Investigation of the biological activities of 2´,4´ –dihydroxy-6´-methoxy-3´,5´-dimethyl-chacone (DMC) derivatives *via* EGFR inhibition pathway in non-small cell lung cancer

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Abstract

Lung cancer is the second most common cancer in the Thai population. Currently, chemotherapy and targeted therapies for lung cancer treatment result in side effects for patients. Therefore, this study investigated a natural compound with anticancer activity, 2´,4´-dihydroxy-6´-methoxy-3´, 5´-dimethylchalcone (DMC), extracted from the seeds of *Syzygium nervosum* A. Cunn. Ex. Dc, a native plant in northern Thailand. This study explores the derivatives DMC-Tyr, DMC-Phe, and DMC-Met. The cytotoxicity against H-460 lung cancer cells was tested using the MTT assay. The anticancer activity of **DMC-Met** was approximately two-fold higher than DMC and had similar activity to currently available drugs. Subsequently, the expression of EGFR protein, which is involved in the mechanism of apoptosis in cancer cells, was studied using a Western blot analysis. NCI-H460 cells showed a significant decrease in EGFR protein expression compared to the control group. These findings suggest that **structural modification of DMC by methionine conjugation** allowed for the induction of apoptosis in lung cancer cells by inhibiting EGFR protein expression.

Introduction

Non-small cell lung cancer is the most common type of lung cancer. It was found about 85 out of every 100 cases of lung cancer. For the several treatment including surgery, radiation, targeted therapy and chemotherapy are available. However, there are some limitations and it causes side effects to normal cells. Therefore, natural compounds with anticancer properties have gained attention as a safer alternative treatment such as DMC, a chalcone from seed of Ma-kiang exhibits biological activity including anticancer activity.

Structural modification of DMC by conjugation with amino acid aims

- To enhance the efficiency of anticancer.
- To improve membrane permeability and better water solubility.

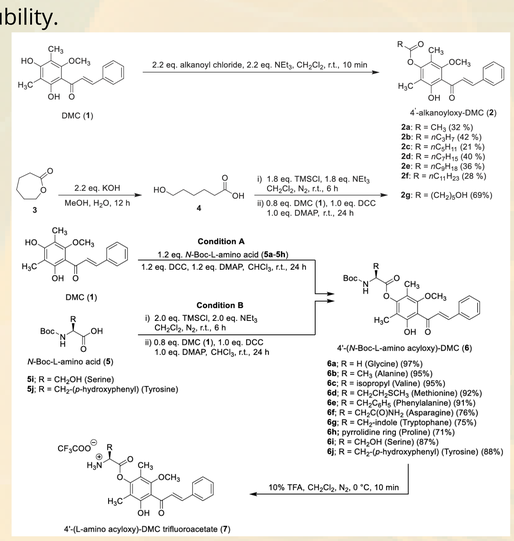


Figure 1. Reagents and conditions for the preparation of DMC derivatives. (N. Khamto *et al.*,2024)

Osimertinib (+): A targeted therapy for lung cancer

EGFR (Growth epidermal factor receptor) is a transmembrane receptor protein that regulates cell growth, survival, and differentiation which is involved in apoptosis pathway.

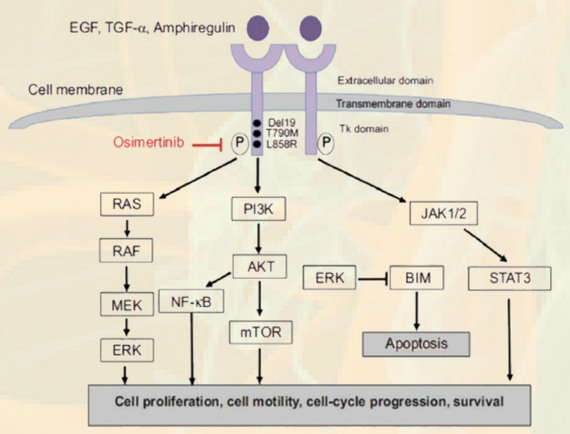


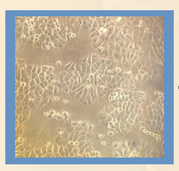
Figure 2. Epidermal growth factor receptor pathway and mechanism of action of Osimertinib (S. Thakur *et al.*,2019)

Proposes of study

- To determine cytotoxicity of DMC and its derivatives on NCI-H460 lung cancer cells
- To investigate the anticancer pathway *via* protein expression of EGFR after treating with DMC and its derivatives on NCI-H460 lung cancer cells

Methodology

Cytotoxicity activity by MTT assay

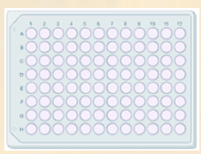


NCI-H460
Non-small cell lung cancer
ATCC - HTB-177

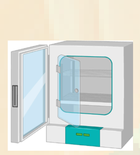
Cell seeding

$$\text{Cell concentration (cells/mL)} = \frac{\text{Total cells counted} \times \text{dilution factor} \times 10^4}{\text{Number of squares counted}}$$

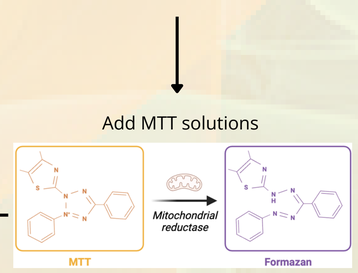
$$\text{Cell volume (µL)} = \frac{\text{(cells/well)}}{\text{Cell conc.}\left(\frac{\text{cell}}{\text{mL}}\right)} \times 1000\ \mu L$$

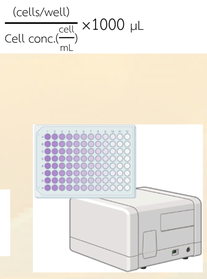


Treatment and incubate for 48 h.



