Exploring the anti-cancer potential of coumarin derivatives targeting COX-2 inhibitors

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ABSTRACT

Overexpression of cyclooxygenase-2 (COX-2) plays a crucial role in cancer therapy. In this study, we investigated the role of two coumarin derivatives with the chlorobenzylamide group in inhibiting COX-2 activity and growth inhibition of cancer cells. The effect of synthesized **2a** and **2b** coumarin derivatives on COX-2 enzyme was investigated in vitro. Besides that, A549, HT-29, MDA-MMB-231, and HeLa tumor cell lines were used to evaluate potential inhibition growth using the MTT assay. According to the results, two synthesized compounds showed an inhibitory effect on COX-2 enzymes. In particular, **2a** exhibited the best activity with an IC_{50} value of 49.54μM on COX-2. The **2a** and **2b** exhibited a noteworthy dose-dependent inhibitory growth activity against all the tested cancer cell lines, indicating their broad-spectrum anti-cancer properties. Based on the in vitro data, we strongly recommend this new coumarin-o-chlorobenzylamide as a preclinical development candidate for novel anti-cancer agents targeting COX-2 inhibition.

1. Introduction

Coumarin and its derivatives have attracted the attention of many researchers due to their various functional properties. Coumarin class compounds have exhibited several biological activities, including anti-inflammatory and anti-cancer properties. Bruna-Haupt et al. (2023) synthesized hydroxylated coumarin to induce antiproliferative effects and apoptosis in HCT-116 and HEK 293 tumor cell lines. Anti-inflammatory effects of the synthesized hybrid structure of curcumin analogs and coumarin were investigated by Ghany et al. (2023). They concluded that a coumarin with 3,4-dimethoxy benzylidene hydrazine inhibits lipopolysaccharide-induced inflammatory responses in macrophage cells. These suggest that coumarin derivatives should be considered a promising drug development candidate.

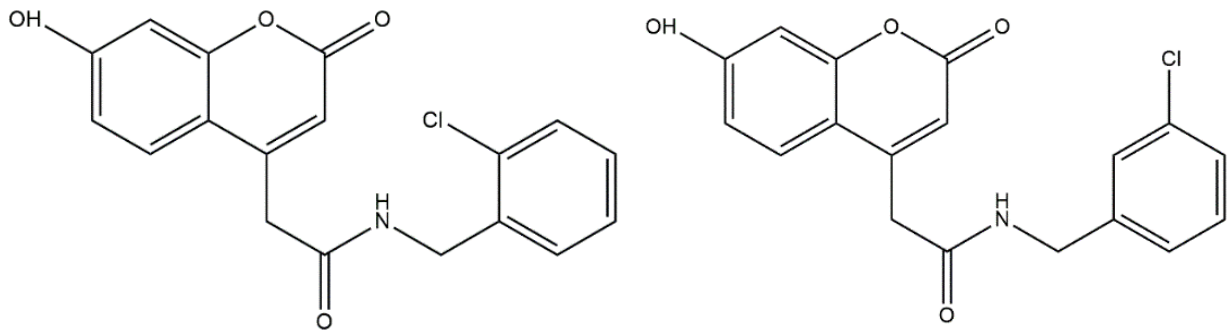
Inflammation is a complex internal process involving activation, recruitment, and action of immune response to repair injured tissue and against infection. Based on much evidence that inflammation is associated with cancer development and progression, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as aspirin reduce incidence and mortality in many cancers in extensive clinical studies (Vane & Botting, 1998). Furthermore, several laboratory and animal studies have demonstrated that the therapeutic effects of NSAIDs may relate to regulating overexpression cyclooxygenase-2 (COX-2) in tumors (Liggett et al., 2014; Zhang et al., 2018;

Zhao et al., 2021). However, these drugs are no "magic bullets," which cause side effects most associated with gastrointestinal symptoms mainly due to the inhibition of another isoform of COX-2, COX-1 (Tolba, 2017). Therefore, developing COX-2 isoform-selective inhibitors can effectively target treatments and reduce the side effects.

