ผลของ guttiferone K, oblongifolin C และ isojacareubin ต่อการแสดงออกของโปรตีนตัวขนส่งยาพี-ไกลโคโปรตีนในเซลล์คาโค-2

นายเชิดศักดิ์ บุญยง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยา (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

EFFECTS OF GUTTIFERONE K, OBLONGIFOLIN C AND ISOJACAREUBIN ON EXPRESSION OF P-GLYCOPROTEIN IN CACO-2 CELLS

Mr. Cherdsak Boonyong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Pharmacology
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2015

Thesis Title	EFFECTS OF GUTTIFERONE K, OBLONGIFOLIN C AND ISOJACAREUBIN ON EXPRESSION OF P-GLYCOPROTEIN IN CACO-2 CELLS
By	Mr. Cherdsak Boonyong
Field of Study	Pharmacology
Thesis Advisor	Associate Professor Suree Jianmongkol, Ph.D.
Thesis Co-Advisor	Associate Professor Rutt Suttisri, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Graduate School

(Associate Professor Sunait Chutintaranond, Ph.D.)

THESIS COMMITTEE

.....Chairman

(Assistant Professor First Lieutenant Naowarat Suthamnatpong, D.V.M., Ph.D.)

.....Thesis Advisor

(Associate Professor Suree Jianmongkol, Ph.D.)

.....Thesis Co-Advisor

(Associate Professor Rutt Suttisri, Ph.D.)

.....Examiner

(Assistant Professor Sireerat Sooampon, D.D.S., Ph.D.)

.....External Examiner

(Pongpun Siripong, Ph.D.)

เชิดศักดิ์ บุญยง : ผลของ guttiferone K, oblongifolin C และ isojacareubin ต่อการแสดงออกของโปรตีนตัวขนส่งยาพี-ไกลโคโปรตีนในเซลล์คาโค-2 (EFFECTS OF GUTTIFERONE K, OBLONGIFOLIN C AND ISOJACAREUBIN ON EXPRESSION OF P-GLYCOPROTEIN IN CACO-2 CELLS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ภญ. ดร.สุรีย์ เจียรณ์มงคล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ภก ดร.รุทธ์ สุทธิศรี, 53 หน้า.

Guttiferone K (GK) และ oblongifolin C (OC) ซึ่งเป็นสารกลุ่ม benzophenone และ isojacaruebin (ISO) ซึ่งเป็นสารกลุ่ม xanthone เป็นสารที่สามารถแยกได้จากเนื้อไม้ของต้นมะดันโดยสารทั้ง 3 ชนิดมีฤทธิ์ต้านมะเร็ง แต่อย่างไรก็ตามยังไม่มีรายงานการศึกษาเกี่ยวกับความสามารถของสารทั้งสามชนิดในการทำให้เกิดการดื้อยามะเร็ง ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของสารทั้งสามชนิดต่อการเพิ่มการแสดงออกของ *MDR1* mRNA จากภาวะเครียดออกซิเดชันและความเกี่ยวข้องกับ MAPKs pathway โดยใช้แบบจำลองเซลล์คาโคทู ผลการศึกษาพบว่าเมื่อให้สารทั้ง 3 ชนิดที่ความเข้มข้น 50 μM เป็นเวลา 24 ชั่วโมงทำให้เซลล์มี ปริมาณ *MDR1* mRNA ที่ตรวจวัดด้วยวิธี qRT-PCR assay เพิ่มขึ้นอย่างมีนัยสำคัญ ซึ่งการเพิ่มการแสดงออกของ *MDR1* mRNA ในการศึกษานี้เกี่ยวข้องกับสารทั้ง 3 ชนิดเหนี่ยวนำให้เกิดภาวะความเครียดออกซิเดชันภายในเซลล์ซึ่งผลดังกล่าวถูกยับยั้งได้เมื่อให้ n-acetyl cysteine (NAC; สารต้านปฏิกิริยาออกซิเดชัน) ร่วมด้วย นอกจากนี้ตัวยับยั้ง MAPK ได้แก่ U0126 (ตัวยับยั้งการทำงานของ ERK1/2/MAPK) และ SB202190 (ตัวยับยั้งการทำงานของ p38/MAPK) มีผลยับยั้งการเพิ่มปริมาณ *MDR1* mRNA ในเซลล์เมื่อให้ร่วมกับสารกลุ่ม benzophenone หรือ xanthone ตามลำดับ ทั้งนี้ผลการศึกษาดังกล่าวสอดคล้องกับปริมาณของ ERK1/2 (p-ERK1/2) หรือ p38 (p-p38) ในรูปแบบที่ถูกเติมหมู่ฟอสเฟตที่เพิ่มขึ้น เมื่อเซลล์ได้รับสารทั้งสามชนิด นอกจากนี้สาร OC และ ISO มีผลเพิ่มการแสดงออกของ *c-JUN/AP*-1 mRNA อีกด้วยในขณะที่สาร GK ไม่มีผลดังกล่าว ผลการวิจัยนี้แสดงให้เห็นว่าสาร GK, OC และ ISO สามารถเพิ่มการแสดงออกของ *MDR1* mRNA ในเซลล์คาโคทูโดยมีส่วนเกี่ยวข้องกับการกระตุ้น MAPK signaling pathway ด้วยการทำให้เกิดภาวะความเครียดออกซิเดชัน ทั้งนี้อาจศึกษาผลของสารทั้งสามชนิดต่อการแสดงออกของ P-gp ในระดับโปรตีนเป็นลำดับต่อไป

สาขาวิชา เภสัชวิทยา ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5587120920 : MAJOR PHARMACOLOGY

KEYWORDS: GUTTIFERONE K, OBLONGIFOLIN C, ISOJACARUEBIN, OXIDATIVE STRESS, MDR1

CHERDSAK BOONYONG: EFFECTS OF GUTTIFERONE K, OBLONGIFOLIN C AND ISOJACAREUBIN ON EXPRESSION OF P-GLYCOPROTEIN IN CACO-2 CELLS. ADVISOR: ASSOC. PROF. SUREE JIANMONGKOL, Ph.D., CO-ADVISOR: ASSOC. PROF. RUTT SUTTISRI, Ph.D., 53 pp.

Two benzophenones, guttiferone K (GK) and oblongifolin C (OC), and a xanthone, isojacaruebin (ISO), were phytochemicals isolated from the wood of *Garcinia schomburgkiana* (Thai name: Ma-dan). These three compounds exhibited cytotoxic activity against several cancer cell lines. However, the capability of these compounds to increase chemo-resistance in cancer cells was not reported. The objective of this study was to investigate the effects of these compounds on the oxidative stress-mediated up-regulation of *MDR1* mRNA in the Caco-2 cells, and the involvement of MAPK pathway. The cells treated with each of these compounds (50 μM) for 24 h had the amount of *MDR1* mRNA increased significantly, as measured by qRT-PCR assay. The up-regulation of *MDR1* in this study was related to oxidative stress generated from cellular exposure to these compounds. Addition of n-acetyl cysteine (NAC; a ROS scavenger) in the culture was able to abolish the inductive effect of GK, OC and ISO on *MDR1* mRNA level. Moreover, MAPK inhibitors including U0126 (an inhibitor of ERK1/2/MAPK activity) and SB202190 (an inhibitor of p-38/MAPK activity) were able to suppress an increase of *MDR1* mRNA levels in the cells treated with benzophenones and xanthone, respectively. These findings were in agreement with the increase of phosphorylated form of either ERK1/2 (p-ERK1/2) or p38 (p-p38) upon treatment of the cells with these three compounds. In addition, OC and ISO, but not GK, increased mRNA of *c-JUN/AP-1* level. These results suggested that GK, OC and ISO were able to up-regulate *MDR1 mRNA* in Caco-2 cells via the activation of oxidative stress-mediated mitogen-activated kinase (MAPK) signaling pathway. Further determination of their inductive effect on P-gp protein level might be pursued.

Field of Study: Pharmacology
Academic Year: 2015

Student's Signature .....
Advisor's Signature .....
Co-Advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my thesis advisor, Associate Professor Dr. Suree Jianmongkol for her invaluable advice, attention, motivation, encouragement, and patience throughout this study.

I would like to thank my co-advisor Associate Professor Dr. Rutt Suttisri Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for providing all three phytochemicals used in this research.

I would like to thank Dr. Piyanuch Wonganan Department of Pharmacology, Faculty of Medicine, Chulalongkorn University, for her guidance on the technique of real-time quantitative PCR (qRT-PCR) in this study.

This work was financially supported by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphisedksomphot Endowment Fund).

Finally, I wish to express my infinite thank to my family for their understanding and ending never support throughout my life. Thank you for always being beside me.

CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

Page

LIST OF ABBREVIATIONS

ABC	= Adenosine triphosphate binding cassette
ADP	= Adenosine diphosphate
ANOVA	= Analysis of variance
AP-1	= Activator protein 1
ATP	= Adenosine triphosphate
BAX	= Apoptosis regulator BAX
BSA	= Bovine serum albumin
BSO	= L-buthionine-(S,R)-sulphoximine
°C	= Degree celsius
Caco-2	= Colorectal adenocarcinoma
CDCFH-DA	= 2′,7′-Dichlorofluorescin diacetate
CO_2	= Carbon dioxide
cm^2	= Square centimeter
DEP	= Diesel exhaust particles
DMEM	= Dulbecco's modified eagle medium
DMSO	= Dimethyl sulphoxide
DNA	= Deoxyribonucleic acid
EDTA	= Ethylenediamine tetraacetic acid
ER	= Endoplasmic reticulum
FBS	= Fetal bovine serum
5-FU	= 5-fluorouracil
GAPDH	= Glyceraldehyde 3-phosphate dehydrogenase
GSH	= Glutathione
g	= gram
HBSS	= Hanks' balanced salt

HCl	= Hydrochloric acid
HRP	= Horseradish peroxidase
h	= hour
kDa	= Kilodalton
MAPK	= Mitogen-activated protein kinase
MDR	= Multidrug resistance
MDR1	= Multidrug resistance protein 1
MTT	= 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
ml	= milliliter
mM	= millimolar
NAC	= N-acetyl-L-cysteine
NF-kB	= Nuclear factor kappa-light-chain-enhancer of activated B cells
NaCl	= Sodium chloride
PARP	= Poly (ADP-ribose) polymerase (PARP)
PBS	= Phosphate buffered saline
PI3K	= Phosphoinositide 3-kinase
PKC	= Protein kinase C
PTEN	= Phosphatase and tensin homologue
PVDF	= Polyvinylidene fluoride
P-gp	= P-glycoprotein
RIPA	= Radioimmunoprecipitation assay
ROS	= Reactive oxygen species
RT-PCR	= Reverse transcription polymerase chain reaction (RT-PCR)
SDS	= Sodium dodecyl sulfate

SEM	= Standard error of mean
sec	= second
V	= Voltage
w/o	= without
µg	= microgram
µl	= microliter
µM	= micromolar

CHAPTER I
INTRODUCTION

Cancer incidence in Thailand has been rising in the last (Khuhaprema and Srivatanakul, 2008). Chemotherapeutic agents are used in standard treatment to cure cancer. Most of these drugs exert their therapeutic actions through generating cytotoxicity and/or anti-proliferative effect in cancer cells. For example, methotrexate and Vinca alkaloids produce cytotoxicity in proliferating cells (Payne and Mike, 2008). Other examples of chemotherapeutic drugs include doxorubicin and mitoxantrone which act through the inhibition of topoisomerase II, DNA intercalation and generation of free radicals in proliferating and resting cells (Alberts et al., 1985; Suzuki et al., 2005).

Long-term treatment with chemotherapeutic drugs may cause multidrug resistance (MDR), which subsequently leads to chemotherapy failure. The MDR phenotype may occur from several mechanisms such as alteration of drug targets, increased DNA repair, reduction of cell apoptosis and overexpression of efflux transporters (Luqmani, 2005). Previous studies have shown that overexpression of drug efflux proteins in the superfamily of the ATP-binding cassette (ABC) transporters including P-glycoprotein (P-gp) is responsible for the development of MDR in cancer therapy. These transporters pump chemotherapeutic drugs out of the cells, leading to reduction of drug-mediated cytotoxicity (Gottesman, 2002). Consequently, the cells become resistant toward these cytotoxic drugs.

Expression of P-gp can be altered by several mechanisms in either transcriptional or translational processes in cancer cells. It was reported that chemotherapeutic drugs including doxorubicin and etoposide increased P-gp expression via oxidative stress or DNA damages in a number of cancer cells (Osborn and Chambers, 1996; Zhou et al., 1999; Sui, Fan and Li, 2012; Cao et al., 2013). Moreover, oxidative stress is associated with the activation of survival signaling

pathways including mitogen-activated kinase (MAPK) pathway (Sui et al., 2012). Activation of the MAPK pathways has been linked to the functions of several transcription factors such as NF-kB or AP-1 (*c-Jun* or *c-Fos*). These transcriptional factors subsequently trigger *MDR1* transcription and translation. Induction of P-gp expression may result in a reduction of intracellular anticancer drugs, leading to drug resistance phenomenon (Scotto and Egan, 1998).

It is commonly known that a number of chemotherapeutic drugs are found and isolated from natural origins such as doxorubicin (from *Streptomyces peucetius*), vincristine (from *Catharanthus roseus*), paclitaxel (from *Taxus brevifolia*) and camptothecin (from *Camptotheca acuminata*) (Lomovskaya et al., 1999; Mans, da Rocha, and Schwartsmann, 2000; Pasqua, Monacelli and Valletta, 2004). These drugs inhibit cell proliferation and induce cytotoxicity in cancer cells. Recently, it was reported that two benzophenones, guttiferone K (GK) and oblongifolin C (OC), as well as a xanthone derivative, isojacareubin (ISO), isolated from the wood of *Garcinia schomburgkiana* (Thai name: Ma-dan) displayed cytotoxic activity against cancer cell lines such as A2780, CHAGO, HT-29, HeLa and SW620 (Mungmee et al., 2013). The cytotoxicity of the benzophenones GK and OC against SW620 colon carcinoma was 100 times more potent than doxorubicin (Mungmee et al., 2013). In addition, the xanthone ISO elicited antiproliferative action against HeLa-C3 cells (Han et al., 2008; Mungmee et al., 2013). It was reported that these GK and OC generated oxidative stress and caused apoptosis in several cancer cell lines (Wu et al., 2015; Xu et al., 2015). However, the effect of these three compounds on chemo-resistance of cancer cells has not been reported. It is possible that these benzopheone and xanthone derivatives may trigger drug resistance as an adaptive survival mechanism of cancer cells through up-regulation of *MDR1* gene.

