Gene Expression Study of Novel Chalcone in Breast Cancer Therapy

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ABSTRACT
The purpose of the current study was aimed to employ gene expression analysis to investigate the impact of chalcone derivatives on breast cancer cell lines. A series of synthesised chalcone derivatives were used for docking studies in order to identify the most effective chalones for cancer therapy. In accordance with the docking score data, the compound A7 ((2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-dichloro-1*H*-benzimidazol-2-yl) prop-2-en-1-one) scored higher than other derivatives in EGFR target while the compound A32 ((2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-fluoro-4-hydroxy-1*H*-1,3-benzimidazol-2-yl) prop-2-en-1-one) in aromatase target. Epidermal growth factor receptor (EGFR) gene expression was studied using the compound A7 and aromatase gene expression were studied using the compound A32. The A549 cell line was used in studies on the expression of the EGFR gene. In order to study the expression of the aromatase gene, MCF-7 cell line was used. A widely prevalent molecule in humans called glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is frequently employed as an endogenous reference gene in investigations of quantitative gene expression. The RTPCR data revealed that A32 did not produce a favourable response in aromatase gene study while A7 treatment with EGFR gene expression is effective against breast cancer.

KEYWORDS: Chalcones, Breast cancer, Gene expression, A549 cell line, GAPDH and Aromatase.

INTRODUCTION

Breast cancer is the consequence of uncontrolled cell proliferation inside the structural components of the breast epithelium. It is the most ubiquitous malignancy in women and one of the three most prevalent malignancies in the globe (with an expected 2.26 million new cases in 2020). Despite the fact that over the past few decades the survival rate for breast cancer has grown, the primary reason for this is early detection.[1] A class of chemicals known as chalcones (1,3-diaryl-2-propen-1-ones) consists of two aryl rings connected by an, alpha and beta-unsaturated ketone molecule. Chalcones (1,3-diphenyl-2-propen-1-one) are a type of flavonoid chemical found in fruits, vegetables, and tea that act as biogenetic precursors to flavonoids and isoflavonoids.[2] They are lipophilic phytochemicals comprised of two aromatic residues (an aldehyde and an acetophenone) linked by a three-carbonyl α, β-unsaturated carbonyl system. It has been demonstrated that chalcone derivatives exhibit a wide range of beneficial features for therapeutic applications, including anticancer, anti-HIV, antimalarial, antioxidant, anti-inflammatory, and anti-allergic activity.[3]

Estrogens are crucial in the emergence of breast cancer. The cytochrome P450 enzyme aromatase (CYP19) is responsible for producing oestrogens. Employing enzyme activity measurement, immunocytochemistry, and RT-PCR analysis, it has been discovered that the expression of aromatase is higher in human breast cancer tissue than in healthy breast tissue. The development of breast tumours by in situ generated oestrogen is more significant than the promotion of breast tumours by circulating oestrogens, as shown by cell culture, animal trials employing breast cancer cells that have been transfected with the aromatase enzyme, and transgenic mouse research.[4] The epidermal growth factor receptor (EGFR) is one of the novel anticancer drugs' first and most significant targets. Overexpression of EGFR is seen in about 50%

of triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) patients. Thus, EGFR inhibitors have been examined in various studies as a potential treatment for breast cancer. Various synthetic medicines effectively inhibited the expression of EGFR.[5]

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)has been shown to be overexpressed in breast, lung, kidney, pancreatic, cervical, and skin carcinomas as compared to normal tissues. A widely prevalent molecule in humans called glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is frequently employed as an endogenous reference gene in investigations of quantitative gene expression.[6]

The purpose of the study is to assess the effectiveness of synthesised chalcone derivatives against breast cancer lines through a gene expression analysis using GAPDH as the reference.

