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MC37, a new mono-carbonyl curcumin analog, induces G2/M cell cycle arrest and mitochondria-mediated apoptosis in human colorectal cancer cells

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ABSTRACT

(E)−1-(3′-fluoro-[1,1′-biphenyl-3-yl)−3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one) (**MC37**), a novel mono-carbonyl curcumin analog, was previously synthesized in our laboratory as a nuclear factor kappa B (NF-κB) inhibitor with excellent cytotoxicity against several cancer cell lines. In this study, our further investigations showed that the potent growth inhibitory activity of **MC37** in human colorectal cancer cells was associated with the arrest of cell cycle progression and the induction of apoptosis. As a multi-targeted agent, **MC37** inhibited the intracellular microtubule assembly, altered the expression of cyclin-dependent kinase 1 (CDK1), and ultimately induced G2/M cell cycle arrest. Moreover, **MC37** collapsed the mitochondrial membrane potential (MMP), increased the Bax/Bcl-2 ratio, activated the caspase-9/3 cascade, and finally led to cancer cells apoptosis, suggesting that the mitochondrial-mediated apoptotic pathway was involved in **MC37**-induced apoptosis. In conclusion, these observations demonstrated that mono-carbonyl curcumin analogs would serve as multi-targeted lead for promising anti-colorectal cancer agent development.

1. Introduction

Colorectal cancer (CRC), the third most common malignant cancer arising from the colon or rectum, is a leading cause of cancer-related mortality worldwide (Kolligs, 2016). Although the improvement of early diagnosis and treatment has increased the survival of patients with advanced CRC, there are still approximately one million new CRC cases and over 500,000 mortalities occur annually (Casagrande et al., 2013). Generally, chemotherapy for CRC is effective in reducing tumor cell growth (Siegel et al., 2014), but the development of chemoresistant CRC results in tumor recurrence and death in patient (Germani et al., 2014). As dietary factors are important modulators of gastrointestinal malignancies, especially CRC (Ullah et al., 2014), exploration of new strategies and novel molecules from dietary agents is urgent for prevention and treatment of CRC.

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)−1,6-heptadiene-3,5-dione) (Fig. 1) is a naturally occurring hydrophobic polyphenol derived from the rhizome of *Curcuma longa* (turmeric). This yellow-colored compound has commonly been used as a food additive or in many traditional medicine remedies for over 2000 years in many Asian countries (Gupta et al., 2013). In the last few decades, in vitro and in vivo studies have demonstrated that curcumin possess various physiological and pharmacological activities including antioxidant, antibacterial, anti-inflammatory, antiviral, antifungal, and anticancer activity (Aggarwal and Harikumar, 2009; Chen et al., 2016b; Gupta et al., 2012b; Rahmani et al., 2014; Zorofchian Moghadamtousi et al., 2014). Particularly, curcumin shows potential chemopreventive and therapeutic value in a wide variety of cancers including lung, breast, hepatic, ovarian, pancreatic, and colon cancer cell lines through modulating all kinds of molecular targets and cell signaling pathways (Aggarwal and

Abbreviations: NF-κB, nuclear factor kaapa B; CDK1, cyclin-dependent kinase 1; MMP, mitochondrial membrane potential; CRC, colorectal cancer; DMEM, Dulbecco's modified Eagle's medium; PMSF, phenylmethylsulfonylfluoride; S.D., standard deviation; PARP, poly-ADP Ribose polymerase; SRB, sulforhodamine B; TNF-α, tumor necrosis factor alpha; DMSO, dimethyl sulfoxide; COX-2, cyclooxygenase 2; FBS, fetal bovine serum; VEGF, vascular endothelial growth factor; TCA, trichloroacetic acid; IL-6, interleukin 6; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel; PVDF, polyvinylidene difluoride; PI, propidium iodide; IL-8, interleukin 8; Notch-1, notch homolog 1; Akt, protein kinase B; FOXO3, forkhead box O3; GSH, glutathione; Nrf2, nuclear factor (erythroid-derived 2)-like 2; CDKs, cyclin-dependent kinases

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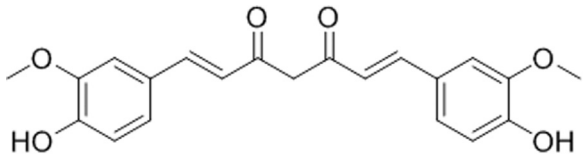
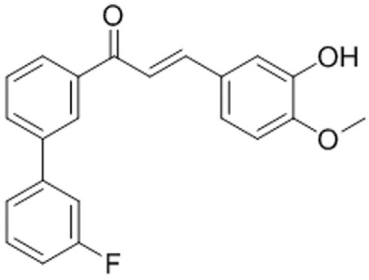


Curcumin



MC37

Fig. 1. Chemical structures of curcumin and **MC37**.

