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Full Length Research Paper

Cytotoxic effect of three novel thiochromanone derivatives on tumor cell in vitro and underlying mechanism

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Thiochromanone derivatives have received extensive attention for their biological activities, but their anti-tumor activities were seldom reported. In this paper, in vitro anti-proliferative activities of three novel thiochromanone derivatives, (z)-3-(chloromethylene)-6-fluorothiochroman-4-one (CMFT), (z)-3-(bromomethylene)-6-fluorothiochroman-4-one (BMFT) and (z)-3-(chloromethylene)-6-chlorothiochroman-4-one (CMCT) were investigated by the method of MTT assay. All the tested chemicals showed cell toxicity to 13 human cell lines (A549, SGC-7901, BGC-823, U937, K562, Hela, MCF-7, HepG-2, A375, LS174T, HT1080, C4-2B and MRC-5), and half maximal inhibitory concentration (IC_{50}) values were between 2.3-36.3 μM. CMFT was chosen as a representative to invested the underlying mechanism. Cell apoptotic ratio was measured by flow cytometry analysis. The results showed that CMFT induced tumor cell apoptosis. Cysteinyl aspartate-specific proteases detection confirmed CMFT increased activity of caspase-8, caspase-9 and caspase-3. Moreover, CMFT could enhance the level of death receptor 3 (DR3). In conclusion, current results suggested novel thiochromanone derivative CMFT could kill tumor cells by inducing tumor cell apoptosis via increasing apoptosis-related factors.

Keywords thiochromanones; antiproliferation; apoptosis; caspases; tubulin.

INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues (Clapp et al., 2008). Currently, a significant proportion of cancers can be cured, especially if they were detected early (Marcu et al., 2010). Surgery, radiation, and chemotherapy play essential roles in treatment, depending on tumor stage and grade (Awasthi et al., 2009; Nonaka et al., 2010). However, chemotherapy is still the main way of treating cancer (Marcu et al., 2010). Chemotherapy is a method of treating cancer with drugs (Cebulla et al., 2009; Saijo et al., 2010; Schlatter et al., 2010). Often, these drugs can destroy cancer cells in proliferation cycle and stop the abnormal tissue from losing control of growth.

Thiochromanones is a compound of high-fat-soluble, low-water-soluble with extensive biological activities (Philipp et al., 1977; Talley et al., 1997; Dodda et al.,

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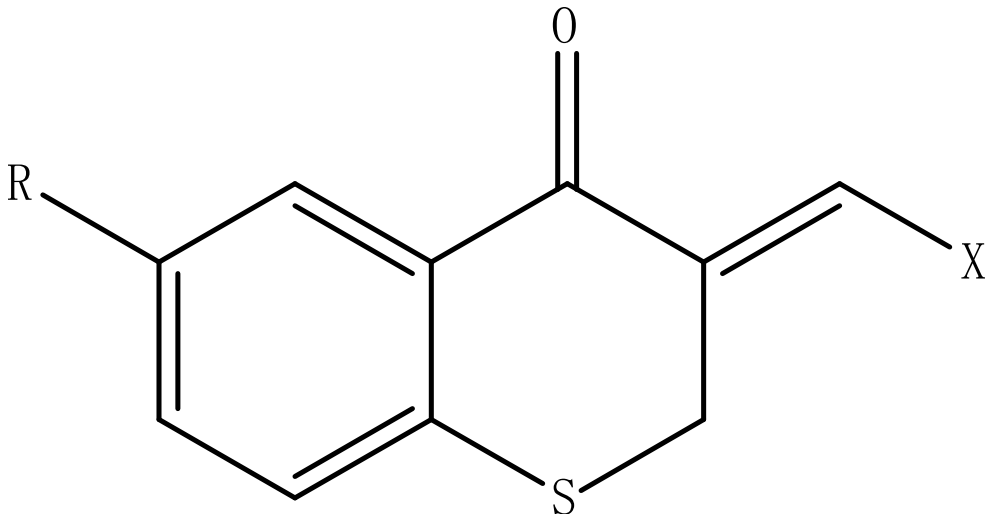

Figure 1. Structural formula of three novel thiochromanone derivatives
R=F, X=Cl (z)-3-(chloromethylene)-6-fluorothiochroman-4-one (CMFT)
R=F, X=Br (z)-3-(bromomethylene)-6-fluorothiochroman-4-one (BMFT)
R=Cl, X=Cl (z)-3-(chloromethylene)-6-chlorothiochroman-4-one (CMCT)

2008). Currently, it has been reported to possess anti-fungal activity, anti-inflammatory response, anti-platelet aggregative activity, and anti-allergic ability (Murguı´a et al., 2008). However, the anti-tumor activities have seldom been reported. Synthesis of more thiochromanones compounds by structural modification, and finding their anti-tumor activities are interesting work for drug researchers. We have synthesized many thiochromanones derivatives (Tian et al., 2010; Li et al., 2010; Fang et al., 2010), showing that different radical groups modify R- and X- creating different activities. In this paper, the anti-tumor effects of three novel synthesized chemicals (Figure 1) on 12 human tumor cell lines were studied. This study showed that all the three novel synthesized chemicals have excellent growth inhibition effects.

Apoptosis occurs during development and aging as a homeostatic mechanism to maintain cell populations in tissues (Norbury et al., 2001). Originally identified through its characteristic cytological morphology, Kerr *et al* (Kerr et al.,1972) reported the concept of apoptosis as being characterized by several ultrastructures such as cell shrinkage, chromatin condensation, budding and apoptotic bodies. Many studies have reported apoptosis as cell death distinct from necrosis. It is well known that several fluorescence dyes, such as Hoechst 33258 specifically binds to DNA and provides a reliable figure of chromatin condensation by fluorescence microscopy. Pyknosis is the result of chromatin condensation and is the most characteristic feature of apoptosis (Elmore et al., 2007). FCM is the most reliable method among those for detecting apoptosis, because apoptosis is confirmed by these characteristic ultrastructures of apoptosis (Otsuki et al., 2000).

