JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 9523–9528 9523
DOI:10.1021/jf102128t

Cytotoxicity of *Monascus* Pigments and Their Derivatives to Human Cancer Cells

YUNQUAN ZHENG,[†] YANWEN XIN,[†] XIANAI SHI,[†] AND YANGHAO GUO*[,†,‡]

[†]Institute of Pharmaceutical Biotechnology and Engineering, College of Biological Science and Technology, Fuzhou University, 2 Xueyuan Road, Fuzhou 350108, China, and [‡]Fujian Key Laboratory of Medical Instrument and Pharmaceutical Technology, Fuzhou University, 523 Gongye Road, Fuzhou 350002, China

Six pigments were separated from *Monascus* product, and some derivatives were chemically synthesized. The cytotoxicity of different *Monascus* pigments to various human cancer cells (SH-SY5Y, HepG2, HT-29, BGC-823, AGS, and MKN45) was evaluated. Rubropunctatin showed the greatest anticancer effect within the tested compounds. The inhibition effect of rubropunctatin was higher than that of taxol on the growth of the human gastric cancer cell SH-SY5Y ($P < 0.05$), BGC-823 ($P < 0.01$), AGS ($P < 0.01$), and MKN45 ($P < 0.05$). On the other hand, its cytotoxicity to the normal human gastric epithelial cell GES-1 was less than that of taxol ($P < 0.01$). The experimental data demonstrated that rubropunctatin was a valuable compound with high anticancer activity, which could offer better therapeutic benefits than taxol. Cell apoptosis stages were assayed by annexin V−EGFP/PI staining experiments using flow cytometry. The data showed that 87.63% of tested BGC-823 cells entered the early phase of apoptosis when treated with 5 μM rubropunctatin for 24 h. A drug concentration-dependent cell apoptosis was observed. The analysis of the relationship between pharmaceutical activity and the chemical structure of the tested compounds led to the conclusion that 6-internal ether, 4-carbonyl, and conjugated double bonds in the tricyclic structure of rubropunctatin were necessary to the anticancer effect, whereas the difference of C_2H_4 in the side chain showed little influence. Rubropunctatin could be supplied as a precursor compound in the development of a new natural anticancer reagent.

KEYWORDS: *Monascus* pigments; anticancer; apoptosis

INTRODUCTION

Red mold rice (RMR) is produced by cultivating *Monascus* species on steamed rice. For centuries, red mold rice has been widely used as a food additive and traditional pharmaceutical in China and Asia (*1*, *2*). *Monascus* pigments are a series of azaphilone compounds exhibiting a wide range of biological activities (*3*). Scientific evidence showed that RMR was proved to be effective for the management of cholesterol, diabetes, cardiovascular disease, and also the prevention of cancer (*4*−*9*).

In recent years, many researchers have paid close attention to the anticancer effects of RMR. The pigments extracted from *Monascus anka* inhibited 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) promoted carcinogenesis in mice(*4*). Ankaflavin showed selective cytotoxicity to HepG2 cells by an apoptosis-related mechanism and showed relatively low toxicity to normal fibroblasts (*5*). Oral administration of monascin inhibited the carcinogenesis of skin cancer initiated by peroxynitrite or ultraviolet light and after the promotion of TPA in the mouse model (*6*). The secondary metabolites produced by *Monascus*, including monacolin K and ankaflavin, have been reported to have antitumor-initiating effects on cancer progression. Ho and Pan reported that the oral administration of RMR extract dramatically inhibited the metastatic ability of murine Lewis lung carcinoma cells in syngeneic C57BL/6 mice (*7*). Hong compared the anticancer effects of monacolin K and RMR extract (without monacolin K) and found that both decreased proliferation of human colon cancer cells and induced apoptosis. The experimental data suggested that other components in red yeast rice (RYR) or a matrix with many metabolites including pigments may be a potent anticancer compound (*8*). It is an essential task for the development of natural anticancer drug to explore the anticancer effect of the purified *Monascus* pigments containing only a single component.

In this study, six *Monascus* pigments were isolated and purified by high-speed counter-current chromatography (HSCCC), and some derivatives were chemically synthesized. To determine which component exhibited the greatest antiproliferative potential, the effects of all prepared compounds on the growth of different human tumor cells, including hepatocellular carcinoma cell HepG2, neuroblastoma cell SH-SY5Y, colon cancer cell HT-29, and gastric adenocarcinoma cell BGC-823, AGS, and MKN45 were compared. Then the apoptotic effect induced by the pigments was examined using flow cytometry. Furthermore, the relationship between chemical structure and anticancer activity of the tested compounds was analyzed.

*Corresponding author (phone +86-591-83720772; fax +86-591-83720772; e-mail yanghaoguo@yahoo.com.cn).

Published on Web 08/13/2010

MATERIALS AND METHODS

Materials. MTT and DMSO were purchased from Sigma (St. Louis, MO). Fetal bovine serum (FBS), RPMI 1640, DMEM, and F12 were obtained from Gibco BRL (Gaithersburg, MD). Annexin V−EGFP/PI Apoptosis Detection Kit was supplied by Nanjing Keygen Biotech. Co., Ltd. (Jiangshu, China). All other reagents were of analytical grade.

