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DDR

Research Article

Synthesis and Activity Evaluation of 3′-Floxuridinyl 4-[3-(3, 5-di-*t*-butyl-4-methoxyphenyl)-3-oxo-propenyl]benzoate: In Vitro and In Vivo as a Potential Dual-Acting Antitumor Prodrug

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT BOBA (4-[3-(3, 5-di-*tert*-butyl-4-methoxyphenyl)-3-oxo-propenyl]benzoic acid), a substituted chalcone derivative, exhibits an excellent inducing differentiation on neoplastic cellular differentiation. FUDR (5-fluoro-2′-deoxyuridine, floxuridine) inhibits DNA biosynthesis and has been used extensively to treat various cancers. In our efforts to find a new dual-action antitumor prodrug, 3′-floxuridinyl 4-[3-(3, 5-di-*t*-butyl-4-methoxyphenyl)-3-oxo-propenyl] benzoate (3′-*O*-BOBA- FUDR) was synthesized, and its antiproliferative activity in vitro and antitumor efficacy in vivo were evaluated. Compared with FUDR, the antiproliferative activity of 3′-*O*-BOBA-FUDR was improved by 3–7-fold. In rat hepatocellular carcinoma xenografts, 3′-*O*-BOBA-FUDR-treated rats had smaller tumors than were found in controls. In addition, the expression of Bcl-2 protein was significantly downgraded, whereas the expression of Bax protein was upregulated in neoplastic tissues. The early apoptotic ratio of 3′-*O*-BOBA-FUDR-treated rat group was increased dose-dependently. These findings strongly support the concept that 3′-*O*-BOBA-FUDR may be a novel and effective dual-action antitumor prodrug. Drug Dev Res 73: 73–81, 2012.

Key words: inducing differentiation; FUDR; antitumor activity; prodrug

INTRODUCTION

Cancer therapy is a great challenge in the fields of medicine and immunology. Finding novel and efficacious compounds has been a high priority for research in pharmaceutical science. Apart from surgery, radiation, immunotherapy, and hormonal therapy, therapies for cancer treatment include cytotoxic drugs as the main form of chemotherapy for cancer. 2′-Deoxy-5-fluorouridine (FUDR) is cytotoxic in vitro against a wide range of cell lines derived from solid tumors and

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has been used in combination with folinic acid as a standard treatment in the adjuvant setting for a variety of carcinomas, including those of the stomach, colon, liver, and breast [Meyerhardt and Mayer, 2005]. However, FUDR exhibits adverse reactions and is rapidly metabolized after administration, particularly in liver [van Laar et al., 1998]. Resistance, rapid elimination (plasma $t_{1/2} = 7.7$ min), and low lipid solubility all contribute to the low efficacy of FUDR [LaCreta, 1987].

To overcome these shortcomings, prodrugs and colloidal carriers for the delivery and targeting of FUDR have been designed. We prepared solid lipid nanoparticles (SLN) of FUDR derivatives and found that the SLN had a desirable hepatocyte-selective targeting with the concentration of FUDR in liver enhanced [Li et al., 2008; Lian et al., 2008]. Urokinase-targeting FUDR conjugates [Vine et al., 2010] and FUDR duplex prodrugs showed that anticancer activity was sequence-dependent [Schott et al., 2009]. The lactosaminated human albumin–FUDR conjugate produces a high concentration of FUDR in hepatic sinusoids and improves FUDR efficacy [Fiume and Stefano, 2010], whereas the Amidon group has studied amino acid ester prodrugs of FUDR for several years [Vig et al., 2003; Landowski et al., 2005; Shin et al., 2006; Tsume et al., 2008]. These investigators found that these prodrugs could enhance intestinal absorption; were resistant to glycosidic bond metabolism; had good solubility, stability, and relatively fast enzymic conversion rates; and improved absorption, tumor selectivity, and efficacy.

It is well known that lipophilic neutral prodrugs of FUDR can cross the cell membrane and potentially liberate the nucleotide intracellularly. Therefore, a number of carboxylic acid ester of FUDR were synthesized and their effects evaluated [Nishizawa et al., 1965; McGuigan et al., 1993; Wang et al., 2002]. Among these, *O*-butanoyl esters were more effective anticancer agents than FUDR based on oral administration experiments that indicated a relative potency order: 3′,5′-di-*O*-butanoyl >3′-*O*-butanoyl >5′-*O*-butanoyl. The greater antitumor efficiency of these esters was due in part to their slower rate of hydrolysis by nonspecific esterases [Yamashita et al., 1988].