Harikumar, 2009; Chen et al., 2016b; Kasi et al., 2016; Li and Zhang, 2014; Rahmani et al., 2014; Tuorkey, 2014). Unfortunately, the utility of curcumin is greatly hindered by its poor bioavailability, which may be due to its poor absorption, rapid metabolism, and rapid systemic elimination (Anand et al., 2007). Therefore, to improve the flaws and increase the anticancer activity of curcumin, extensive effort was continuously devoted to the synthesis of new curcumin analogs (Anand et al., 2008; Dai et al., 2015; Liang et al., 2009; Qudjani et al., 2016; Raghavan et al., 2015; Weng et al., 2015). Previously we have reported that several series of new synthesized curcumin analogs exhibit highly improved anticancer activity compared with the parent compound curcumin in lung, breast, liver, nasopharynx, and colon cancer cell lines (Qiu et al., 2010; Zuo et al., 2012a, 2012b). In the present study, we further investigated the anticancer effect of one mono-carbonyl curcumin analog **MC37** (Fig. 1) ((E)–1-(3′-fluoro-[1,1′-biphenyl]–3-yl)–3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one) and explored the underlying mechanisms for **MC37**-mediated cell cycle arrest and apoptosis in CRC cells.

2. Material and methods

2.1. Reagents and chemicals

The mono-carbonyl curcumin analog **MC37** was synthesized in the same manner as we previously reported (Zuo et al., 2012b). Stock solution of **MC37** (10 mM) was prepared in dimethyl sulfoxide (DMSO) and freshly diluted with complete medium to obtain working solutions. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco BRL (Burlington, Ontario, Canada). Annexin V-FITC/PI apoptosis kit (catalog no. AP101) was a product from Liankebio Corporation (Guangzhou, China). Mitochondrial membrane potential detection kit was purchased from Beyontime (Jiangsu, China). Tubulin polymerization assay kit (Cytoskeleton, catalog no. BK011P) and Cellomics cytoskeletal rearrangement kit were purchased from Univ-Bio Corporation (Shanghai, China). β-actin, CDK1, Cdc 25C, Cyclin B1, cleaved caspase-9, cleaved caspase-3, and cleaved PARP-1 antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA). Antibodies for Bad, Bcl-xl, Bax, and Bcl-2 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other chemicals were of molecular biology grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture

Human colon carcinoma cell line SW480 and HCT-8 were maintained in DMEM medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cells in the exponential growth phase were used for experiments.

2.3. Cell viability assay

The effect of compounds on growth of cancer cells was determined using sulforhodamine B (SRB) assay according to the procedure of our previous reports (Zuo et al., 2012a, 2012b). In brief, cells were seeded in 96-well plates at a density of 5000 per well and treated with desired concentrations of compounds for 48 h. Then cells were fixed with ice-cold trichloroacetic acid (TCA), washed five times and air-dried, followed by stained with SRB dye for 30 min. The bound SRB dye was dissolved in Tris base solution (pH 10.4), and the optical density at 515 nm was recorded on a TECAN infinite® M200 pro multimode reader. The cytotoxic ability of compounds was evaluated by IC_{50}, which was obtained from the does-response curve in GraphPad Prism.

2.4. Cell cycle analysis

Flow cytometric cell cycle analysis was performed as described in a previous paper (Zhou et al., 2013a). Cancer cells were seeded in six-well plates at a density of 3×10^5 per well and treated with compounds in various concentrations for 24 h. Then cells were collected by centrifugation at 1000*g* 5 min, washed three times with PBS, and resuspended in 70% ethanol overnight at 4 °C. After cells were resuspended in PBS containing 40 μg/ml RNase A and 50 μg/ml propidium iodide (PI) at 37 °C for 30 min, Nuclear DNA content was analyzed using a Beckman Coulter EPICS-XL flow cytometer. Data analysis was carried out using EXPO32 software.

2.5. Western blotting analysis

Treated and untreated cells were washed with cold PBS twice and lysed with lysis buffer (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mg/ml leupeptin, 1 mM phenylmethylsulfonylfluoride (PMSF)) for 30 min at 4 °C. Cell lysates were centrifuged at 12,000g for 10 min at 4 °C. Aliquots of the supernatants were determined for total protein content by BCA method. Equal amounts of protein sampled were denatured by boiling in Laemmli buffer, subjected to sodium dodecyl-sulphate polyacrylamide gel (SDS-PAGE) and transferred electrophoretically onto polyvinylidene difluorde (PVDF) membranes. Following blocking with 5% skimmed milk for 2 h, membranes were incubated overnight at 4 °C with the primary antibodies. After washing three times, membranes were incubated with HRP-conjugated secondary antibodies for 2 h. Protein bands were visualized using the SuperSignal West Pico Chemiluminescent Substrate (Pierce) according to the manufacturer's description.

2.6. Immunofluorescence microscopy studies

Cellomics cytoskeletal rearrangement kit was used, and the experimental protocol with slight modification was followed. SW480 cells were seeded in a confocal culture dish at 1×10^5 cells/dish and grown for 24 h. After treated with **MC37** for 24 h, cells were placed on ice for 1 h to make the microtubule depolymerized. Subsequently cells were preincubated in 37 °C incubator for 0, 3, 6, 9, and 15 min to observe