Cell Lines and Culture. HepG2 (human hepatocellular carcinoma cell), SH-SY5Y (human neuroblastoma cell), HT-29 (human colon cancer cell), BGC-823 (human gastric adenocarcinoma cell), AGS (human gastric adenocarcinoma cell), and MKN45 (human gastric adenocarcinoma cell) were purchased from the Cell Resource Center of Shanghai Biological Sciences Institute (Chinese Academy of Sciences, Shanghai, China). The human gastric mucosal epithelial cell line GES-1 was a gift of Prof. Feifei S. (Fujian Medical University, Fuzhou, China). BGC-823 and HepG2 were cultured in RPMI 1640 supplemented with 10% FBS. MKN45, HT-29, SH-SY5Y, and GES-1 were grown in DMEM plus 10% FBS. AGS cells were maintained in F12 medium with the same supplement. All of the cells were incubated in a humidified incubator at 37 °C with 5% CO_2 and 95% air.

Preparation of *Monascus* Pigments. Steamed rice was inoculated with *Monascus* sp. strain FZU04 and then cultivated at 33 °C (humidity = 50−60%) for 8 days until the rice was completely red. The fermented rice (RMR) were dried and finely ground (diameter < 0.15 mm). Then the resulting powder (100 g) was extracted with 1 L of ethanol/water solution (7:3, v/v). The extract was collected and concentrated under reduced pressure. A HSCCC method was developed to separate six *Monascus* pigments, including rubropunctatamine, monascorubramine, monascin, ankaflavin, rubropunctatin, and monascorubrin, from the crude extract. Six eluted fractions were collected and subjected to crystallization. Six crystallized *Monascus* pigments were obtained separately.

The HSCCC separation was performed with a TBE-300B high-speed counter-current chromatography apparatus manufactured by Tauto Biotech Co., Ltd. (Shanghai, China). The apparatus was equipped with three preparative coils (diameter of tube, 1.9 mm; total volume, 260 mL) and a 20 mL sample loop. The β values of this column range from 0.5 to 0.8 ($\beta = r/R$, where r is the distance from the coil to the holder shaft and R is the revolution radius or the distance between the holder shaft and central axis of the centrifuge). The selected solvent system that consisted of *n*-hexane, methanol, and water (10:7.5:2.5, v/v/v) was used for the separation of five fractions: the red pigments (mixture of rubropunctatamine and monascorubramine), monascin, rubropunctatin, ankaflavin, and monascorubrin. Then the red pigments were separated by the solvent system that consisted of *n*-hexane, ethyl acetate, methanol, and water with the volume ratio of 2.5:7.5:5:5 for rubropunctatamine and monascorubramine. When the column was totally filled with the two phases, only the lower phase was pumped at a flow rate of 3.0 mL/min, and at the same time, the HSCCC apparatus was run at a revolution speed of 900 rpm. The temperature was controlled at 25 °C in the separation procedure. The effluent from the outlet of the column was continuously monitored at 254 nm.

Identification of *Monascus* Pigments. The purity of six pigments obtained was determined by HPLC (*10*), and the chemical structure was identified by HPLC-MS and NMR spectroscopy.

An Agilent 1100 series ion trap LC/MS systems (Agilent, USA) was used to determine the molecular weight of *Monascus* pigments. The mass spectrometer was equipped with an electrospray ionization (ESI) source. The ESI conditions were as follows: capillary voltage, 3.5 kV; nebulizer pressure, 40 psi; drying gas flow, 10 mL/min; temperature, 350 °C. The mass range was from m/z 100 to 400.

^{1}H and ^{13}C NMR spectra were measured with an Inova-500 M NMR spectrometer (Varian, USA) in $CDCl_3$ operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. Tetramethylsilane (TMS) was employed as an internal standard.

MTT Cell Proliferation Assay. Cells (1×10^4 per cell) were seeded in 100 μL of medium into sterile 96-well plates. After 24 h of incubation, the medium in the 96-well plate was discarded and replaced with 100 μL of medium that contained tested components at final concentrations of 0, 3, 15, 30, 60, 120, 180, and 240 μM (with 0.4% DMSO), each in sextuplicate. These plates were then incubated in a 37 °C humidified incubator with 5% CO_2 for 24 h. The media in these plates were discarded, and then 200 μL of MTT solution (500 μg of MTT in 1 mL of fresh medium) was added. The culture was then incubated for 4 h to convert MTT to formazan. Thereafter, the supernatant was aspirated, and 200 μL of DMSO was added to dissolve the formazan. The optical density (OD) was determined at 570 nm. Percentage of inhibition was calculated using the following formula: growth inhibition (%) = $1 - (OD_{test}/OD_{control}) \times 100\%$.

Cell Apoptosis Determined by Flow Cytometry. Early and late apoptotic changes in BGC-823 cells were determined using an Annexin V−EGFP/PI Apoptosis Detection Kit. The operation was performed as recommended by the manufacturer (Keygen, China). Briefly, cells (5×10^5) were collected and washed twice with PBS and suspended in 500 μL of binding buffer (adding 5 μL of annexin V−EGFP and 5 μL of PI). Thereafter, the samples were incubated in the dark for 10 min at room temperature. Flow cytometry was carried out on a flow cytometer system (Bechman Coulter, USA). The number of annexin V−EGFP-positive and PI-positive of cells in each field was determined by counting the cells directly.