Hypothesis

Two benzophenones, guttiferone K (GK) and oblongifolin C (OC), and a xanthone, isojacareubin (ISO), might be able to up-regulate expression of *MDR1* gene in colorectal adenocarcinoma cells via oxidative stress mechanism. Increase in *MDR1* expression was likely to be associated with oxidative stress-mediated activation of mitogen-activated kinase (MAPK) signaling pathway.

Objective

The objectives of this study were

1. To study the effects of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on the expression of *MDR1* gene in colorectal adenocarcinoma cells (Caco-2 cells).
2. To investigate the mechanism of these three compounds on oxidative stress-mediated expression of *MDR1* in Caco-2 cells, and their association with mitogen-activated kinase (MAPK) signaling pathway.

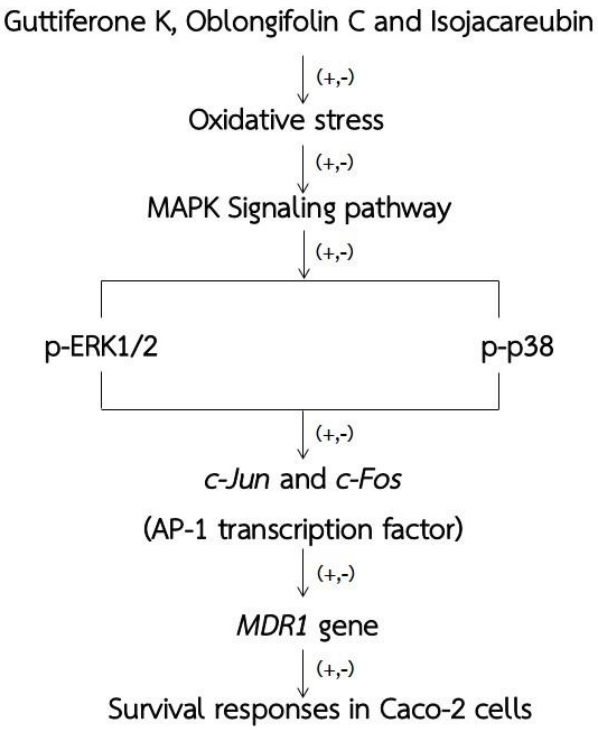
Scope of study

The present study would provide preliminary data on the effect of guttiferone K, oblongifolin C and isojacareubin on the expression of *MDR1* gene in colorectal adenocarcinoma cells (Caco-2 cells). The findings would provide an understanding on the oxidative stress-mediated expression of *MDR1* gene and the involvement of mitogen-activated kinase signaling pathway.

The study was divided into 2 parts as follows:

1. Investigation of the cytotoxic effects of GK, OC and ISO on Caco-2 cells viability.
2. Determination of the effects of GK, OC and ISO on stress-induced up-regulation of *MDR1* gene and its association with mitogen-activated kinase (MAPK) signaling pathway including ERK1/2 or p-38 activity.

Conceptual framework



CHAPTER II

LITERATURE REVIEW

Phytochemicals and anti-cancer activities

Chemotherapeutic drugs are used in a standard treatment of cancer. Their mechanisms of action can be divided into (1) cell cycle-specific action; and (2) cell cycle-nonspecific action. The cycle-specific drugs such as 5-fluorouracil and methotrexate killed proliferating cells only in the S phase. Vinca alkaloids arrest cancer cells at the M phase (Payne and Mike, 2008). The cells cycle-nonspecific drugs such as cyclophosphamide, cisplatin and doxorubicin exert their action during the proliferating or resting phase of cancer cells through several mechanisms including inhibition of topoisomerase II enzyme, intercalation of DNA or generation of reactive oxygen species (ROS) (Alberts et al., 1985; Suzuki et al., 2005).

A number of chemotherapeutic agents have been discovered and isolated from natural origin such as doxorubicin from *Streptomyces peucetius (family Streptomycetaceae)*, vincristine from *Catharanthus roseus (family Apocynaceae)*, paclitaxel from *Taxus brevifolia (family Taxaceae)* and camptothecin from *Camptotheca acuminata (family Nyssaceae)* (Lomovskaya et al., 1999; Mans et al., 2000; Pasqua et al., 2004). In addition, other natural products have been reported to show anti-cancer effects in several studies. For example, beauvericin from *Beauveria bassiana (family Cordycipitaceae)* induced apoptosis in colon cancer cells through ROS production, leading to mitochondrial damage (Prosperini et al., 2013). Resveratrol from grapes also caused autophagy and apoptosis in colon cancer cells via ROS generation (Miki et al., 2012). Rotenone from the roots and backs of the *Derris* induced apoptosis in breast cancer cells by generation of ROS and activation of the MAPKs pathways including JNK and p38 (Deng, Huang and Lin, 2010).

Three phytochemicals including guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) were isolated from the wood of *Garcinia schomburgkiana* (Thai name: Ma-dan, *family Clusiaceae*). These compounds exerted their cytotoxic activity in several cancer cell lines such as A2780, CHAGO, HT-29, HeLa and SW620 (Mungmee et al., 2013). The cytotoxicity of the benzophenones GK and OC against SW620 colon carcinoma was 100 times more potent than doxorubicin (Mungmee et al., 2013).

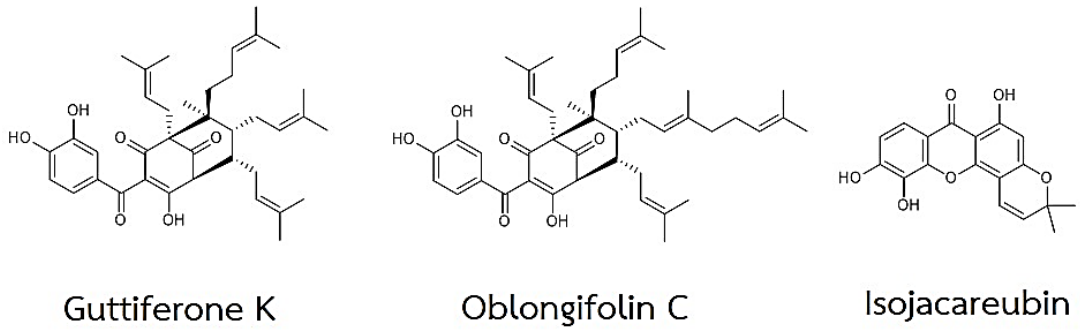


Figure 1. Chemical structures of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO).

The benzophenone GK has been reported to arrest cell cycle at G_0/G_1 by down-regulating cyclins D1, D3 and cyclin-dependent kinases 4 and 6 in human colorectal cancer cells (Kan et al., 2013). This compound was able to stimulate chromatin condensation, poly (ADP-ribose) polymerase (PARP) and induce the caspases-3, -8 and -9 mediated apoptosis. Furthermore, GK decreased tumor volume in a xenograft mouse model either when it was used alone or in combination with 5-fluorouracil (Kan et al., 2013). In human papillomavirus cell lines, GK induced autophagy and sensitized apoptosis through the accumulation of reactive oxygen species and activation of JNK/MAPK pathway (Wu et al., 2015).

Recently, it was reported that benzophenone OC could activate pro-apoptotic BAX protein and increased cytochrome C-release from mitochondria, leading to apoptotic cell death (Feng et al., 2012). In addition, OC was able to induce ER-stress and inhibit DNA repair in colon carcinoma HCT116 cells and in Bax/Bak-deficient mouse embryonic fibroblasts cells. It was suggested that OC exerted its actions via activation of JNK/MAPK pathway and up-regulation of the transcription factor CHOP (Xu et al., 2015). The xanthone ISO was reported to be inhibitory against cell signaling involving protein kinase C and was able to suppress both proliferating and metastasis of hepatoma cells (Yuan et al., 2015). It was shown that xanthone ISO decreased tumor volume and caused apoptosis in hepatoma xenograft mouse model (Yuan et al., 2015).

A number of compounds with chemical structure related to GK, OC and ISO elicited cytotoxic action in several cancer cell lines. For example, the benzophenone guttiferone A induced apoptotic cell death in hepatoma HepG2 cells by generating ROS and increasing mitochondrial membrane permeability (Pardo-Andreu et al., 2011). Another benzophenone, cambogin, caused apoptotic cell death in breast cancer MCF7 cells through oxidative stress mediated activation of -JNK/MAPK pathway (Shen et al., 2015). The synthetic caged xanthone cluvenone induced cellular stress and apoptosis in T-cell acute lymphoblastic leukemia CEM cells by activation of p38/MAPK and NrF2 stress response pathways (Batova et al., 2010).

Role of P-gp expression in cancer cells

P-gp is an ATP binding cassette (ABC) transporter (170 kDa) encoded by the *MDR1* gene (*ABCB1*). This transporter was first identified from its involvement with multidrug-resistance (MDR) in cancer cells. Chemotherapeutic drugs including doxorubicin and vinblastine are known P-gp substrates with ability to up-regulate the *MDR1* gene in cancer cells (Wang et al., 2005). An increase of P-gp expression can cause less accumulation of intracellular drug and the cells become resistant toward chemotherapy (Gottesman, 2002). An overexpression of P-gp has been linked to up-regulation of *MDR1* gene at either transcriptional level or translational processes in cancer cell lines (Gottesman, 2002). For instance, doxorubicin increased *MDR1* gene expression by activation of ERK1/2/MAPK transduction pathway-mediated the transcription factor of Y-box binding protein in B-cell lymphoma (Shen et al., 2011). In addition, an increase of P-gp level in human gastric cancer cells that were resistant to vincristine treatment involved the activation of the p-38/MAPK pathway (Guo et al., 2008).

Roles of oxidative stress in the expression of *MDR1* gene

Oxidative stress has been suggested to play a role in the expression of *MDR1* gene in several cancer cell lines (Osborn and Chambers, 1996; Ledoux et al., 2003; Sugihara et al., 2007). It was reported that rapid up-regulation of *MDR1* gene was associated with either extracellular or intracellular stress caused by carcinogen, chemotherapy, glucose deprivation, and hypoxia (Scotto and Egan, 1998). For example, L-buthionine-(S,R)-sulfoximine (BSO), a known GSH synthesis inhibitor, at the concentration of 200 μM decreased intracellular GSH and increased reactive oxygen species (ROS) after 24-hour treatment in rat brain microvascular endothelial cells. Concurrently, BSO treatment increased P-gp function and expression in these cells (Hong et al., 2006). Another study reported that benzo(a)pyrene and benzo(e)pyrene (50 μM) increased P-gp expression possibly through oxidative stress mechanism in Caco-2 cells after 72-hour exposure (Sugihara et al., 2007). Doxorubicin, a known cytotoxic drug, at 3 μM, was able to increase P-gp expression in human hepatoma HepG2 cells after 24-hour exposure. The effect of doxorubicin on P-gp expression was abolished in the presence of 0.5 mM NAC (an antioxidant and radical scavenger) (Komori et al., 2014). An increase of P-gp expression has been suggested to be associated with the activation of survival signaling pathways such as phosphoinositide 3-kinase (PI3K), protein kinase C (PKC) and mitogen activated protein kinases (MAPKs) including JNK, ERK1/2 and p38 in human cancer cells as shown in Figure 2 (Sui et al., 2012).

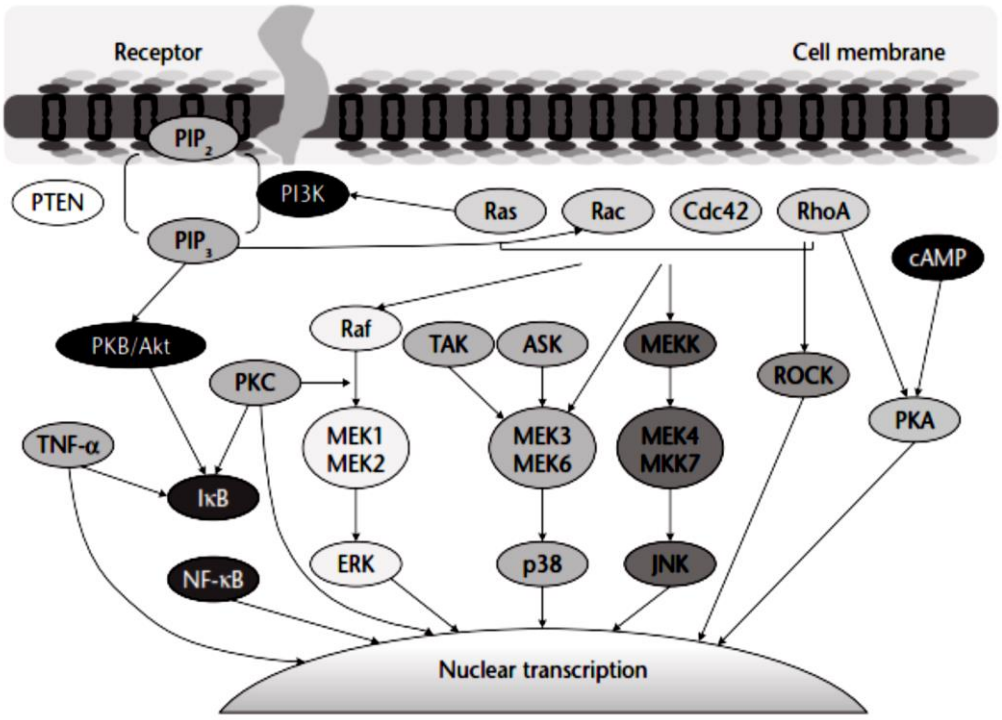


Figure 2. Proposed signal transduction pathways and transcription factors involving with P-gp-mediated multiple drug resistance in human MDR cancer cells (Sui et al., 2012).