Agents that induce cell differentiation or inhibit cell proliferation represent another approach for cancer treatment [Sachs, 1987]. All-*trans*-retinoic acid (RA) can induce cell differentiation or suppress cell proliferation in tumor cell lines, including melanoma, neuroblastoma, leukemia, breast tumor, epithelial cancer, and papillomavirus-induced cancer [Smith et al., 1992; Khan et al., 1993]. A prodrug combining RA with FUDR may exhibit a dual-action antitumor effect. From a series of *O*-retinoyl esters of FUDR, the 3′-*O*-retinoyl FUDR prodrug was the most potent anticancer agent as assessed by in vitro cell differentiation and tumor growth delay [Xia et al., 1999]. From a series of substituted chalcones, BOBA (4-[3-(3, 5-di-*t*-butyl-4-methoxyphenyl)-3-oxo-propenyl] benzoic acid; Fig. 1) displayed differentiation activity comparable to that of RA [Guo et al., 1997]. The EC_{50} values of BOBA and RA for HL-60 cell line were 86 nM and 100 nM, repectively. Compared with RA, BOBA is easy to synthesize and exhibits more chemical stability. The combination of FUDR and BOBA may represent a novel dual-action prodrug, functioning as an antimetabolite and differentiation inducer. The aim of the present research was to synthesize 3′-*O*-BOBA-FUDR and evaluate its activity in vitro and in vivo as a potential antitumor dual action prodrug.

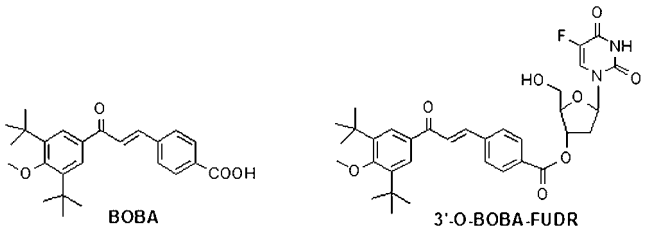

Fig. 1. Structures of BOBA and 3′-*O*-BOBA-FUDR.

MATERIALS AND METHODS

FUDR was kindly supplied by the Zhejiang Haizheng Pharmaceutical Company (Taizhou, China). BOBA was prepared in our laboratory. Trityl chloride was purchased from Alfa Aesar. 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma (St. Louis, MO). Solvents were purchased from SCRC (Xi'an, China). Flash column chromatography was performed on silica gel.

All melting points were determined on a Beijing micromelting-point apparatus, and the thermometer was uncorrected. ^{1}H-NMR spectra were recorded in $CDCl_3$ at 300 MHz on a Bruker NMR spectrometer with tetramethylsilane (TMS) as an internal reference. All chemical shifts are reported in parts per million (ppm). Mass spectra were performed on a Shimadzu GC-MS-QP2010 instrument.

The SD male rats weighing 220 ± 25 g were purchased from Experiment Animal Center of Xi'an Jiaotong University College of Medicine and were raised in the same place. The experimental protocol was approved by the Ethics Committee of Xi'an Jiaotong University.

Synthesis of 3′-*O*-BOBA-FUDR

The synthetic route for 3′-*O*-BOBA-FUDR is outlined in Figure 2.

5′-*O*-tritylfloxuridine (1)

To a three-necked flask equipped with a thermometer were added floxuridine (0.98 g, 3.99 mmol) and pyridine (20 ml), trityl chloride (1.56 g, 5.60 mmol), and 4-dimethylaminopyridine (0.05 g). The mixture was stirred under N_2 at room temperature for 12 h, then heated to 100°C and stirred for another 5 h, cooled to room temperature, and poured into ice water (100 ml). The resulting mixture was extracted with chloroform (40 ml × 4). The combined organic layers were washed sequentially with hydrochloric acid (2 M, 40 ml × 2), saturated sodium bicarbonate solution (40 ml × 2), and brine (40 ml × 2), dried over sodium sulfate and were concentrated in vacuo to give a residue that was purified by silica gel column chromography (chloroform/methanol = 20:1) to give 1.65 g of the title compound as a white solid. Mp = 115–116°C ([Xia et al., 1999], Mp = 149–151°C); Yield = 84.4%. ^{1}H NMR δ: 8.32 (s, 1H, -NH), 7.81 (d, 1H, =CH), 7.30–7.43 (m, 15H, Ph-H), 6.27 (t, 1H, H-1′), 4.55 (m, 1H, H-3′), 4.15 (m, 1H, H-4′), 4.06 (d, 2H, H-5′), 2.24–2.49 (m, 2H, H-2′). MS: m/z = 489 $[M+1]^+$, 243.

5′-*O*-trityl-3′-floxuridinyl 4-[3-(3,5-di-*t*-butyl-4-methoxyphenyl)-3-oxo-propenyl] benzoate (2).