Based on this knowledge, we reported the synthesis of some new coumarin derivatives with expected activities activities (Dam et al., 2024). Continuing our efforts to understand the structure-function relationships in cancer diseases, we are interested in exploring more of two coumarin derivatives, including coumarin-o-chlorobenzylamide and coumarin-m-chlorobenzylamide as COX-2 inhibitors and other cancer cells lines (Figure 1).

Figure 1

Chemical Structure of Compounds Used in this Study



coumarin-o-chlorobenzylamide coumarin-m-chlorobenzylamide

Source. The data are from "Synthesis and anticancer evaluation of novel coumarin derivatives" by T. T. H., Dam, D. P. A., Chan, M. T., Phan, T. K. L., To, T., Vo, T. S., Phan, V., Hoang, T. T. H., Le, T. Y., Vu, D. D., Vo, and P. H. Nguyen, 2024, *ChemistrySelect*, 9(37), Article e202403030.

2. Material and methods

2.1. Materials

For evaluating biological activities, we have used synthesized coumarin derivatives. In our previous study, two new coumarin derivatives, **2a** and **2b**, were designed and synthesized via the reaction of the mixture of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid with Hexafluorophosphate Azabenzotriazole Tetramethyl Uranium (HATU), Triethylamine (Et3N) and 2-chlorobenzylamine. The coumarin synthesis process involved the addition of 2-(7-hydroxy-2-oxo-2H-chromen-4-yl) acetic acid, HATU, and Et3N to a solution of 2-chlorobenzylamine in THF at 25°C for 2h (Dam et al., 2024). Faculty of Chemistry provided the **2a** and **2b** compounds, Ho Chi Minh City University of Education, Ho Chi Minh City, Vietnam.

2.2. In vitro COX inhibition assay

COX inhibition assays were performed using the COX-2 enzyme for testing synthetic compounds (Kulmacz & Lands, 1983). Briefly, COX-2 (10 units/well) was mixed with 1μM hematin and each sample at 37°C for 10min. Then, 20mM TMPD and 100μM arachidonic acid in 0.1% DMSO was added to start the reaction. The reaction mixture was incubated at 37°C for another 10min with shaking before the amount of PGF2α produced was measured at 450nm. The enzyme activity is the ratio (%) inhibitory activity compared to the control. The concentration of tested compounds needed that cause inhibition of 50% COX-2 enzyme activity is defined as IC_{50} value. The percent inhibition curves were plotted using a minimum of five determinations for

each inhibitor concentration, and IC_{50} values were computed from the semilogarithmic plots. A linear regression analysis was performed using GraphPad.

2.3. Cell culture

The human lung cancer A549 (ATCC# CCL-185™), human colorectal cancer HT-29 (ATCC# HTB-38™), human breast cancer MDA-MMB-231 (ATCC# HTB-26™), and human cervical cancer HeLa (ATCC# CRM-CCL-2™) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). HeLa was cultured in Dulbecco's Minimum Essential Medium (DMEM; WelGENE Inc., Daegu, Korea). A549, HT-29, and MDA-MMB-231 were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (WelGENE Inc.). Both mediums were supplemented with 10% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL of Gibco Amphotericin B and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

2.4. Antiproliferative activity assay

In vitro, antiproliferation activities were assessed with A549, HT-29, MDA-MMB-231, and HeLa cell lines treated with the synthesized compounds (Mosmann, 1983). The cells were seeded at a final 5 × 104 cells/well concentration into 96-well plates. After adherence for 24 hours, a series of synthesized compounds (at a final concentration of 1.95 - 1000μM) were added to each well and continued incubation for another 24h. Then, the cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml) for 4 hours to produce formazan crystal. To dissolve the crystal, 100μL DMSO replaced the medium. The absorbance at 570nm was read on a micro-plate reader (Synergy LX, BioTek, USA). The untreated cells were set as 100% activity, while the relative activity was calculated using the equation:

$$\% \text{ Relative activity} = (OD_{\text{treated cell}}/OD_{\text{untreated cell}}) \times 100 \quad (1)$$

The IC_{50} was calculated according to the dose-dependent curves.