The MAPK pathway has been proposed to be a major signaling pathway responsible for P-gp expression (Duan et al., 2012; Saksena et al., 2013; Priyamvada et al., 2016). The pathway has been known to activate cellular oxidative stress (Gaitanaki et al., 2003; Matos et al., 2005). For instance, diesels exhaust particles (DEPs; 50μg/ml) increased expression of P-gp protein in primary isolated brain capillaries after 6-hour exposure (Hartz et al., 2008). The effect of DEPs was linked to an increase of cellular oxidative stress and activation of the JNK/MAPK pathway (Hartz et al., 2008). In colon cancer Caco-2 cells, sodium nitroprusside (NOx donors) at the concentration of 0.1 mM increased the function and expression of P-gp after 24-hour treatment possibly through activation of PI3K, PKC, Akt and p38/MAPK pathways (Duan et al., 2012). The effect of doxorubicin on P-gp overexpression was related to activation of ERK1/2/MAPK transduction pathway and transcription factor of Y-box binding protein in B-cell

lymphoma (Shen et al., 2011). Long-term treatment with keratinocyte growth factor-2 increased P-gp expression in Caco-2 cells through activation of ERK1/2/MAPK pathway (Saksena et al., 2013). In addition, a blockade of the MEK-ERK-RSK signaling pathway was able to suppress P-gp expression in HT-15, SW620-14, MCF-7/MDR and MDA-MB-231/MDR cell lines (Katayama et al., 2007).

Several studies have proposed that activator protein-1 (AP-1) is one of the transcription factors of *MDR1* gene, as shown in Figure 3A (Scotto, 2003). AP-1 is either heterodimer (c-JUN, c-FOS) or homodimer (c-JUN, c-JUN) which can bind to the *MDR1* promoter region, as shown in Figure 3B. Recently, it was reported that an increased *MDR1* gene expression in the vinblastine-resistant Caco-2 cells involved the activation of several transcription factors including AP-1 (*c-JUN, c-JUN*) and *NF-kB* (Chen, Bian and Zeng, 2014). In addition, suppression of *MDR1* gene was observed when the cells were treated with either a c-JUN inhibitor (SP600125) or an NF-kB inhibitor (pyrrolidine dithiocarbamate) (Chen et al., 2014). Furthermore, it was shown that the increase in *MDR1* gene expression was related to activation of p-38/MAPK pathway and AP-1 in vincristine-resistant human gastric cells (SGC7901/VCR) (Guo et al., 2008). In addition, treating the cells with SB202190, a p38/MAPK inhibitor, resulted in a decrease of P-gp expression level (mRNA) in SGC7901/VCR cells (Guo et al., 2008). In colon cancer Caco-2 cells, *Lactobacillus acidophilus* culture supernatant was able to increase P-gp function and expression via activation of ERK1/2/MAPK-mediated AP-1 (Priyamvada et al., 2016).

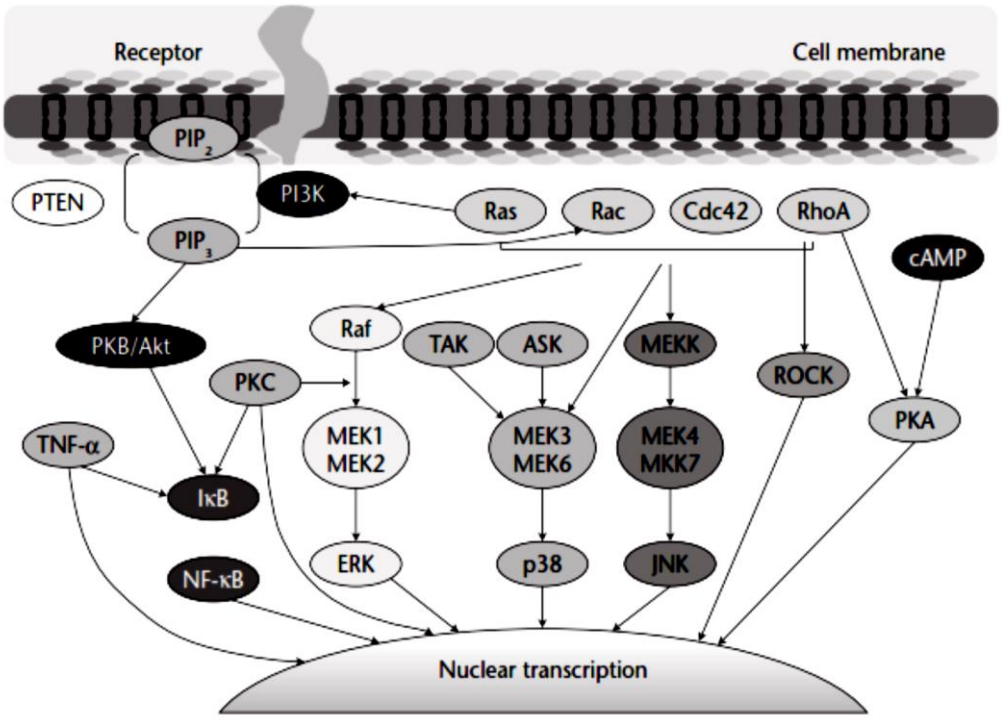


Figure 2. Proposed signal transduction pathways and transcription factors involving with P-gp-mediated multiple drug resistance in human MDR cancer cells (Sui et al., 2012).

The MAPK pathway has been proposed to be a major signaling pathway responsible for P-gp expression (Duan et al., 2012; Saksena et al., 2013; Priyamvada et al., 2016). The pathway has been known to activate cellular oxidative stress (Gaitanaki et al., 2003; Matos et al., 2005). For instance, diesels exhaust particles (DEPs; 50μg/ml) increased expression of P-gp protein in primary isolated brain capillaries after 6-hour exposure (Hartz et al., 2008). The effect of DEPs was linked to an increase of cellular oxidative stress and activation of the JNK/MAPK pathway (Hartz et al., 2008). In colon cancer Caco-2 cells, sodium nitroprusside (NOx donors) at the concentration of 0.1 mM increased the function and expression of P-gp after 24-hour treatment possibly through activation of PI3K, PKC, Akt and p38/MAPK pathways (Duan et al., 2012). The effect of doxorubicin on P-gp overexpression was related to activation of ERK1/2/MAPK transduction pathway and transcription factor of Y-box binding protein in B-cell

lymphoma (Shen et al., 2011). Long-term treatment with keratinocyte growth factor-2 increased P-gp expression in Caco-2 cells through activation of ERK1/2/MAPK pathway (Saksena et al., 2013). In addition, a blockade of the MEK-ERK-RSK signaling pathway was able to suppress P-gp expression in HT-15, SW620-14, MCF-7/MDR and MDA-MB-231/MDR cell lines (Katayama et al., 2007).

Several studies have proposed that activator protein-1 (AP-1) is one of the transcription factors of *MDR1* gene, as shown in Figure 3A (Scotto, 2003). AP-1 is either heterodimer (c-JUN, c-FOS) or homodimer (c-JUN, c-JUN) which can bind to the *MDR1* promoter region, as shown in Figure 3B. Recently, it was reported that an increased *MDR1* gene expression in the vinblastine-resistant Caco-2 cells involved the activation of several transcription factors including AP-1 (*c-JUN, c-JUN*) and *NF-kB* (Chen, Bian and Zeng, 2014). In addition, suppression of *MDR1* gene was observed when the cells were treated with either a c-JUN inhibitor (SP600125) or an NF-kB inhibitor (pyrrolidine dithiocarbamate) (Chen et al., 2014). Furthermore, it was shown that the increase in *MDR1* gene expression was related to activation of p-38/MAPK pathway and AP-1 in vincristine-resistant human gastric cells (SGC7901/VCR) (Guo et al., 2008). In addition, treating the cells with SB202190, a p38/MAPK inhibitor, resulted in a decrease of P-gp expression level (mRNA) in SGC7901/VCR cells (Guo et al., 2008). In colon cancer Caco-2 cells, *Lactobacillus acidophilus* culture supernatant was able to increase P-gp function and expression via activation of ERK1/2/MAPK-mediated AP-1 (Priyamvada et al., 2016).

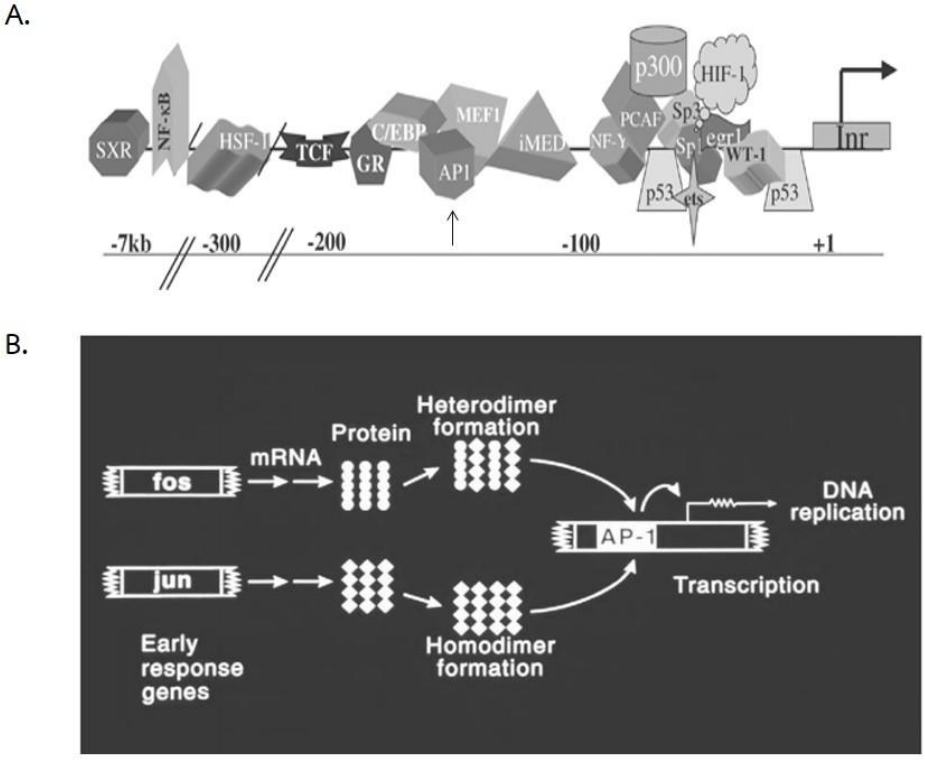


Figure 3. (A) Activator protein-1 (AP-1) as a transcription factor of *MDR1* gene (Scotto, 2003). (B) The AP-1 transcription factor binds to the promoter region on *MDR1* gene, leading to an increase transcription process (Shukla and Mossman, 2008).

CHAPTER III

MATERIAIS AND METHODS

1. Materials

1.1 Test compounds

Two benzophenones, guttiferone K (GK) and oblongifolin C (OC), and a xanthone, isojacareubin (ISO), were obtained from Assoc. Prof. Rutt Suttisri, Ph.D., Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. These compounds were isolated from the wood of *Garcinia schomburgkiana* (Thai name: Ma-dan), using the purification and identification processes as previously described (Mungmee, Sitthigool, Buakeaw, andSuttisri, 2013). Each purified compound was dissolved in 99.9% dimethyl sulfoxide (DMSO) and stored at -20 $^{\circ}$C until use. The final concentration of DMSO in each experiment was less than 0.5% (v/v).

1.2 Chemicals

The chemicals were obtained from Sigma company (St Louis, MO, USA). These chemicals included bovine serum albumin (BSA), Bradford reagent, calcein acetoxymethyl (calcein-AM), ethylenediamine tetraacetic acid (EDTA), glycine, glycerol, Hanks' balanced solution (HBSS), 2′,7′-dichlorofluorescin diacetate (DCFH-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent), N-acetyl-L-cysteine (NAC), non-essential amino acids, penicillin G sodium, protease inhibitor cocktail, SB202190, streptomycin sulfate, Triton X-100, 0.04% tryptan blue, trypsin, Tween 20, U0126 and verapamil.

Dulbecco's modified eagle medium and fetal bovine serum (FBS) were purchased from Gibco Life Technologies (Grand Island, NY, USA).

Polyvinylidine difluoride (PVDF) transfer membranes were purchased from Pall Gelman Laboratory (Pensacola, FL, USA).

ImProm-II™ reverse transcription system was purchased from Promega (Madison, WI, USA).

SsoFast™ EvaGreen® supermix was purchased from Bio-rad. (Berkeley, CA, USA).

Super signal West Pico chemiluminescent substrates and TRIzol® Reagent were purchased from Pierce Biotechnology (Rockford, IL, USA).

The mouse monoclonal anti-GAPDH and secondary goat anti-mouse IgG (H&L) horseradish peroxidase (HRP) were purchased from Calbiochem (San Diego, CA, USA).

The mouse monoclonal anti-ERK1/2 and anti-phosphorylated ERK1/2, the rabbit monoclonal anti-p38 and anti-phosphorylated p38, secondary goat anti-rabbit IgG (H&L) horseradish peroxidase (HRP) were purchased from Cell Signaling Technology (Beverly, MD, USA).

All other chemicals and solvents used throughout this study were commercially available.