To a solution of compound 1 (0.49 g, 1 mmol) in anhydrous THF (10 ml) was added BOBA (0.44 g, 1.12 mmol), N, N′-dicyclohexylcarbodimide (0.41 g, 1.99 mmol) and 4-dimethylaminopyridine (0.05 g). The mixture was stirred under N_2 at room temperature for 11 h. The solid was filtered off and the filtrate concentrated in vacuo to give a residue, which was purified by silica gel column chromatography (chloroform/methanol = 100:1) to produce 0.44 g of the title compound as an oil. Yield = 51.0%. ^{1}H NMR δ 9.55 (d, 1H, -NH), 8.10 (d, 2H, Ar-H), 7.95 (s, 1H, Ar-H), 7.90 (d, 1H, =CH), 7.80 (d, 1H, =CH, J = 15.6 Hz), 7.44 (d, 2H, Ar-H), 7.34 (d, 1H, =CH, J = 15.0 Hz), 7.29–7.36 (m, 15H, Ar-H), 6.44 (t, 1H, H-1′), 5.74 (m, 1H, H-3′), 3.74 (s, 3H, -OCH_3), 3.69 (m, 1H, H-4′), 3.45 (d, 2H, H-5′), 2.53–2.78 (2m, 2H, H-2′), 1.48 (s, 18H, t-Bu). MS: m/z = 865 $[M+1]^+$, 377, 243.

3′-floxuridinyl 4-[3-(3,5-di-t-butyl-4-methoxyphenyl)-3-oxo-propenyl] benzoate (3′-*O*-BOBA-FUDR)

Compound 2 (0.44 g) was added to a solution of 80% aqueous solution of acetic acid (10 ml). The mixture was heated at reflux for 30 min, cooled to room temperature. Ethyl acetate (100 ml) was added. The organic layer was washed with water, saturated sodium bicarbonate solution (30 ml × 3) and brine (30 ml × 3), dried over sodium sulfate, and concentrated in vacuo to give a residue, which was purified by silica gel column chromatography (chloroform/methanol = 30:1) to give the title compound as an oil, which was recrystallized from ethanol to produce a light yellow solid (0.25 g). Mp = 128–130°C; yield = 78.8%; purity = 97.8% (HPLC). IR (KBr): 3465, 2960, 2358, 1716, 1704, 1652, 1606, 1558 cm^{-1}. ^{1}H NMR δ 8.58 (s, 1H, NH), 8.10 (d, 2H, Ar-H), 7.94 (s, 2H, Ar-H), 7.87 (d, 1H, =CH), 7.79 (d, 1H, =CH, J = 15.9 Hz), 7.73 (d, 2H, Ar-H), 7.57 (d, 1H, =CH, J = 15.6 Hz), 6.45 (t, 1H, H-1′), 5.62 (m, 1H, H-3′), 4.32 (m, 1H, H-4′), 4.06 (m, 2H, H-5′), 3.74 (s, 3H, -CH_3), 2.26 (m, 2H, H-2′), 1.48 (s, 18H, t-Bu). MS: m/z = 623 $[M+1]^+$, 377.

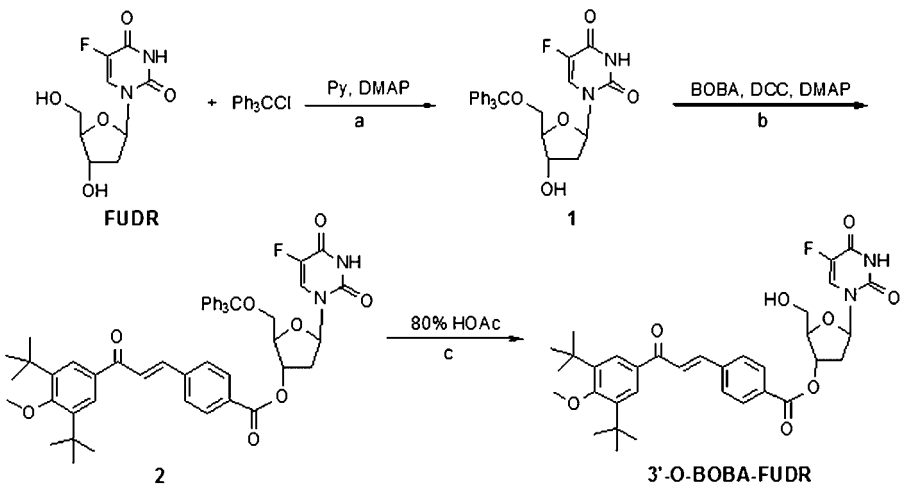

Fig. 2. Synthesis of 3′-*O*-BOBA-FUDR. Conditions: (a) rt, 3 h; 100°C, 5 h; 84.4% (b) rt, 11 h; 51.0%; (c) reflux, 30 min, 78.8%.