The mechanisms of apoptosis are highly sophisticated and complex. Apoptosis is a cellular suicide mechanism that occurs by extrinsic or intrinsic mechanisms. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events (Elmore et al., 2007; Wyllie et al., 2010). The extrinsic signaling pathway is mediated by transmembrane death receptors of the CD95/TRAIL/DR3, whose combination triggers assembly and recruitment of multiprotein complexes that activate caspase 8 (Walczak et al., 2000; Evan et al., 2001). The intrinsic signaling pathway involves the mitochondrion where it releases cytochrome c into the cytosol to activate caspase 9. Thus, caspase 8 is activated when specific extracellular ligands of the tumor necrosis factor family bind to their receptors (the extrinsic apoptosis pathway), whilst caspase 9 is activated at the mitochondrial membrane (the intrinsic pathway) (Wyllie et al., 2010; Evan et al., 2001). Each pathway activates its own initiator caspase (8, 9, 10) which in turn will activate the executioner caspase-3 (Elmore et al., 2007).

In this research, we have studied how the three compounds caused cell death, and the possible relationship between the compounds and apoptosis, as well as what probable factors they stimulated.

EXPERIMENTAL

Materials

The human cell lines used in the experiment include tumor cell lines A549, SGC-7901, BGC-823, U937, K562, Hela, MCF-7, HepG-2, A375, LS174T, HT1080 and C4-2B； and human normal embryonic lung fibroblast (MRC-

5). All of them were gifts from Dr. Jingxiang Zhao of Academy of Military Medical Sciences, maintained by our laboratory.

Culture medium RPMI-1640, DMEM and FBS were purchased from Introgen Corporation (GIBCOTM, Grand Island, NY, USA). Trypsin 1:250 and MTT were purchased from Amresco (Solon, OH, USA). Annexin V-FITC Apoptosis Detection Kit 1 was purchased from BD Pharmingen Co (San Diego, CA, USA). Human Caspase-8, Caspase-9, Caspase-3 activity assay ELISA Kit were purchased from Beyotime Institute of Biotechnology. Human DR3 ELISA Kit was purchased from Boster biological technology company. Other reagents and solvents used in the experiments were of either analytic grade or reagent grade as appropriate.

CMFT, BMFT and CMCT were synthesized by Medicine Quality Analysis and Control Laboratory of Hebei Province in Hebei University.

Preparation of Testing Chemicals

The testing chemicals, CMFT, BMFT and CMCT were dissolved individually in dimethyl sulfoxide (DMSO) as stock solutions (8 mol/L) and later mixed with treatment medium to final concentrations (80, 40, 20, 10 and 5 μM), and maintaining DMSO at 0.2%. Anti-tumor drug Cisplatin was also diluted to final concentration (40 μM) with treatment medium containing 0.2% DMSO.

Determination of Cell Proliferation

The cells were grown in culture medium with glutamine supplemented with 10% (v/v) heat-inactivated FBS and 1% penicillin (100 U/mL), streptomycin (100 μM), 2-mercaptoethanol (50 μM), and sodium pyruvate (1 mmol/L) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C(Xiao et al.,2007).

The cells were harvested in logarithmic phase, and seeded in 96-well plate at a density of 1×10^4 cells/well (100 μL), and incubated for 12 h, then treated by adding serial dilutions of testing chemicals or Cisplatin, cell-free control were added medium containing 1‰ DMSO. After 24 h, 10 μL of MTT (5 mg/mL) was added and cells were incubated for additional 4 h. The medium was removed and 100 μL of DMSO was added. Subsequently, the absorbance at a wavelength of 570 nm was read with Bio-Tek microplate Reader. Each experiment was repeated three times.

The Time Course Effect of CMFT Treatment on Hela Cells

Treated Hela cells with CMFT (40 μM), and removed the treatment medium at 10, 20, 30, 40, 50, 60 and 70 min, respectively, washed the wells with PBS 3 times, continuously cultured the cells in DMEM culture medium for 24 h, and determined the growth inhibition rate by MTT assay.

Apoptotic Cells Analysis by Flow Cytometry

In order to investigate the potential effects of CMFT on apoptosis level, Hela cells were seeded in 6-well plate and incubated with CMFT (20, 40 μM) for 24 h. Acquisitions were treated with FITC Annexin V Apoptosis Detection Kit 1 (BD 556547) and analysis was performed using FACS Calibur Cytometer. These assays include Annexin V for the detection of early apoptosis and propidiumiodide (PI) as an indicator of late apoptosis or necrosis(Vo et al.,2010; Keese et al.,2010). Data acquisition and analysis were processed with Cell Quest Pro.

Fluorescence Staining by Hoechst 33258

Hela Cells were plated in 6-well plate and incubated overnight, and then the cells were exposed to CMFT (20 μM) for 24 h. The treatment medium was poured off and covered cells with Hoechst 33258 (100 μL), and then the cells were incubated for 30 min at 37°C,after which the treatment medium was poured off again and the cells were washed with PBS. At last, the treated cells were observed by the Reflected Fluorescence Observation System (OLYMPUS IX51).