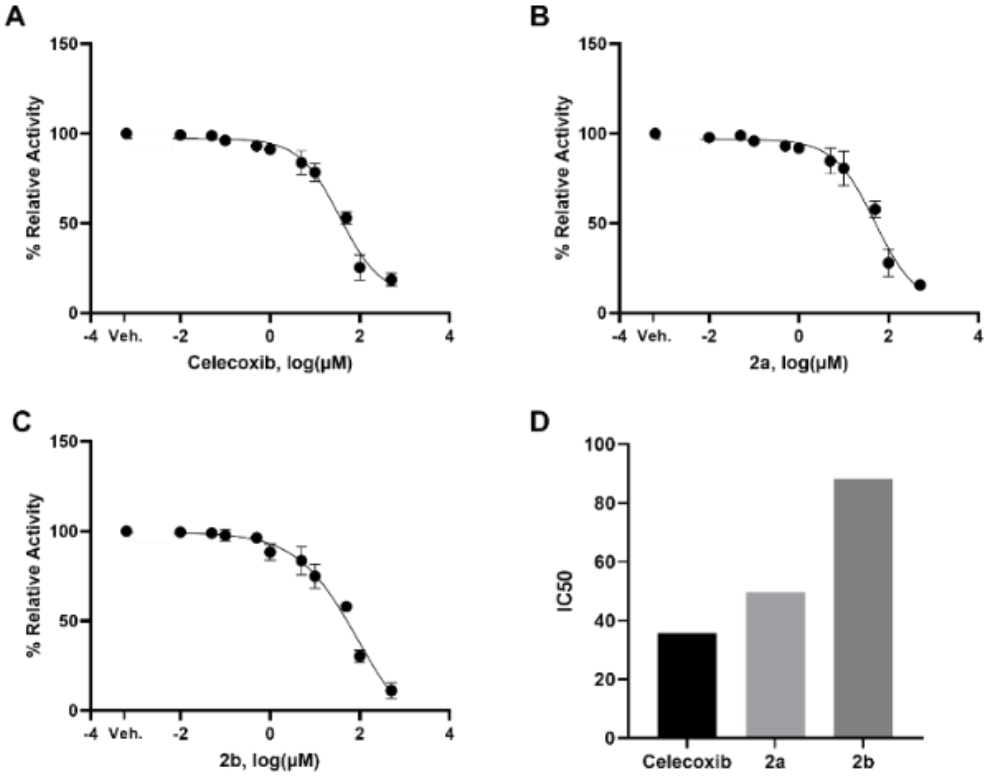
3. Results and discussion

3.1. In vitro cyclooxygenase activity

A new phenolic of coumarin with o-chlorobenzylamide and m-chlorobenzylamide group at C-4 having various substituents was synthesized, and the COX-2 inhibitory activity of these compounds was determined. COX-2 inhibition assay was performed according to the principal metabolism of prostaglandin H through cyclooxygenase (Kulmacz & Lands, 1983). Within the examined compounds **2a**, **2b,** and celecoxib as a well-known drug, results of COX-2 inhibition indicate that compounds **2a** and **2b** display a dose-dependent efficacy. The IC_{50} values for compound **2a** were 49.54μM, while the IC_{50} values for compound **2b** were 88.33μM. The IC_{50} values for celecoxib were 1.38 and 2.47-fold lower than **2a** and **2b**, respectively. These results demonstrated that the presence of chlorobenzylamide on the ortho position of the C-4 reduces the activity of COX-2 in vitro activities, which are essential for the synthesis of inflammatory mediators (Wong, 2019). It is important to note that the limited evidence in the literature available to date is linked to the inhibition action of coumarin with chlorobenzylamide group on COX-2 for its involvement in the antiproliferation of tumor cell, as demonstrated for the first time in the present study.

