Biological Activities of 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethyl-chalcone (DMC) Derivatives *via* PARP1 Inhibition in Human Colorectal Cancer HCT116 Cells

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Abstract

Currently, colorectal cancer ranks as the third most common cancer in Thailand. Colorectal cancer develops from atypical cells that proliferate and form tumors. Chemotherapeutic drugs are widely used for cancer treatment, such as Cisplatin, Osimertinib Doxorubicin. However, these drugs can cause side effects on normal human cells. Previous studies have reported phytochemical compound properties that inhibit cancer cell proliferation. This study focuses on a natural compound with anticancer activities, called 2',4'-dihydroxy-6'-methoxy-3',5'-dimethyl-chalcone (DMC), which is found in *Syzygium nervosum* A. Cunn. ex. DC. DMC is a chalcone compound, and its chemical structure has been modified to enhance its anticancer efficacy. This study aimed to evaluate the anticancer activity of DMC and its derivatives, including 4'-*O*-acryloyloxy (DMC1) and 2',4'-*O*-diacryloyloxy (DMC2), on the viability of human colorectal cancer HCT116 cells using the MTT assay. The half maximal inhibitory concentration (IC_{50}) values were determined and compared with Osimertinib. The results showed that DMC, DMC1, DMC2, and Osimertinib inhibited the growth of colorectal cancer cells, with IC_{50} values of 22.94 ± 1.70, 2.54 ± 0.17, 0.86 ± 0.19, and 2.65 ± 0.09 μM, respectively. The findings indicated DMC1 and DMC2 exhibited approximately 10-fold and 26-fold greater inhibitory effects when compared with DMC. To confirm the mechanism of action related to apoptosis, the expression of apoptosis-related proteins such as poly (ADP-ribose) polymerase 1 (PARP1) in HCT116 colorectal cancer cells was analyzed using Western blot analysis. The results revealed the upregulation of cleaved PARP1 which is a marker Protein of apoptotic cell death. In conclusion, DMC and its derivatives inhibit the proliferation of colorectal cancer cells through caspase-dependent apoptotic pathways.

Introduction

Cancer in 2022

Cancer statistics in 2022 indicate that colorectal cancer is among the most prevalent malignancies globally and in Thailand, underscoring its importance as a major public health concern and the need to strengthen prevention, screening, diagnosis, and treatment strategies.

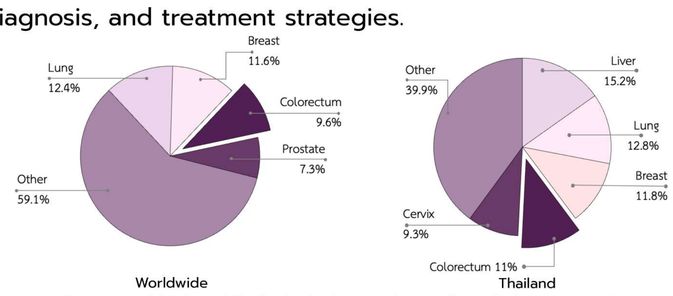


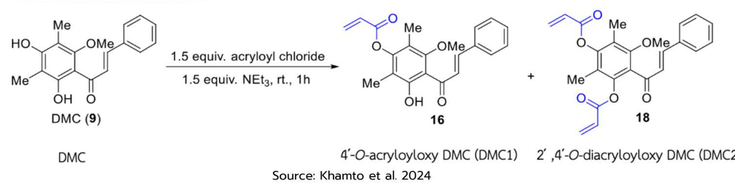
Figure 1. Worldwide and Thailand absolute number incidence both sexes in 2022

Natural compound

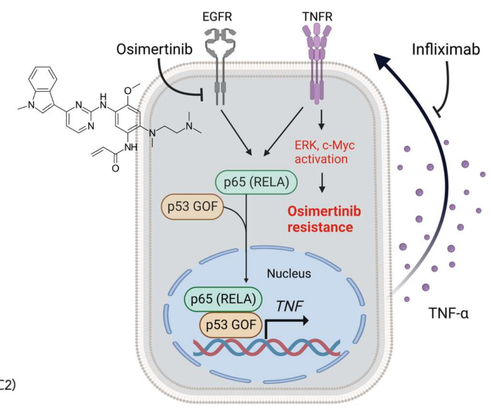
Natural compounds are important sources of bioactive molecules for anticancer drug development. Ma-kiang exhibits diverse biological activities, including anticancer effects. Previous studies reported that the derivatives DMC1 and DMC2 were chemically developed from DMC, a chalcone with anticancer potential, by introducing acryloyl groups to enhance biological activity and anticancer efficacy.