1.3 Experimental instruments

1. Autoclave: Hirayama, Saitama, Japan
2. CFX96 Touch™ Real-Time PCR Detection System: Bio-rad, Hercules, CA, USA
3. GE ImageQuant LAS 4000: GE Healthcare Life-Sciences Ltd., Branch, Taiwan
4. Hot air oven: MEMMERT, Buchenbach, Germany
5. Humidified carbon dioxide incubator: Forma Scientific, Marietta, OH, USA
6. Inverted microscope: Axiovert 135, Zeiss, Konstanz, Germany

7. Microplate reader: Wallac 1420 Perkin-Elmer Victor 3, Perkin Elmer lnc., Waltham, Massachusetts, USA
8. Multiwell plates: Corning, New York, NY, USA
9. NanoDrop™ 2000/2000c Spectrophotometers: Thermo Scientific, Wilmington, UK
10. OmniPAGE mini vertical systems: Cleaver Scientific, Warwickshire, UK
11. Orbital shaker: OS-20, Biosan, Riga, Latvia
12. pH meter: CG 842, Schott, Hofheim, Germany
13. Refrigerated centrifuge: Z 383K, Hermle Labortechink, Burladingen, Germany
14. Tissue culture flasks: Corning, New York, NY, USA
15. Vortex mixer: mode K550-GE. Scientific Industries, New York, NY, USA
16. Water bath: WB22, Memmert, Hannover, Germany

1.4 Cell culture

Human colon adenocarcinoma Caco-2 cells (ATCC® HTB-37™) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were maintained in DMEM (supplemented with 2.5 mM L-glutamine, 100 U/L penicillin-streptomycin mixtures, 1% nonessential amino acid, and 10% fetal bovine serum) at 37 ^{o}C in a controlled atmosphere at 5% CO_2 and 90% relative humidity. The cells (passage number 51 to 74) at the seeding density of 6.0 x 10^3 cells/cm^2 were fed with fresh medium every 2 days and sub-cultured every 3-4 days (at approximately 70% confluence), using 0.25% trypsin solution containing 1 mM EDTA.

2. Methods

2.1 Assay for cell viability

2.1.1 Trypan blue dye exclusion assay

The cells were cultured at the seeding density of 6.25×10^4 cells/cm^2 for 24 h. Then, the cells were treated with GK, OC and ISO at various concentrations (0-50 μM) for 4 h in the CO_2 incubator. After treatment, the cells were trypsinized to produce a single-cell suspension. Then, they were stained with 0.04% trypan blue and counted under the microscope. The viable cells would be those unstained by trypan blue.

2.1.2 MTT assay

The cells were cultured at seeding density of 1.56×10^4 cells/cm^2 for 24 h prior to treatment with various concentrations (from 0-20 μM) of GK, OC or ISO. After 24-72 h treatment period, the cells were washed and incubated with MTT solution (0.5 mg/ml in free-serum medium) in the CO_2 incubator for 4 h. The intracellular formazan crystals were dissolved with DMSO and quantified spectrophotometrically at 570 nm with a microplate reader.

2.2 Determination of reactive oxygen species (ROS)

The effect of the three compounds on ROS generation was measured by the 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) assay. DCFH-DA is a non-fluorescent compound which will be converted by the intracellular esterases into DCFH. DCFH is converted to highly fluorescent DCF upon oxidation (Yoshioka et al., 2006). In this study, the cells were cultured at seeding density of 6.25×10^4 cells/cm^2 for 24 h prior to incubation with 100 μM DCFH-DA in free-serum media. After 30 min incubation period, the cells were treated with GK, OC or ISO at 50 μM for another 1 h. Then, the cells were washed with ice-cold PBS and lysed with 1% Triton X-100. The fluorescence intensity of DCF was measured by a microplate reader at the excitation and emission wavelengths of 485 and 535 nm, respectively.

2.3 Real-time polymerase chain reaction

The mRNA contents of *MDR1* and activator protein 1 (AP-1) (including *c-JUN* and *c-FOS*) were determined by a real-time reverse transcription PCR (qRT-PCR) (Chen, Bian, andZeng, 2014). The cells were cultured at seeding density of 1.3×10^4 cells/cm^2 for 14 days. Then, the cells were treated with GK, OC or ISO at 50 μM for 24 h. In some experiments, the cells were pretreated with either NAC (a ROS scavenger; 10 mM), U0126 (an ERK1/2 MAPK inhibitor; 10 μM) or SB202190 (an p-38 MAPK inhibitor; 10 μM) for 60 min prior to addition of GK, OC or ISO.

After treatment, total RNA were extracted using TRIzol® Reagent kit, according to the manufacturer's instructions and kept at -80 °C. The amounts of RNA samples were quantified using a NanoDrop™ 2000/2000c spectrometer at the wavelengths 260 and 280 nm. In this study, 1 μg of RNA was reversely transcribed to cDNA by ImProm-II™ reverse transcription system.

The qPCR reactions were performed using SsoFast™ EvaGreen® Supermix kit. The specific primers were listed in Table 1. The conditions of qPCR were as follows: an initial denaturing at 95 °C for 2 min, 40 cycles of denaturing at 95 °C for 5 sec, annealing and extension at 60 °C for 5 sec. The mRNA expression of *MDR1, c-JUN* or *c-FOS* was normalized to *GAPDH* and calculated using by the $2^{\Delta\Delta_{CT}}$ method (Livak and Schmittgen, 2001).

Table 1. Primers used for real-time reverse transcription PCR (qRT-PCR) analyses

Gene	Oligonucleotides	Sequences (5'-3')	Product size (bp)	References
c-JUN	Forward Reverse	CCCCAAGATCCTGAAACAGA CCGTTGCTGGACTGGATTAT	168	Chen et al., 2014
c-FOS	Forward Reverse	GGGCAAGGTGGAACAGTTAT AGTTGGTCTGTCTCCGCTTG	138	Chen et al., 2014
MDR1	Forward Reverse	CCCATCATTGCAATAGCAGG TGTTCAAACTTCTGCTCCTGA	167	Egashira, et al., 1999
GAPDH	Forward Reverse	AGGTCGGAGTCAACGGATTTGGT ATGGCATGGACTGTGGTCATGAGT	530	Van der Linden et al., 2014

2.4 Western blot analysis

Activities of ERK1/2 and p-38 were determined from their phosphorylated forms by western blot analysis. The cells were cultured at seeding density of 1.3×10^4 cells/cm^2. On day 14 after seeding, the cells were treated with GK, OC or ISO at 50 μM for 24 h. Then, the cells were washed with PBS three times and their proteins were extracted with ice-cold RIPA lysis buffer (50 mM Tris-HCl pH 6.8, 150 mM NaCl, 1% Tritron-X, 0.5% sodium deoxycholate and 0.1% SDS) supplemented with protease inhibitor cocktails (1:100). Then, cells'lysate was centrifuged at 18,000x g for 15 min at 4 °C. The supernatant was discarded. The amount of protein in precipitated samples was quantified using Bradford reagent® and measured spectrophotometrically at 595 nm.

Subsequently, 40 μg of total protein samples were denatured by boiling in sample buffer (60 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol and 0.01% bromophenol blue) at 95 °C for 5 min. After the denaturation process, the protein samples were separated using a 12% SDS-polyacrylamide gel electrophoresis at constant voltage of 200 V for 90 min and transferred to PVDF membrane at constant voltage of 60 V for 120 min. After protein transfer, the membranes were blocked with 5% BSA in TBS-T (50 mM Tris-base, 150 mM NaCl and 0.05% Tween 20) for 30 min at room temperature. After blocking, the membranes were probed with primary antibodies for ERK1/2 (1:2000), p-ERK1/2 (1:1000), p-38 (1:2000), p-p-38 (1:1000) or GAPDH (1:2000) at 4 °C overnight. Subsequently, the membranes were washed for 7 min with TBS-T three times and incubated with corresponding HRP-conjugated secondary antibody in TBS-T for 60 min at room temperature. After incubation, the membranes were washed and developed using the Super signal West Pico chemiluminescent substrates. The membranes were visualized by a GE ImageQuant LAS 4000. The activities of ERK1/2 and p-38 were normalized to GAPDH and shown as relative fold induction.

2.4 Data analysis

Data were expressed as mean ± SEM obtained from three to seven separated experiments (N=3-7). Statistical analyses were performed by either the Student's *t* test or one-way analysis of variance (ANOVA), followed by the post-hoc Dunnett's test. Differences were considered statistically significance when $P < 0.05$.

CHAPTER IV

RESULTS

4.1 Effect of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on cell viability

As shown in Figure 4, the cytotoxic effects of GK, OC and ISO in Caco-2 cells were concentration dependent, as measured by the trypan blue dye exclusion assay. Of the three compounds, the xanthone ISO was the most potent cytotoxic agent whereas the benzophenone GK was the least potent one. ISO reached its maximal cytotoxicity against the cells at the concentration of 25 μM. At this concentration, treatment with ISO for 4 h decreased the viability of caco-2 cells to 40%.

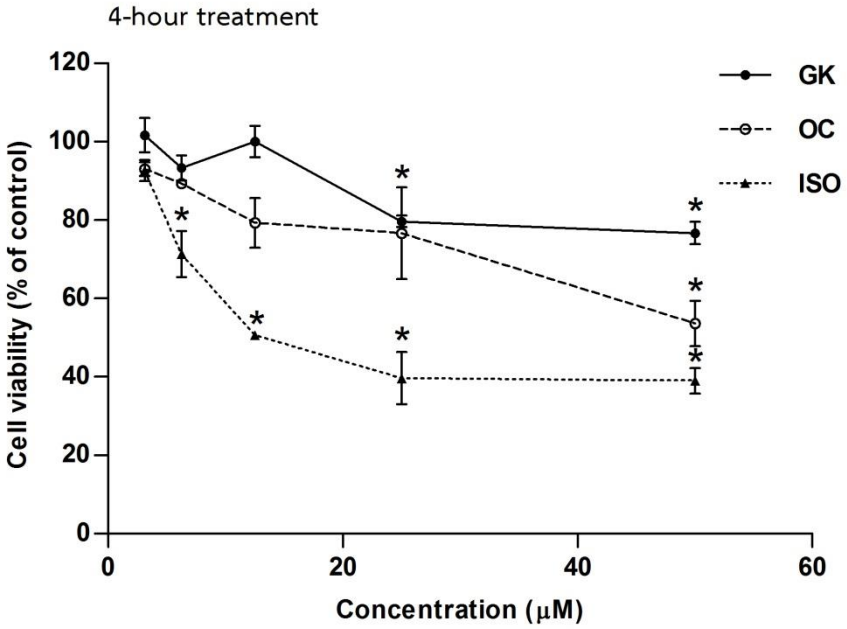


Figure 4. Cytotoxicity of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) against Caco-2 cells after 4-hour treatment. Data are presented as the mean ± SEM (N=3). **P*< 0.05 compared with the control group.

As shown in Figure 5, the three compounds were able to inhibit the proliferation of Caco-2 cells in time-dependent manner, as measured by MTT assay. At the concentration up to 20 μM, neither GK nor OC was toxic to the cells after 24-hour exposure. However, their cytotoxicity markedly increased when the treatment periods were extended to 48 and 72 h. Treatment of the cells with ISO at the concentration up to 10 μM for 24 h decreased cell viability in concentration dependent manner. The cell viability further decreased when the treatment periods were extended to 48 and 72 h. The IC_{50} values of each treatment were shown in Table 2.

Table 2. IC_{50} values of cytotoxic effect of GK, OC and ISO against Caco-2 cells after the treatment periods of 24, 48, 72 hours.

Compounds	IC_{50} (μM)		
	24 h	**48 h**	**72 h**
Guttiferone K	N/A	N/A	9.46 ± 0.94
Oblongifolin C	N/A	10.41 ± 0.09	6.12 ± 0.60
Isojacareubin	N/A	5.09 ± 0.60	3.49 ± 0.66

N/A; not applicable

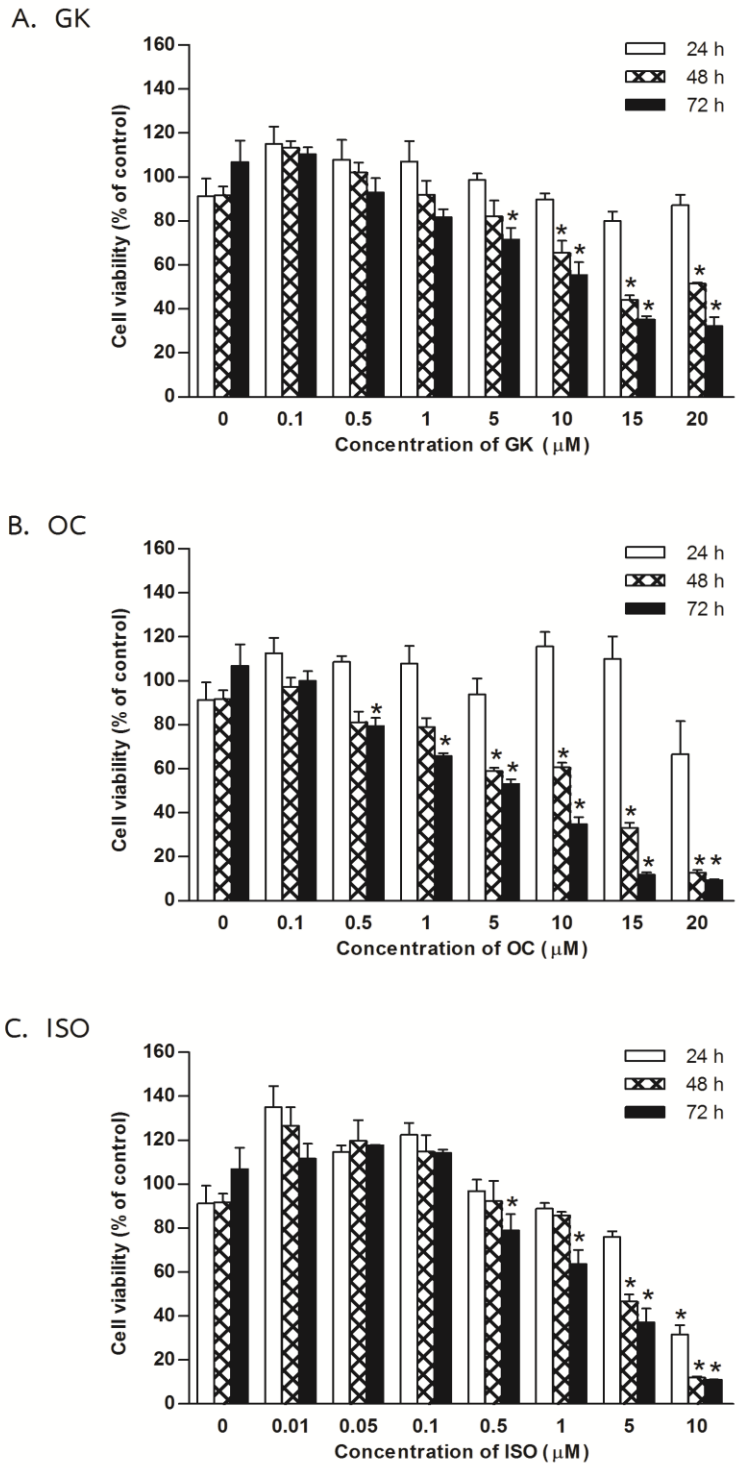


Figure 5. Antiproliferative effects of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) against Caco-2 cells after treatment periods of 24, 48 and 72 h. Data represent the mean ± SEM (N=3). **P*< 0.05 compared with untreated group.