Antiproliferative Activity In Vitro

Two different human cancer cell lines, SMMC-7721 (human hepatoma cell line) and MDA-MB-231 (human breast cancer cell line), were used in the in vitro assay. Approximately 5×10^4 cells, containing RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), were added into each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. Test compounds were added to each well at the indicated final concentrations. The concentrations ranged from 10 nM to 100 μM. The plate was incubated at 37°C for 48 h. Fresh MTT was added to each well at a final concentration of 5 μg/ml and incubated with cells at 37°C for 4 h. The medium was removed and 150 μl of DMSO was added. Optical densities (OD_{570}) were recorded in a microplate reader. Results were expressed as IC_{50} values ($n = 5$) and were calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

Antitumor Activity In Vivo

Establishment of experimental animal models

SD rat were inoculated sc in the bilateral armpit and groin with hepatocellular carcinoma cells (Walker-256) (1.5×10^7 cells per ml, 0.5 ml for each rat). Three weeks after inoculation, solid tumors had grown to ~2 cm in diameter. The sc tumor tissue was removed, cut into small pieces of 1-mm size, and kept in saline for future use. Male rats were anesthetized, and an abdominal incision was made exposing the left lateral lobe of liver. On the liver surface, a 1-cm-long tunnel was cut under the liver capsule, and tumor pieces inserted. A wet cotton swab was pressed on the cut for 3 min to prevent the tumor pieces from overflowing. The lobe of the liver was placed in the abdominal cavity, which was sutured. Ten days after inoculation, solid tumors grew in the liver to ~1-cm diameter. The rats were used in the experiment.

Grouping and administration

All tumor-bearing rats were randomly divided into five groups, 10 rats per group. In the positive group, FUDR was administered i.p. at 30 mg/kg once a day for 5 days. In the negative control group, the same volume of saline was administered i.p. 3′-*O*-BOBA-FUDR was dissolved in DMSO/alcohol/water (30:30:40) and administered i.p. at 3.75, 7.5, 15 mg/kg for the low-, middle-, and high-dosage groups once a day for 5 days, respectively. Rats were sacrificed on day 12. Body weight, neoplasm, and volume of neoplasm were measured and inhibitory rates for tumor volume and weight calculated.

Inhibitory rates for tumor volume and tumor weight

Vernier calipers were used to determine the longest diameter (a) and shortest diameter (b) of the tumor. The formula

$$v = ab^2/2 \quad (1)$$

was used to calculate the tumor volume.

Volume inhibition rate

$$= (1 - \text{mean tumor volume of test group} / \text{saline group mean tumor volume}) \times 100\%. \quad (2)$$

Tumor weight inhibition rate

$$= (1 - \text{mean tumor weight of experimental group} / \text{average tumor weight of saline group}) \times 100\%. \quad (3)$$

Immunohistochemistry

Tumor tissues were removed from each group and fixed overnight in 10% neutral formalin, followed by gradient alcohol dehydration, and paraffin-embedding, and were cut into slices. All slices were blinded. Bcl-2 and Bax protein immunohistochemical staining was performed according to the manufacturer's kit instructions. A medical pathology image analysis system was used for data collection and analysis.

Identification of Apoptosis

Approximately 1 g of fresh tumor tissue was added to a Petri dish containing phosphate buffered saline (PBS) solution, digested for 30 min (0.25% trypsin), and the process terminated by FBS. Treated tissues were filtered through 200-mesh nylon, centrifuged at 1,000 rpm for 5 min, washed twice with PBS, and filtered again through with 300-mesh nylon mesh and centrifuged at 1,000 rpm for 5 min. The supernatant was discarded. RPMI 1640 culture medium was added and single-cell suspensions made. These were centrifugated at 4°C at 1,000 rpm for 5 min and washed twice with 250 μl ice-cold PBS. The number of the cells was adjusted to 1×10^6/ml. DNA staining of cells was performed with Annexin-V/PI double staining according to the manufacturer's kit instructions.

Statistical Analysis

All data were statistically analyzed by the SPSS10.0 software; monofactor analysis of variance and the q-test were carried out.

RESULTS AND DISCUSSION

Synthesis of 3′-*O*-BOBA-FUDR

To synthesize 3′-BOBA ester of FUDR, the 5′-hydroxy of FUDR requires selective protection.

Reaction of trityl chloride and FUDR produced 5′-*O*-trityl FUDR (**1**) in the presence of pyridine and DMAP. Esterification of BOBA and **1** was carried out in the presence of dicyclohexylcarbodiimide (DCC) and DMAP. In this process, DCC was converted to dicyclohexylurea, which proved difficult to remove from the reaction mixture. As a result, compound **2** was purified by using silica gel column chromatography twice. The *O*-Trityl group can be removed in acidic condition. Refluxing of **2** in 80% acetic acid gave 3′-*O*-BOBA-FUDR as a white solid. The structure of 3′-*O*-BOBA-FUDR was characterized by ^{1}H NMR, MS, and IR.

