84 % of As(III) was oxidated to As(V), whereas only approximately 2 % of As(III) was methylated to methylated arsenicals in the presence of a high As(III) concentration. Its marked ability to biocatalyze inorganic arsenic encouraged us to investigate its capability to transform organic compounds. We confirmed this conjecture and achieved approximately 32 % conversion of geniposide to genipin. Although this rate is moderate, our demonstration of its capability to hydrolyze geniposide is really inspiring.

Genipin is a natural iridoid compound and the aglycone of geniposide found in the fruits of gardenia. Genipin has been shown to possess anti-inflammatory, anti-diabetic, anti-tumor, anti-thrombotic, anti-oxidative, anti-angiogenic, and neurotrophic activities (Nam et al. 2010). In addition to various

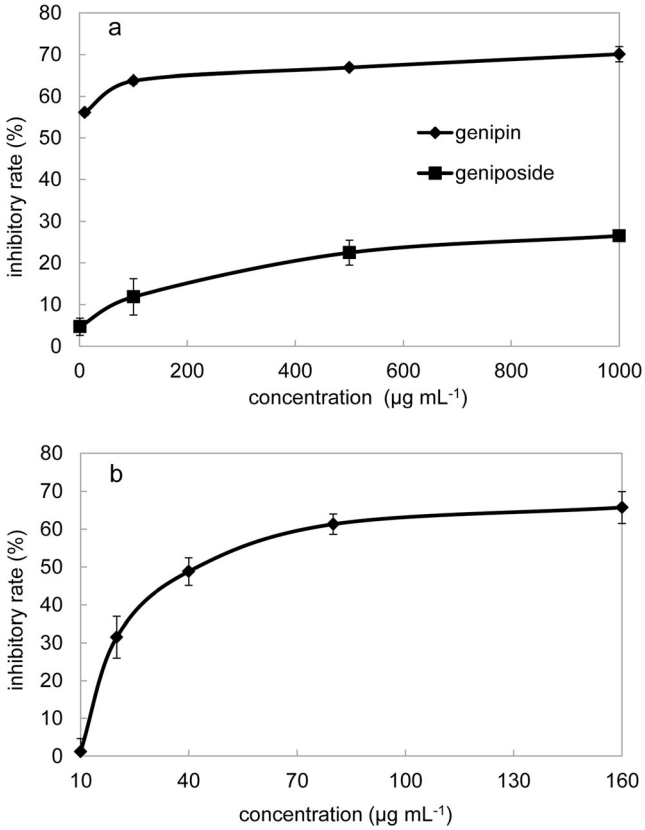

Fig. 5 Cytotoxicity of genipin on two photosynthetic cyanobacteria. The MTT assay was used to assess the cytotoxicity of genipin and geniposide against *Synechocystis* sp. PCC 6803 (**a**) and the cytotoxicity of genipin against *Microcystis* sp. PCC 7806 (**b**). The incubation times before the addition of MTT were 48 h (**a**) and 72 h for (**b**). The results are presented as the means±SD (n=4 for **a**, n=3 for **b**)

pharmacological effects, genipin is also used to prepare blue colorants in the food industry and as a cross-linking reagent for biological tissue fixation (Khanal et al. 2012). Despite its great value in various applications, its concentration in gardenia fruits is rather low (approximately 0.005–0.01 %), and it is difficult to extract genipin directly from gardenia fruits. The common method for the synthesis of genipin is the hydrolyzation of geniposide by β-glucosidase. However, the low productivity and high product cost of this method have limited the applications of genipin. Using the fast-growing and environmentally friendly *Synechocystis* sp. PCC 6803 as a biocatalyst may be an inexpensive, alternative approach for the synthesis of genipin.

Synechocystis sp. PCC 6803 has been successfully cultivated in a 25-L carboy, which can be treated as a pilot-scale bioreactor, for lipid extraction (Sheng et al. 2011). It has also been cultivated in a 20-L carboy for long-term (more than 130 days), semi-continuous operation. Although nutrient limitation occurs during this operation, it can be avoided by balancing the delivery rates of CO_2, N, P, and other components (Kim et al. 2013). To the best of our knowledge, larger-scale photobioreactors for the cultivation of *Synechocystis* sp. PCC 6803 and longer-term operation have not been reported publicly. However, large-scale (1,000 L and 2,000,000 L) photobioreactors for the cultivation of algae and cyanobacteria have already been put into use (Rupprecht et al. 2006). Given that *Synechocystis* sp. PCC 6803 is a representative cyanobacteria, there is no doubt that these huge photobioreactors can serve as suitable bioreactors for the cultivation of *Synechocystis* sp. PCC 6803. Because the scale of photobioreactors is the key problem that needs to be considered in the large-scale cultivation of *Synechocystis* sp. PCC 6803, the solution to this problem leads to the hypothesis that *Synechocystis* sp. PCC 6803 presents the requested productivity for large-scale application.