Caspase-8, -9 and -3 Activity Assays

Caspase-8, Caspase-9 and Caspase-3 were measured by the Human Caspase-8, Caspase-9 and Caspase-3 ELISA Kit following the manufacturer's instruction. Briefly, following treatment with different concentrations of CMFT (0, 5, 10, 20 μM) or 0.2% DMSO for 24 h, Hela cells were trypsinized, harvested and lyzed in 150 μL of ice-cold lysis buffer and incubated on ice for 15 min. The samples were centrifuged at 14,000g for 15 min to collect the cytosol extract. To determine caspase 3, 8 or 9 activities, identical amounts of cytosolic protein in 10 μL volume were aliquoted into a 96-well plate and 40 μL Sample Diluent was added to each well. The reaction was initiated by adding 100 μL of a HRP-conjugate reagent to each well and the plate covered with an adhesive strip was incubated for 1 h at 37°C. Aspirate each well and wash. Add chromogen solution A 50 μL and chromogen solution B 50 μL to each well and the plate was incubated for 15 min at 37°C. At the end of incubation add 50 μL Stop Solution to each well and the plate was read at 450 nm by Bio-Tek microplate Reader.

DR3 Level Detection

Hela cells were seeded in 6-well plate, incubated for 12h then exposed to 0, 5μM, 10μM, 20μM CMFT or 0.2% DMSO for 24 h. Cells were trypsinized, harvested and lyzed in 150 μL of ice-cold lysis buffer and incubated on ice for 15 min. The samples were centrifuged at 14,000 g for 15 min to collect the supernatant. DR3 was measured using Human DR3 ELISA Kit following the manufacturer's protocol.

Data Processing and Statistical Analysis

Data were expressed as mean ± SEM. Statistical significance was assessed with ANOVA followed by Bonferroni's *t*-test using the StatView program (Abacus Concepts, Inc., Berkeley, CA, USA). A value of $P < 0.05$ was considered statistically significant, and $P < 0.01$ was considered extremely statistically significant.

Tubulin Activity Analysis

Based on the documents (Shelanski et al.,1973; Cleveland et al.,1979), tubulin was extracted and isolated in advance, and then referring to previous report (Thomas et al.,1982), ATP solution was put into the purified tubulin (last concentration:100 mol/L), then seeded into 96-well plate and kept under 37°C.Detected the OD data of tubulin per 3 min, and down regulated the temperature to 0°C when the OD data remained unchanged. The plate was read at 350nm per 3min.

RESULTS

Antiproliferative Effect of Three Target Chemicals on 13 Cell Lines

The results showed that CMFT, BMFT and CMCT inhibited cell proliferation on not only all tested human tumor cell lines, but also human normal embryonic lung fibroblast MRC-5 in a concentration–dependent manner. The inhibition rate was close to 100% when the concentration was 80 μM. The inhibition effects of the chemicals on 13 cells were much higher than cisplatin at the same concentration (40 μM) (Table 1). The data demonstrated a statistically significance between the same concentration of tested chemicals and cisplatin.

The half maximal inhibitory concentration (IC_{50}) values of three test chemicals on 13 human cell lines were between 2.3-36.3μM (Figure 2).

CMFT Transitory Contaction Inducing Proliferative Inhibition on Hela Cells

CMFT was chosen as a representative of three chemicals to see cell response in different time point. The data showed Hela cells were very sensitive to CMFT (40μM), even only 10 minutes treatment resulted in 93.8% growth inhibition. There were no significant difference in inhibition rate when extend treatment time (10min-70min), which suggested that CMFT could quickly connect to specific targets of tumor cells, and trigged cell death process irreversibly (< 10 min) (Figure 3).

Apoptosis Induction of CMFT on Hela and HepG-2 Cell Lines

Cells were seeded in 6-well plate and incubated with CMFT (40μM) for 24 h. Acquisitions were treated with FITC Annexin V Apoptosis Detection Kit 1 (BD 556547) and analysis were performed using FACS Calibur Cytometer. The percentage of apoptotic cells obviously increased when the concentration of CMFT was 40μM. The apoptotic cells (LR quadrant) increased to 21.42% in Hela cells, and increased to 38.20% in HepG-2 cells (Figure 4a). The statistics data of apoptosis induced by CMFT were shown in Figure 4b.

The cells treated with 20 μM CMFT for 24 h showed morphological characteristics of apoptotic cells with the condensed chromatin, the nuclear fragmentation, the apoptotic body and brighter green fluorescent (Figure 5).

Apoptosis-related Proteins Analysis

Cells were incubated with 5, 10 and 20μM CMFT or DMSO (0.2%). The expression of DR3 had a concentration-dependent relationship with CMFT. Studies on the death receptors revealed that CMFT concentration dependently increased DR3 expression in Hela cells. At 20μM the level of DR3 increased 123.7% compared with control in Hela cells, and increased 61.2% in HepG-2 cells (Figure 6d). CMFT also increased caspase-8, caspase-9 and caspase-3 activities in Hela cells and HepG-2 cells. Caspase-8 activity was increased 186.7% in Hela cells and 132.2% in HepG-2 cells (Figure 6b). Caspase-9 activity was increased 280.6% in Hela cells and 183.3% in HepG-2 cells (Fig.6 c). Caspase-3 activity was increased 246.5% in Hela cells and 536.8% in HepG-2 cells (Figure 6a). The above data suggested that CMFT triggers apoptosis of cancer cells.