In their studies, Xia et al. [1999] indicated that 3′-*O*-retinoyl-FUDR was the most promising anticancer agent with a broad spectrum of anticancer activities relative to 3′, 5′-di-*O*-retinoyl-FUDR and 5′-*O*-retinoyl-FUDR, as well as to FUDR, RA alone, or in physical combination. Thus, 3′-*O*-BOBA-FUDR was the only compound synthesized in this work. 3′-*O*-BOBA-FUDR and FUDR were compared under similar conditions.

Antiproliferative Activity In Vitro

FUDR was used as positive control. The results expressed as IC_{50} values are summarized in Table 1. As listed in Table 1, the antiproliferative activity of 3′-*O*-BOBA-FUDR was improved over that of FUDR by 3–7-fold.

Antitumor Activity In Vivo

Inhibitory rate of tumor volume and weight

No significant weight loss was observed in all the dosage groups. Tumor volume and weight were measured on day 12 (Table 2). 3′-*O*-BOBA-FUDR reduced the average volume and weight of transplanted tumors with a consistent inhibition ratio for effects on tumor weight and volume within groups. The antitumor efficacy of 3′-*O*-BOBA-FUDR at 7.5 mg/kg was greater than that of FUDR at 30 mg/kg, and the efficacy was dose-dependent.

Tumor Bcl-2, Bax protein immunohistochemical staining

Bcl-2 and Bax proteins in the cell cytoplasm were yellow or brown in color. Bcl-2 protein was expressed in a high percentage in the saline group. With increasing doses of 3′-*O*-BOBA-FUDR, Bcl-2 expression was significantly diminished. Expression of Bax protein was the lowest in the saline group. With the increasing doses of 3′-*O*-BOBA-FUDR, Bax expression gradually increased. The integral optical density value of Bcl/Bax expression in the tumor tissue is summarized in Table 3. The results demonstrate that the differentiation activity of 3′-*O*-BOBA-FUDR is significantly higher than that of FUDR. This is likely because 3′-*O*-BOBA-FUDR is hydrolyzed to BOBA and FUDR in rats and BOBA has excellent differentiation-inducing properties. Immunohistochemistry results (Figs. 3 and 4) showed that 3′-*O*-BOBA-FUDR decreased the expression of Bcl-2 protein and increased the expression of Bax protein in tumor cells.

TABLE 1. Antiproliferative Activity of 3′-*O*-BOBA-FUDR ($\bar{x} \pm s$, $n = 5$)

Drug	Cell	IC_{50} (μM)
3′-*O*-BOBA-FUDR	SMMC-7721	12.4±3.2
	MDA-MB-231	7.1±2.1
FUDR	SMMC-7721	86.0±8.5
	MDA-MB-231	23.2±4.5

TABLE 3. Integral Optical Density of Bcl/Bax Expression in Tumor Tissue ($\bar{x} \pm s$, $n = 10$)

Group	Dosage (mg/kg)	Bcl (OD)	Bax (OD)
Saline	5 ml/kg	37.58±7.17	13.84±3.76
FUDR	30.0	23.46±4.91*	29.36±6.64*
3′-*O*-BOBA-	3.75	28.34±6.39*,‡	22.86±5.39*,‡
FUDR	7.50	12.04±4.34*,†	37.86±8.65*,‡
	15.0	6.20±2.24*,†	48.08±9.71*,†

*$P<0.01$ vs saline group; †$P< 0.01$; ‡$P< 0.05$ vs FUDR group.

TABLE 2. Inhibitory Effect on Tumor Volume and Weight ($\bar{x} \pm s$, $n = 10$)

Groups	Dosage (mg/kg)	Average vol (cm^3)	Inhibition in vol (%)	Average wt (mg)	Inhibition in wt (%)
Saline	5 ml/kg	2.93±0.45	–	2515.5±452.5	–
FUDR	30.0	2.00±0.38*	31.7	1747.6±405.3*	30.5
3′-*O*-BOBA-FUDR	3.75	2.52±0.45	13.9	2164.6±368.3	13.9
	7.50	1.57±0.42*,‡	46.3	1358.2±343.8*,‡	46.0
	15.0	0.95±0.23*,†	67.6	842.4±237.8*,†	66.5

*$P< 0.01$ vs saline group; †$P< 0.01$; ‡$P< 0.05$ vs FURR group.