Ma-kiang
(*Syzygium nervosum* A. Cunn. ex. DC.)
Found in Asia: Vietnam China and Northern Thailand
Biological Activities: Antibacterial, Antidiabetic, Anti-inflammatory, Antiviral and Anticancer



Source: Khamto et al. 2024



Osimertinib induces DNA damage *via* EGFR inhibition, activating the apoptotic pathway and resulting in caspase3–mediated PARP1 cleavage. The chalcone-based derivatives DMC1 and DMC2, modified with acryloyl groups, demonstrate potential to enhance apoptosis-related signaling associated with the PARP1 pathway.

Objectives

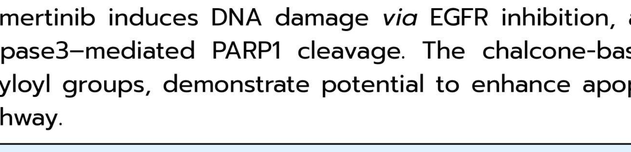
1. To evaluate the inhibitory effects of DMC, DMC1 and DMC2 on the proliferation of human colon cancer HCT116 cells
2. To elucidate the mechanism of action of the candidate compounds by analyzing the PARP1 expression which apoptosis-related is proteins using Western blot analysis.

Methodology

Cytotoxicity

1. Thaw cells (Human colorectal Carcinoma: HCT116 from American Type Culture Collection (ATCC) and subculture in cell culture flask.
2. Seed cells (5,000 cells/well) and treat at a different concentrations (DMC, DMC1, DMC2 and Osimertinib) in 96- well plates.
3. Add MTT solution
4. Measure the absorbance at Wavelengths of 550 nm and 620 nm using a microplate reader.

SDS-PAGE



SDS-PAGE was subsequently performed to confirm the presence of proteins and to evaluate their separation based on molecular weight.

Western blotting

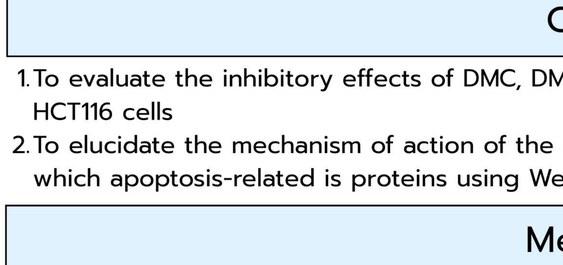


Figure 2. Method for Western blotting.

To confirm the mechanism of action related to apoptosis, the expression of apoptosis-related proteins was investigated, with particular focus on poly (ADP-ribose) polymerase 1 (PARP1), a well-established substrate of caspases. During caspase-dependent apoptosis, PARP1 is cleaved into characteristic fragments, and the accumulation of cleaved PARP1 serves as a biochemical hallmark of apoptotic cell death. Therefore, examining PARP1 expression in HCT116 colorectal cancer cells provides molecular evidence that the tested compounds trigger apoptotic signaling pathways rather than causing nonspecific cytotoxicity.

Results and Discussion

Cytotoxicity: MTT assay

To evaluate the effects of DMC, DMC1, and DMC2 on the viability of HCT116 colorectal cancer cells, an MTT assay was performed. The half maximal inhibitory concentration (IC_{50}) values were determined and compared with those of Osimertinib.

