Biological activity of chalcone and their derivatives in inhibiting HepG2 liver cancer cells *via* regulation of integrin expression

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Abstract

Liver cancer is the highest incidence among the Thai population and arises from abnormal proliferation of hepatocytes, which leads to tumor formation and metastasis. Although targeted therapy with osimertinib and chemotherapy as doxorubicin has shown high efficacy in cancer cell growth. This targeted therapy drugs continue to cause side effects in patients. Consequently, recent research has been conducted on natural extracts with anticancer properties. This study focuses on 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone (DMC), a flavonoid derived from Ma-kiang (*Syzygium nervosum* A. Cunn. Ex.DC.), a plant commonly found in Northern Thailand. In addition, structural modification of DMC was performed to coumarin derivatives with anticancer activity. The objective of this study was to evaluate the biological activities of DMC and its derivatives in HepG2 inhibition by MTT assay, and to compare their inhibitory efficacy with anticancer drugs. The results showed that the half-maximal inhibitory concentration (IC_{50}) values for doxorubicin, osimertinib, DMC, coumarin C12, and coumarin C10 were 9.96 ± 3.06, 1.33 ± 0.09, 0.15 ± 0.01, 13.59 ± 2.60, and 8.79 ± 0.92 μM, respectively. However, future studies will be investigated for molecular mechanisms of action by analyzing the expression of integrin-related signaling proteins *via* Western blot analysis to evaluate the role of integrin receptors in mediating liver cancer cell apoptosis.

Introduction

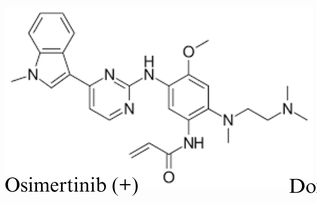
Liver cancer is one of the leading causes of cancer-related death worldwide and ranks first in both incidence and mortality in Thailand. Hepatocellular carcinoma (HCC) is the most common type. Current treatments, including chemotherapy and targeted therapy such as doxorubicin and osimertinib, are effective but limited by toxicity and adverse effects. Natural bioactive compounds have attracted attention as safer alternatives. 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethyl chalcone (DMC), isolated from *Syzygium nervosum*, has shown anticancer potential. Structural modification of DMC has led to coumarin derivatives such as coumarin C10 and C12; however, their effects on liver cancer cells remain unclear.Therefore, this study aimed to investigate their effects on liver cancer cells.

Structural modification of natural extracts or bioactive compounds aims to

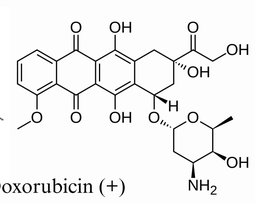
- To increase biological activities, including anti-cancer properties.



Syzygium nervosum A. Cunn. ex DC.



Osimertinib (+)
A Targeted Drug Therapy



Doxorubicin (+)
A Chemotherapy Drug Therapy

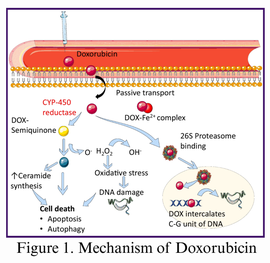


Figure 1. Mechanism of Doxorubicin

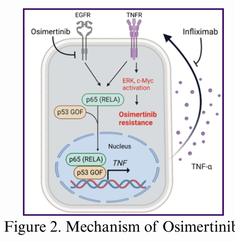
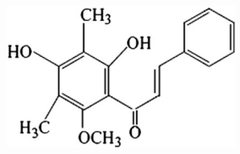


Figure 2. Mechanism of Osimertinib

Objectives

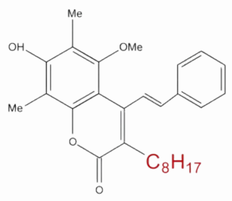
- To investigate cytotoxicity of DMC and its derivatives against HepG2 liver cancer cells
- To evaluate and compare the inhibitory effects of DMC and its derivatives on HepG2 cell proliferation with doxorubicin and osimertinib.
- To determine the effect of chalcone and its derivatives on integrin β-1 expression, which involves in Liver cancer cell death pathway