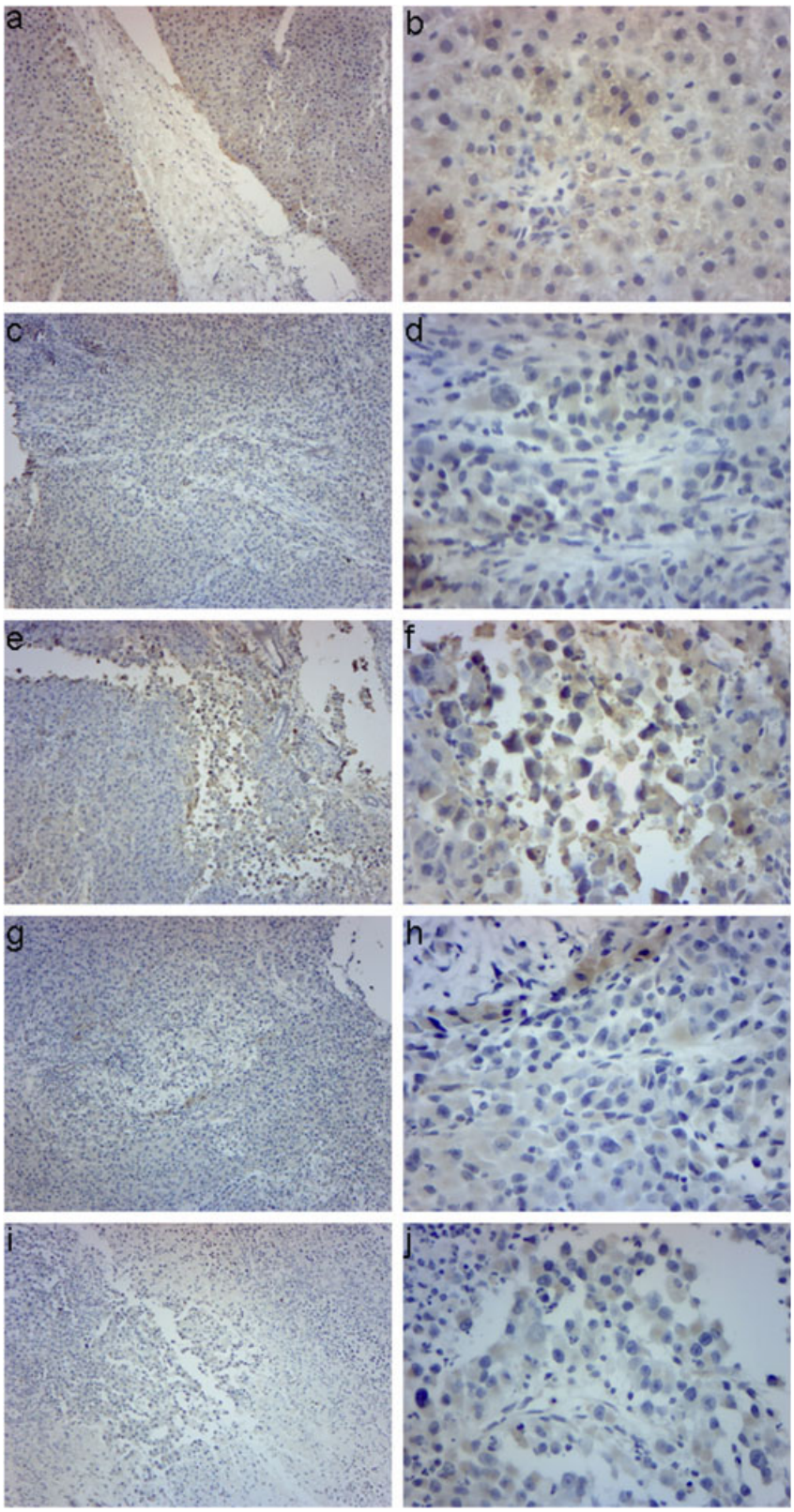

Fig. 3. Expression of Bcl-2 in rat transplanted tumor tissues. a: saline control (SP 100); b: saline control (SP 400); c: FUDR (SP 100); d: FUDR (SP 400); e: 3′-*O*-BOBA-FUDR (low-dosage) (SP 100); f: 3′-*O*-BOBA-FUDR (low-dosage) (SP 400); g: 3′-*O*-BOBA-FUDR (middle-dosage) (SP 100); h: 3′-*O*-BOBA-FUDR (middle-dosage) (SP 400); i: 3′-*O*-BOBA-FUDR (high-dosage) (SP 100); j: 3′-*O*-BOBA-FUDR (high-dosage) (SP 400) [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com].