In addition, the cytotoxic effects of these compounds were determined in the cells grown for 14 days by the tryptan blue dye exclusion assay. As shown in Figure 6, at the concentration of 50 μM none of the compounds elicited toxic action on cell viability after 24 h treatment. When visualized under a microscope, the cell morphology remained unchanged, after treatment (Figure 7).

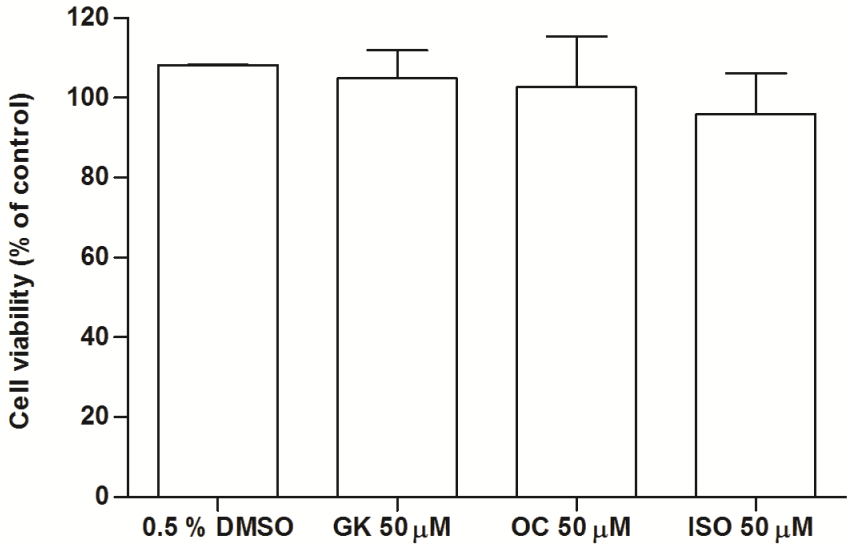


Figure 6. Cytotoxicity of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) in Caco-2 cells aged 14 days after 24-hour treatment. Data represent the mean ± SEM (N=3). **P*< 0.05 compared with the control group.

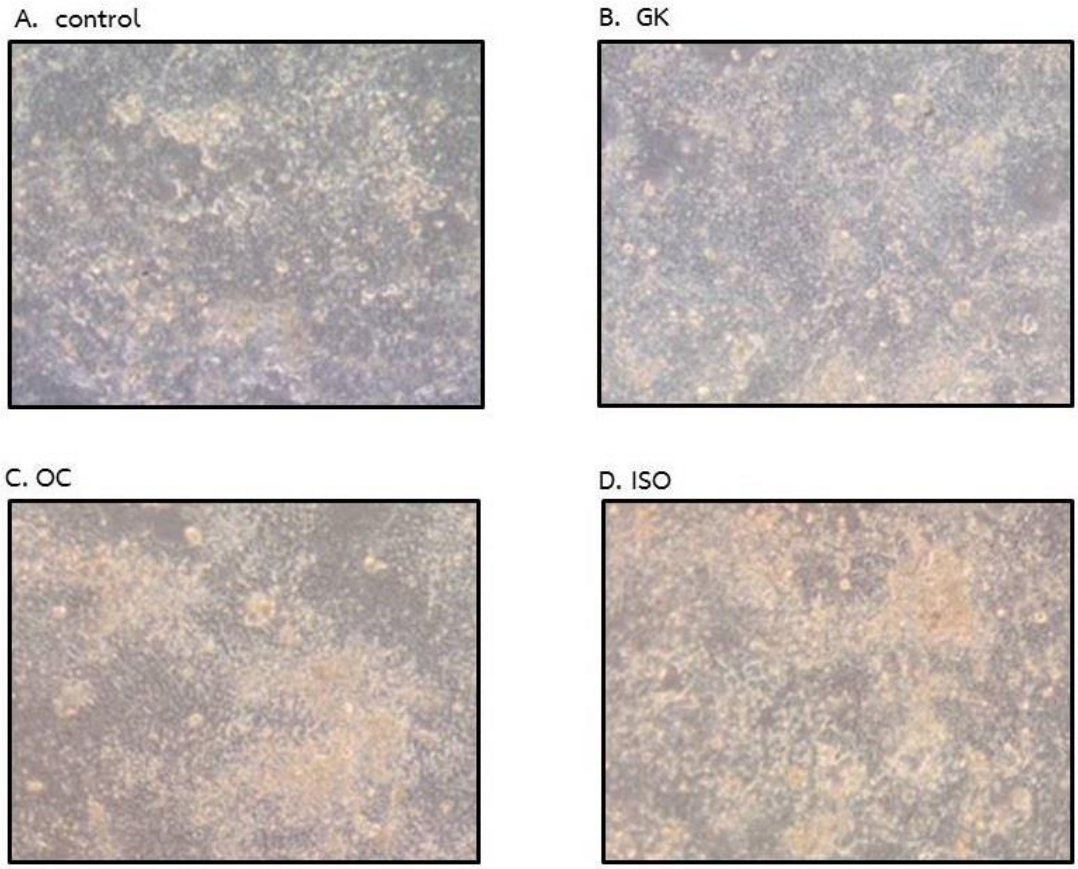


Figure 7. Morphology of Caco-2 cells after 24-hour treatment with guttiferone K (GK), oblongifolin C (OC) and isojacarubin (ISO) at the concentration of 50 μM.

4.2 Effect of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on the generation of reactive oxygen species (ROS)

As shown in Figure 8, the benzophenone OC or the xanthone ISO at the concentration of 50 μM significantly increased ROS level in Caco-2 cells after 1-hour treatment. By contrast, the benzophenone GK at equimolar concentration had no effects on ROS level within the cells.

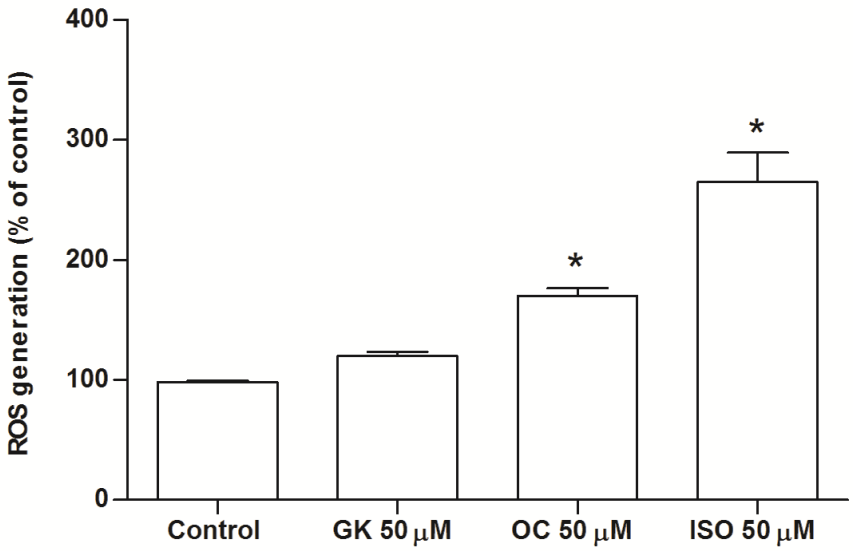


Figure 8. Effect of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on ROS level in Caco-2 cells after 1-hour treatment. Data are expressed as the percentage of the control group. Each bar represents the mean ± SEM (N=3-4). **P*< 0.05 compared with the control group.

4.3 Effect of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on the expression of *MDR1* gene

As shown in Figure 9, the three compounds (at 50 μM) significantly increased the relative amount of mRNA of *MDR1* gene in Caco-2 cells after 24 h treatment. The presence of 10 mM NAC, a ROS scavenger, significantly abolished the stimulatory effects of the benzophenone OC and the xanthone ISO on *MDR1* mRNA level. In addition, NAC was also able to suppress the benzophenone GK-mediated overexpression of *MDR1* mRNA (*P*= 0.057).

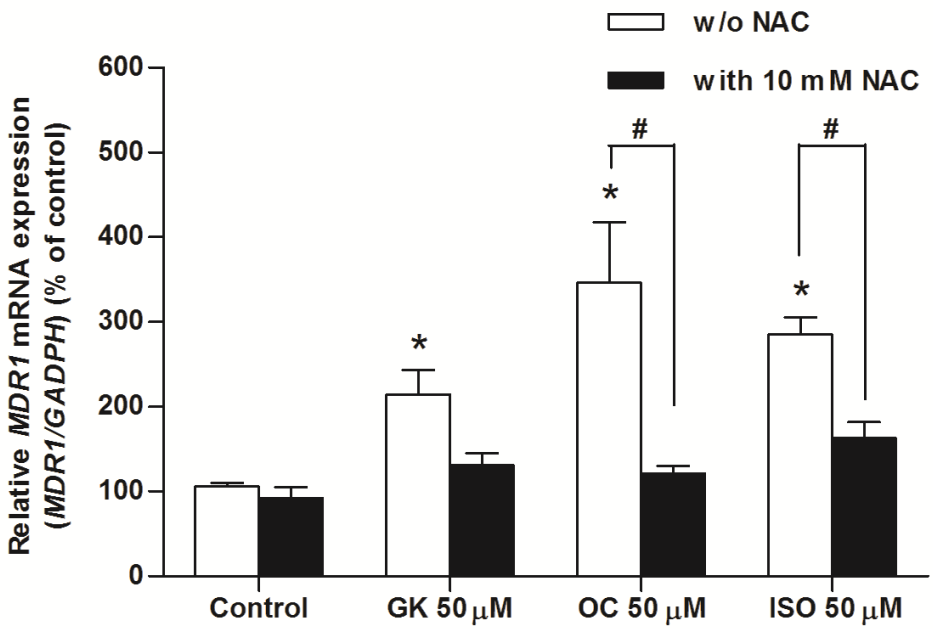


Figure 9. Relative level of *MDR1* mRNA after treatment with guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) in the absence or presence of 10 mM NAC. Data are expressed as the percentage of the control group. Each bar represents the mean ± SEM (N=3-5). *P< 0.05 indicated statistically significant difference from the control group; #P< 0.05 indicated statistically significant difference between treatment in the presence and absence of NAC.

4.4 Involvement of the mitogen-activated protein kinases (MAPKs) pathway in GK/OC/ISO-mediated *MDR1* expression

MAPKs pathway including ERK and p-38 is related to survival mechanism in the response of cells to stress. The presence of specific inhibitors such as U0126 or SB202190 in the MAPK pathway definitely affected GK/OC/ISO-mediated *MDR1* expression. As shown in Figure 10A, U0126, which can specifically inhibit ERK1/2 activity, was able to suppress an increase in the mRNA levels of the cells treated with either of the two benzophenones GK and OC. On the contrary, U0126 had no effect on the xanthone ISO-mediated overexpression of *MDR1* mRNA. These findings suggested that both benzophenones might increase *MDR1* gene expression at the transcriptional level through activation of ERK1/2/MAPK pathway. Although U0126 could not suppress ISO-mediated increase of *MDR1* mRNA, another MAPK inhibitor SB202190, demonstrated otherwise. SB202190 is a known specific inhibitor of p38/MAPK activity. As seen in Figure 10B, the cells treated with ISO and SB202190 had a significantly lesser amount of *MDR1* mRNA than those treated with ISO alone. Moreover, SB202190 had no effect on the benzophenone-mediated overexpression of *MDR1*. Hence, these findings suggested that the mechanism in which the xanthone ISO mediated an increase of *MDR1* mRNA might involve the p38/MAPK pathway, but not the ERK1/2/MAPK pathway.

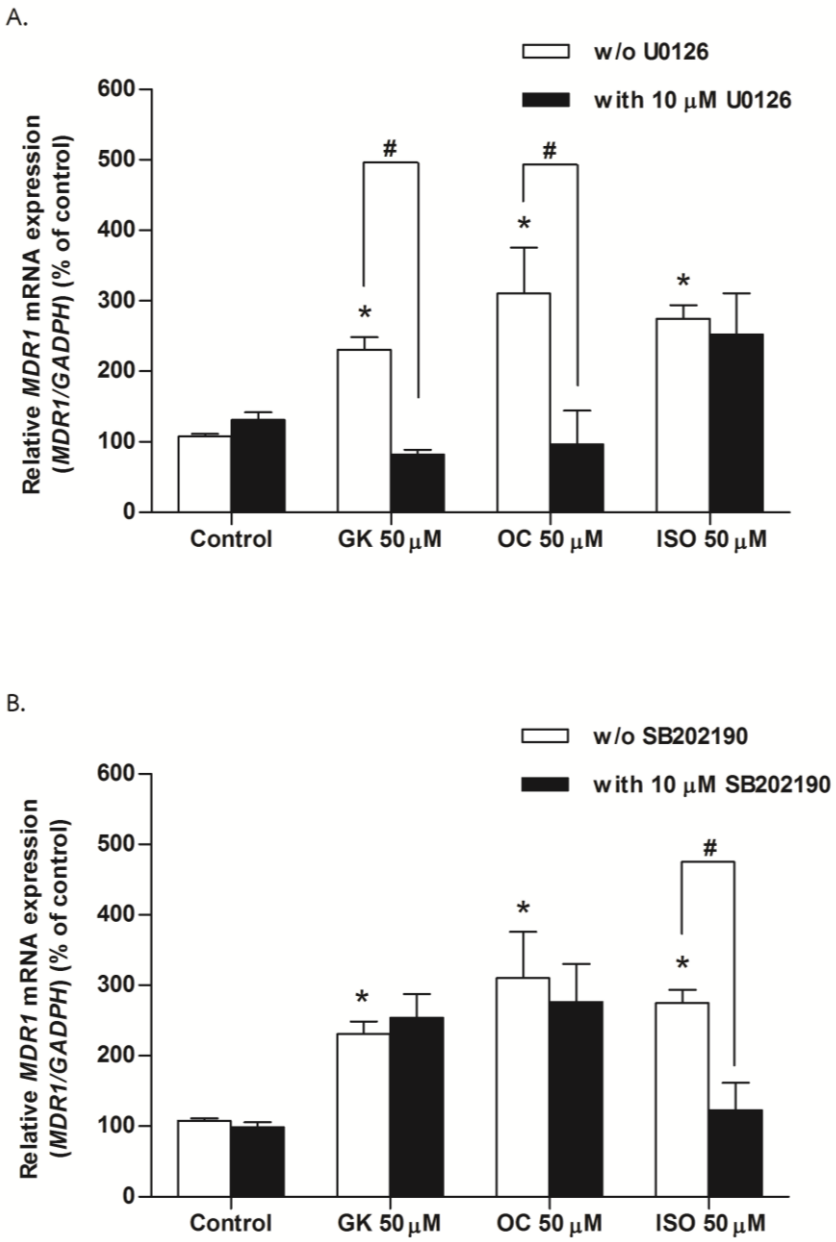


Figure 10. Relative *MDR1* mRNA level in the Caco-2 cells after treatment with guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) in the absence and presence of 10 μM U0126 or SB202190. Data are presented as the percentage of the control group. Each bar represents the mean ± SEM (N=3-7). *P< 0.05 indicated statistically significant difference from the control group; $^{\#}P$< 0.05 indicated statistically significant difference between the treatment in the presence and absence of specific inhibitors of MAPKs pathway.