Table 1. Inhibition ratio of CMCT，BMFT,CMFT and CDDP on 13 human cell lines(%)

	CON（μM）	Cell lines												
		A549	SGC-7901	BGC-823	U937	K562	Hela	MCF-7	HepG-2	A375	LS174T	HT1080	C4-2B	MRC-5
CMCT	5.0	32.11±1.22	19.79±6.96	18.30±1.41	43.36±2.31	57.78±4.52	29.35±0.63	18.04±1.12	38.52±9.63	18.87±10.80	21.78±2.28	15.56±6.57	21.46±8.84	10.50±4.42
	10.0	61.90±3.44	52.02±7.47	36.81±0.85	57.82±3.39	83.30±0.62	46.31±3.01	43.84±0.06	61.35±2.93	48.55±1.28	50.96±4.47	45.06±0.94	30.69±1.83	28.05±3.02
	20.0	69.99±7.16**	74.52±1.15**	50.87±3.88 *	67.49±3.65 **	84.27±0.01 **	64.11±0.22 **	57.91±2.45 *	76.22±0.90 **	54.22±1.05 **	73.60±1.75 **	64.72±0.19 **	56.60±6.98 *	59.25±5.44 *
	40.0	86.04±1.88**	81.47±0.33	74.92±0.41 *	71.34±6.21	87.00±1.85	79.26±1.31	67.79±0.97 **	88.13±1.70	69.05±0.29 **	80.79±2.09 **	70.39±2.55 **	81.01±6.17 **	64.27±3.17 *
BMFT	5.0	25.42±1.46	28.64±2.11	14.09±2.17	56.11±7.86	30.97±2.74	22.69±6.22	23.49±6.89	26.11±5.27	12.89±2.99	21.99±6.76	-0.64±1.24	31.26±12.08	-2.08±3.85
	10.0	49.68±3.07	43.90±6.68	24.12±0.86	64.98±4.16	79.64±4.78	50.65±2.14	40.92±7.38	39.70±1.73	32.70±6.63	50.00±6.89	16.26±15.12	48.75±2.74	15.22±7.45
	20.0	63.25±1.40**	70.28±12.06 *	31.71±0.20 *	77.53±0.81 **	81.99±4.33 **	64.64±0.41 *	51.92±4.32 *	76.57±0.23 **	59.10±3.14 **	67.25±0.97 **	48.70±3.52 **	49.95±2.59 *	28.58±2.83 *
	40.0	82.00±2.09**	90.15±1.76	57.08±0.78 *	93.62±2.39	84.68±2.76	73.04±2.29	67.81±4.65 *	90.77±2.84	70.90±1.75 **	71.01±3.57 **	63.25±3.32 **	73.36±2.05 **	52.75±1.03 *
CMFT	5.0	22.27±16.37	20.19±2.05	13.34±2.09	61.47±1.29	42.35±0.71	32.18±2.12	16.31±3.34	35.79±5.01	29.54±8.75	28.65±11.76	14.35±8.10	12.62±4.65	7.09±0.57
	10.0	62.83±1.83	36.40±2.39	44.32±2.48	68.45±1.05	79.28±5.00	54.59±3.72	27.51±7.74	58.31±0.76	46.67±11.06	61.05±5.13	38.54±1.83	25.07±5.63	27.91±1.60
	20.0	71.40±7.79**	76.80±2.06 *	49.92±1.84 *	78.85±1.75 **	81.01±5.04 **	80.03±5.36 **	48.83±3.98 *	78.31±5.72 **	61.48±3.03 **	72.44±0.37 **	62.06±1.38 **	46.85±12.21 **	50.39±1.80 *
	40.0	87.04±1.79**	89.25±3.74	75.61±0.51 *	92.96±0.74	83.56±3.16	88.69±2.40	67.98±2.44 *	92.07±4.24	76.93±3.82 **	82.43±1.31 **	71.31±1.87 **	72.59±5.00 **	69.00±2.67 **
CDDP	40.0	10.36±0.98	58.97±3.67	47.03±3.01	63.47±0.38	17.91±4.65	60.55±3.73	38.25±2.17	10.48±2.67	41.55±2.70	25.37±1.88	10.25±2.88	18.59±8.87	10.02±2.12
	80.0	96.81±0.86	97.29±0.36	92.46±0.25	93.06±4.41	90.19±1.03	94.14±0.19	89.24±2.33	95.61±0.62	94.95±2.41	87.65±0.99	77.23±3.76	89.94±4.61	79.78±7.50

Three chemicals were tested for anti-proliferative effects against 13 human cell lines (A549, SGC-7901, BGC-823, U937, K562, Hela, MCF-7, HepG-2, A375, LS174T, HT1080, C4-2B & MRC-5) by the MTT assay. The inhibition effects of the chemicals on 13 cell lines were much higher than cisplatin at the same concentration (40 μM). * $P<0.05$ was considered statistically significant. ** $P<0.01$ was considered extremely statistically significant.