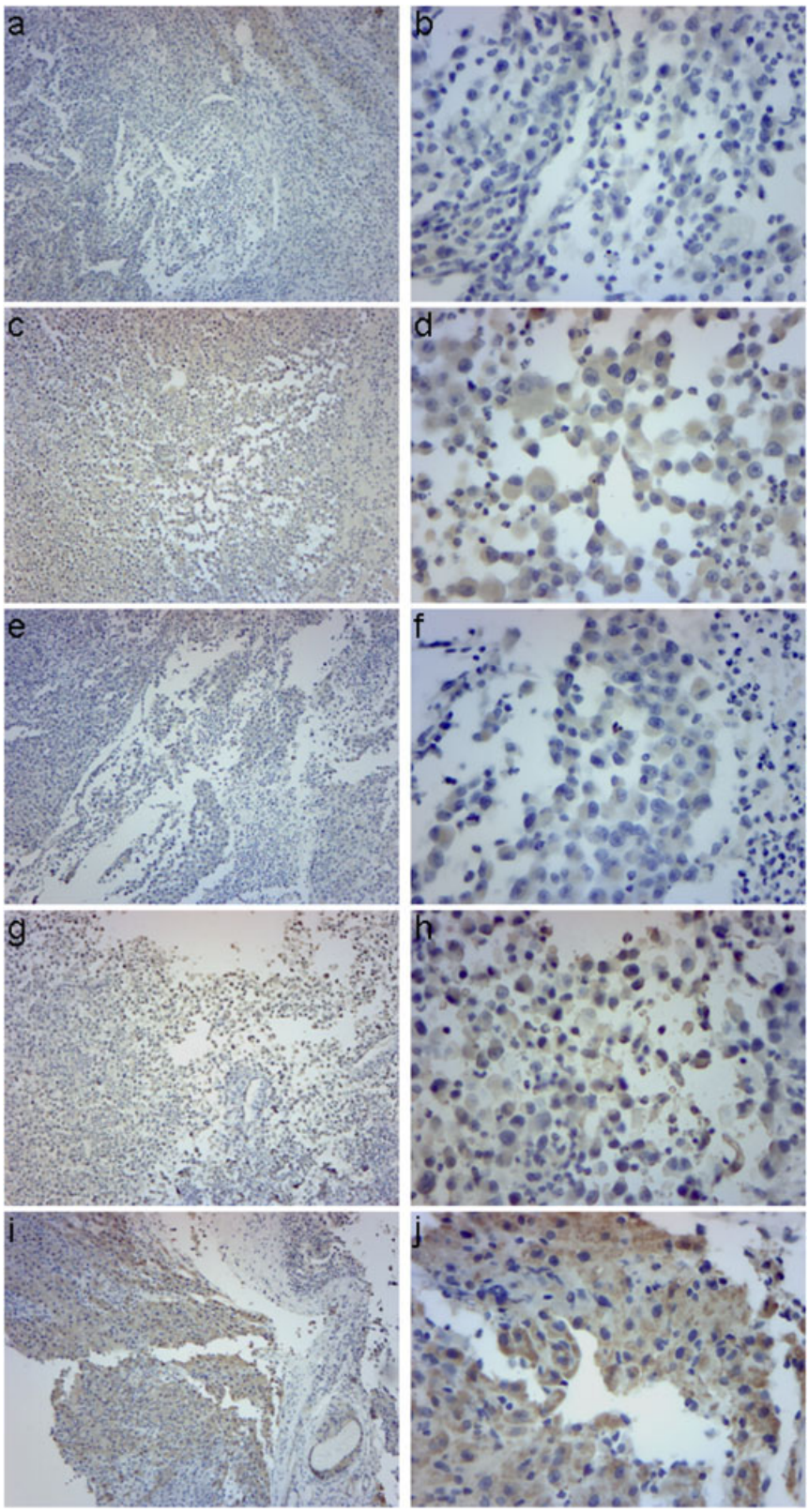

Fig. 4. Expression of Bax in rat transplanted tumor tissues. a: saline control (SP 100); b: saline control (SP 400); c: FUDR (SP 100); d: FUDR (SP 400); e: 3′-*O*-BOBA-FUDR (low-dosage) (SP 100); f: 3′-*O*-BOBA-FUDR (low-dosage) (SP 400); g: 3′-*O*-BOBA-FUDR (middle-dosage) (SP 100); h: 3′-*O*-BOBA-FUDR (middle-dosage) (SP 400); i: 3′-*O*-BOBA-FUDR (high-dosage) (SP 100); j: 3′-*O*-BOBA-FUDR (high-dosage) (SP 400) [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com].

Apoptosis Detected by Flow Cytometry

The early apoptotic ratios of transplanted tumor cells in each group were detected by flow cytometry (Table 4). Early apoptotic ratios are not fully identical ($P<0.01$) with the apoptotic ratio in saline controls being lower than the FUDR group ($P<0.01$). In the three different 3′-*O*-BOBA-FUDR dose groups, the ratio was improved with increasing dose. In middle- and high-dose groups, the ratio was higher than the FUDR group. In addition, the ratio appeared to be dose-dependent. Figure 5 shows the apoptotic ratio of transplanted tumor tissues in each group.

