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Synthesis and cytotoxic activity of selected dual COX-2 and 5-LOX inhibitors in HeLa and MIA PaCa-2 human cancer cell lines

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Abstract: Among novel cancer chemotherapy approaches, the use of cyclooxygenases (COXs) and lipoxygenases (LOXs) inhibitors represents a promising mean for cancer treatment showing lesser toxicity comparing to the currently used cytotoxic drugs. This study detailed the synthesis of three novel compounds: 1ME, BHTK-AA, and IBU-Ac, each with the capability to concurrently inhibit both COX-2 and 5-LOX. Subsequently, we assessed their effectiveness in inhibiting the proliferation of HeLa cervical and MIA PaCa-2 pancreatic cancer cells. The IC_{50} values for both examined cell lines were approximately 40 μM, indicating the promising inhibitory potential of the IBU-Ac compound in both types of cancer cells. This finding is positioned to stimulate further investigation into the potential application of IBU-Ac against these particular types of cancers, while also advocating its use in combination with standard anti-cancer protocols, i.e., chemoterapeutics or radiation therapy. The results of this work are also advocating the development and refinement of dual COX-2 and 5-LOX inhibitors, thus improving their efficacy and safety.

Keywords: cancer, dual COX-2 and 5-LOX inhibitors, cytotoxicity

1. Introduction

In the context of tumor microenvironment, inflammation is currently acknowledged as one of the fundamental characteristics of cancer. Epidemiological studies have shown that individuals afflicted with chronic inflammatory conditions face an elevated likelihood of developing cancer, suggesting that inflammation is, to some extent, a causative factor rather than a consequence of cancer development [1]. The enzyme families cyclooxygenase (COX) and lipoxygenase (LOX) are accountable for the metabolism of arachidonic acid. During this metabolic pathway, secondary products such as eicosanoids, encompassing prostaglandins and leukotrienes can serve as potent agents in fostering inflammation [2,3]. Both COX-2 and 5-LOX are co-expressed, up-

regulated and the eicosanoids generated by these enzymes perform pivotal functions in the progression of cancer such as proliferation, angiogenesis, invasion and metastasis [2].

Dual COX-2 and 5-LOX inhibitors are being considered as promising and innovative anti-inflammatory agents, representing a potential strategy in cancer therapy. This approach of combined inhibition addresses certain limitations associated with selective COX-2 inhibitors, while also sparing the gastrointestinal mucosa [4, 5]. The primary objective of this study was to synthesize and assess the cytotoxic potential of novel compounds capable of simultaneously inhibiting both COX-2 and 5-LOX. The focus of this research was on investigating the inhibitory effects of three inhibitors on two types of cancer cells: cervical and pancreatic carcinoma.

2. Experimental

2.1 *Synthesis of 1ME*

1ME (Figure 1A) was synthesized through a series of three sequential steps. In the initial phase, 2-sulfobenzoic anhydride (0.01357 mol) was dissolved in 8 ml of methanol, followed by the addition of 6 ml of methanolic ammonia. The resulting product was collected, air-dried overnight, and subsequently utilized in the next step without any additional purification. The compound obtained from the preceding step (0.0129 mol) was dissolved in 2 ml of dimethyl formamide, and subsequently, 16 ml of thionyl chloride was introduced. The reaction mixture was subjected to reflux heating overnight, cooled within an ice bath, and then combined with crushed ice (approximately 200 ml). The ensuing product was extracted using dichloromethane, with subsequent evaporation to yield the acid chloride. To a solution of the acid chloride (0.004 mol) in 13 ml of tetrahydrofuran, a solution of hydroxylamine hydrochloride (0.008 mol) in 4 ml of water was added. The final product (1ME) was extracted using dichloromethane and then subjected to recrystallization from ethyl acetate.

2.2 Synthesis of BHTK-AA

A solution of 3,5-di-tert-butyl-4-hydroxybenzoic acid (0.42 mmol) was prepared by dissolving it with allylamine (0.5 mmol, 1.2 eq) in dimethylformamide (6 mL) at room temperature. Following this, EDC (0.63 mmol), HOBt (0.63 mmol), and TEA (0.84 mmol) were introduced into the reaction mixture. The resulting mixture was stirred overnight at room temperature. Subsequently, the product was subjected to further purification using preparative thin-layer chromatography (TLC) with a solvent mixture of dichloromethane and methanol in a ratio of 9.5:0.5 v/v. The chemical structure of BHTK-AA is presented in Figure 1B.