Activation of ERK1/2/MAPK and p-38/MAPK pathways following treatment with GK, OC and ISO was determined with western blot analysis. As shown in Figures 11 and 12, the expression of ERK 1/2 in Caco-2 cells was significantly shifted into the phosphorylated form (p-ERK1/2) upon treatment with GK and OC, but not with ISO. On the other hand, the extent of phosphorylated form of p38 (p-p38) increased significantly in the presence of ISO, but not GK nor OC.

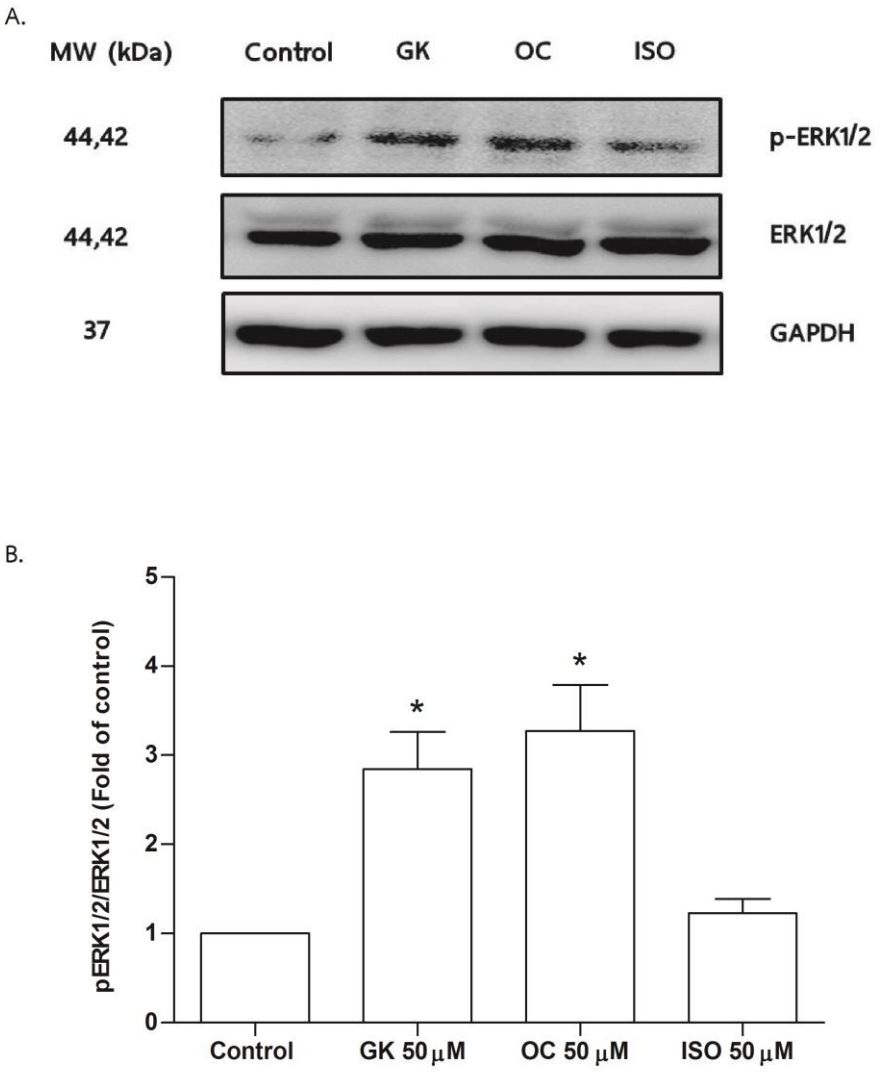


Figure 11. Effects of GK, OC or ISO on the expression of ERK1/2 and its phosphorylated form (p-ERK1/2) in Caco-2 cells. (A) Immunoblots of ERK1/2 and phosphorylated ERK1/2. (B) Densitometrical analysis of immunoblots calculated as the ratio of phosphorylated ERK1/2 to ERK1/2. Each bar represents the mean ± SEM obtained from separated experiments (N=3). **P*< 0.05 compared with the control group.

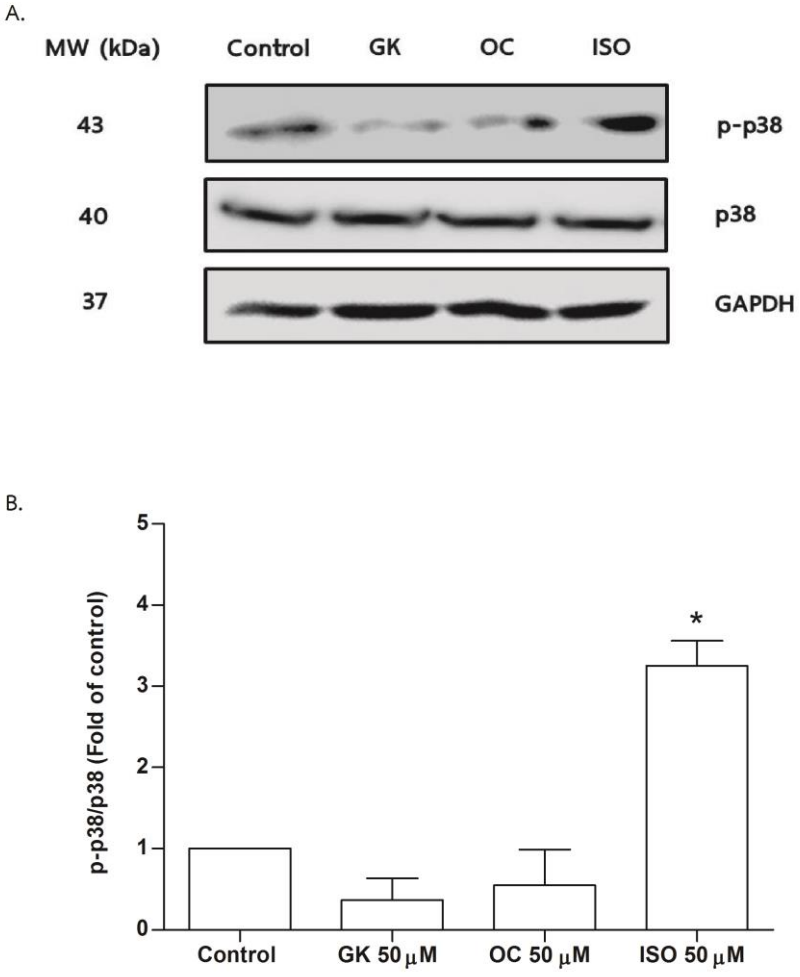


Figure 12. Effects of GK, OC or ISO on the expression of p38 and its phosphorylated form (p-p38) in Caco-2 cells. (A) Immunoblots of p38 and phosphorylated p38. (B) Densitometrical analysis of immunoblots calculated as the ratio of phosphorylated p38 to p38. Each bar represents the mean ± SEM obtained from separated experiments (N=3). **P*< 0.05 compared with the control group.

4.5 Effect of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on transcription factor activator protein 1 (AP-1)

AP-1 is a transcription factor of *MDR1* gene and a downstream target of the MAPKs-pathway. AP-1 is composed of either homodimers (c-JUN/c-JUN) or heterodimers (c-JUN/c-FOS). As shown in Figure 13A, the benzophenone OC and the xanthone ISO at the concentration of 50 μM significantly increased the mRNA level of *c-JUN* compared with the control group. By contrast, the benzophenone GK at equimolar concentration had no effect on mRNA level of *c-JUN*. Furthermore, none of these compounds affected the mRNA level of *c-FOS* under similar treatment condition (Figure 13B). These results further indicated the involvement of *c-JUN* in the up-regulation of *MDR1* gene expression in Caco-2 cells treated with either OC or ISO. Moreover, it was likely that the effect of GK on an increase of *MDR1* mRNA was not related to AP-1 activity.

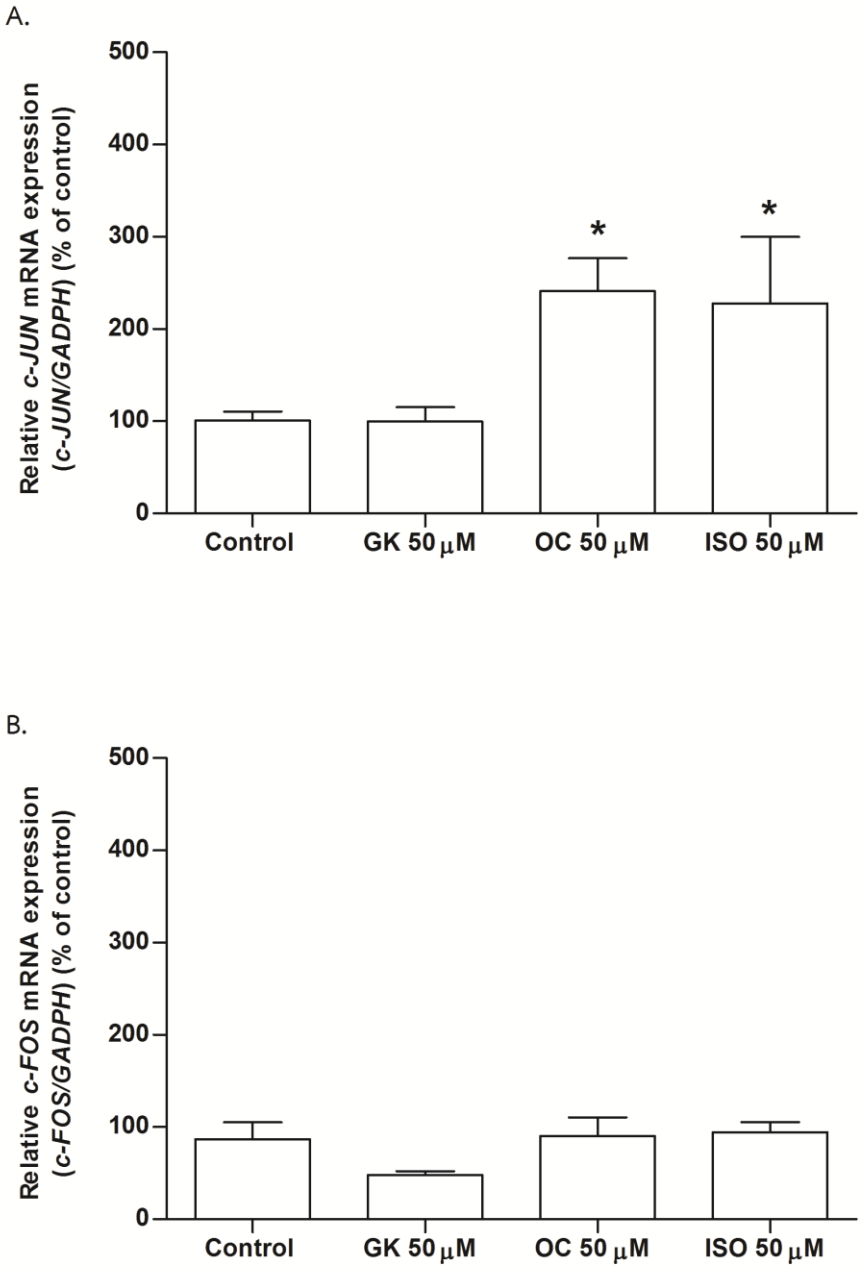


Figure 13. Effect of guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) on AP-1 (*c-JUN* or *c-FOS* gene) expression. Data are expressed as the percentage of the control group. Each bar represents the mean ± SEM (N=3-4). **P*< 0.05 compared with the control group.