Figure 2

Concentration-Response Curves for the Inhibition of COX-2 Activities by Celecoxib (A), 2a (B), 2b (C) and IC_{50} Value



Source. Data analysis result of the research

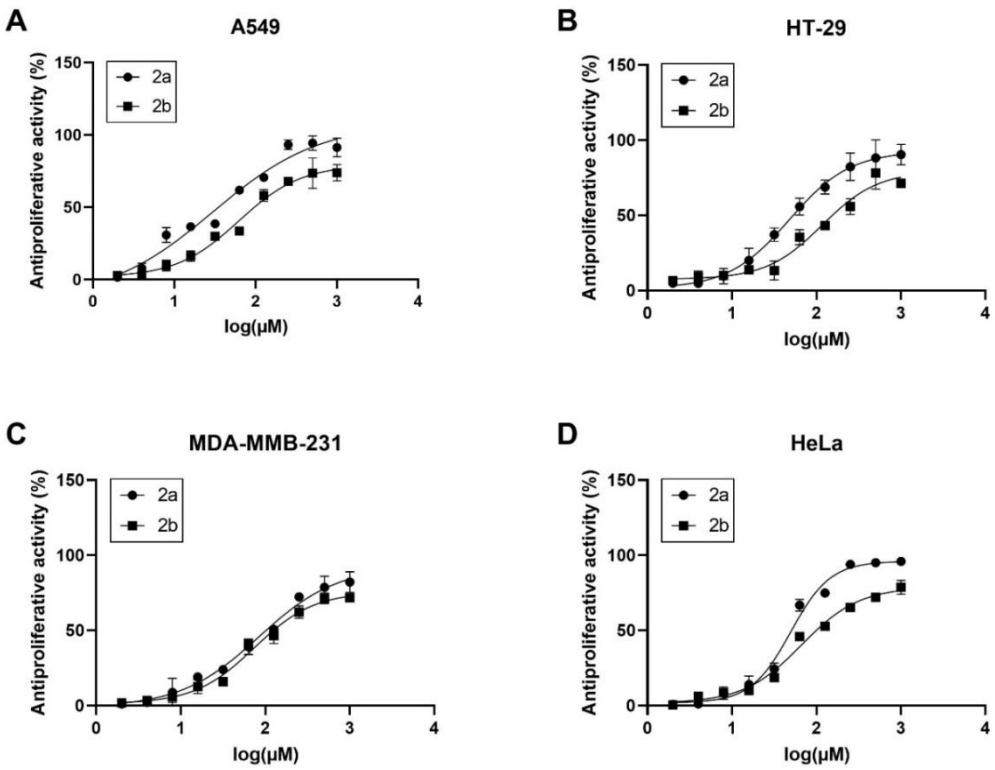
3.2. In vitro anti-cancer study

The synthesized compounds, **2a** and **2b**, have been screened against human lung cancer (A549), human colorectal cancer (HT-29), human breast cancer (MDA-MMB-231), and human cervical cancer cells (HeLa) cell lines. As a result of the examinations, two compounds had high efficiency and broad spectrum in decreasing the survival rate of tested cancer cells, summarized in Figure 2. Compound **2a** exhibited the most potent growth inhibition activity. When the concentration of compound **2a** is 1.95 - 1000μM, the IC_{50} values of A549, HT-29, MDA-MMB-231, and HeLa were observed for 29.89μM, 47.52μM, 83.98μM, and 49.49μM, respectively. The traditional anti-cancer screening strategies are used in vitro cell-based screening assays, animal systems, and bioinformatics approaches (Singh et al., 2021). In cell-based screens in cancer research, the targeted compounds are tested with a range of concentrations to identify nonspecific growth inhibitory against human tumor cell lines (Boyd, 1997). Among accepted cell-based screening methods, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay is the earliest and simplest method to dye the live cell membranes.

These preliminary results demonstrated that compound **2a** could prevent cancer cell growth. Consistent with these findings, the anti-cancer was strongly related to anti-inflammation, which was concentration-dependently reserved with compound **2a**. The design and synthesis of coumarin derivatives have been a subject of consistent interest as this promises to develop as a preclinical anti-inflammatory drug.

