5,10-Dihydro-7,8-Dimethyl Alloxazine as an Anticancer Agent from Lumichrome of Riboflavin

Shafia Farhana Etu¹, Ali Alqahtani² and Nazmul Qais¹*

¹Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh
²Department of Pharmacology, College of Pharmacy, King Khalid University, Guraiger, Abha, Saudi Arabia

Abstract
Scientists are continuing their relentless endeavor to find a remedy for cancer or therapeutic agents which will minimize the growing burden of this seemingly incurable disease to a great extent. From literature review, we got the insight that a photoproduct of riboflavin named lumichrome has anticancer effect against lung cancer and this demands working on this compound and evaluation of anticancer activity. The present study represents the potential anticancer property of lumichrome and its synthesized derivative 5,10-dihydro-7,8-dimethyl alloxazine against liver, breast and colorectal cancer. Cytotoxic activity was investigated by in vitro sulforhodamine B (SRB) assay against HepG2 (Hepatocellular carcinoma), MCF-7 (Breast Adenocarcinoma), Colo-205 (Colorectal cancer) cell lines. IC₅₀ values were between 7.7 to 23.9 μg/ml. HepG2 cell line was most sensitive to lumichrome as well as its synthesized derivative with IC₅₀ values of 7.7 and 17.2 μg/ml respectively. The mechanisms of actions of these compounds are thought to involve induction of apoptosis and interference with the transcription process. Future studies will be required to confirm their mechanism of actions (Graphical Abstract).

Keywords
Anticancer, Lumichrome, 5,10-dihydro-7,8-dimethyl alloxazine, Breast cancer, Liver cancer, Colorectal cancer

Introduction
Cancer is still considered as one of the most dreaded diseases in the world. In 2020, an estimated 19.3 million new cancer patients were reported by the International Agency for Research on Cancer (IARC). It is predicted that this number of cancer patients will rise up to 28.4 million in the next two decades, mostly from developing countries [1]. Breast cancer, liver cancer and colorectal cancer are some commonly occurring cancers. Of almost 9.9 million cancer patients, breast cancer accounted for 2.1 million, liver cancer for 0.9 million, and colorectal cancer for 1.7 million cases.
Chemistry

Lumichrome was purchased from a commercial source named Sigma Aldrich. The compound, 5,10-dihydro-7,8-dimethyl alloxazine 2 (Figure 1) was prepared from lumichrome 1 by its reduction with sodium borohydride in THF [4]. Doxorubicin for cytotoxicity test, was purchased from Beacon Pharmaceuticals Ltd., Dhaka, Bangladesh.

Cell culture

There are HepG2 (Hepatocellular carcinoma), MCF-7 (Breast Adenocarcinoma), and Colo-205 (Colorectal cancer) cell lines in Nawah Scientific Inc., (Mokattam, Cairo, Egypt). Culture cells were preserved there in RPMI media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO₂ atmosphere at 37 °C.

Cytotoxicity test

The sulforhodamine B (SRB) assay is now more commonly used than the formazan-based assays because of its more simplicity, sensitivity and resolution [7, 8]. The assay is based on the interaction of the anionic pink aminoxanthine dye sulforhodamine B (SRB) with basic amino acids of the viable cells. The amount of dye taken up depends on the number of cells and more intense color as well as greater absorbance is produced when the cells are lysed after fixation [9]. Therefore cytotoxic effects of the test compounds on HepG2 (Hepatocellular carcinoma), MCF-7 (Breast Adenocarcinoma), Colo-205 (Colorectal cancer) cell lines were determined using the SRB assay.

1. 100 μL cell suspension (5 × 10⁴ cells) of each type of culture (HepG2, MCF-7, Colo-205) was taken in 96-well plates and incubated for 24 h in complete media.
2. Another aliquot of 100 μL media containing the test compound at various concentrations ranging from 0.01 μg/ml to 100 μg/ml were added in cells.
3. Fixation of cells was carried out by changing media with 150 μL of 10% TCA solution after 72h of exposure of the test compound and incubated for 1 h at 4 °C.
4. Cells were then washed 5 times with distilled water to remove TCA solution and incubated for 10 min. in a dark place by adding 70 μL SRB solution (0.4% w/v) at
Figure 2: Percentages of cell viability of A) HepG2, B) MCF-7 & C) Colo-205 cancer cell lines at five different concentrations: 0.01 μg/ml, 0.1 μg/ml, 1 μg/ml, 10 μg/ml and 100 μg/ml. Test compounds and standard were examined against cancer cells in triplicate.
Results and Discussion

Through the SRB Assay, cytotoxic effects of the test compounds against HepG2, MCF-7, Colo-205 cancer cell lines were determined while taking doxorubicin as a standard. It is clear that both lumichrome and 5,10-dihydro-7,8-dimethyl alloxazine have cytotoxic effects against all three cell lines. At 100 μg/ml, test compounds significantly reduced the percentages of the cell viability, accounting for less than 6% at every cell line. Compared to MCF-7 and Colo-205 cell lines, the viability of HepG2 cell lines decreased gradually with the increase of concentration of lumichrome and 5,10-dihydro-7,8-dimethyl alloxazine (Figure 2A). However lumichrome had better cytotoxic profile than 5,10-dihydro-7,8-dimethyl alloxazine and lowered the viability of HepG2, MCF-7 and Colo-205 cell lines more in accordance with the rise of concentration (Figure 2B and Figure 2C). The cell inhibition (IC_{50}) results are shown in Table 1. IC_{50} values determined from cytotoxic profile of lumichrome against HepG2, MCF-7, Colo-205 cancer cell lines were 7.7 μg/ml, 15.3 μg/ml, 17.6 μg/ml and it was 17.2 μg/ml, 23.9 μg/ml and 19.7 μg/ml in case of 5,10-dihydro-7,8-dimethyl alloxazine respectively. Although IC_{50} values of test compounds against HepG2 cell lines were close to the value of standard doxorubicin but against MCF-7 and Colo-205 cell lines more than 100 times higher than standard.

From the cytotoxic property profile (Figure 2) of lumichrome and 5,10-dihydro-7,8-dimethyl alloxazine against HepG2, MCF-7, Colo-205 cancer cell lines it was observed that at highest concentration the cytotoxic effects of both lumichrome and 5,10-dihydro-7,8-dimethyl alloxazine were comparable to doxorubicin. After 10 μg/ml, the inhibitory effect of lumichrome against all three cancer cell lines rapidly increased (Figure 2). The most potent cytotoxic activity was expressed by lumichrome against liver cancer (HepG2 cell line) with the IC_{50} value of 7.7 μg/ml compared to standard (IC_{50} = 3.4 μg/ml). On the other hand, 5,10-dihydro-7,8-dimethyl alloxazine also had its most cytotoxic activity against liver cancer with the IC_{50} value of 17.2 μg/ml. Therefore, as expected, 5,10-dihydro-7,8-dimethyl alloxazine possessed anticancer potential but lower than its precursor lumichrome. It has already been speculated that lumichrome provides its cytotoxic effect against lung cancer through induction of apoptosis by activation of tumor suppressor p53 protein as well as attenuation of cancer stem cells properties by suppression of protein kinase B (AKT) function and β-catenin level [6]. On the other hand, 5,10-dihydro-7,8-dimethyl alloxazine (2) may give its anticancer effect through formation of a non-functional analog of RNA during transcription and targeting multiple oncogenic proteins [5]. Further wet lab testing is required to understand their mechanism of actions.

Conflict of Interest

The author(s) declare that this article has no any type of conflict of interest.

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