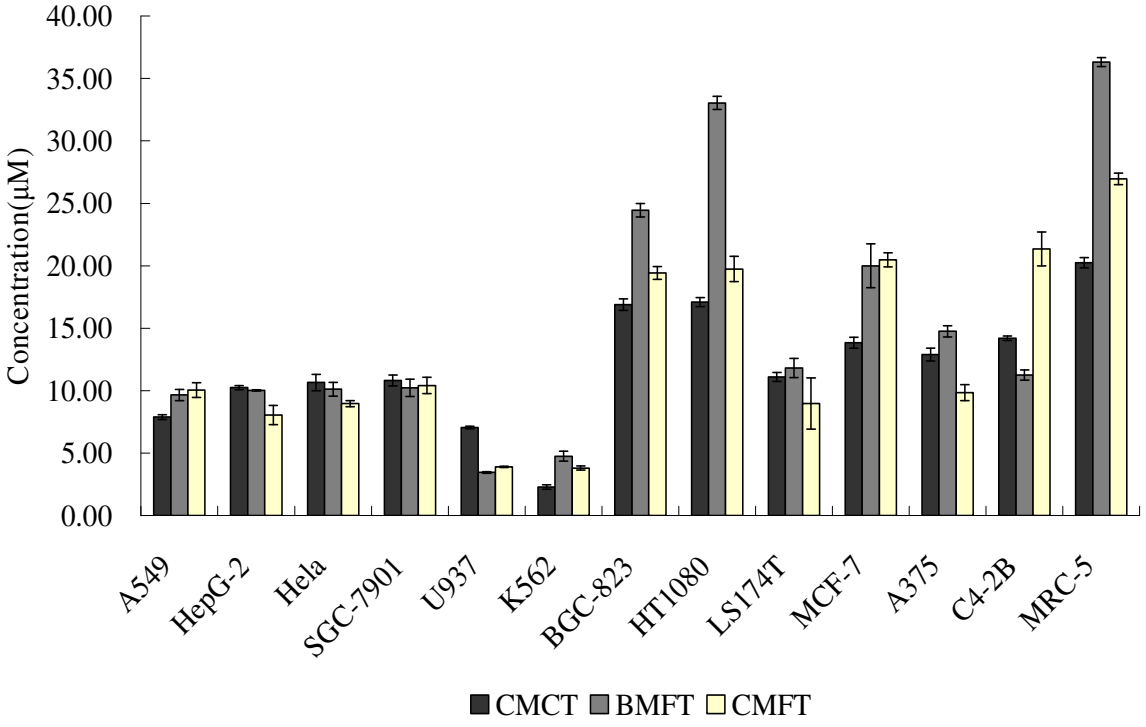

Figure 2. The IC_{50} of three novel thiochromanone derivatives
Comparison of IC_{50} values of CMFT, BMFT and CMCT. The IC_{50} values of three tested chemicals on 13 human cells were between 2.3-36.3 μM.

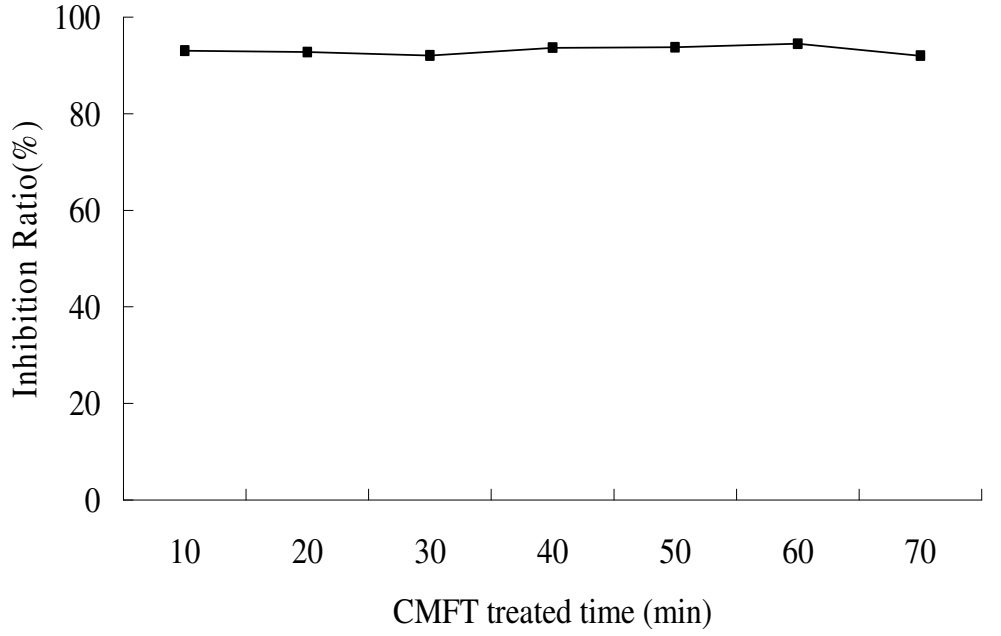

Figure 3. The inhibition ratio of CMFT on Hela cells in different treating time
Incubated Hela cells with CMFT (40μM), and removed the treatment medium at 10, 20, 30, 40, 50, 60 and 70 min respectively, then determined the inhibition rate.