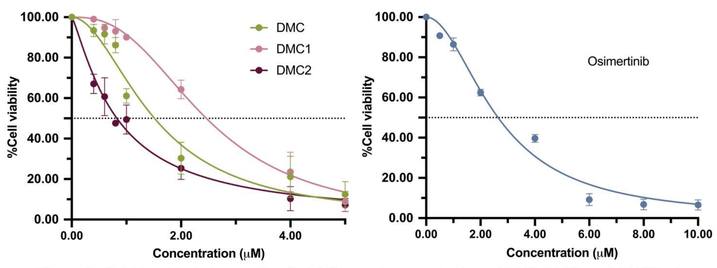


Figure 3. Relationship between %cell viability and concentration of DMC, DMC1 and DMC2 when compared with Osimertinib

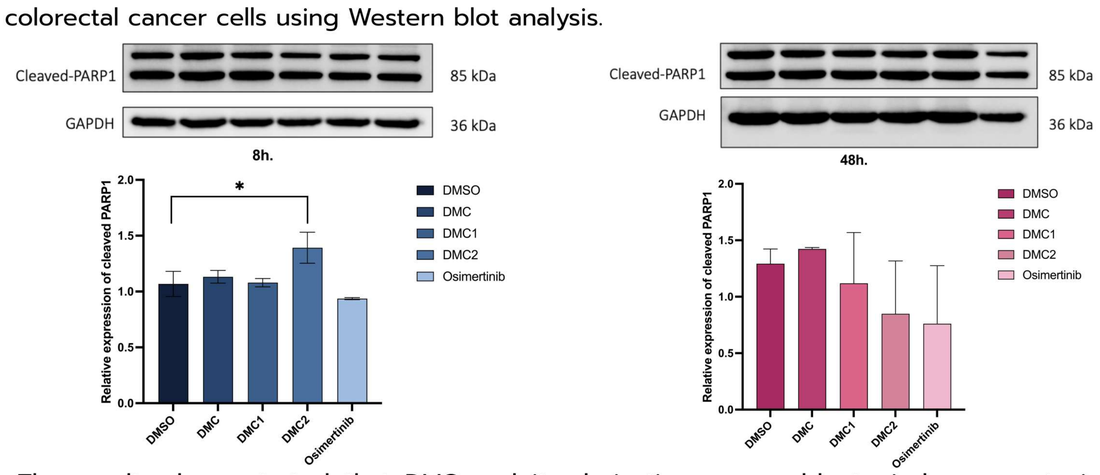
Table1. Anti-cancer activity of DMC derivatives on human HCT116 colon cancer cell line

Compound	IC_{50} (μM)
DMC	22.94 ± 1.70
DMC1	2.54 ± 0.17
DMC2	0.86 ± 0.19
Osimertinib	2.65 ± 0.09

The DMC derivatives exhibited enhanced biological activity against HCT116 colorectal cancer cells following chemical structure modification through substitution with an acryl group. DMC1 and DMC2 showed 9.03-fold and 26.67-fold increases in activity, respectively, compared with DMC. The half maximal inhibitory concentration (IC_{50}) values of DMC, DMC1, DMC2, and Osimertinib were 22.94 ± 1.70, 2.54 ± 0.17, 0.86 ± 0.19, and 2.65 ± 0.09 μM, respectively.

PARP1 expression: Western blot analysis

To confirm the mechanism of action related to apoptotic cell death, the expression of apoptosis-associated proteins, particularly poly (ADP-ribose) polymerase 1 (PARP1), was analyzed in HCT116 colorectal cancer cells using Western blot analysis.



The results demonstrated that DMC and its derivatives were able to induce apoptosis in HCT116 colorectal cancer cells, as evidenced by the increased expression of cleaved PARP1, a key marker of caspase-dependent apoptosis. At 8 h, of drug treatment cleaved PARP1 expression levels were observed, particularly in the DMC2-treated group, which showed the highest expression. These findings indicate that the compounds are capable of activating apoptotic cell death pathways *via* the down regulation of PARP1

Conclusions

- Chemical structure natural compounds DMC1 and DMC2 substituted with acryl groups, exhibited inhibitory effects on the proliferation of human colorectal cancer HCT116 cells, indicating that modification of functional groups enhanced their anti-cancer activity.
- DMC2 showed the highest anti-cancer activity, as indicated by the lowest IC_{50} value, which was approximately 27-fold lower than that of the parent of compound (DMC) and approximately 3-fold lower than that of the chemotherapeutic drug Osimertinib.
- An increased expression of cleaved PARP1 was observed in HCT116 cells treated with DMC2, suggesting the activation of apoptotic signaling.

Acknowledgments

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