In accordance with the proven anti-tumor activity of genipin described above, we demonstrate the concentration-dependent and time-dependent inhibitory effects of genipin on the growth of the human hepatocellular carcinoma cell line BEL-7402. The result that genipin has a stronger effect than geniposide is identical to the results reported by Khanal et al. (2012). These researchers confirmed that genipin induces the apoptosis of HepG2 cells through ROS/JNK signaling. The results from assays on FaO rat hepatoma cells and human hepatocarcinoma Hep3B cells conducted by another research group also confirmed the hepatoprotective activity of genipin (Kim et al. 2005). Taken together, the results show that genipin may have broad-spectrum cytotoxicity against different types of hepatoma cells and thus possesses marked hepatoprotective activity.

As described above, many studies have reported the desirable pharmacological effects of genipin in many aspects. However, few studies have investigated the antimicrobial effect of genipin, which has been shown to have sporicidal efficacy (Reich and Akkus 2013) and anti-fungal properties (Lelono et al. 2009). In addition, a crude extract of Zhizi is thought to possess antibacterial activity in China. Given that genipin is the metabolite of geniposide and may function as the main bioactive compound of gardenia, the antibacterial activity of genipin was tested on *E. coli* DH-5α cells in this study, and geniposide was also tested for comparison. The results show the high inhibitory activity of genipin on the growth of *E. coli* DH-5α cells. Some studies have investigated the antibacterial properties of films containing genipin-crosslinked chitosan, and their results suggest that chitosan contributes a large share to the antimicrobial properties of such films (Liu et al. 2012). According to the results presented here, genipin may also play a role in the antibacterial activity of genipin-crosslinked reagents. To the best of our knowledge, this study provides the first direct demonstration of the antibacterial activity of genipin and geniposide.

During the biotransformation period, the color of the algal cell suspension changed from blue-green to yellowish-brown. This interesting phenomenon attracted our interest, and it was assumed that the algal cells were dead as a consequence of the cytotoxicity of genipin against *Synechocystis* sp. PCC 6803 cells. To validate this hypothesis, genipin was subjected to the

MTT assay to assess the cytotoxicity of genipin against *Synechocystis* sp. PCC 6803. This positive result prompted us to test the cytotoxicity of genipin against another cyanobacterium, *Microcystis* sp. PCC 7806. *Microcystis* sp. PCC 7806, which is also well known as *Microcystis aeruginosa* PCC 7806, is one of the most common bloom-forming cyanobacteria in freshwater ecosystems worldwide (Alexova et al. 2011; Humbert et al. 2013). Our assays on these two cyanobacteria demonstrate that genipin may be potentially used for the control of toxic algae blooms.

In conclusion, our data demonstrate that *Synechocystis* sp. PCC 6803 has the ability to transform organic compounds and may be a potential biocatalyst for the transformation of glycosides and transglucosylation similarly to β-glucosidase. It also provides an alternative, inexpensive method for the production of genipin. We then provided the first demonstration of the antibacterial activity of genipin and geniposide. In addition, we provide the first report of the cytotoxicity of genipin against photosynthetic cyanobacteria, which offers new insight for the control of water blooms.

Acknowledgments The authors would like to thank Prof. Huang Fang of the Chinese Academy of Sciences for providing the cyanobacteria strain *Synechocystis* sp. PCC 6803.