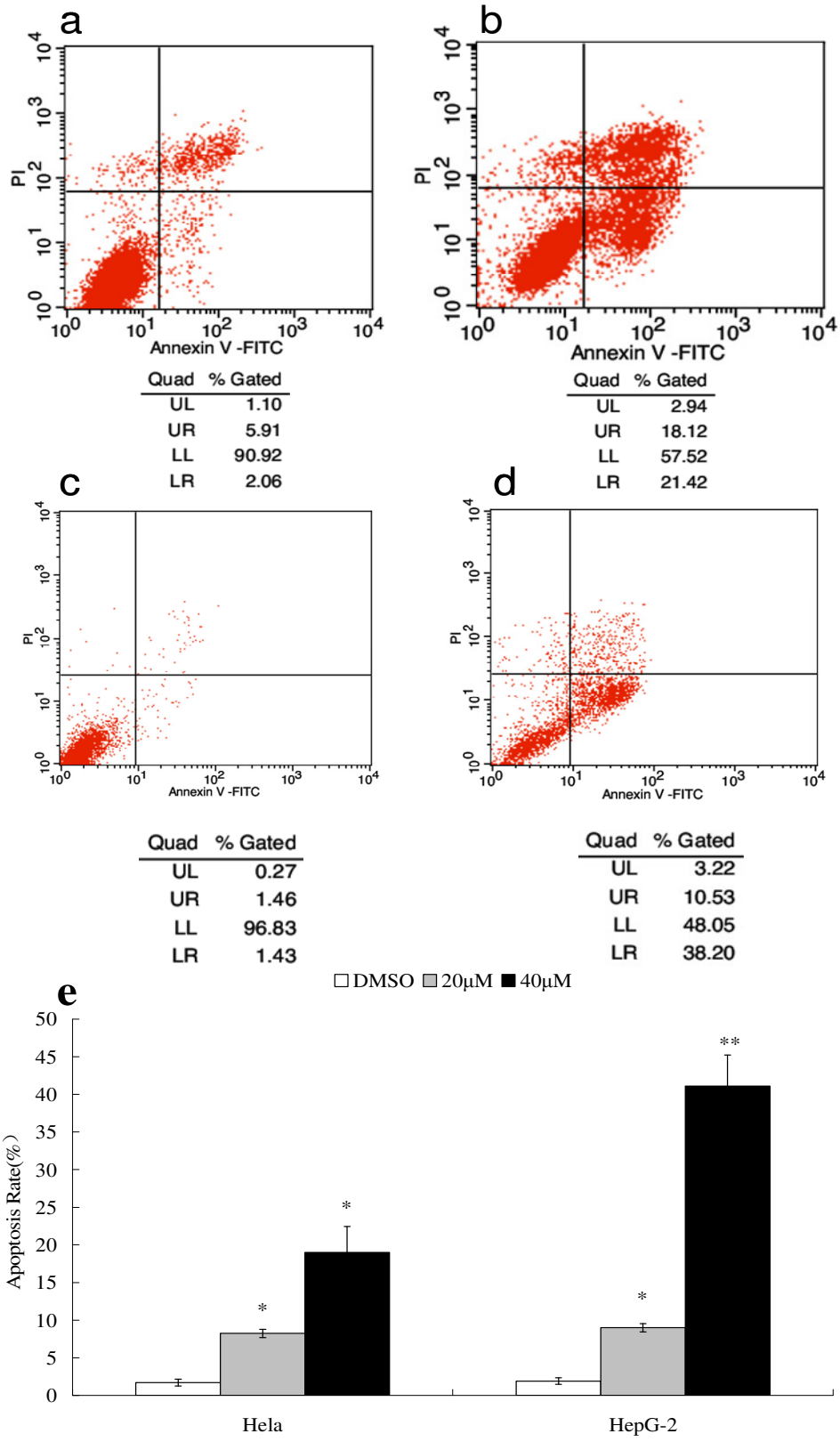

Figure 4. Apoptotic cells analysis by flow cytometry.
a. Hela cells treated with DMSO. b. Hela cells treated with CMFT. c. HepG-2 cells treated with DMSO. d. HepG-2 cells treated with CMFT. e. CMFT concentration dependently induced apoptosis in Hela and HepG-2 cells detected by flow cytometry. Results are expressed as a percentage of total tested cells and are the mean ± SEM. of three independent experiments. * $P < 0.05$, ** $P < 0.01$, compared with DMSO-treated cultures.

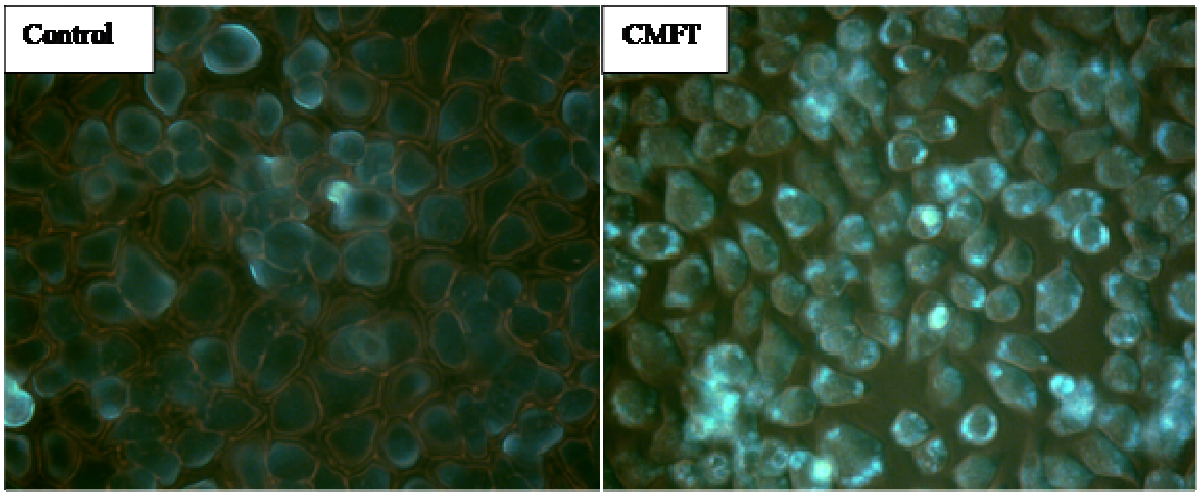

Figure 5. The changes of CMFT on Hela cell morphology by fluorescence staining
Compared with control groups, cells treated with CMFT(20μM) revealed morphological changes from a full circle to a crenated shape, and the cells stained by Hoechst 33258 appeared compact or cloddy pulverescent chromatoid nucleolus.

