Evaluation of Cytotoxicity of Synthetic Heterocyclic Compounds Against Lung Cancer Cell Line

Woranittha Laiad [1], Torsak Luanphaisarnnont [2] and Panchika Prangkio [1]

[1] Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

[2] Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

*Corresponding author's e-mail address: panchika.p@cmu.ac.th

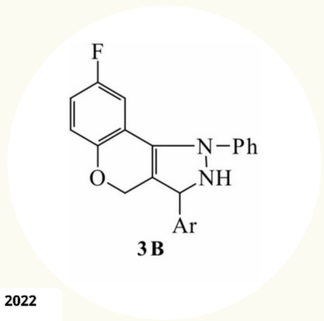
Abstract

Lung cancer is the most diagnosed cancer and the leading cause of death worldwide. Despite advances, current therapies are limited by resistance, toxicity, and poor selectivity. Therefore, the development of synthetic compounds with high efficacy and cancer cell specificity is essential. Benzopyrano[4,3-c]pyrazolines and their derivatives have been reported to exhibit anti-lung-cancer activity. This study investigated the anticancer activity of four synthetic heterocyclic compounds (TLWP01, TLWP07, TLWP08, and TLWP09) against lung cancer cells using the MTT assay. These compounds differ in methoxy substitution positions on the benzopyran ring. TLWP01 is the parent compound lacking substituents, whereas TLWP07, TLWP08, and TLWP09 are methoxy-substituted derivatives containing a methoxy (–OCH_3) group that may affect interactions with biological targets. Half-maximal inhibitory concentration (IC_{50}) values were determined. Gefitinib was used as a reference drug and showed a mean IC_{50} value of 6.661 ± 1.477 μM. In A549 lung cancer cells, TLWP08 exhibited the highest cytotoxic activity (IC_{50} = 8.273 ± 0.784 μM), followed by TLWP01 (8.784 ± 2.558 μM) and TLWP07 (11.792 ± 2.458 μM), while TLWP09 showed no inhibitory effect within the tested concentration range. Selectivity was assessed in normal HEK293T cells, where IC_{50} values were not determined. Overall, %cell viability decreased with increasing compound concentration but remained higher than the control due to cell detachment. Notably, TLWP08 showed higher cytotoxicity in A549 cells than in HEK293T cells, indicating favorable selectivity. Benzopyrano[4,3-c]pyrazolines derivatives, particularly TLWP08, therefore demonstrate potential as lead compounds for anti-lung-cancer drug development. Further studies are required to confirm their mechanisms and safety.