Statistical Analysis. The results were expressed as the mean ± standard deviation (SD) from three independent experiments. A significant difference from the respective controls for each experimental test condition was assessed using Student's unpaired t test, with P values of < 0.01 (*) or < 0.05 (**) being regarded as statistically significant.

RESULTS

Purification and Identification of *Monascus* Pigments. There are yet no available commercial *Monascus* pigments with high purity. The prepared pigments should be identified by HPLC, HPLC-MS, and NMR.

Six pigment components were prepared separately from *Monascus* products. The purity of each component was analyzed according to the previously reported determination method of HPLC fingerprint profile of the metabolites in the *Monascus* product(*10*). As shown in **Figure 1**, a high purity of > 98.5% was achieved.

O1: UV−vis (ethanol), λ_{max} 213, 247, 286, and 473 nm; ESI-MS, m/z 355.2 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz), δ 0.89 (3H, t, H-19), 1.305−1.334 (4H, m, H-17−18), 1.61 (2H, m, H-16), 1.71 (3H, s, H-10), 1.96 (3H, d, H-13), 2.94 (2H, m, H-15), 6.05 (1H, d, H-11), 6.15 (1H, s, H-8), 6.59 (1H, dq, H-12), 6.89 (1H, s, H-9), 7.87 (1H, s, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz), δ 13.927 (C-19), 18.721 (C-10), 22.524 (C-13), 23.379 (C-18), 28.291 (C-16), 31.377 (C-17), 41.580 (C-15), 85.758 (C-3a), 104.154 (C-8), 109.544 (C-4a), 113.228 (C-9), 116.283 (C-11), 122.356 (C-1), 136.358 (C-12), 141.584 (C-8a), 152.760 (C-5), 156.398 (C-7), 169.192 (C-2), 171.625 (C-9a), 190.777 (C-14), 197.398 (C-4).

Y1: UV−vis (ethanol), λ_{max} 231, 291, and 387 nm; ESI-MS, m/z 359.2 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz), δ 0.89 (3H, t, H-19), 1.25−1.35 (4H, m, H-17−18), 1.45 (3H, s, H-10), 1.63 (2H, m, H-16), 1.88 (3H, d, H-13), 2.43 (1H, m, H-15), 2.63, 3.23 (2H, m, H-9), 2.98−3.06 (H, m, H-9a), 3.49 (1H, d, H-1), 4.73, 5.05 (2H, d, H-5), 5.27 (1H, s, H-8), 5.90 (1H, d, H-11), 6.51 (1H, dq, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz), δ 13.885 (C-19), 17.730 (C-13), 18.489 (C-10), 22.402 (C-18), 22.787 (C-16), 29.455 (C-9), 31.152 (C-17), 42.870 (C-9a), 42.908 (C-15), 54.900 (C-1), 63.833 (C-5), 83.149 (C-3a), 103.265 (C-8), 114.010 (C-11), 124.377 (C-12), 135.443 (C-4a), 150.715 (C-8a), 160.495 (C-7), 169.474 (C-2), 189.793 (C-4), 202.464 (C-14).

R1: UV−vis (ethanol), λ_{max} 304, 413, and 524 nm; ESI-MS, m/z 354.1 $[M + H]^+$; ^{1}H NMR ($CDCl_3$, 500 MHz), δ 0.90 (3H, t, H-19), 1.360−1.388 (4H, m, H-17−18), 1.640−1.700 (2H, m, H-16), 1.806 (3H, s, H-10), 2.05 (3H, d, H-13), 2.86 (2H, m, H-15), 6.39 (1H, d, H-11), 7.05 (1H, dq, H-12), 6.75 (1H, s, H-8), 6.77 (1H, s, H-9), 9.25 (1H, s, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz), δ 14.026 (C-19), 19.206 (C-10), 22.517 (C-13), 24.565 (C-18), 29.459 (C-16), 31.858 (C-17), 41.580 (C-15), 86.852 (C-3a), 104.346 (C-8), 109.952 (C-4a), 113.625 (C-9), 116.283 (C-11), 121.982 (C-1), 123.435 (C-12), 141.584 (C-8a), 153.120 (C-5), 156.458 (C-9a), 169.346 (C-7), 174.291 (C-2), 191.234 (C-4), 197.345 (C-14).