Add MTT solutions

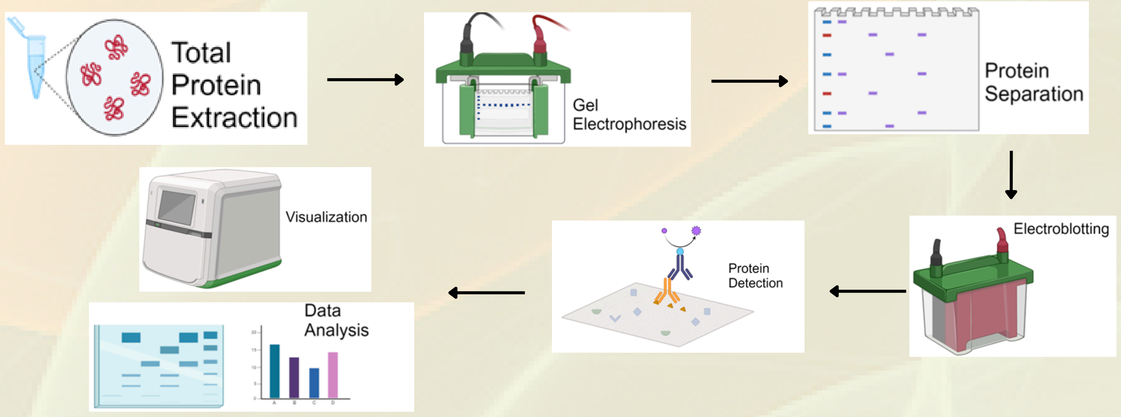




Measurement

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

Protein expression by Western blot analysis



Results and discussion

Cytotoxicity activity by MTT assay

Cytotoxicity activity by MTT assay was performed after treated for with DMC and DMC-Met for 48 h and compared with Doxorubicin and Osimertinib as the reference controls. The results showed DMC-Met demonstrated the strongest cytotoxic effect on NCI-H460 lung cancer cells.

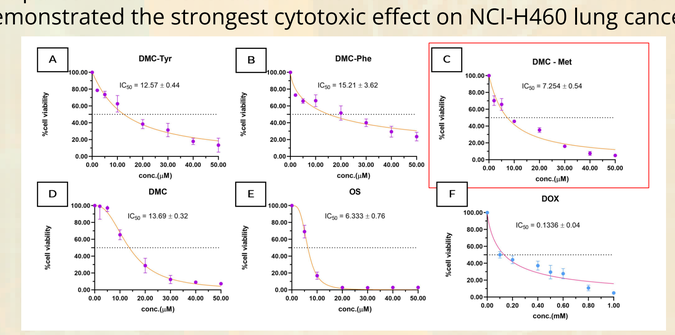


Figure 3: Graph representing the relationship between drug concentration and % cell viability on non-small cell lung cancer (H460 cells). The cytotoxicity activity of the compounds was expressed as IC50 values.

Table 1: Cytotoxicity activity of DMC and its derivative on non-small cell lung cancer (H460 cells)

Compounds	IC_{50} (µM)
DMC-Tyr	12.57 ± 0.44
DMC-Phe	15.21 ± 3.62
DMC-Met	7.25 ± 0.54
DMC	13.69 ± 0.32
Doxorubicin (+)	0.13 ± 0.04
Osimertinib (+)	6.33 ± 0.76

Protein expression by Western blot analysis

EGFR protein expression of H-460 cells after treated with DMC and DMC-Met for 48 h compared with Doxorubicin and Osimertinib as the reference controls and DMSO as a negative control. The result showed the down regulation of EGFR after treatment with DMC-Met in H-460 lung cancer cells.

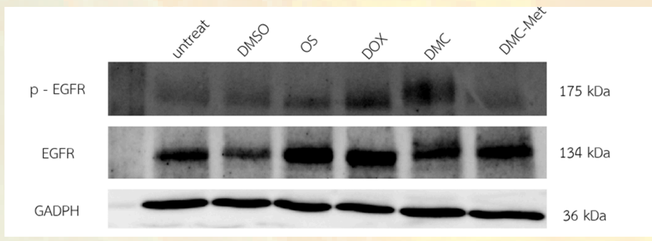


Figure 4. Western blot analysis of the target protein expression of H-460 cells after treat for 48 h.

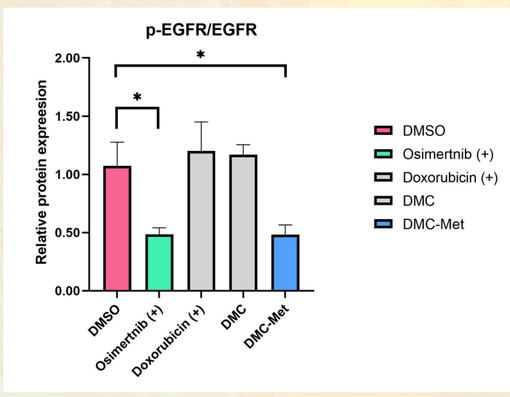


Figure 5. Relative p-EGFR/EGFR protein expression in H-460 cells following treatment with 1% DMSO (control group) and Osimertinib, Doxorubicin (reference controls) DMC, and DMC-Met were analyzed by Western blotting. Data are presented as mean ± SD. (*$p < 0.05$) when were compared with the control group.

Conclusions

- The modified DMC structure with Methionine amino acid demonstrated cytotoxic activity comparable to the reference control (Osimertinib) and approximately two-fold greater than DMC.
- DMC-Met can induce apoptosis in lung cancer cells by downregulation epidermal growth factor receptor (EGFR) protein expression.

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