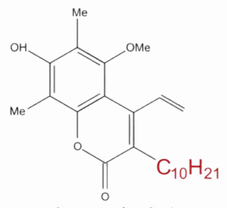
DMC

Chemical structures of DMC and Its Derivatives

All compounds were elucidated using spectroscopic techniques, as previous studies (Khamto et al., 2021).



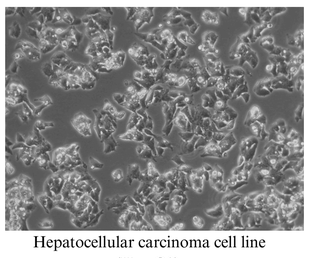
Coumarin C12



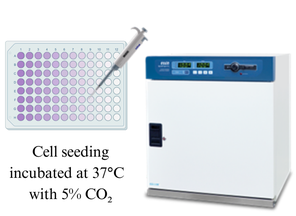
Coumarin C10

Methodology

Anti-cancer activity and Cytotoxicity by MTT assay



Hepatocellular carcinoma cell line (Hep G2)

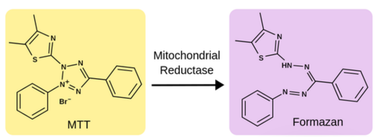


Cell seeding incubated at 37°C with 5% CO_2

24 hours

Treatment
Osimertinib and Doxorubicin as positive (+) controls

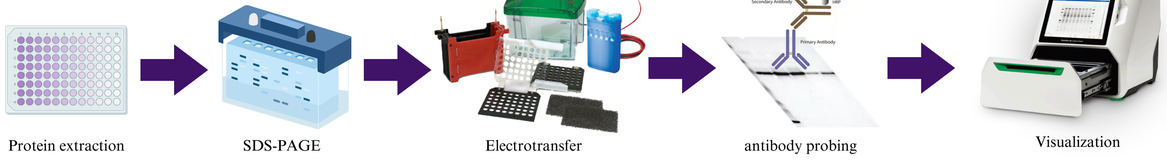
48 hours



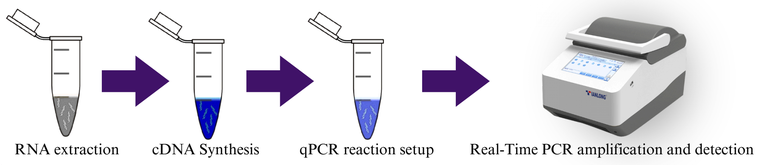
Measurement

$$\% \text{ Cell viability} = \frac{A_{550-620}\,(\text{control}) - A_{550-620}\,(\text{treated cell})}{A_{550-620}\,(\text{control})} \times 100$$

SDS-PAGE and Western blot analysis



Gene Expression Analysis by Real-Time RT-PCR (qPCR)



Results and discussion

Anti-cancer activity and Cytotoxicity by MTT assay

Anti-proliferative activity of DMC and its derivatives in HepG2 cells assessed by MTT assay compared with doxorubicin and osimertinib

