

CYTOTOXIC ACTIVITY OF 4H-CHROMENES ON COLO 205 HUMAN COLON
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Abstract: 4H-chromenes are synthetic benzopyran derivatives which have various pharmacological activities. This study aimed to investigate and compare the cytotoxic activity of three selected 4H-chromenes (compound I, II, and III) against human colon adenocarcinoma cell line (COLO 205) using MTT assay. Cells were treated with increasing concentrations of compound I, II, and III from 20 to 200 μ M and incubated for 24 hours. The results were presented as the percentage of viable cells compared to control (untreated cells). At the concentration from 20 to 200 μ M, compound I, II and III showed a significant decline in % mean viability on COLO 205 cells from 85.63% to 2.01%, 89.18% to 1.31%, and 68.03 % to 7.42%, respectively. Compound I, II and III significantly exhibited cytotoxic activity against COLO 205 cells with IC_{50} of 26, 30, and 150 μ M, respectively.

Keywords: 4H-chromenes, cytotoxic activity, human colon adenocarcinoma cell line (COLO 205)

บทคัดย่อ: 4H - chromenes เป็นอนุพันธ์สังเคราะห์ benzopyran ที่มีฤทธิ์หลากหลายทางเภสัชวิทยา การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาและเปรียบเทียบความเป็นพิษต่อเซลล์มะเร็งลำไส้ใหญ่มนุษย์ (COLO 205) ของสาร 4H - chromene ที่คัดเลือกมา 3 ชนิด (สาร I, II, และ III) ด้วยวิธี MTT assay ใช้ความเข้มข้น 20 -200 ไมโครโมลาร์ ทดสอบเป็นเวลา 24 ชั่วโมง ผลที่ได้นำเสนอเป็นเปอร์เซ็นต์การมีชีวิตเมื่อเทียบกับกลุ่มควบคุม (เซลล์ที่ไม่ได้ใส่สารตัวอย่าง) พบว่าสาร I, II, และ III ที่ระดับความเข้มข้น 20 -200 ไมโครโมลาร์ ได้ค่าเปอร์เซ็นต์การมีชีวิตของเซลล์ COLO 205 เท่ากับ 85.63% - 2.01%, 89.18% - 1.31%, และ 68.03 % - 7.42% ตามลำดับ การทดสอบความเป็นพิษต่อเซลล์มะเร็งลำไส้ใหญ่ COLO 205 ของสาร I, II, และ III พบว่ามีค่า IC_{50} เท่ากับ 26, 30, และ 150 ไมโครโมลาร์

คำสำคัญ: 4H-chromenes, ฤทธิ์ความเป็นพิษต่อเซลล์, เซลล์มะเร็งลำไส้ใหญ่

INTRODUCTION

Heterocycles such as chromans and chromenes are important classes of benzopyran derivatives found in many natural products (Pratap and Ram, 2014). Particularly, 4H-chromene derivatives show various pharmacological properties such as spasmolytic, diuretic, anticoagulant, anticancer, and antianaphylactic activities (Thomas and Zachariah, 2013; Daigle *et al.*, 2011). For this reason, the synthesis of 4H-chromene derivatives has recently received great interest, and until now various methods have been developed for synthesizing 4H-chromenes, such as the cyclo-addition of phenols with ketones or propargylic alcohols (Xi *et al.*, 2013).

Currently, the incidence of colorectal cancer is less than the incidence of breast cancer, but more than the incidence of lung and liver cancer. It has been found in both male and female patients at similar rates and most often found in middle-aged people (aged 40 year-old and above) (American Cancer Society, 2015). Consequently, studies have been focused on diverse substances particularly in modification of none chromophore to generate more effective drug-able substances that have anticancer activity and one of those substances are 4H-chromenes derivatives (Li *et al.*, 2012). The pharmacological effects of 4H-chromenes derivatives were reported in recent studies, including spasmolytic, diuretic, anticoagulant, anticancer, and anti-anaphylactic activities (Pratap and Ram, 2014; Thomas and Zachariah, 2013; Gore and Desai, 2014). In addition, more recent studies have been conducted in several types of cancer cell line, such as breast cancer, lung cancer and leukemia cell. However, study in colon cancer cell line has not been yet.

This study aimed to investigate the cytotoxic activity of 4H-chromenes derivatives against human colon adenocarcinoma cell line (COLO 205) as well as to compare the cytotoxic activity on normal murine fibroblast (L929).

MATERIALS AND METHODS

4H-Chromene derivatives

Three selected 4H-chromene derivatives were provided from Chulabhorn Research Institute.