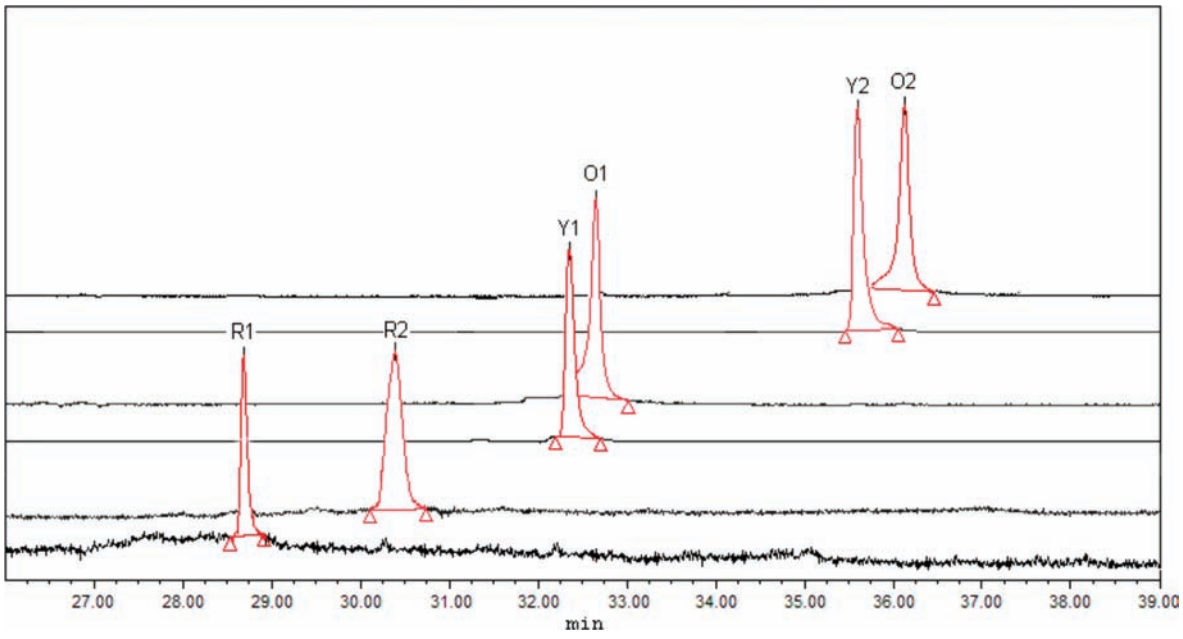


Figure 1. HPLC chromatogram of six purified *Monascus* pigments. The elution gradient was as follows [eluent A (water/HAC = 100:10), eluent B (acetonitrile/HAC = 100:10)]: 0 min, 80% A and 20% B; 25 min, 50% A and 50% B; 26 min, 15% A and 85% B; flow rate, 1 mL/min; detected by PDA detector at 390 nm.

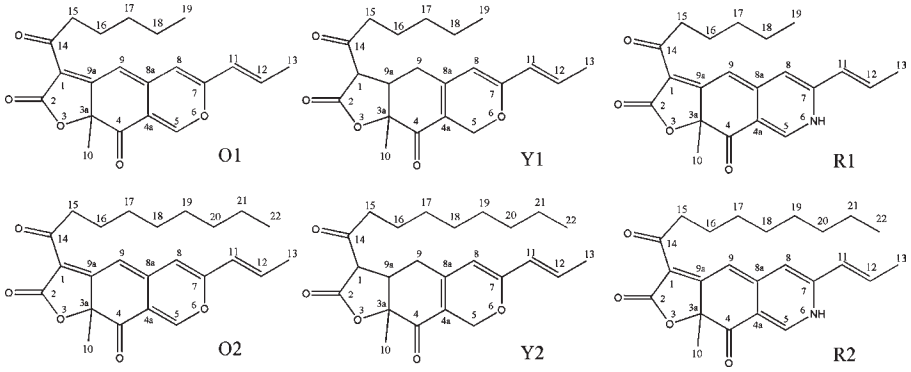


Figure 2. Chemical structures of *Monascus* pigments.

The determined MS and NMR data demonstrated that Y1, O1, and R1 were monascin, rubropunctatin, and rubropunctatamine, separately (*11*−*14*). Their structures are shown in **Figure 2**. The other three components (Y2, O2, and R2) were ankaflavin, monascorubrin, and monascorubramine, which were homologues of Y1, O1, and R1, respectively. There was a common difference of C_2H_4 in the chemical structure of the side chain between both homologues.

Inhibition Effect of Y1, O1, and R1 on Proliferation of Different Cancer Cell Lines. The effects of Y1, O1, and R1 at different concentrations on the proliferation of the human cancer cell lines (HepG2, SH-SY5Y, HT-29, BGC-823, AGS, and MKN45) were tested separately using the MTT assay. As shown in **Figure 3**, O1 was demonstrated to have the greatest in vitro anticancer potency among the tested compounds. The inhibition effects of the yellow pigment Y1 and the red pigment R1 on the tested cells were much less than that of the orange pigment O1. After treatment by O1 for 24 h, IC_{50} values against the human gastric adenocarcinoma cells (BGC-823, AGS, and MKN45) were below 15 μM and those against the other three cells (HepG2, SH-SY5Y, and HT-29) ranged from 30 to 45 μM (**Table 1**).

Taxol, an efficient natural anticancer drug (with activity against a number of cancers in the human breast, ovary, lung, and gastrointestin) was used as a positive control in the present work. The inhibition effects of the tested compounds on the cell growth of different cancer cell lines are compared in **Table 1**. As shown in **Table 1**, the inhibition effect of O1 was higher than that of taxol on the growth of SH-SY5Y ($P < 0.05$), BGC-823 ($P < 0.01$), AGS ($P < 0.01$), and MKN45 ($P < 0.05$).

