Evaluation of Cytotoxic Properties of Lumichrome-derived compounds on Breast Adenocarcinoma (SkBr3), Colorectal Cancer (BRAF), and Liver Carcinoma (Hep3B)

INTRODUCTION
One of the most feared illnesses in the world is cancer. More than 28.4 million people will be diagnosed with cancer in the next two decades, most of them from developing nations [1]. Many people are diagnosed with a variety of different types of cancer each year. Lung cancer was the top cause of death among the almost 9.9 million cancer fatalities, followed by colorectal and liver cancer [2]. However, the number of women diagnosed with breast cancer (2.3 million) has surpassed the number of women diagnosed with lung cancer for the first time [2]. To combat this increasing mortality, cancer cells' developing resistance to anticancer treatments, and the unpleasant side effects of many medications, scientists have always devoted their time and energy to finding new chemotherapeutic agents with higher safety and effectiveness.
Irradiation of riboflavin in neutral or mildly acidic solutions results in the formation of lumichrome, which is one of the essential riboflavin derivatives [3, 4]. Because alloxazine 2 (Figure 1) may be synthesized from lumichrome, the structure of lumichrome is thought stimulating. The reduction of lumichrome1 with sodium borohydride in THF results in compound 2 [4]. The design of alloxazine 2 seems to be very intriguing (Fig.1). The ring on the right side of its structure is identical to uracil, a pyrimidine base found in RNA and other nucleic acids (Fig. 1). Because of this resemblance, alloxazine two may be integrated into RNA during transcription, resulting in the formation of a non-functional analog of RNA, which, in turn, may result in the suppression of protein synthesis and, ultimately, cell development [5]. Lumichrome1 has also been linked to a reduction in the risk of lung cancer [6]. Alloxazine 2 was researched further to compare its anticancer activity with lumichrome1, which was also tested for anticancer activity. In this article, the actions of the chemicals against three distinct cancer cell lines are discussed: Hep3B (liver cancer), SKBR3 (breast adenocarcinoma), and BRAF (colorectal cancer).

Figure 1. Lumichrome-derived Alloxazine

Materials and methods

Anticancer Agents and Cell Culture
Lumichrome chemicals were bought from Sigma Aldrich. It was made by reducing lumichrome(1) with sodium borohydride in THF [4]. The ATCC (http://www.lgcstandards-atcc.org/) provided the Hep3B (liver cancer), SKBR3 (breast adenocarcinoma), and BRAF (colorectal cancer) cell lines. The culture cells were kept in RPMI medium with 100 ng/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum at 37 °C in a humidified, 5% CO₂ environment.

Cytotoxicity Test
Because of its greater simplicity, sensitivity, and resolution, the MTT test has become more widely utilized than the formazan-based assays in recent years [6,7,8]. Based on the interaction of the anionic pink aminoxanthine dye sulforhodamine B (SRB) with the essential amino acids of viable cells, the test determines whether or not the cells are viable. The quantity of dye taken up by the cells is dependent on the number of cells present. After fixation, more vivid color and increased absorbance are generated [9]. As a result, the MMT assay was used to detect the cytotoxic effects of the test compounds on Hep3B (liver cancer), SKBR3 (breast adenocarcinoma), and BRAF (colorectal cancer) cell lines.

The cells were incubated in a complete medium for 24 hours after being suspended in 100L (5x103 cells) in 96-well plates of each kind of culture (HepG2, SKBR3, and BRAF). The cells were then given another aliquot of 100L medium containing the test chemical at different doses ranging from 0.1g/ml to 100g/ml. Using 150 mL of 10% TCA solution after 72 hours of exposure to the test chemical and incubating the cells for 1 hour at four °C, fixation of the cells could be accomplished. To remove the TCA solution, cells were washed five times with distilled water and incubated for 10 minutes at room temperature with 70L SRB solution (0.4 percent w/v), added at the end of the incubation period. In the last step, plates were allowed to air-dry overnight after washing three times.
with 1 percent acetic acid. Then, the protein-bound SRB stain was dispersed by adding 150 mL of TRIS to the plates (10mM). It was necessary to measure the absorbance at 540 nm with the help of a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany).

All studies were carried out in triplicate, each in a separate location. The data were reported as the mean plus standard error of the mean. To calculate IC_{50} values using concentration-response curves, the following software was used: SPSS version 26.0 (IBM) for statistical analysis and an E-max model equation was used to calculate the following results:

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\% \text{ Cell viability} = (100 - F) \times \left(1 - \frac{[D^m]}{K^m + [D^m]}\right) + F
\]

For example, in this equation, D=the concentration of test compound utilized, F=the residual, unaffected portion, K=the test compound concentration that reduces the maximal inhibition rate by 50%, and m=a Hill-type coefficient. To calculate the IC50, researchers looked at the absorbance of the test sample and the control sample's absorbance [10].

**Results and discussion**

The MTT assay was used to investigate the cytotoxic effects of the test substances on HepG2, SKBR3, and BRAF cancer cell lines, while Valrubicin was used as a control. All three cell lines were shown to be cytotoxic to lumichrome and alloxazine. There were substantial reductions in less than 6 percent viability at 100g/ml for all cell lines tested. The vitality of Hep3B cell lines reduced when the quantity of lumichrome and alloxazine increased, compared to SKBR3 and BRAF cell lines (Fig. 2). However, lumichrome showed a superior cytotoxic profile than alloxazine and affected the viability of HepG2, SKBR3, and BRAF cell lines more following the increase in concentrations. (Fig. 3 and 4).

![Figure 2. Cell Viability (%) of Hep3B (liver carcinoma).](image)

![Figure 3. Cell Viability (%) of SkBr3 (Breast adenocarcinoma).](image)
The authors may be performed. For example, the synthesis of other analogs and examination of their anticancer activity quickly rose at 10 g/ml (Figure 2). For liver cancer (Hep3B cell line), lumichrome has the highest IC50 value of 8.9 g/ml compared to the standard (IC50=3.4 g/ml). With an IC50 value of 16.6 g/ml, alloxazine was likewise the most effective in killing liver cancer cells. Alloxazine, on the other hand, had a weaker anticancer potential than its precursor lumichrome, as predicted. By activating the tumor suppressor p53 protein and suppressing the protein kinase B (AKT) activity, lumichrome can inhibit lung cancer stem cells’ ability to increase, as well as its ability to induce apoptosis. A non-functional RNA analog is formed during transcription by alloxazine (2), which has been shown to have an anticancer impact by targeting numerous oncogenic proteins [5]. The mechanism of action of these drugs has to be studied further in the laboratory.

**Conclusion**
Because the global burden of cancer is in no way diminishing, novel medicines are required to increase cancer patient survival. Although lumichrome showed the most promising results against liver cancer, this research also offers evidence that both lumichrome and alloxazine had considerable cytotoxic effects against three primary malignancies: breast, liver, and colorectal cancers. To develop Valrubicin-like, more effective anticancer chemicals, more studies on lumichrome and alloxazine may be conducted. For example, the synthesis of other analogs and examination of their anticancer activity may be performed.

**Conflict of Interest**
The authors declare that this article has no type of conflict of interest.
Acknowledgment
The experimentations were conducted at the Shaheed Benazir Bhutto University, Dir Upper KP, Pakistan.

REFERENCES