2.3 Synthesis of IBU-Ac

The synthesis of IBU-Ac (Figure 1C) was carried out in the following manner. 4'-isobutylacetophenone (17 mmol) and hydroxylamine hydrochloride (35 mmol) were dissolved in a mixture of ethanol (25 mL) and pyridine (25 mL), then heated at 50°C for 2 hours. The solution was evaporated to yield an oxime compound. This oxime (5 mmol)

was dissolved in ethanol (10 mL) and cooled to 0°C. Borane-pyridine complex (15 mmol, 3 eq) was added via syringe under a nitrogen, followed by the addition of 6M HCl (5 mL) after 10 minutes. The resulting mixture was subjected to ethyl acetate extraction, yielding compound C. To a solution of TEA (7.8 mmol) and compound C (2.6 mmol) in dichloromethane (10 mL) at 0°C, acetyl chloride (5.73 mmol) was added. The mixture was then introduced to 2M HCl (10 mL), and the organic layer was evaporated to obtain compound D. Compound D (0.36 mmol) was dissolved in 2-propanol (1 mL), and a solution of lithium hydroxide (207 mg/mL) in water (0.5 mL) was added. The resulting product was extracted using 2M HCl (5 mL) and ether (10 mL), followed by further purification through preparative TLC using an ethyl acetate – cyclohexane solvent mixture with a ratio of 15:1 v/v.

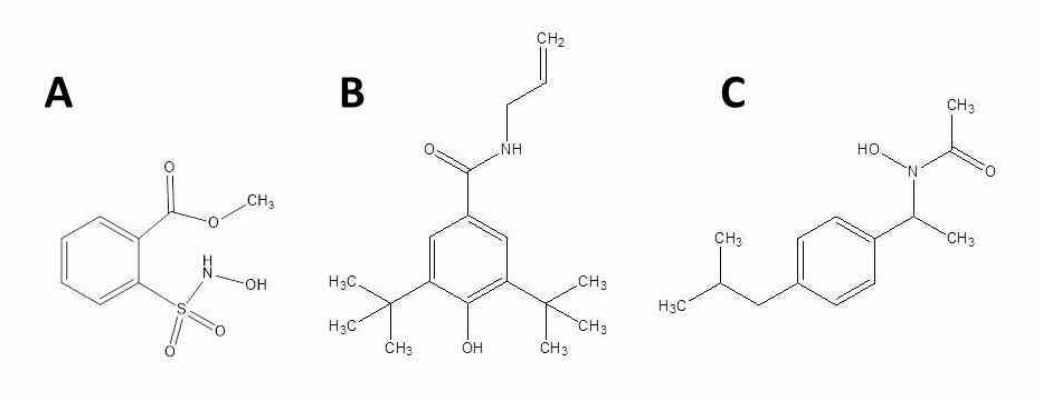

Figure 1. Chemical structure of 1ME (A), BHTK-AA (B) and IBU-Ac (C).

2.4 Cytotoxic activity of 1ME, BHTK-AA and IBU-Ac

For the evaluation of cytotoxic activity of synthesized compounds the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was applied according to procedure described elswere [6]. Briefly, HeLa and MIA PaCa-2 cells (ATCC, Manassas, VA, USA) were grown in 96-well plates, treated with increasing concentrations of tested compounds for 72 h and then incubated with MTT (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) until blue formazan crystals were formed. The water-insoluble formazan produced is then solubilized by a dimethyl sulfoxide (DMSO). Subsequently, the absorbance was measured by a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, PerkinElmer, Turku, Finland) at a wavelength of 550 nm. The IC_{50} values of each inhibitor are given in Table 1. The results have shown that only after application of IBU-Ac 50% inhibition in both cell lines can be achieved. However, the observed IC_{50} values did not differ much between HeLa and MIA PaCa-2 cells being around 40 μM. As presented in Table 1, 1ME and BHTK-AA did not have a significant impact on the viability of tested cell lines.

Table 1. Cytotoxic activity of 1ME, BHTK-AA and IBU-Ac estimated by MTT assay with corresponding IC_{50} values.

Cell line	1 ME IC_{50} (μM)	BHTK-AA IC_{50} (μM)	*IBU-Ac* IC_{50}(μM)
HeLa	>100	>100	39.89±0.61
MIA PaCa-2	>100	>100	46.80±0.29

3. Conclusions

The results of this preliminary study suggest that among three newly synthesized dual COX-2 and 5-LOX inhibitors, IBU-Ac acts as a potent inhibitor against cervical and pancreatic carcinoma. The aim of future experiments will be to investigate more deeply the mechanisms that underlie the response to this agent and to test its utility in conjunction with other anti-cancer modalities.

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