A normal human gastric epithelial cell GES-1 was also included in parallel experiments. IC_{50} values of O1 and taxol were 108.38 ± 9.65 and 80.03 ± 11.96 μM, respectively, which showed that O1 possessed less cytotoxicity than taxol for the normal gastric epithelial cell ($P < 0.01$). The experimental data demonstrated that rubropunctatin (O1) was a valuable component with high anticancer activity, which could offer better therapeutic benefits than taxol.

Induction of Apoptosis As Evidenced by Phosphatidylserine Externalization. In attempt to elucidate whether the loss in cell viability induced by O1 was associated with apoptosis, we performed the annexin V−EGFP/PI staining experiment to examine the occurrence of phosphatidylserine externalization onto the cell surface, as shown in **Figure 4**.

In the control group, the cell was not treated with O1 and 97.57% of cells were in normal condition ($EGFP^-/PI^-$). When BGC-823 cells were incubated with 5 μM O1 for 24 h, a substantial amount of cells (84.63%) was primarily in the early phase of apoptosis ($EGFP^+/PI^-$). Treatment with a higher concentration of O1,

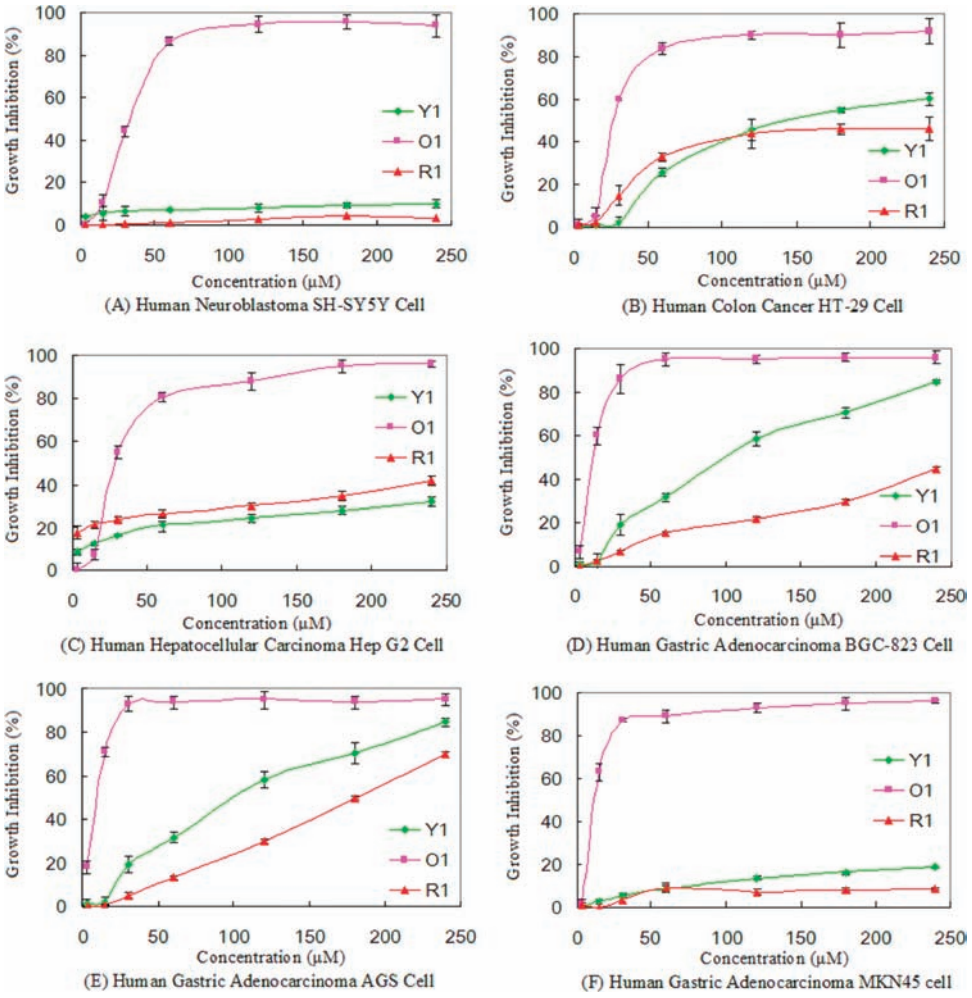


Figure 3. Growth inhibition of Y1, O1, and R1 on various cancer cell lines. Cells were seeded in 96-well plates and incubated with different concentrations of Y1, O1, and R1 (3, 15, 30, 60, 120, 180, and 240 μmol/L) for 24 h at 37 °C in an atmosphere of 5% CO_2. Growth inhibition was determined by MTT assay. Each value represents the mean ± SD of six replicates.