CHAPTER V
DISCUSSION

Derivatives of benzophenones and xanthones including guttiferone K (GK), oblongifolin C (OC) and isojacareubin (ISO) could be found in and isolated from the wood of *Garcinia schomburgkiana* (Thai name: Ma-dan). These three compounds displayed cytotoxic property against cancer cell lines such as A2780, CHAGO, HT-29, HeLa and SW620 (Mungmee et al., 2013). In this study, the acute cytotoxicity and anti-proliferative actions of GK, OC and ISO were further investigated in colorectal adenocarcinoma (Caco-2 cells). At the concentrations of greater than 25 μM, these compounds interfered with the measurement utilizing formazan dye in the MTT assay. Hence, trypan blue dye exclusion assay was selected to evaluate their cytotoxicity. The MTT assay could still be applied effectively in experiments in which the concentrations of these phytochemicals were less than 20 μM. The results demonstrated that the xanthone ISO was more potent than the benzophenones GK and OC in causing cell death after 4-hour treatment. All three compounds were able to inhibit the proliferation of Caco-2 cells in concentration- and time-dependent manners. Of the three compounds in this study, the xanthone ISO was the most potent anti-proliferative agent. Recently, this xanthone was reported as able to inhibit the proliferation and metastasis of HepG2 hepatoma cells (Yuan et al., 2015). In addition, this compound, at the concentration of 5 mg/kg given by tail vein injection, could decrease tumor volume through induction of apoptosis in hepatoma xenograft mouse model (Yuan et al., 2015). The benzophenone OC (60 mg/kg, i.p.) has also been demonstrated to suppress tumor growth in melanoma xenograft mouse model by inducing apoptosis (Feng et al., 2012), whereas the benzophenone GK was reported to arrest G0/G1 phase of certain HT-29 cell lines (Kan et al., 2013). In addition, this compound (10 mg/kg, i.p.) was able to decrease the tumor volume when used alone

or in combination with 5-fluorouracil in colon tumor mouse model (Kan et al., 2013). Therefore, these compounds might have therapeutic potential as anticancer agents.

Overexpression of drug efflux pumps has been known as one of the major mechanisms responsible for multidrug-resistance (MDR) in cancer. Involvement of *MDR1* was first identified from MDR in cancer cells. An increase of P-gp expression can lessen intracellular drug accumulation, and therefore the cells become resistant toward chemotherapy (Gottesman, 2002). Chemotherapeutic drugs from natural origins such as doxorubicin (from *Streptomyces peucetius*) and vinblastine (from *Catharanthus roseus*) are known P-gp substrates and inducers (Osborn and Chambers, 1996; Zhou et al., 1999; Sui et al., 2012; Cao et al., 2013). Prolonged exposure to these drugs leads to increased P-gp expression in several cancer cells such as KB-3, MCF-7 and Caco-2 cells (Wang et al., 2005; Silva et al., 2011; Cao et al., 2013). In addition, a number of compounds with chemical structures related to GK, OC and ISO have been shown to up-regulate P-gp expression in several cell lines. For example, hyperforin, a phloroglucinol derivative, at the concentration of 1 μM increased the expression and function of P-gp in colon adenocarcinoma LS 180 cell lines after 24- and 48-hour treatment (Tian et al., 2015). The xanthone mangiferin, at the concentration up to 200 μM, was able to increase P-gp expression after 72-hour exposure in human kidney HK-2 cell lines (Chieli et al., 2010). Moreover, dihydroxylated xanthones, at the concentration of 25 μM, increased the function and expression of P-gp after 24-hour treatment in Caco-2 cells (Silva et al., 2014b).

In this study, the effects of three phytochemicals on the up-regulation of *MDR1* gene were investigated in Caco-2 monolayers grown for 14 days. At this age, the cells were already differentiated and expressed P-gp at appreciable levels. It was reported that the expressed level of *MDR1* mRNA was stable when the Caco-2 cells were in the differentiation phase (Goto et al., 2003). In addition, the Caco-2 monolayers at the same age have been used to study the effects of several chemical compounds on the

up- or down-regulation of P-gp expression (Zrieki, Farinotti and Buyse, 2008; Wongwanakul et al., 2013).

The chemical-mediated up-regulation of *MDR1* gene and P-gp protein depends on both concentration of the compound and duration of exposure. For example, colchicine at concentration range of 0.1 to 100 μM could increase the P-gp expression in Caco-2 cells in concentration- dependent manner after 24-hour treatment (Silva et al., 2014a). Cadmium (5 mM) was able to increase P-gp expression in PT cells in time-dependent manner after 24- to 72-hour treatment (Thevenod et al., 2000). In this study, significant increase of *MDR1* mRNA was observed in the Caco-2 cells treated with either doxorubicin or vinblastine (50 μM) for 24 h. In these regards, the effects of benzophenones GK, OC and a xanthone ISO on *MDR1* expression were at the concentration of 50 μM, these three compounds had no cytotoxic effect on the 14-day old Caco-2 cells after 24-hour exposure. Moreover, each compound was able to increase mRNA of *MDR1* gene to about 2 - 3.5 times of the control. Hence, the results suggested that all these compounds could up-regulate *MDR1* mRNA.

Oxidative stress have been linked the induction of *MDR1* gene and P-gp protein expression in various cell culture models such as Caco-2, hepG2, KB-3 and KBV1 cancer cell lines (Osborn and Chambers, 1996; Ledoux et al., 2003; Hong et al., 2006; Sugihara et al., 2007; Komori et al., 2014). For example, L-buthionine-(S,R)-sulfoximine (BSO), at concentration 200 μM, increased P-gp function and expression through oxidative stress for 24-hour in rat brain microvascular endothelial cells (Hong et al., 2006). In Caco-2 cell lines, the benzo(a)pyrene and benzo(e)pyrene (50 μM) could generate ROS and increased P-gp expression after 72-hour exposure (Sugihara et al., 2007). Doxorubicin (3 μM) was able to generate oxidative stress and up-regulated P-gp expression after 24-hour treatment in human hepatoma HepG2 cells (Komori et al., 2014). In this study, the results showed that OC and ISO (50 μM) could significantly generate oxidative stress after 1-hour exposure in Caco-2 cells by about 1.7 - 2.7 times of the control, but

benzophenone GK had no effect on cellular ROS level as measured by dichlorofluorescein diacetate (DCFH-DA) assay. The presence of 10 mM NAC (a ROS scavenger) prevented the up-regulation of *MDR1* mRNA level caused by 24-hour treatment with these phytochemicals. Thus, it was likely that all three compounds could increase *MDR1* mRNA level through oxidative stress mediated mechanisms. In this study, DCFH-DA probes might not be able to effectively measure ROS generated from 1-hour treatment with GK. It was possible that the amount of ROS in this treatment might be below the limit of DCFH-DA detection. Upon 24-hour treatment, GK might gradually generate enough ROS to induce an up-regulation of *MDR1*. In addition, the amount of mRNA extracted from the GK treated cells was significantly lower than those extracted from cells treated with the other two compounds. This observation might relate to the lower amount of ROS produced in GK-treated cells.

Overexpression of P-gp have been suggested to be associated with the activation of survival signaling pathways which include mitogen-activated kinase (MAPK), nuclear factor kappa-B (NF-kB), phosphoinositide 3-kinase (PI3K) and protein kinase C (PKC) (Sui et al., 2012). The MAPK pathway has been proposed to be a major signaling pathway responsible for P-gp expression (Duan et al., 2012; Saksena et al., 2013; Priyamvada et al., 2016). The MAPK pathway including ERK and p-38 has been shown to be activated by cellular oxidative stress (Gaitanaki et al., 2003; Matos et al., 2005). For example, sodium nitroprusside (0.1 mM) was able to increase the function and expression of P-gp in Caco-2 cells after 24-hour treatment by activation of PI3K, PKC, Akt and p38/MAPK pathways (Duan et al., 2012). Long-term treatment with keratinocyte growth factor-2 could increase P-gp expression in Caco-2 cells via activation of ERK1/2/MAPK pathway (Saksena et al., 2013). Hence, it was likely that cellular stress generated from GK, OC and ISO might be responsible for the up-regulation of *MDR1* mRNA through mechanism involving MAPKs pathway. Moreover, inhibitors of two specific isoforms of MAPK affected the *MDR1* up-regulation induced

by benzophenones and xanthone differently. U0126, an inhibitor of ERK1/2/MAPK activity, was able to suppress an increase of *MDR1* mRNA level in the cells treated with either benzophenones, but not with the xanthone ISO. On the contrary, ISO-mediated increase of *MDR1* mRNA was inhibited by SB202190, an inhibitor of p38/MAPK activity. These findings were in agreement with increasing phosphorylated form of either ERK1/2 (p-ERK1/2) or p38 (p-p38) upon treatment of the cells with benzophenones or xanthone, respectively. Taken together, it was likely that the benzophenones were able to induce an overexpression of *MDR1* mRNA via oxidative stress-mediated ERK1/2/MAPK pathway, whereas the xanthone induced *MDR1* mRNA through the stress-activated p38/MAPK pathway.

AP-1 is a transcription factor of *MDR1* gene and a downstream target of the MAPKs-pathway (Whitmarsh and Davis, 1996; Guo et al., 2001; Monje, Marinissen and Gutkind, 2003; Scotto, 2003; Zhou et al., 2007). AP-1 is composed of either homodimers (c-JUN/c-JUN) or heterodimers (c-JUN/c-FOS). Recently, it was shown that an increase *MDR1* gene expression in the vinblastine-resistant Caco-2 cells involved activation of several transcription factors including AP-1 (c-JUN, c-JUN) and NF-kB (Chen et al., 2014). Furthermore, increased expression of *MDR1* mRNA was shown to be related to the activation of p-38/MAPK pathway-mediated AP-1 in vincristine-resistance human gastric cancer cells (SGC7901/VCR) (Guo et al., 2008). In addition, treating the cells with a p38/MAPK inhibitor resulted in decreased P-gp expression level (mRNA) in SGC7901/VCR cells (Guo et al., 2008). In Caco-2 cells, *L. acidophilus* culture supernatant could increase the function and expression of P-gp by activation of ERK1/2/MAPK-mediated AP-1 (Priyamvada et al., 2016). In this study, the benzophenone OC and the xanthone ISO (50 μM) significantly increased mRNA level of *c-JUN* after 24-hour treatment while another benzophenone, GK, had no such effect. However, none of these compounds affected the mRNA level of *c-FOS* under similar treatment condition. Thus, it was likely that OC and ISO were able to up-regulate

MDR1 mRNA through the activation of MAPK-mediated homodimers AP-1. The benzophenone GK had no effect on AP-1 although it up-regulated *MDR1* mRNA via activation of ERK1/2/MAPK, therefore it might have effect on other *MDR1* transcription factors such as NF-kB. It has been reported that activation of ERK1/2/MAPK signaling pathway could cross-talk with NF-kB signaling pathway at the transcription level, leading to an increase of *MDR1* mRNA level in MCF-7/Adr cells (Zhang et al., 2012). Hence, the GK-mediated up-regulation of *MDR1* gene should be investigated further for its involvement with NF-KB signaling pathway.

In conclusion, these results suggested that GK, OC and ISO could induce the up-regulate of *MDR1* mRNA in colorectal adenocarcinoma cells (Caco-2 cells). The effects of these compounds were associated with oxidative stress-mediated mitogen-activated kinase (MAPK) signaling pathway. Further determination of their induction effect on P-gp protein level should be pursued.

REFERENCES

REFERENCES

Alberts, D. S., Peng, Y. M., Bowden, G. T., Dalton, W. S., and Mackel, C. (1985). Pharmacology of mitoxantrone: mode of action and pharmacokinetics. Invest New Drugs 3(2): 101-107.

Batova, A., Altomare, D., Chantarasriwong, O., Ohlsen, K. L., Creek, K. E., Lin, Y. C., Messersmith, A., Yu, A. L., Yu, J., and Theodorakis, E. A. (2010). The synthetic caged *Garcinia* xanthone cluvenone induces cell stress and apoptosis and has immune modulatory activity. Mol Cancer Ther 9(11): 2869-2878.

Cao, B., Li, M., Zha, W., Zhao, Q., Gu, R., Liu, L., Shi, J., Zhou, J., Zhou, F., Wu, X., Wu, Z., Wang, G., and Aa, J. (2013). Metabolomic approach to evaluating adriamycin pharmacodynamics and resistance in breast cancer cells. Metabolomics 9(5): 960-973.

Chen, Q., Bian, Y., and Zeng, S. (2014). Involvement of AP-1 and NF-kappaB in the up-regulation of P-gp in vinblastine resistant Caco-2 cells. DMPK 29(2): 223-226.

Chieli, E., Romiti, N., Rodeiro, I., and Garrido, G. (2010). In vitro modulation of ABCB1/P-glycoprotein expression by polyphenols from *Mangifera indica*. Chem Biol Interact 186(3): 287-294.

Deng, Y. T., Huang, H. C., and Lin, J. K. (2010). Rotenone induces apoptosis in MCF-7 human breast cancer cell-mediated ROS through JNK and p38 signaling. Mol Carcinog 49(2): 141-151.

Duan, R., Hu, N., Liu, H. Y., Li, J., Guo, H. F., Liu, C., Liu, L., and Liu, X. D. (2012). Biphasic regulation of P-glycoprotein function and expression by NO donors in Caco-2 cells. Acta Pharmacol Sin 33(6): 767-774.

Egashira, M., Kawamata, N., Sugimoto, K., Kaneko, T., and Oshimi, K. (1999). P-glycoprotein expression on normal and abnormally expanded natural killer cells and inhibition of P-glycoprotein function by cyclosporin A and its analogue, PSC833. Blood 93(2): 599-606.

Feng, C., Zhou, L. Y., Yu, T., Xu, G., Tian, H. L., Xu, J. J., Xu, H. X., and Luo, K. Q. (2012). A new anticancer compound, oblongifolin C, inhibits tumor growth and promotes apoptosis in HeLa cells through Bax activation. Int J Cancer 131(6): 1445-1454.

Gaitanaki, C., Konstantina, S., Chrysa, S., and Beis, I. (2003). Oxidative stress stimulates multiple MAPK signalling pathways and phosphorylation of the small HSP27 in the perfused amphibian heart. J Exp Biol 206(Pt 16): 2759-2769.

Goto, M., Masuda, S., Saito, H., and Inui, K. (2003). Decreased expression of P-glycoprotein during differentiation in the human intestinal cell line Caco-2. Biochem Pharmacol 66(1): 163-170.

Gottesman, M. M. (2002). Mechanisms of cancer drug resistance. Ann Rev Med 53: 615-627.

Guo, X., Ma, N., Wang, J., Song, J., Bu, X., Cheng, Y., Sun, K., Xiong, H., Jiang, G., Zhang, B., Wu, M., and Wei, L. (2008). Increased p38-MAPK is responsible for chemotherapy resistance in human gastric cancer cells. BMC Cancer 8(1): 1-9.