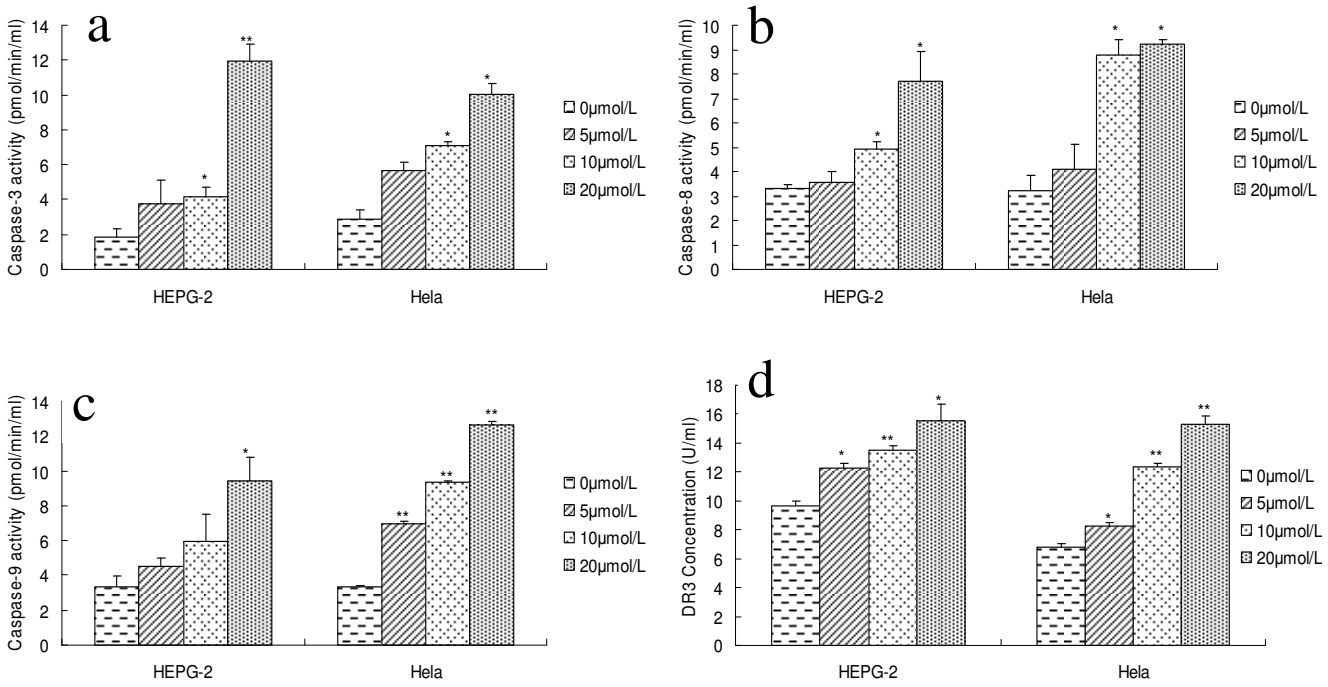

Figure 6. Effects of CMFT on release of caspase-3, -8, -9 and DR3
Representative histograms of Caspase-3 (a), Caspase-8 (b), Caspase-9 (c) activity and DR3(d) concentration in HepG-2 and Hela cells.*P < 0.05, ** P< 0.01, vs. the DMSO groups.

The Effects of Thiochromanones on Tubulin

The effects of thiochromanones (CMFT, BMFT and CMCT) were tested as well (Figure 7).The results showed that the absorbance values of tubulin with treatment of thiochromanones were lower than that of controlled group, and performed a concentration dependently manner, the higher the concentration, the smaller the absorbance value. It suggested that CMFT, BMFT and CMCT could inhibit tubulin polymerize to microtubule, and resulting in the decrease of turbidity, thus, the absorbance values reduced.