Table 1. Cytotoxicity of O1 and Taxol on Various Cancer Cell Lines and Normal GES-1 Cell Line[a]

cell type	cell line	IC_{50} of O1 (μM)	IC_{50} of taxol (μM)
cancer cell	SH-SY5Y	30.95 ± 6.26*	35.46 ± 4.57
	HepG2	44.19 ± 5.23	37.53 ± 4.93
	HT-29	36.69 ± 4.08	33.07 ± 2.28
	BGC-823	12.57 ± 1.82**	21.65 ± 1.93
	AGS	7.98 ± 0.71**	20.57 ± 2.53
	MKN45	14.27 ± 1.39*	17.95 ± 2.34
normal cell	GES-1	108.38 ± 9.65**	80.03 ± 11.96

[a] Cells were treated with various concentrations of O1 and taxol for 24 h and then determined by MTT proliferation assays. Results are expressed as mean ± SD of three different experiments.

however, induced a shift of the cell population to late apoptotic/necrotic stage ($EGFP^+/PI^+$); 94.91% of cells were determined in the late apoptotic/necrotic stage when treated with 40 μM O1 for 24 h. The results indicate obviously that O1 induced cell apoptosis of BGC-823 and that cell apoptosis was dose-dependent.

Relationship between the Chemical Structure and Anticancer Activity of *Monascus* Pigments. A series of *Monascus* pigments and the derivatives were prepared in this work. Y1, O1, R1, and O2 were separated from RMR, as shown in **Figures 1** and **2**. By way of Schiff base formation (*15*), O3 and O4 were produced, when glutamic acid and carbamide reacted with O1 separately. O5 was the derivative produced by reduction of the carbonyl group at position 4 (*16*) (**Figure 5**).

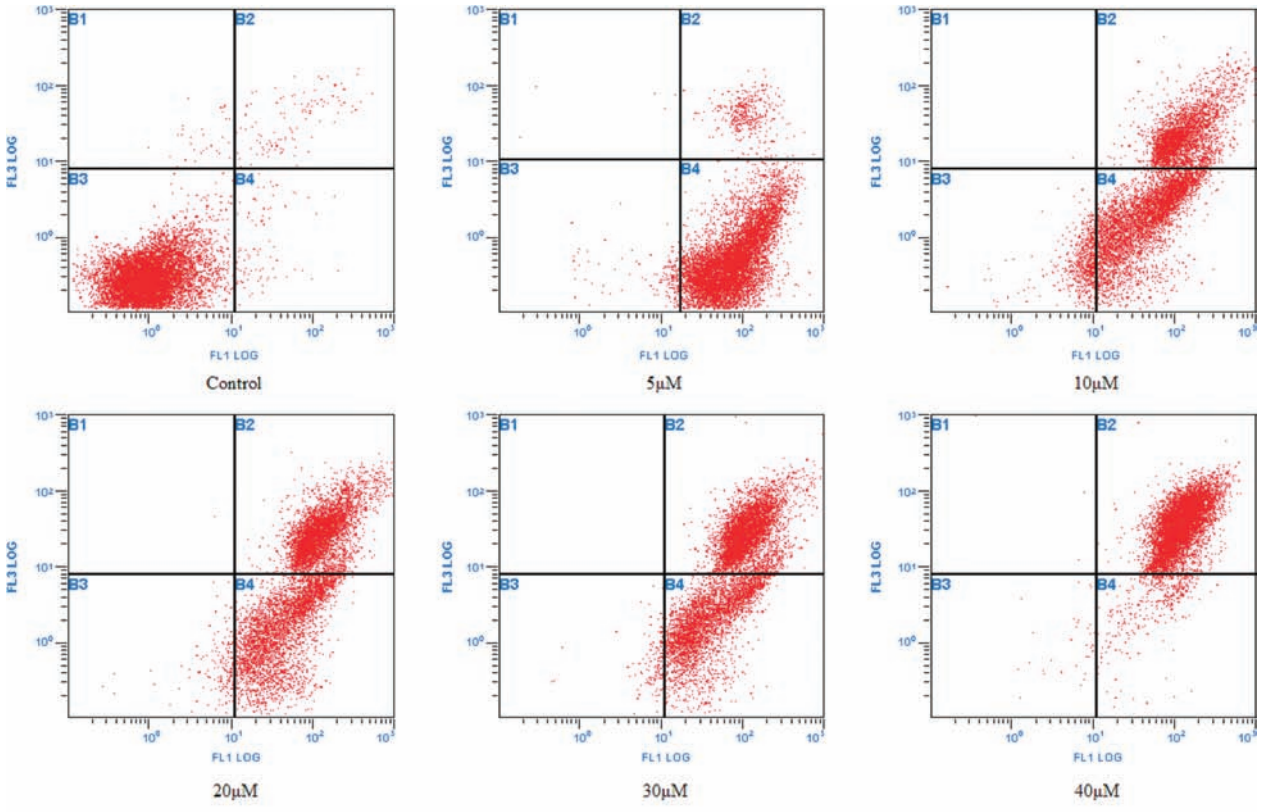
The prepared compounds of different concentrations (3, 15, and 30 μM) were used to explore their anticancer effects. The influence of different chemical structures on the proliferation of the human gastric adenocarcinoma cell BGC-823 was analyzed using MTT assay (**Figure 6**).

O1 and O2, a pair of orange pigments, displayed similar inhibition effects on the proliferation of BGC-823 at the same concentration applied. The experimental data indicated that the difference of C_2H_4 in the chemical structure of the side chain showed little influence on the anticancer effect.