References

Akao T, Kobashi K, Aburada M (1994) Enzymic studies on the animal and intestinal bacterial metabolism of geniposide. Biol Pharm Bull 17:1573–1576. doi:10.1248/bpb.17.1573

Alexova R, Fujii M, Birch D, Cheng J, David-Waite T, Ferrari BC, Neilan BA (2011) Iron uptake and toxin synthesis in the bloom-forming *Microcystis aeruginosa* under iron limitation. Environ Microbiol 13: 1064–1077. doi:10.1111/j.1462-2920.2010.02412.x

Deng Y, Guan M, Xie X, Yang X, Xiang H, Li H, Zou L, Wei J, Wang D, Deng X (2013) Geniposide inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma. Int Immunopharmacol 17:561–567. doi:10.1016/j.intimp.2013.06.028

Faramarzi MA, Adrangi S, Yazdi MT (2008) Microalagal biotransformation of steroids. J Phycol 44:27–37. doi:10.1111/j.1529-8817.2007.00446.x

Fu Y, Liu B, Liu J, Liu Z, Liang D, Li F, Li D, Cao Y, Zhang X, Zhang N, Yang Z (2012) Geniposide, from *Gardenia jasminoides* Ellis, inhibits the inflammatory response in the primary mouse macrophages and mouse models. Int Immunopharmacol 14:792–798. doi:10.1016/j.intimp.2012.07.006

Ghasemi Y, Rasoul Amini S, Fotooh Abadi E (2011) The biotransformation, biodegradation, and bioremediation of organic compounds by microalgae. J Phycol 47:969–980. doi:10.1111/j.1529-8817.2011.01051.x

Hsu HY, Yang JJ, Lin SY, Lin CC (1997) Comparisons of geniposidic acid and geniposide on antitumor and radioprotection after sublethal irradiation. Cancer Lett 113:31–37. doi:10.1016/s0304-3835(96)04572-7

Humbert JF, Barbe V, Latifi A, Gugger M, Calteau A, Coursin T, Lajus A, Castelli V, Oztas S, Samson G, Longin C, Medigue C, Marsac NT (2013) A tribute to disorder in the genome of the bloom-forming freshwater cyanobacterium *Microcystis aeruginosa*. PLoS One 8: e70747. doi:10.1371/journal.pone.0070747

Kang MJ, Khanal T, Kim HG, Lee DH, Yeo HK, Lee YS, Ahn YT, Kim DH, Jeong HG, Jeong TC (2012) Role of metabolism by human intestinal microflora in geniposide-induced toxicity in HepG2 cells. Arch Pharm Res 35:733–738. doi:10.1007/s12272-012-0418-y

Khanal T, Kim HG, Choi JH, Do MT, Kong MJ, Kang MJ, Noh K, Yeod HK, Ahn YT, Kang W, Kim DH, Jeong TC, Jeong HG (2012) Biotransformation of geniposide by human intestinal microflora on cytotoxicity against HepG2 cells. Toxicol Lett 209:246–254. doi:10.1016/j.toxlet.2011.12.017

Kim BC, Kim HG, Lee SA, Lim S, Park EH, Kim SJ, Lim CJ (2005) Genipin-induced apoptosis in hepatoma cells is mediated by reactive oxygen species/c-Jun NH_2-terminal kinase-dependent activation of mitochondrial pathway. Biochem Pharmacol 70:1398–1407. doi:10.1016/j.bcp.2005.07.025

Kim CY, Kim J (2007) Preparative isolation and purification of geniposide from gardenia fruits by centrifugal partition chromatography. Phytochem Anal 18:115–117. doi:10.1002/pca.958

Kim HW, Vannela R, Rittmann BE (2013) Responses of *Synechocystis* sp. PCC 6803 to total dissolved solids in long-term continuous operation of a photobioreactor. Bioresour Technol 128:378–384. doi:10.1016/j.biortech.2012.10.046

Koo HJ, Lim KH, Jung HJ, Park EH (2006) Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. J Ethnopharmacol 103:496–500. doi:10.1016/j.jep.2005.08.011

Lelono RAA, Tachibana S, Itoh K (2009) Isolation of antifungal compounds from *Gardenia jasminoides*. Pak J Biol Sci 12:949–956. doi:10.3923/pjbs.2009.949.956

Li Y, Jin W, Jing W, Yao M, Tang Y, Lu F (2014) Enzymolysis and Microbial Transformation of Geniposide in Gardenia Jasminoides into Genipin by *Aspergillus niger*, p 981-992. *In* Zhang TC, Ouyang P, Kaplan S, Skarnes B (ed), Proceedings of the 2012 International Conference on Applied Biotechnology (ICAB 2012), Springer Berlin Heidelberg. doi:10.1007/978-3-642-37922-2_105