Figure 3

*Antiproliferative Activity of **2a** and **2b** on The Survival of A549 (A), HT-29 (B), MDA-MMB-231 (C), and Hela (D) Cancer Cells. Cells Were Incubated with Different Concentrations in 24h, and Its Proliferation Was Estimated by MTT Assay (Mean ± SD, N = 3)*



Source. Data analysis result of the research

4. Conclusion and recommendations

Here, we synthesized coumarin with chlorobenzylamide and evaluated its inhibitory potential against the COX-2 enzyme and the proliferation of cancer cells. The initial evaluation showed compound **2a** exhibited the highest dual anti-inflammatory activity with anti-cancer activity. To investigate novel agents in more detail, an intracellular damage-associated molecular patterns analysis would be possible.

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References

Boyd, M. R. (1997). The NCI in vitro anti-cancer drug discovery screen: Concept, implementation, and operation, 1985 - 1995. In *Anti-cancer drug development guide: Preclinical screening, clinical trials, and approval* (pp. 23-42). Springer.

Bruna-Haupt, E. F., Perretti, M. D., Garro, H. A., Carrillo, R., Machín, F., Lorenzo-Castrillejo, I., Gutiérrez, L., Vega-Hissi, E. G., Mamberto, M., Menacho-Marquez, M., Fernández, C. O., García, C., & García, C. R. (2023). Synthesis of structurally related coumarin derivatives as antiproliferative agents. *ACS Omega*, *8*(29), 26479-26496.

Dam, T. T. H., Chan, D. P. A., Phan, M. T., To, T. K. L., Vo, T., Phan, T. S., Hoang, V., Le, T. T. H., Vu, T. Y., Vo, D. D., & Nguyen, P. H. (2024). Synthesis and anticancer evaluation of novel coumarin derivatives. *ChemistrySelect, 9*(37), Article e202403030.

Ghany, L. M. A., Beshay, B. Y., Youssef Moustafa, A. M., Maghrabi, A. H. A., Ali, E. H. K., Saleem, R. M., Zaki, I., & Ryad, N. (2023). Design, synthesis, anti-inflammatory evaluation, and molecular modelling of new coumarin-based analogs combined curcumin and other heterocycles as potential TNF-α production inhibitors via upregulating Nrf2/HO-1, downregulating AKT/mTOR signalling pathways and downregulating NF-κB in LPS induced macrophages. *Journal of Enzyme Inhibition and Medicinal Chemistry, 38*(1), Article 2243551.

Kulmacz, R. J., & Lands, W. E. (1983). Requirements for hydroperoxide by the cyclooxygenase and peroxidase activities of prostaglandin H synthase. *Prostaglandins, 25*(4), 531-540.

Liggett, J. L., Zhang, X., Eling, T. E., & Baek, S. J. (2014). Anti-tumor activity of non-steroidal anti-inflammatory drugs: Cyclooxygenase-independent targets. *Cancer Letters, 346*(2), 217-224.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1/2), 55-63.

Singh, M., Patra, S., & Singh, R. K. (2021). Common techniques and methods for screening of natural products for developing of anti-cancer drugs. In *Evolutionary diversity as a source for anti-cancer molecules* (pp. 323-353). Elsevier.

Tolba, R. (2017). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). In *Treatment of chronic pain conditions: A comprehensive handbook* (pp. 77-79). Springer.

Vane, J. R., & Botting, R. M. (1998). Anti-inflammatory drugs and their mechanism of action. *Inflammation Research, 47*(2), 78-87.

Wong, R. S. (2019). Role of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in cancer prevention and cancer promotion. *Advances in Pharmacological and Pharmaceutical Sciences, 2019*(1), Article 3418975.

Zhang, Z., Chen, F., & Shang, L. (2018). Advances in antitumor effects of NSAIDs. *Cancer Management and Research, 10*(1), 4631-4640.

Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y., & Li, Y. (2021). Inflammation and tumor progression: Signaling pathways and targeted intervention. *Signal Transduction and Targeted Therapy, 6*(1), Article 263.