R1, O3, and O4 were derivatives of O1. Reaction of amino compounds (ammonia, glutamic acid, and carbamide) with O1 gave water-soluble pigments (R1, O3, and O4). The oxygen atom at position 6 was displaced by a nitrogen atom in the derivative. The inhibition of R1, O3, or O4 on cell growth of BGC-823 was significantly low compared with O1. The result indicated that the chemical structure of 6-internal ether was necessary to the anticancer activity.

O1 was reduced by sodium borohydride to give the product O5. O5 had a hydroxyl group instead of a carbonyl group at position 4. After treatment with O5 for 24 h, no cytotoxicity on BGC-823 cells was observed and, on the contrary, the proliferation of BGC-823 was promoted.

Y1 was also a reduced product of O1. Y1 and O1 varied only in the number of conjugated double bonds in the tricyclic structure.



Label	Control (%)	5μM (%)	10μM (%)	20μM (%)	30μM (%)	40μM (%)
Normal (AV^- PI^-)	97.57	3.24	2.36	1.92	1.78	0.41
Early apoptosis (AV^+ PI^-)	0.95	84.63	59.29	41.61	39.58	4.65
Late apoptosis/Necrosis (AV^+ PI^+)	1.11	12.04	38.35	56.45	58.64	94.91
Others (AV^- PI^+)	0.37	0.09	0.01	0.02	0.00	0.03

Figure 4. Annexin V−FITC/PI staining of BGC-823 cells treated with rubropunctatin for 24 h. BGC-823 cells were treated with different concentrations of rubropunctatin (0, 5, 10, 20, 30, and 40 μmol/L) for 24 h at 37 °C in an atmosphere of 5% CO_2. Cells collected were subjected to annexin V−EGFP/PI staining and analyzed by flow cytometry. The table summarizes the proportion of cells in each stage of apoptosis.

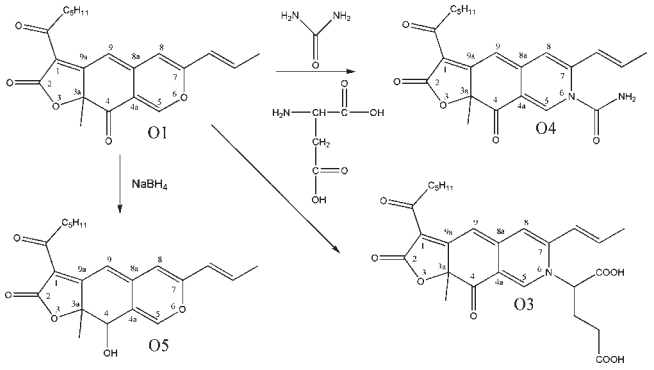


Figure 5. Chemical structures of *Monascus* pigment derivatives.

Y1 showed low cytotoxicity on BGC-823 cells, which manifested that multiple conjugated double bonds in the tricyclic structure of O1 were important to the anticancer activity.

DISCUSSION

The main pigments produced by *Monascus* spp. comprised rubropunctatin (O1), monascorubrin (O2), monascin (Y1), ankaflavin (Y2), rubropunctatamine (R1), and monascorubramine (R2), which could be divided into three groups, O1 and O2, Y1 and Y2, and R1 and R2. In the present work, we evaluated the cytotoxicity of different *Monascus* pigments to various human

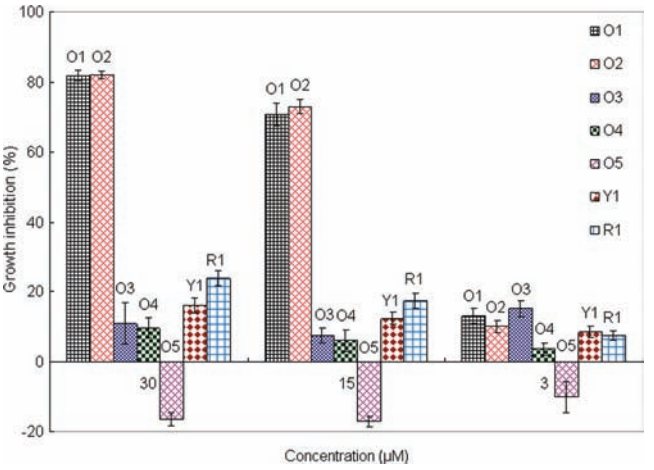


Figure 6. Growth inhibition of *Monascus* pigments and derivatives on BGC-823. Cells were seeded in 96-well plates and incubated with different concentrations of Y1, O1, and R1 (3, 15, and 30 μmol/L) for 24 h at 37 °C in an atmosphere of 5% CO_2. Growth inhibition was determined by MTT assay. Each value represents the mean ± SD of six replicates.

cancer cell lines. Both orange pigments, O1 and O2, showed the greatest inhibition effect on the proliferation of the tested human cancer cells. It was notable that O1 and O2 exhibited a significant antigastric cancer activity. The inhibition effect of O1 was higher

than that of taxol on the growth of the human gastric cancer cells SH-SY5Y ($P < 0.05$), BGC-823 ($P < 0.01$), AGS ($P < 0.01$), and MKN45 ($P < 0.05$). On the other hand, O1's inhibition of cell proliferation on normal human gastric epithelial cell GES-1 was less than that of taxol ($P < 0.01$).