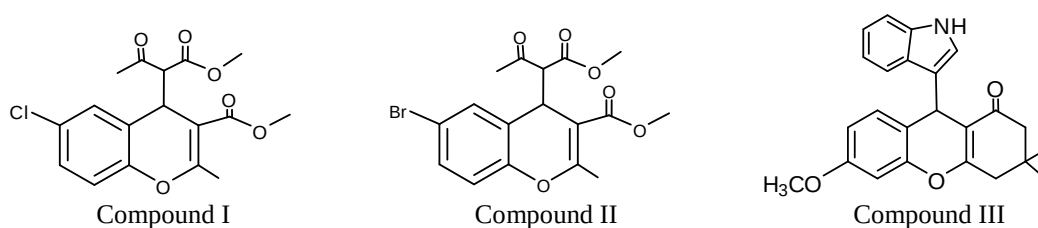


Figure. 1 Structures of compounds I-III, *Compound I*; 6-chloro-4-(1-methoxy-1,3-dioxobutan-2-yl)-2-methyl-4H-chromene-3-carboxylate, *compound II*; 6-bromo-4-(1-methoxy-1,3-dioxobutan-2-yl)-2-methyl-4H-chromene-3-carboxylate, and *compound III*; 9-(1H-indol-3-yl)-6-methoxy-3,3-dimethyl-2,3,4,9-tetrahydro-1H-xanthen-1-one.

Human colon cancer cells (COLO 205) cell line

COLO 205, which maintain characteristics of colon epithelial cell derived from ascites metastatic site in culture, were routinely incubated with Roswell Park Memorial Institute (RPMI) 1640 medium containing L-glutamine and phenol red, supplemented with 10% (v/v) fetal bovine serum, 0.1% penicillin-streptomycin and hepes (complete RPMI). The cells were cultured at 37°C in humidified air containing 5% CO₂.



Figure 2. Morphology of COLO 205 cells at 80% confluent.

Mouse fibroblast (L929) cell line

L929 which maintain characteristics of fibroblast derived from mouse adipose in culture were routinely incubated with Dulbecco's Modified Eagle medium (DMEM), medium containing L-glutamine and phenol red, supplemented with 10% (v/v) fetal bovine serum, 0.1% penicillin-streptomycin and hepes (complete DMEM). The cells were cultured at 37°C in humidified air containing 5% CO₂.

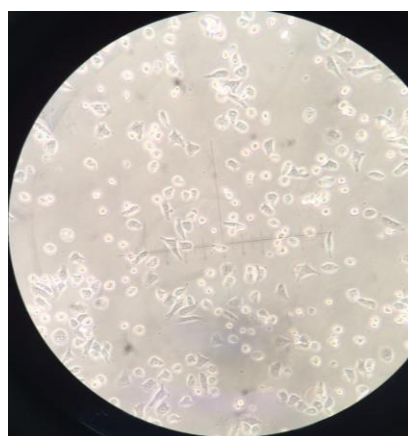


Figure 3. Morphology of L929 cells at 40% confluent.

Investigation of the cytotoxic activity of compound I, II and III on COLO 205 cells and L929

COLO 205 cells were treated separately with the three selected 4H-chromenes; compound I, II and III at concentration of 20, 40, 60, 80, 100, and 200 µM. L929 Cells were treated separately with compound I, II and III at concentrations of 5 – 320 µM. Both cells were incubated for 24 hours at 37°C. Cell viability was determined by using MTT assay after the incubation was completed (Tominaga *et al.*, 1999; Berridge *et al.*, 2005).

MTT solution was freshly prepared in incubation medium at the concentration of 0.2 mg/ml MTT solution of which 500 µL was added to each well of 24-well plates. Then, the plates were incubated for 45 minutes at 37°C in an incubator. At the end of the incubation, the MTT solution was removed and 125 µL of DMSO was added to each well and the plate

was gently shaken to dissolve the formazan crystals. Two 50 μ L aliquots were then transferred to wells of a 96 well plate so that the absorbance (540 nm) will be measured in duplicate (Berridge *et al.*, 2005).

Data and Statistical Analysis

Results were represented as mean percentage viability, IC_{50} and standard deviation. Data was analyzed and graphed with Microsoft Excel 2007 and GraphPad Prism, version 6.0. Significant differences between the mean values within the group were determined by using a Student's t test or one-way analysis of variance (ANOVA) followed by a Tukey test for further comparison. The level of significant was set up at $P \leq 0.05$. All graphs were drawn using GraphPad Prism version 6.0.

RESULTS AND DISCUSSION

Effects of 4H-chromenes compound I, II, and III on cellular viability of L929

The result showed that there was no significant change in mean percentage of cell viability of both constituents at the concentration of 5 μ M to 160 μ M. However, at the concentration of 320 μ M, all compounds showed cytotoxic effects on L929 mouse normal fibroblast with a significant difference compared to untreated cells (Fig. 4).