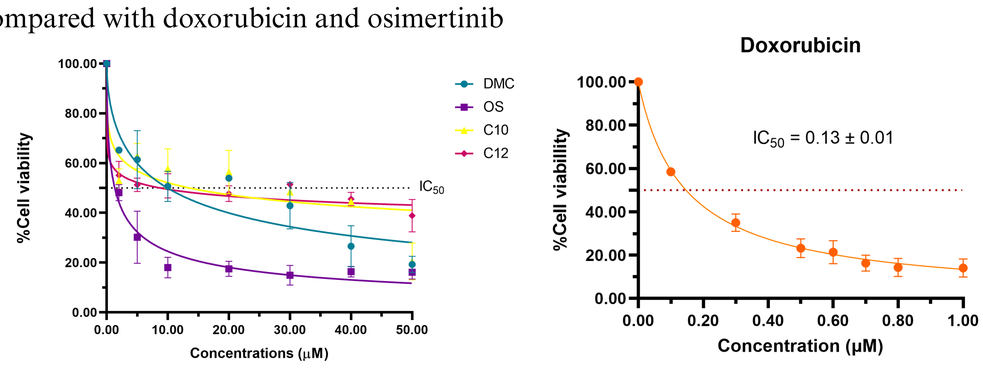


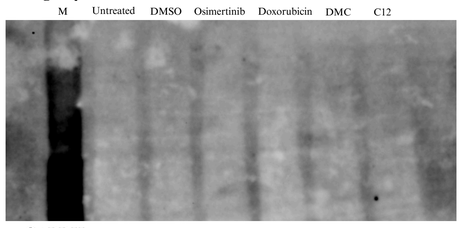
Figure 3. Relationship between drug concentration and %cell viability on HepG2 cell line and the anti-proliferative activity of the candidate compounds were expressed as IC_{50} values.
*Values represent mean ± SD from three independent experiments (n = 3)

Table 1 : Anti-proliferative activity of DMC and its derivatives on HepG2 cell lines

Compounds	IC_{50} (μM)
Doxorubicin	0.13 ± 0.01
Osimertinib	1.33 ± 0.61
DMC	9.96 ± 3.31
Coumarin C10	13.59 ± 0.76
Coumarin C12	8.79 ± 1.54

Western blot analysis

- **Integrin β-1**



- **GAPDH**

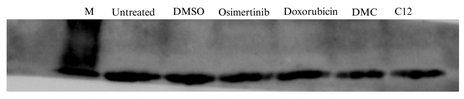


Figure 4. Western blot analysis of target protein expression after treatment.

Gene Expression Analysis by Real-Time RT-PCR (qPCR)

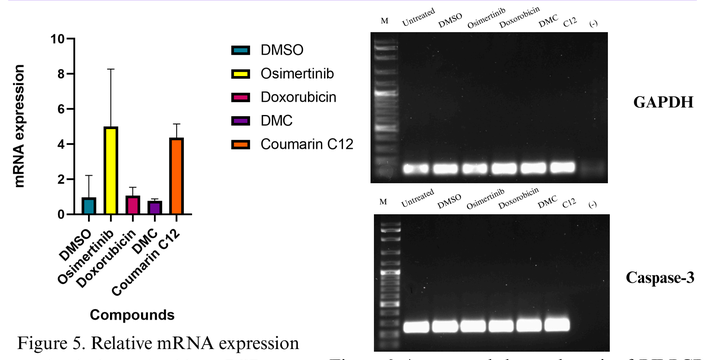


Figure 5. Relative mRNA expression levels determined by q-PCR.
*Values represent mean ± SD from two independent experiments (n = 2)

Figure 6. Agarose gel electrophoresis of RT-PCR products showing mRNA expression levels of Caspase-3 and GAPDH

Conclusions

- Structural modification of the chalcone compound isolated from Ma-kiang led to the generation of coumarin derivatives with enhanced anticancer activity. These findings suggest that chemical structure modification of DMC improves its anticancer potential.
- Coumarin C12 exhibited slightly greater anticancer activity, as indicated by its lowest IC_{50} value. Compared with the DMC, Coumarin C12 demonstrated an approximately 1.13-fold lower IC_{50} value, indicating enhanced anticancer potency following structural modification.
- qPCR analysis show that treatment with Coumarin C12 significantly upregulated Caspase-3 mRNA expression compared with negative control group. This suggests that the enhanced anticancer activity of C12 may be associated with the induction of apoptosis at the transcriptional level.

Acknowledgements

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