Cell apoptosis stages were determined by annexin V−EGFP/PI staining experiments using flow cytometry. The proportion of annexin V-stained cells signifying both the early and late apoptotic cells increased with the concentration of rubropunctatin applied. It manifested the loss in cell viability after treatment with the pigment component could be associated with cell apoptosis.

The relationship between pharmaceutical activity and chemical structure of different *Monascus* pigments and derivatives showed that 6-internal ether, 4-carbonyl, and conjugated double bonds in the tricyclic structure of rubropunctatin were necessary to the anticancer effect, whereas the difference of C_2H_4 in the side chain showed little influence. The experimental data demonstrated that rubropunctatin could be supplied as a potential precursor compound in the development of a new natural anticancer reagent.

LITERATURE CITED

(1) Jůzlová, P.; Martínková, L.; Křen, V. Secondary metabolites of the fungus *Monascus*: a review. *J. Ind. Microbiol. Biotechnol.* **1996**, *16*, 163–170.

(2) Lin, Y.; Wang, T.; Lee, M.; Su, N. Biologically active components and nutraceuticals in the *Monascus*-fermented rice: a review. *Appl. Microbiol. Biotechnol.* **2008**, *77*, 965–973.

(3) Osmanova, N.; Schultze, W.; Ayoub, N. Azaphilones: a class of fungal metabolites with diverse biological activities. *Phytochem. Rev.* **2010**, *9* (2), 315−342.

(4) Yasukawa, K.; Takahashi, M.; Natori, S.; Kawai, K.; Yamazaki, M.; Takeuchi, M.; Takido, M. Azaphilones inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mice. *Oncology* **1994**, *51*, 108–112.

(5) Su, N.; Lin, Y.; Lee, M.; Ho, C. Ankaflavin from monascus-fermented red rice exhibits selective cytotoxic effect and induces cell death on hep G2 cells. *J. Agric. Food Chem.* **2005**, *53*, 1949–1954.

(6) Akihisa, T.; Tokuda, H.; Yasukawa, K.; Ukiya, M.; Kiyota, A.; Sakamoto, N.; Suzuki, T.; Tanabe, N.; Nishino, H. Azaphilones, furanoisophthalides, and amino acids from the extracts of *Monascus* pilosus-fermented rice (red-mold rice) and their chemopreventive effects. *J. Agric. Food Chem.* **2005**, *53*, 562–565.

(7) Ho, B.; Pan, T. The *Monascus* metabolite monacolin K reduces tumor progression and metastasis of Lewis lung carcinoma cells. *J. Agric. Food Chem.* **2009**, *57*, 8258–8265.

(8) Hong, M.; Seeram, N.; Zhang, Y.; Heber, D. Anticancer effects of Chinese red yeast rice versus monacolin K alone on colon cancer cells. *J. Nutr. Biochem.* **2008**, *19*, 448–458.

(9) Knecht, A.; Humpf, H. Cytotoxic and antimitotic effects of N-containing *Monascus* metabolites studied using immortalized human kidney epithelial cells. *Mol. Nutr. Food Res* **2006**, *50*, 406–412.

(10) Zheng, Y.; Xin, Y.; Guo, Y. Study on the fingerprint profile of Monascus products with HPLC-FD, PAD and MS. *Food Chem.* **2009**, *113*, 705–711.

(11) Fielding, B.; Holker, J.; Jones, D.; Powell, A.; Richmond, K.; Robertson, A.; Whalley, W. The chemistry of fungi. Part XXXIX. The structure of monascin. *J. Chem. Soc.* **1961**, 4579–4589.

(12) Haws, E.; Holker, J.; Kelly, A.; Powell, A.; Robertson, A. The chemistry of fungi. Part XXXVII. The structure of rubropunctatin. *J. Chem. Soc.* **1959**, *70*, 3598–3610.

(13) Kumasaki, S.; Nakanishi, K.; Nishikawa, E.; Ohashi, M. Structure of monascorubrin. *Tetrahedron* **1962**, *18*, 1171–1184.

(14) Teng, S.; Feldheim, W. Analysis of anka pigments by liquid chromatography with diode array detection and tandem mass spectrometry. *Chromatographia* **1998**, *47*, 529–536.

(15) Hajjaj, H.; Klaebe, A.; Loret, M.; Tzedakis, T.; Goma, G.; Blanc, P. Production and identification of *N*-glucosylrubropunctamine and *N*-glucosylmonascorubramine from *Monascus ruber* and occurrence of electron donor-acceptor complexes in these red pigments. *Appl. Environ. Microbiol.* **1997**, *63*, 2671–2678.

(16) Martin, E.; Kurek, P.; Schumacher, E.; Rohrbach, R. Reduced monascus pigment derivatives as yellow food colorants. U.S. Patent 5,013,564, 1991.

Received for review June 4, 2010. Revised manuscript received July 27, 2010. Accepted August 2, 2010. We are grateful for funds provided by the Fujian Key Lab of Medical Instrument and Pharmaceutical Technology, China (No. 09002).