Guo, Y. S., Hellmich, M. R., Wen, X. D., and Townsend, C. M., Jr. (2001). Activator protein-1 transcription factor mediates bombesin-stimulated cyclooxygenase-2 expression in intestinal epithelial cells. J Biol Chem 276(25): 22941-22947.

Han, Q. B., Yang, N. Y., Tian, H. L., Qiao, C. F., Song, J. Z., Chang, D. C., Chen, S. L., Luo, K. Q., and Xu, H. X. (2008). Xanthones with growth inhibition against HeLa cells from *Garcinia xipshuanbannaensis*. Phytochemistry 69(11): 2187-2192.

Hartz, A. M., Bauer, B., Block, M. L., Hong, J. S., and Miller, D. S. (2008). Diesel exhaust particles induce oxidative stress, proinflammatory signaling, and P-glycoprotein up-regulation at the blood-brain barrier. FASEB J 22(8): 2723-2733.

Hong, H., Lu, Y., Ji, Z. N., and Liu, G. Q. (2006). Up-regulation of P-glycoprotein expression by glutathione depletion-induced oxidative stress in rat brain microvessel endothelial cells. J Neurochem 98(5): 1465-1473.

Kan, W. L., Yin, C., Xu, H. X., Xu, G., To, K. K., Cho, C. H., Rudd, J. A., and Lin, G. (2013). Antitumor effects of novel compound, guttiferone K, on colon cancer by p21Waf1/Cip1-mediated G(0) /G(1) cell cycle arrest and apoptosis. Int J Cancer 132(3): 707-716.

Katayama, K., Yoshioka, S., Tsukahara, S., Mitsuhashi, J., and Sugimoto, Y. (2007). Inhibition of the mitogen-activated protein kinase pathway results in the down-regulation of P-glycoprotein. Mol Cancer Ther 6(7): 2092-2102.

Khuhaprema, T., and Srivatanakul, P. (2008). Colon and rectum cancer in Thailand: An overview. Jpn J Clin Oncol 38(4): 237-243.

Komori, Y., Arisawa, S., Takai, M., Yokoyama, K., Honda, M., Hayashi, K., Ishigami, M., Katano, Y., Goto, H., Ueyama, J., Ishikawa, T., and Wakusawa, S. (2014). Ursodeoxycholic acid inhibits overexpression of P-glycoprotein induced by doxorubicin in HepG2 cells. Eur J Pharmacol 724: 161-167.

Ledoux, S., Yang, R., Friedlander, G., and Laouari, D. (2003). Glucose depletion enhances P-glycoprotein expression in hepatoma cells: Role of endoplasmic reticulum stress response. Cancer Res 63(21): 7284-7290.

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4): 402-408.

Lomovskaya, N., Otten, S. L., Doi-Katayama, Y., Fonstein, L., Liu, X. C., Takatsu, T., Inventi-Solari, A., Filippini, S., Torti, F., Colombo, A. L., and Hutchinson, C. R. (1999). Doxorubicin overproduction in *Streptomyces peucetius*: Cloning and characterization of the dnrU ketoreductase and dnrV genes and the doxA cytochrome P-450 hydroxylase gene. J Bacteriol 181(1): 305-318.

Luqmani, Y. A. (2005). Mechanisms of drug resistance in cancer chemotherapy. Med Princ Pract 14 Suppl 1: 35-48.

Mans, D. R., da Rocha, A. B., and Schwartsmann, G. (2000). Anti-cancer drug discovery and development in Brazil: Targeted plant collection as a rational strategy to acquire candidate anti-cancer compounds. Oncologist 5(3): 185-198.

Matos, T. J., Duarte, C. B., Goncalo, M., and Lopes, M. C. (2005). Role of oxidative stress in ERK and p38 MAPK activation induced by the chemical sensitizer DNFB in a fetal skin dendritic cell line. Immunol Cell Biol 83(6): 607-614.

Miki, H., Uehara, N., Kimura, A., Sasaki, T., Yuri, T., Yoshizawa, K., and Tsubura, A. (2012). Resveratrol induces apoptosis via ROS-triggered autophagy in human colon cancer cells. Int J Oncol 40(4): 1020-1028.

Monje, P., Marinissen, M. J., and Gutkind, J. S. (2003). Phosphorylation of the carboxyl-terminal transactivation domain of c-Fos by extracellular signal-regulated kinase mediates the transcriptional activation of AP-1 and cellular transformation induced by platelet-derived growth factor. Mol Cell Bio 23(19): 7030-7043.

Mungmee, C., Sitthigool, S., Buakeaw, A., and Suttisri, R. (2013). A new biphenyl and other constituents from the wood of *Garcinia schomburgkiana*. Nat Prod Res 27(21): 1949-1955.

Osborn, M. T. and Chambers, T. C. (1996). Role of the stress-activated/c-Jun NH2-terminal protein kinase pathway in the cellular response to adriamycin and other chemotherapeutic drugs. J Biol Chem 271(48): 30950-30955.

Pardo-Andreu, G. L., Nunez-Figueredo, Y., Tudella, V. G., Cuesta-Rubio, O., Rodrigues, F. P., Pestana, C. R., Uyemura, S. A., Leopoldino, A. M., Alberici, L. C., and Curti, C. (2011). The anti-cancer agent guttiferone-A permeabilizes mitochondrial membrane: ensuing energetic and oxidative stress implications. Toxicol Appl Pharmacol 253(3): 282-289.

Pasqua, G., Monacelli, B., and Valletta, A. (2004). Cellular localisation of the anti-cancer drug camptothecin in Camptotheca acuminata Decne (Nyssaceae). Eur J Histochem 48(3): 321-327.

Payne, S., and Miles, D. Mechanisms of anticancer drugs Scott-Brown's Otorhinolaryngology: Head and Neck Surgery 7th ed. Florida: CRC Press, 2008.

Priyamvada, S., Anbazhagan, A. N., Kumar, A., Soni, V., Alrefai, W. A., Gill, R. K., Dudeja, P. K., and Saksena, S. (2016). *Lactobacillus acidophilus* stimulates intestinal P-glycoprotein expression via a c-Fos/c-Jun dependent mechanism in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 310(8): 599-608.

Prosperini, A., Juan-Garcia, A., Font, G., and Ruiz, M. J. (2013). Beauvericin-induced cytotoxicity via ROS production and mitochondrial damage in Caco-2 cells. Toxicol Lett 222(2): 204-211.

Saksena, S., Priyamvada, S., Kumar, A., Akhtar, M., Soni, V., Anbazhagan, A. N., Alakkam, A., Alrefai, W. A., Dudeja, P. K., and Gill, R. K. (2013). Keratinocyte growth factor-2 stimulates P-glycoprotein expression and function in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 304(6): 615-622.

Scotto, K. W. (2003). Transcriptional regulation of ABC drug transporters. Oncogene 22(47): 7496-7511.

Scotto, K. W., and Egan, D. A. (1998). Transcriptional regulation of MDR genes. Cytotechnol 27(1): 257-269.

Shen, H., Xu, W., Luo, W., Zhou, L., Yong, W., Chen, F., Wu, C., Chen, Q., and Han, X. (2011). Upregulation of mdr1 gene is related to activation of the MAPK/ERK signal transduction pathway and YB-1 nuclear translocation in B-cell lymphoma. Exp Hematol 39(5): 558-569.

Shen, K., Xie, J., Wang, H., Zhang, H., Yu, M., Lu, F., Tan, H., and Xu, H. (2015). Cambogin induces caspase-independent apoptosis through the ROS/JNK pathway and epigenetic regulation in breast cancer cells. Mol Cancer Ther 14(7): 1738-1749.

Shukla, A., and Mossman, B. T. Cell signaling by oxidants: pathways leading to activation of mitogen-activated protein kinases (MAPK) and activator protein-1 (AP-1) In M. Sadis ed. Cambridge, Massachusetts : Academic Press, 2008.

Silva, R., Carmo, H., Dinis-Oliveira, R., Cordeiro-da-Silva, A., Lima, S. C., Carvalho, F., Bastos Mde, L., and Remiao, F. (2011). In vitro study of P-glycoprotein induction as an antidotal pathway to prevent cytotoxicity in Caco-2 cells. Arch Toxicol 85(4): 315-326.

Silva, R., Carmo, H., Vilas-Boas, V., Barbosa, D. J., Palmeira, A., Sousa, E., Carvalho, F., Bastos Mde, L., and Remiao, F. (2014a). Colchicine effect on P-glycoprotein expression and activity: in silico and in vitro studies. Chem Biol Interact 218: 50-62.

Silva, R., Sousa, E., Carmo, H., Palmeira, A., Barbosa, D. J., Gameiro, M., Pinto, M., Bastos Mde, L., and Remiao, F. (2014b). Induction and activation of P-glycoprotein by dihydroxylated xanthones protect against the cytotoxicity of the P-glycoprotein substrate paraquat. Arch Toxicol 88(4): 937-951.

Sugihara, N., Toyama, K., Okamoto, T., Kadowaki, M., Terao, K., and Furuno, K. (2007). Effects of benzo(e)pyrene and benzo(a)pyrene on P-glycoprotein-mediated transport in Caco-2 cell monolayer: A comparative approach. Toxicol In Vitro 21(5): 827-834.

Sui, H., Fan, Z. Z., and Li, Q. (2012). Signal transduction pathways and transcriptional mechanisms of ABCB1/Pgp-mediated multiple drug resistance in Human Cancer Cells. J Int Med Res 40(2): 426-435.

Sukhai, M., and Piquette-Miller, M. (2000). Regulation of the multidrug resistance genes by stress signals. J Pharm Pharm Sci 3(2): 268-280.

Suzuki, F., Hashimoto, K., Kikuchi, H., Nishikawa, H., Matsumoto, H., Shimada, J., Kawase, M., Sunaga, K., Tsuda, T., Satoh, K., and Sakagami, H. (2005). Induction of tumor-specific cytotoxicity and apoptosis by doxorubicin. Anticancer Res 25(2a): 887-893.

Thevenod, F., Friedmann, J. M., Katsen, A. D., and Hauser, I. A. (2000). Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-kappaB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. J Biol Chem 275(3): 1887-1896.

Tian, R., Koyabu, N., Morimoto, S., Shoyama, Y., Ohtani, H., and Sawada, Y. (2005). Functional induction and de-induction of P-glycoprotein by St. John's wort and its ingredients in a human colon adenocarcinoma cell line. Drug Metab Dispos 33(4): 547-554.

Van der Linden, A., Blokker, B. M., Kap, M., Weustink, A. C., Robertus, J. L., Riegman, P. H., and Oosterhuis, J. W. (2014). Post-mortem tissue biopsies obtained at minimally invasive autopsy: an RNA-quality analysis. PLoS One 9(12): e115675.

Wang, Y. H., Li, Y., Yang, S. L., and Yang, L. (2005). Classification of substrates and inhibitors of P-glycoprotein using unsupervised machine learning approach. J Chem Inf Model 45(3): 750-757.

Whitmarsh, A. J., and Davis, R. J. (1996). Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. J Mol Med 74(10): 589-607.

Wongwanakul, R., Vardhanabhuti, N., Siripong, P., and Jianmongkol, S. (2013). Effects of rhinacanthin-C on function and expression of drug efflux transporters in Caco-2 cells. Fitoterapia 89: 80-85.

Wu, M., Lao, Y., Xu, N., Wang, X., Tan, H., Fu, W., Lin, Z., and Xu, H. (2015). Guttiferone K induces autophagy and sensitizes cancer cells to nutrient stress-induced cell death. Phytomedicine 22(10): 902-910.

Xu, W., Cheng, M., Lao, Y., Wang, X., Wu, J., Zhou, L., Zhang, Y., Xu, H., and Xu, N. (2015). DNA damage and ER stress contribute to oblongifolin C-induced cell killing in Bax/Bak-deficient cells. Biochem Biophys Res Commun 457(3): 300-306.

Yoshioka, Y., Kitao, T., Kishino, T., Yamamuro, A., and Maeda, S. (2006). Nitric oxide protects macrophages from hydrogen peroxide-induced apoptosis by inducing the formation of catalase. J Immunol 176(8): 4675-4681.

Yuan, X., Chen, H., Li, X., Dai, M., Zeng, H., Shan, L., Sun, Q., and Zhang, W. (2015). Inhibition of protein kinase C by isojacareubin suppresses hepatocellular carcinoma metastasis and induces apoptosis in vitro and in vivo. Sci Rep 5: 12889.

Zhang, J., Lu, M., Zhou, F., Sun, H., Hao, G., Wu, X., and Wang, G. (2012). Key role of nuclear factor-kappaB in the cellular pharmacokinetics of adriamycin in MCF-7/Adr cells: the potential mechanism for synergy with 20(S)-ginsenoside Rh2. Drug Metab Dispos 40(10): 1900-1908.

Zhou, H., Gao, J., Lu, Z. Y., Lu, L., Dai, W., and Xu, M. (2007). Role of c-Fos/JunD in protecting stress-induced cell death. Cell Prolif 40(3): 431-444.

Zhou, R., Vitols, S., Gruber, A., Liliemark, J., Wang, Y., and Liliemark, E. (1999). Etoposide-induced DNA strand breaks in relation to p-glycoprotein and topoisomerase II protein expression in leukaemic cells from patients with AML and CLL. Br J Haematol 105(2): 420-427.

Zrieki, A., Farinotti, R., and Buyse, M. (2008). Cyclooxygenase inhibitors down regulate P-glycoprotein in human colorectal Caco-2 cell line. Pharm Res 25(9): 1991-2001.

VITA

Mr. Cherdsak Boonyong was born on June 15, 1989 in Trang, Thailand. In 2009, he received his Bachelor degree in Biomedical Science from Rangsit University. After graduation, he enrolled in the Master's degree program in Pharmacology at the Graduate School, Chulalongkorn University.

Poster Presentation

1. Cherdsak Boonyong, Rutt Suttisri and Suree Jianmongkol P. 2014. Phytochemicals from Garcinia schomburgkiana as potential cytotoxic agents for colon cancer. The 36th Congress on Pharmacology of Thailand. March 27-28, 2014, Siriraj Hospital, Bangkok, Thailand.