Effects of compound I, II and III on normal mouse fibroblasts (L929)

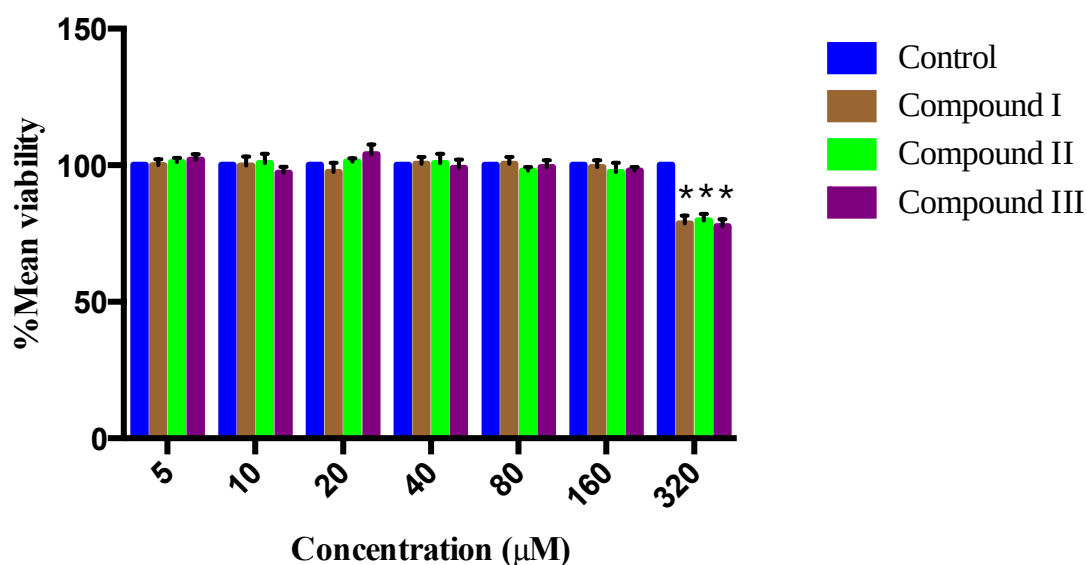


Figure 4. The bar chart indicated the mean percentage of cell viability and standard deviation of 4H-chromenes compound I, II, and III on L929 cells after 24- hour incubation. $N = 3$, $*P \leq 0.05$ vs. untreated cells.

Effects of 4H-chromenes compound I, II, and III against COLO 205

The result showed that there was a notable increase in mean percentage inhibition from 18% (20 μ M) to 95% (60 to 200 μ M of compounds I and II). Compound III showed less potency compared to compound I and II. Additionally, compound I at the concentration greater than 80 μ M showed the percentage inhibition about 95%. This experiment demonstrated that compound I with chloride and compound II with bromide substitution, respectively showed a potent toxicity to COLO 205 cells at increasing concentrations with the IC_{50} of 26 and 30 μ M, respectively. In addition, compound III had the IC_{50} of 160 μ M. (Fig.5).

Log-dose inhibition curve of compound I, II and, III on COLO 205

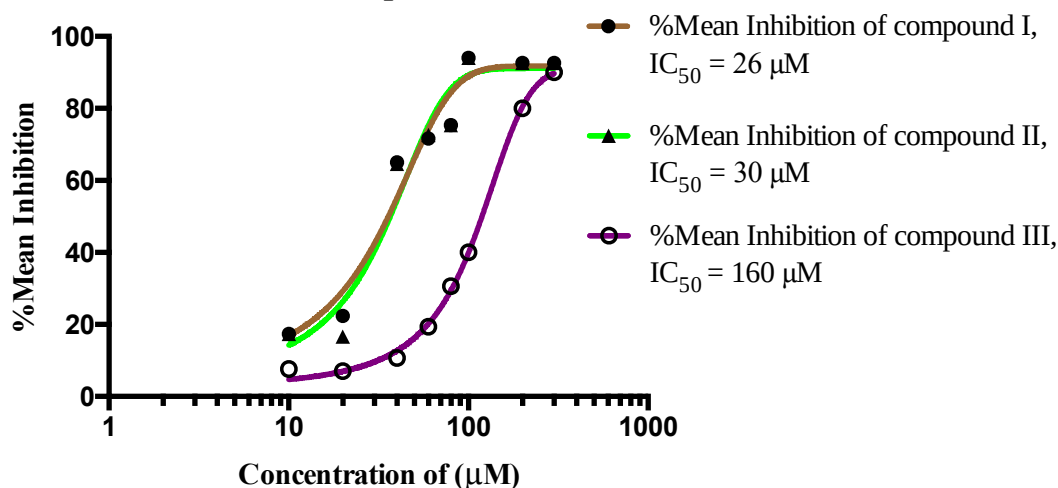


Figure 5. Dose-inhibition curve showed 50% inhibition concentration of compound I, II, and III on COLO 205 at 26, 30 and, 160 μM , respectively.

CONCLUSION

This study showed that 4H-chromene derivatives with different substitution groups at the concentration of 5 μM to 160 μM were not toxic to normal mouse fibroblast (L929 cells). In addition, with the non-toxic concentration these compounds have a variation of significant cytotoxic activity on human colon cancer (COLO 205 cells), which represented as IC_{50} . Compound I and II with halogen substitution exhibited a strong potency with IC_{50} of 26 and 30 μM , respectively. Whereas, compound III showed less potency compared to compound I and II. Further investigation is still required to study the cytotoxic activity particularly in molecular level.

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