Anticancer Activity of Chalcones and Its Derivatives in NCI-H460 Lung Cancer Cells

Natthida Khamma[1], Kraikrit Utama[3,4], Atchara Janthong[2], Puttinun Meepowpan[1,4], Padchanee Sangthong[1,4]

[1] Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand
[2] Program in Biotechnology, Multidisciplinary and Interdisciplinary School, Chiang Mai University, Chiang Mai, 50200, Thailand
[3] Office of Research Administration, Chiang Mai University, Chiang Mai, 50200, Thailand
[4] Center of Excellence in Materials Science and Technology, Chiang Mai University, Chiang Mai, 50200, Thailand

Abstract

Lung cancer remains a leading cause of mortality worldwide, with rising incidence in Thailand due to risk factors like smoking and pollution. Phytochemicals play a key role in anticancer research, including *Syzygium nervosum* (Makiang), a fruit plant from northern Thailand. Its primary compound, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC), exhibits antioxidant, anti-inflammatory, and anticancer properties. This study explores semi-synthetic chalcone derivatives (**C4-C12**) from *S. nervosum*, particularly 7-O-acylated-4-hydroxycoumarin, for their anti-lung cancer effects in NCI-H460 cells. **C4** and **C10** showed potent antiproliferative activity with IC_{50} of 11.87 ± 0.58 and 9.98 ± 0.57 µM, respectively. Confocal microscopy with acridine orange (AO) and propidium iodide (PI) staining confirmed increased apoptosis in treated cells. These findings highlight the potential of 7-O-acylated-4-hydroxycoumarin derivatives as promising anti-lung cancer agents, warranting further investigation into their cell-death mechanisms in *in vitro* and *in vivo* models.

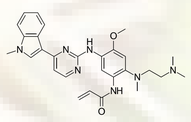
Introduction

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases. Cancer treatment varies by types and stages, commonly involving surgery, chemotherapy, radiation therapy, and targeted therapy. While advancements like Osimertinib have improved outcomes, chemotherapy and targeted therapies often cause severe adverse effects, necessitating the search for safer alternatives, such as phytochemicals. DMC, a chalcone derived from *Syzygium nervosum* (Ma-kiang), exhibits diverse biological activities, including anticancer potential.

Structural Modification of Phytochemicals aims

- To increase biological activities, including anti-cancer properties.
- To reduce cytotoxicity in normal cells.

Syzygium nervosum's fruits



Osimertinib (+) : A Targeted Therapy for Lung Cancer

Objectives

1. To determine anti-lung cancer and cytotoxicity of chalcone and its derivatives
2. To investigate cell damage pathways including apoptosis cell death

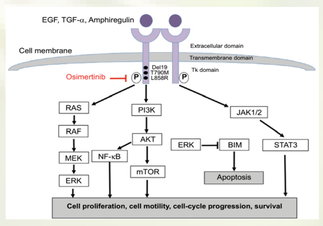
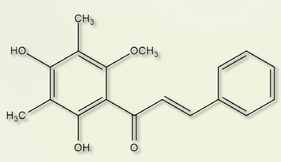


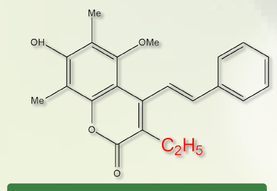
Figure 1. EGFR pathway and mechanism of action of Osimertinib : (Santarpia *et al*.,2017)

Chemical structures of Chalcones and Its Derivatives

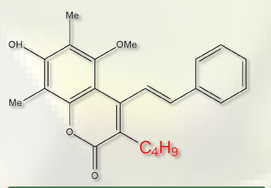
- All compounds were elucidated using spectroscopic techniques, as previous studies (Khamto *et al*., 2021)



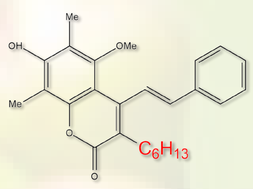
DMC



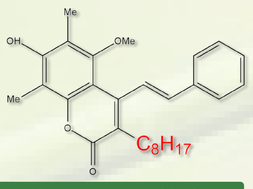
Coumarin C4



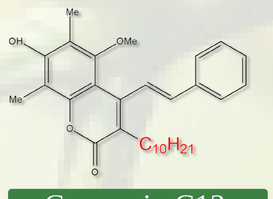
Coumarin C6



Coumarin C8



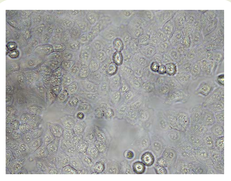
Coumarin C10



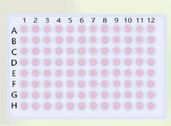
Coumarin C12

Methodology

• Anti-proliferative activity by MTT assay

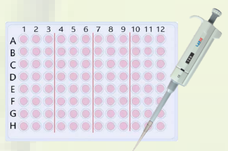


Human lung cancer cell lines (NCI-H460)



Cell seeding

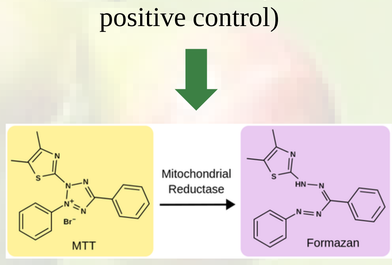
5% CO_2, 37°C



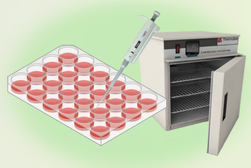
Treatment (Osimertinib (20.01 µM) as positive control)

Measurement

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

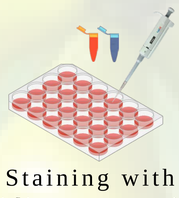


• Apoptosis assay by Confocal Microscopy



Cell seeding

Treatment



Staining with acridine orange (AO) propidium iodide (PI) staining

Observation and Measurement

Results and discussion

• Anti-proliferative activity by MTT assay

Anti-proliferative activity by MTT assay of H460 cells after treatment with DMC and its derivative when compared with Osimertinib.