MATERIAL AND METHODS

Experimental Details

Protein Preparation

The RCSB PDB website was used to obtain the target's crystal structure of Aromatase (PDB ID: 3S7S) and EGFR (PDB ID: 3POZ)

Ligand Preparation

CHEMSKETCH was used to prepare the ligand's structure

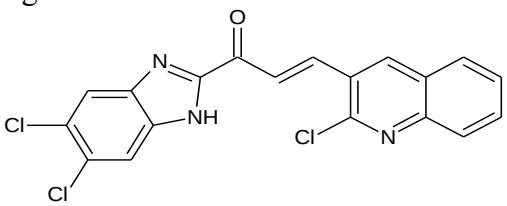

Fig no.1: (2*E*)-3-(2-chloroquinolin-3-yl)-1-(5,6-dichloro-1*H*-benzimidazol-2-yl) prop-2-en-1-one

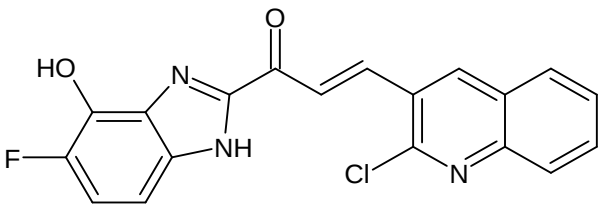

Fig no.2: (2*E*)-3-(2-chloroquinolin-3-yl)-1-(5-fluoro-4-hydroxy-1*H*-1,3benzimidazol-2-yl) prop-2-en-1-one

Docking software

The ligands were docked with Schrodinger (Maestro version 13.1.141)

RNA isolation

RNA isolation from tissues (100mg) was performed by using Trizol (Thermo Scientific) by following the manufacturer's protocol. The integrity of isolated RNA was checked on agarose gel (1.5% w/v).

cDNA preparation

Preparation of cDNA was done by using PrimeScript™. 1st strand cDNA Synthesis Kit(Takara, Cat No. 6110A) by following the manufacturer's protocol. 2 microgram total RNA along with the equal amount of random hexamers and oligo dT primers were used for synthesis of cDNA.

Reverse Transcriptase PCR conditions

cDNA Synthesis 42°C for 60 mins at 1 Cycle, Enzyme Inactivation 95°C for 5 mins at 1 Cycle

Primer details

Gene GAPDH
Forward (5`-3`)
CGGAGTCAACGGATTTGGTCGTAT
Reverse (5`-3`)
AGTCTTCTCCATGGTGGTGAATAC
Product (bp) ~300
Gene Aromatase
Forward (5`-3`)
GAATATTGGAAGGATGCACAGACT
Reverse (5`-3`)
GGGTAAAGATCATTTCCAGCATGT
Product (bp) ~300
Gene EGFR
Forward (5`-3`)
CGAAGGCGCCACATCGTTC
Reverse (5`-3`)
GAGGAGATCTCGCTGGCAG
Product (bp) ~340

PCR conditions

Reaction Mixture (10 µl), Template DNA 10 mg, Forward Primer 0.25 µM, Reverse Primer 0.25 µM, Emerald Amp Master Mix 5 µl, Nuclease Free Water Volume makeup 10 µl, Initial Denaturation 2 minutes at 98°C for aromatase and 30 seconds at 98°C for EGFR. Annealing 30 seconds at 59.2°C for Aromatase,

30 seconds at 59.2°C for EGFR and 30 seconds at 59.2°C for GAPDH, Extension 20 seconds at 72°C and Final Extension 5 minutes at 72°C

Gel Analysis of Amplified Products

PCR product was analyzed in 1.5% agarose gel electrophoresis. Gel was run for 30 mins at 90V.

Gel Imaging and Densitometric Analysis of the Data

Gel images were capture in gel documentation system and densitometric analysis was carried out using Image J software, (from NCBI). Data were normalized with the GAPDH expression.

RESULTS AND DISCUSSION

Molecular Docking

Various chalcone derivatives were selected for docking research against the targets aromatase and EGFR. The studies were conducted using Schrodinger software. According to the docking data, Compound A32 exhibited the highest binding energy in aromatase, with a binding energy of 8.85 kcal/mol, whereas Compound A7 demonstrated good binding energy (8.11 kcal/mol) in EGFR. So, it was decided to use compound A7 to conduct a gene expression study against EGFR and compound A32 to do so against aromatase.