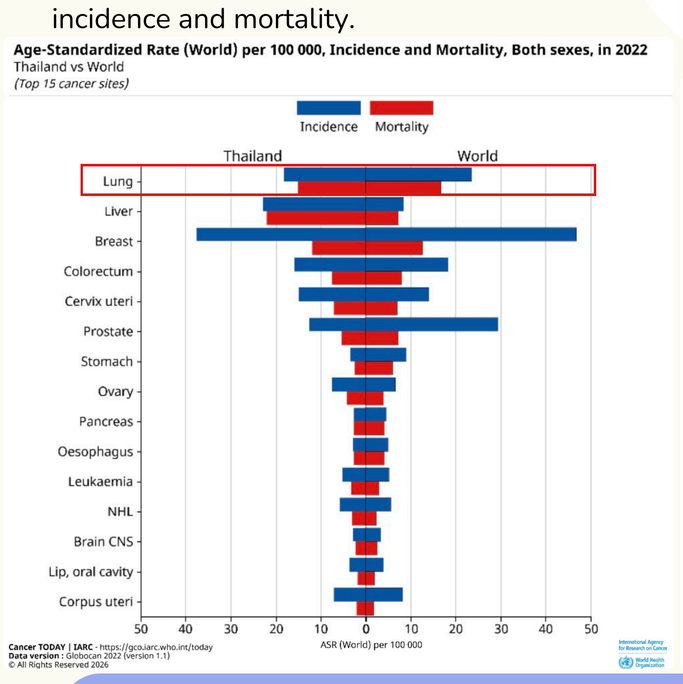
Introduction

Lung Cancer

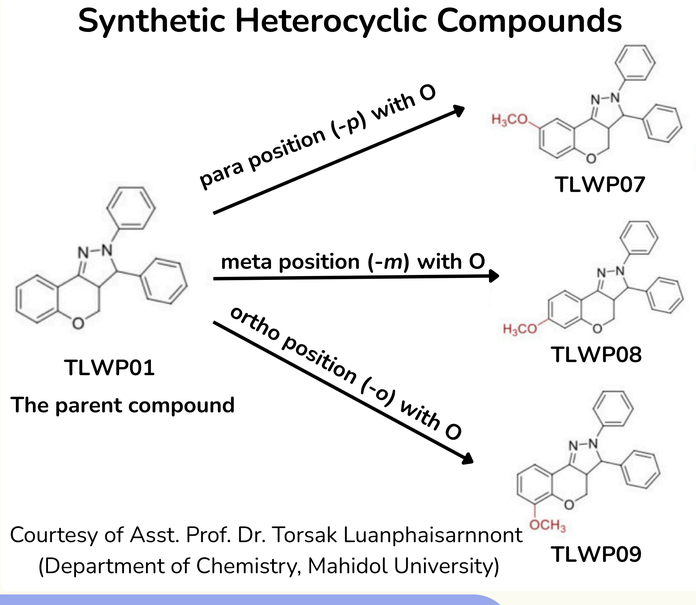
- Leading cause of cancer incidence and mortality worldwide.
- In Thailand, lung cancer ranks among the top three cancers with increasing incidence and mortality.



- **3-aryl-8-fluoro-2-phenyl benzopyrano[4,3-c]pyrazoline (3B)** reported to exhibit anticancer activity against lung cancer cells.



Synthetic Heterocyclic Compounds



Courtesy of Asst. Prof. Dr. Torsak Luanphaisarnnont (Department of Chemistry, Mahidol University)

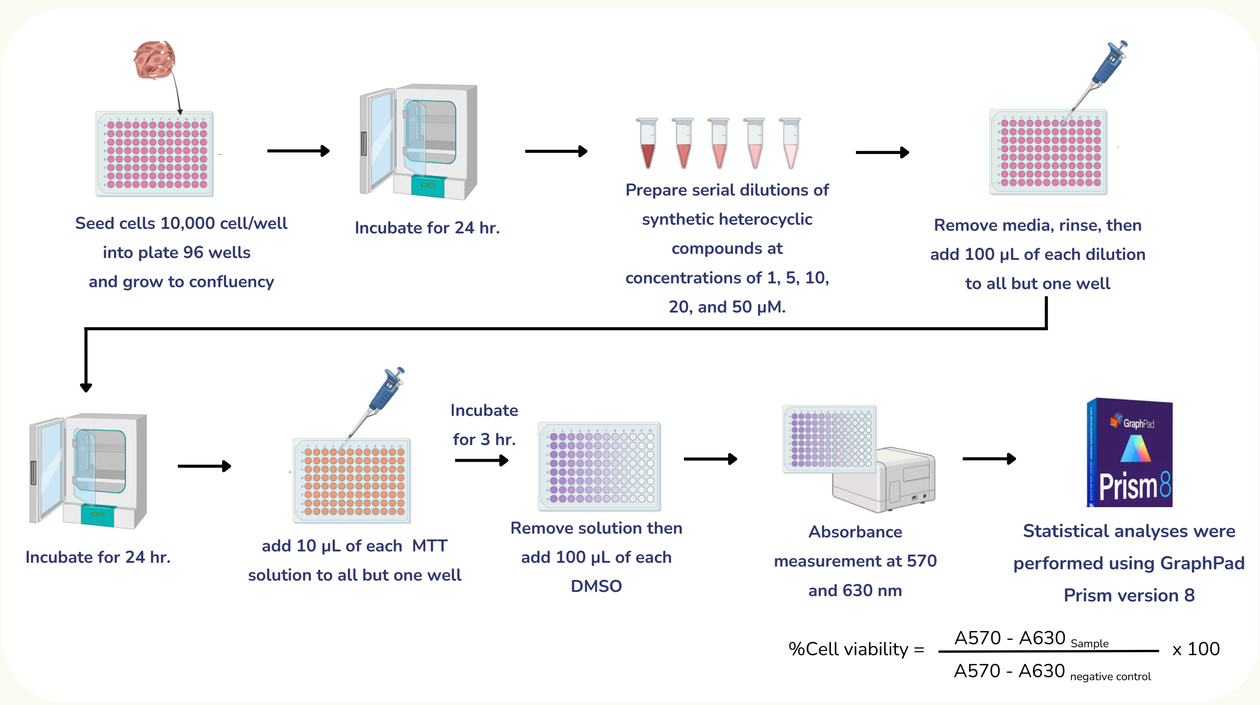
Objectives

- To investigate the cytotoxic effects of synthetic heterocyclic compounds against the lung cancer cell line A549.
- To evaluate the selectivity of synthetic heterocyclic compounds through comparative analysis of their cytotoxic effects on normal HEK293T cells and lung cancer A549 cells.