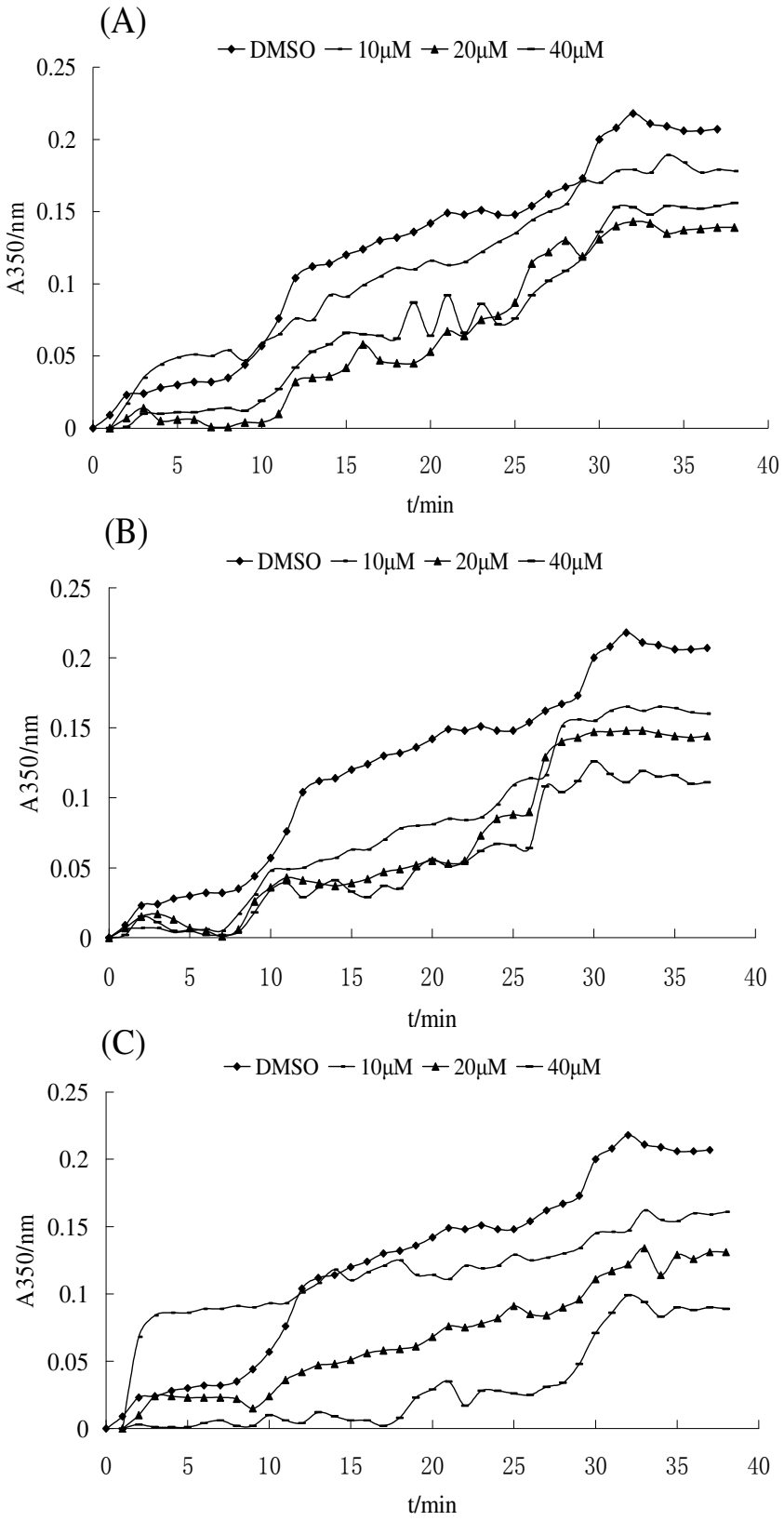

Figure 7. Effects of thiochromanones on tubulin
The absorbance values of tested groups:(A) BMFT,(B) CMCT and (C) CMFT were lower than that of DMSO groups obviously, and performed a concentration-dependent manner.

DISCUSSION

Thiochromanones were reported in many reviews for their extensive activities. Yang *et al* (Li et al., 2010; Huang et al., 2012) have designed and synthesized series of thiochromanones to search for more potential active chemicals. Our present studies demonstrated CMFT, BMFT and CMCT all exhibited noticeable anti-tumor activities at low concentrations (The IC_{50} were between 2.3-36.3 μM). So we can postulate that the excellent antiproliferative effects were most likely due to the structural modification of 3-substituent and 6-substituent. The groups –F, –Cl, and –Br were the key elements. From the comparison of IC_{50} values, we can find that the average IC_{50} values of BMFT, CMFT and CMCT for 12 tumor cells are 13.2, 12.1 and 11.2 μM respectively, thus, we can evaluate the activities as follows: BMFT<CMFT<CMCT.

Multiple mechanisms have been reported for the anti-neoplastic agents inducing cancer cells death. Apoptosis is a major mechanism of cell death induced in different signal apoptosis pathway, such as DNA damage, cell cycle arrest (protein synthesis, DNA replication, and tubulin-assisted process), inactivation of DNA polymerase, cellular membrane damage and alteration in signal transduction pathways involved in apoptosis (Clarke et al., 2005). For example, Cisplatin is one of the most effective chemotherapeutic agents that binds covalently to purine DNA bases and mediates cellular apoptosis. Many papers reported that cisplatin-induced apoptosis was associated with caspase-3 activation (Wang et al., 2008).The eukaryotic cell cycle is viewed as a cyclical progression through four phases: G1, S, G2 and M. Anti-mitotic agents often arrest the cell cycle in M phase. Microtubules as a major cytoskeleton component in all eukaryotic cells play essential roles such as maintenance of cell polarity, intracellular traffic, organization, and cell motility. Paclitaxel, a diterpenoid compound initially isolated from Taxus brevifolia, is widely used against malignant epithelial tumors, which mainly targets β-tubulin at the molecular level and stabilizes microtubule dynamics to induce microtubule polymerization (Lee et al., 2007; Shord et al., 2009). Figure 7 showed that the tested thiochromanones groups had lower absorbance values than the normal tubulin group at the same point in time, and showed concentration-dependent manners, the higher the concentration, the smaller the absorbance value. This indicated that the thiochromanones could inhibit tubulin from polymerizing to microtubule, and resulting in the decrease of turbidity, thus, the absorbance values reduced. Tubulin was synthesized in G2 phase, and then polymerized to microtubule, and last evolved into spindles in M phase. The spindles towed chromosome to undergo mitosis. The thiochromanones could inhibit the formation of microtubule, and thus blocked the mitosis, restraining the cell proliferation and inducing apoptosis.

Chunliu Yang *et al* (Yang et al., 2011) reported CMFT could inhibit the growth of subcutaneous transplanted H_{22} sarcoma in mice obviously, and could prolong the life of mice with S_{180} ascites tumor. In current studies, we used different methods to explore the effective targets of the test chemicals against rapid cancer cells growth. Firstly, the CMFT treatment could inhibit proliferation of Hela cells dramatically in a short time (<10min), implied that the target of CMFT might be the functional membrane or intracellular proteins which were vital for the cell. Secondly, CMFT could also increase the expression of DR3. Thirdly, CMFT triggered caspase cascade by activating caspase-8, caspase-9 and caspase-3, which suggested that CMFT induced cancer cell apoptosis by multiple apoptotic signaling pathways which involve in DR3, caspase-8, caspase-9 and capase-3. Fourthly, all investigated chemicals including CMFT, BMFT and CMCT inhibited tubulin polymerization.

CONCLUSION

In summary, we reported that three novel thiochromanone derivatives could significantly inhibit 12 tumor cell lines and MRC-5 growth in vitro, indicating that the three compounds have poor selectivity. The representative chemical CMFT induced cell apoptosis by activating DR3, caspase-8, caspase-9 and caspase-3. The anti-cancer effect of these chemicals comes from inducing apoptosis of tumor cells by triggering caspase cascade and increasing expression of death receptor 3 (DR3), however, another thiochromanone (Huang et al., 2012) was reported could increase the expression of tumor necrosis factor receptor 1(TNFR1). It suggested that different groups substitute different positions could trigger different receptors. Although the target for these drugs was not clear, the result suggested tubulin was possible target. Further studies have been underway for certifying these conjectures.

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