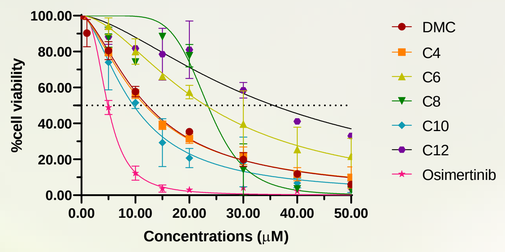


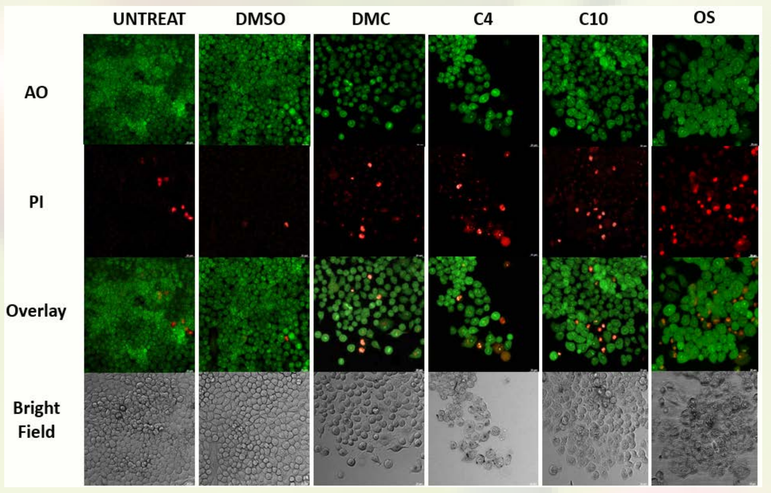
Figure 2. Graph representing the relationship between drug concentration and %cell viability on H460 cells. The anti-proliferative activity of the compound was expressed as IC_{50} values.

Table 1 : Anti-proliferative activity of DMC and its derivatives on non-small cell lung cancer H460 cell lines

Compounds	IC_{50} (µM)
DMC	12.20 ± 1.22
Coumarin C4	11.87 ± 0.58
Coumarin C6	24.12 ± 2.58
Coumarin C8	22.39 ± 2.09
Coumarin C10	9.98 ± 0.57
Coumarin C12	35.34 ± 1.64
Osimertinib (+)	4.27 ± 1.11

Expressed as the mean ± standard deviation (SD) of triplicate experiments
[b] Positive control (+)
IC_{50}, half-maximal inhibitory concentration

• Apoptosis assay by Confocal Microscopy



- DMC and its derivatives, Coumarin C4 and Coumarin C10, induced apoptotic cell death in H460 cells, as indicated by the increased red fluorescence compared to the untreated and 1% DMSO control groups.

Remarks
DMC = 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone
C4 = Coumarin C4
C10 = Coumarin C10
OS = Osimertinib (+)

Figure 3. Observation of apoptotic cell death after treatment with DMC and its derivatives using acridine orange (AO) and propidium Iodide (PI) staining under Confocal Microscopy.

Conclusions

- Coumarin C4 and C10 demonstrated higher antiproliferative activity in H460 lung cancer cell lines, with an IC_{50} value of 11.87 ± 0.58 and 9.983 ± 0.57 µM respectively, subsequent to the structural modification of the phytochemical.
- Coumarin C4 induced cell death, as observed through fluorescent microscopy when compared to control groups, comparable to osimertinib.

Acknowledgements

The author would like to offer the special thank to Assoc. Prof. Dr. Padchanee Sangthong (Adivisor), Assoc. Prof. Dr. Puttinun Meepowpan provided for the DMC and modified DMC structures, Dr. Kraikrit Utama and Ms. Atchara Janthong for their professional guidance and valuable support. Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

References

- Utama K. et al. Effects of 2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone from *Syzygium nervosum* Seeds on Antiproliferative, DNA Damage, Cell Cycle Arrest, and Apoptosis in Human Cervical Cancer Cell Lines. Molecules **2022**, 27(4), 1154;
- Utama K. et al. 2′,4′-Dihydroxy-6′-methoxy-3′,5′-dimethylchalcone and Its Amino Acid-conjugated Derivatives Induce G0/G1 Cell Cycle Arrest and Apoptosis *via BAX/BCL2* Ratio Upregulation and *in Silico* Insight in SiHa Cell Lines. European Journal of Pharmaceutical Sciences **2023**, 184, 106390.
- Khamto et al. Synthesis, Cytotoxicity Evaluation and Molecular Docking Studies on 2',4'-Dihydroxy-6'-methoxy-3',5'-dimethylchalcone Derivatives 2021, 31433-31447.
- Santarpia et al. Osimertinib in the Treatment of Non-small-cell lung cancer: Design, Development and Place in Therapy 2017, 10.2147.