Liu Y, Chen W, Kim HI (2012) Mechanical and antimicrobial properties of genipin-crosslinked chitosan/poly (ethylene glycol) IPN. J Macromol ScI, Part B, Phys 51:1069–1079. doi:10.1080/00222348.2011.625889

Lu M, Qiao Q, Pang C, Wang Y (2002) Effect of macroporous resin in separation of gardenoside. Zhong Cao Yao. 33:794-795. doi:10.3321/j.issn:0253-2670.2002.09.014

Ma T, Huang C, Zong G, Zha D, Meng X, Li J, Tang W (2011) Hepatoprotective effects of geniposide in a rat model of nonalcoholic steatohepatitis. J Pharm Pharmacol 63:587–593. doi:10.1111/j.2042-7158.2011.01256.x

Nam KN, Choi YS, Jung HJ, Park GH, Park JM, Moon SK, Cho KH, Kang C, Kang I, Oh MS, Lee EH (2010) Genipin inhibits the inflammatory response of rat brain microglial cells. Int Immunopharmacol 10:493–499. doi:10.1016/j.intimp.2010.01.011

Quan LH, Min JW, Jin Y, Wang C, Kim YJ, Yang DC (2012) Enzymatic biotransformation of ginsenoside Rb1 to compound K by recombinant β-glucosidase from *Microbacterium esteraromaticum*. J Agric Food Chem 60:3776–3781. doi:10.1021/jf300186a

Rather MY, Mishra S, Verma V, Chand S (2012) Biotransformation of methyl-β-D-glucopyranoside to higher chain alkyl glucosides by cell bound β-glucosidase of *Pichia etchellsii*. Bioresour Technol 107:287–294. doi:10.1016/j.biortech.2011.11.061

Reich MS, Akkus O (2013) Sporicidal efficacy of genipin: a potential theoretical alternative for biomaterial and tissue graft sterilization. Cell Tissue Bank 14:381–393. doi:10.1007/s10561-012-9335-z

Rupprecht J, Hankamer B, Mussgnug JH, Ananyev G, Dismukes C, Kruse O (2006) Perspectives and advances of biological H_2 production in microorganisms. Appl Microbiol Biotechnol 72:442–449. doi:10.1007/s00253-006-0528-x

Sheng J, Vannela R, Rittmann BE (2011) Evaluation of methods to extract and quantify lipids from *Synechocystis* PCC 6803. Bioresour Technol 102:1697–1703. doi:10.1016/j.biortech.2010.08.007

Tseng TH, Chu CY, Wang CJ (1994) Comparison of geniposide and its acetylated derivative for the inhibition of aflatoxin B_1-induced DNA-repair synthesis in rat primary hepatocyte. Oncol Rep 1: 165–168. doi:10.3892/or.1.1.165

Verma A, Prasad KN, Singh AK, Nyati KK, Gupta RK, Paliwal VK (2010) Evaluation of the MTT lymphocyte proliferation assay for the diagnosis of neurocysticercosis. J Microbiol Methods 81:175–178. doi:10.1016/j.mimet.2010.03.001

Wang J, Hou J, Zhang P, Li D, Zhang C, Liu J (2012) Geniposide reduces inflammatory responses of oxygen-glucose deprived rat microglial cells via inhibition of the TLR4 signaling pathway. Neurochem Res 37:2235–2248. doi:10.1007/s11064-012-0852-8

Wang X, Zhang A, Yan G, Sun W, Han Y, Sun H (2013) Metabolomics and proteomics annotate therapeutic properties of geniposide: targeting and regulating multiple perturbed pathways. PLoS One 8:e71403. doi:10.1371/journal.pone.0071403

Yang YS, Zhang T, Yu SC, Ding Y, Zhang LY, Qiu C, Jin D (2011) Transformation of geniposide into genipin by immobilized β-glucosidase in a two-phase aqueous-organic system. Molecules 16: 4295–4304. doi:10.3390/molecules16054295

Yin XX, Chen J, Qin J, Sun GX, Rosen BP, Zhu YG (2011) Biotransformation and volatilization of arsenic by three photosynthetic cyanobacteria. Plant Physiol 156:1631–1638. doi:10.1104/pp.111.178947

Zhang A, Sun H, Wang X (2013) Recent advances in natural products from plants for treatment of liver diseases. Eur J Med Chem 63:570–577. doi:10.1016/j.ejmech.2012.12.062