Methodology

MTT assay

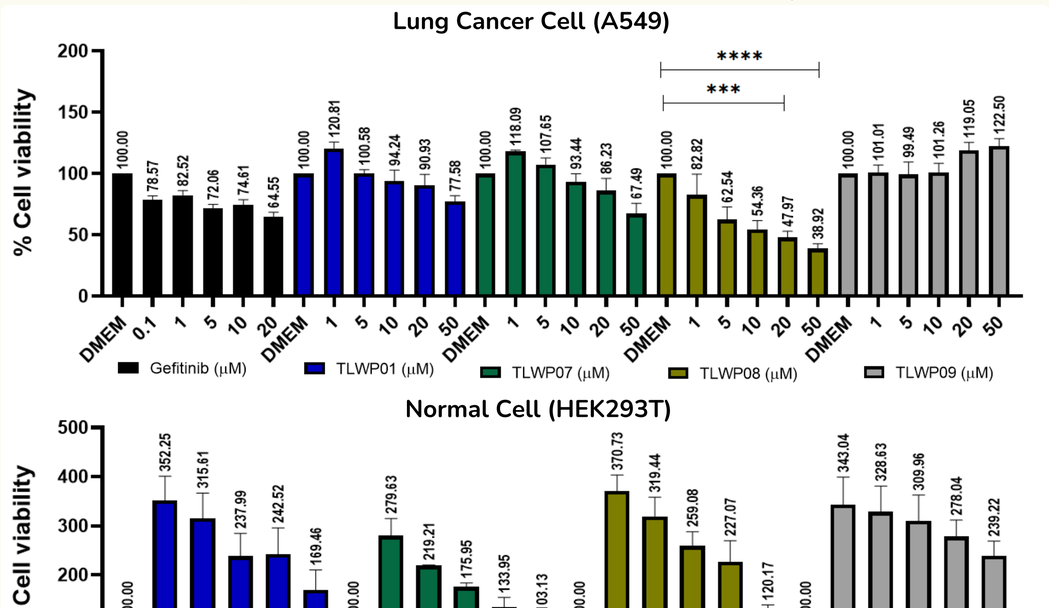
- Cell Line Used: Lung Cancer Cell (A549) and Normal Cell (HEK293T)
- Reference Drug: Gefitinib
- Tested Compounds: TLWP 01,07,08 and 09 (Synthetic Heterocyclic Compounds)



Results & Discussion

TLWP08 exhibited the strongest cytotoxic effect against A549 cells

- All synthetic heterocyclic compounds exhibited cytotoxic activity against A549 lung cancer cells, with distinct selectivity profiles when compared to normal cells.
- TLWP08 demonstrated the strongest anticancer activity, showing the lowest IC_{50} value in A549 cells (8.273 ± 0.784 μM).
- The IC_{50} values in A549 cells were ranked as follows: TLWP08 (8.273 ± 0.784 μM) > TLWP01 (8.784 ± 2.558 μM) > TLWP07 (11.792 ± 2.458 μM), while TLWP09 showed no inhibitory effect within the tested concentration range.



Compounds	% Cell viability at maximum concentration (50 μM)	
	Lung Cancer Cell (A549)	Normal Cell (HEK293T)
TLWP01	77.58	169.46
TLWP07	67.49	103.13
TLWP08	38.92	120.17
TLWP09	122.50	239.22

Figure 1. Cell viability of A549 and HEK293T cells after treatment with 50 μM of the synthetic heterocyclic compounds (TLWP01, TLWP07, TLWP08 and TLWP09) for 24 hours. Data are represented as mean ± SEM (n = 3), $p < 0.001$ (***) and $p < 0.0001$ (****) compared with control group (DMEM).

Conclusion

- Synthetic heterocyclic compounds exhibited cytotoxic activity against lung cancer cells, with TLWP08 showing the strongest potency.
- Methoxy substitution at the meta position relative to the oxygen atom of the pyran ring showed the highest cytotoxic and anticancer activity. However, its position on the synthetic heterocyclic compound is crucial, with TLWP08 showing the most favorable selectivity toward A549 lung cancer cells compared to normal HEK293T cells, suggesting that optimal methoxy placement affects anticancer potential.

Acknowledgement

- Drug Discovery Research Laboratory, Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University
- Asst. Prof. Dr. Torsak Luanphaisarnnont, Department of Chemistry, Faculty of Science, Mahidol University

References

- El-Fotooh Hammam, A. G., Abd El-Salam, O. I., Mohamed, A. M., & Abdel Hafez, N. (2005). Novel fluoro substituted benzo[b]pyran with anti-lung cancer activity. In Indian Journal of Chemistry (Vol. 44).