TABLE 4. Apoptotic Ratio of Transplanted Tumor Cells ($\bar{x} \pm s$, $n = 10$)

Group	Dosage (mg/kg)	Apoptosis (%)
Saline	5 ml/kg	2.27 ± 1.13
FUDR	30.0	$29.34 \pm 6.24^{*}$
3′-*O*-BOBA-FUDR	3.75	$20.03 \pm 5.31^{\ddagger}$
	7.50	$34.46 \pm 10.48^{\dagger}$
	15.0	$56.67 \pm 15.14^{\dagger,\S}$

$^{*}P<0.01$ vs saline group; $^{\dagger}P<0.01$ vs FUDR group; $^{\ddagger}P<0.05$ vs FUDR group; $^{\S}P<0.01$ vs 3′-*O*-BOBA-FUDR (middle-dosage) group.

CONCLUSIONS

Compared with FUDR, 3′-*O*-BOBA-FUDR exhibits a higher inducing differentiation action and antitumor efficacy in vivo and antiproliferative activity in vitro. These findings strongly support the assumption that 3′-*O*-BOBA-FUDR is an effective dual-action antitumor prodrug.

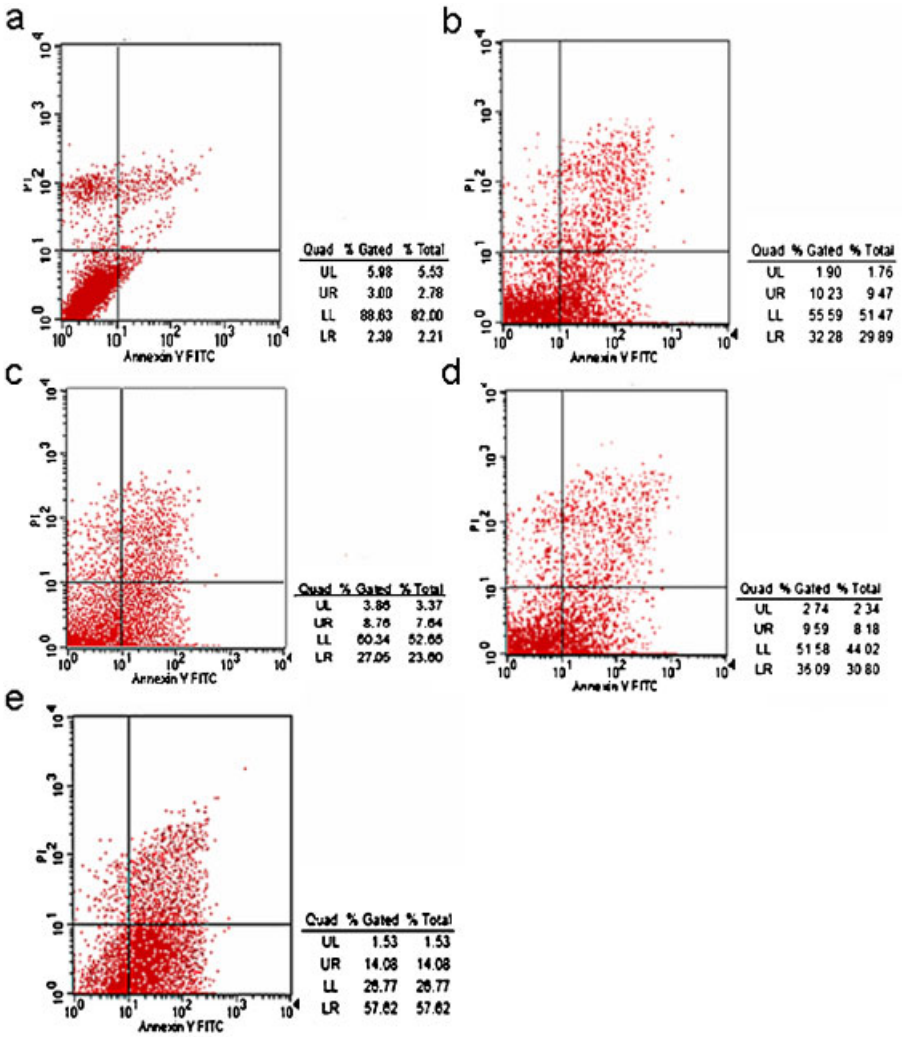

Fig. 5. Apoptotic ratios of rat transplanted tumor tissues. a: saline control; b: FUDR (SP 100); c: 3′-*O*-BOBA-FUDR (low-dosage); d: 3′-*O*-BOBA-FUDR (middle-dosage); e: 3′-*O*-BOBA-FUDR (high-dosage) [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com].

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