Gene expression study

The rate-limiting enzyme in oestrogen production is aromatase. A number of benign and malignant hormone-dependent diseases, such as breast and endometrial malignancies, as well as their initial development and progression, are largely influenced by oestrogen.[7]

The gene expression investigation was conducted using compound A32 based on the docking study. The compound A32 and the reference standard glyceraldehyde-3-phosphate dehydrogenase were selected for the analysis of aromatase gene expression using the MCF-7 cell line (GAPDH). RT-PCR studies showed that the compound A32 was not down regulated the enzyme as expected. The findings shown in figures No. 3 and 4.

EGFR is found in a wide range of human cancers, including lung, head and neck, colon, pancreatic, breast, ovary, bladder, and kidney tumours, as well as gliomas. In several human malignancies, EGFR expression and cancer prognosis have been studied.[8]

In research on the EGFR gene's expression, the A549 cell line was employed. A549 cell line and the reference standard glyceraldehyde-3-phosphate dehydrogenase were used to evaluate the molecule A7's effect on EGFR gene expression (GAPDH). The enzyme was downregulated by compound A7, according to RT-PCR research. The results depicted in the figure no.5&6.

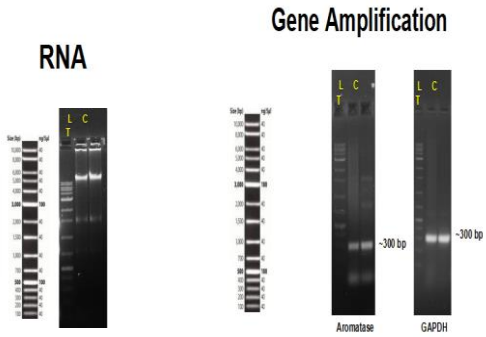

Fig no.3: Aromatase Gene Expression Analysis in MCF-7 Cell Line

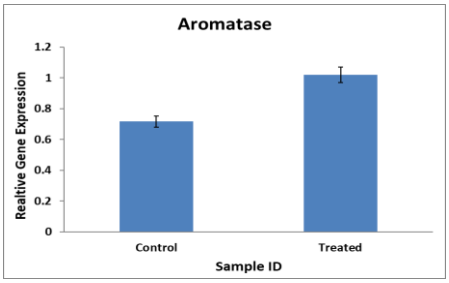

Fig no.4: Graphical representation of aromatase treated with compound A32

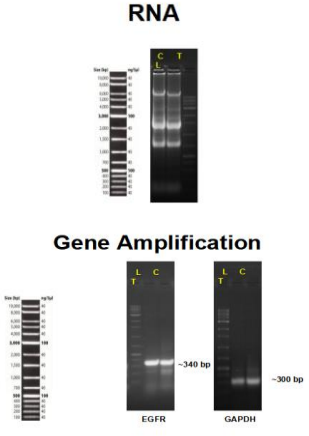

Fig no.5: EGFR Gene Expression Analysis in A549 Cell Line

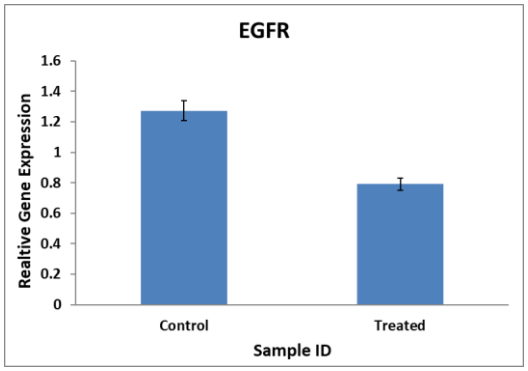

Fig no.6: Graphical representation of EGFR treated with compound A7

CONCLUSION:

The purpose of the study was to find the best chalcone targets for the development of selective aromatase modulators or inhibitors and to prevent the synthesis of oestrogen in the targeted tissues by studying the response of aromatase gene regulation in breast cancer cell lines (MCF-7). Moreover, the impact of epidermal growth factor gene regulation in breast cancer cell line (A549) was also studied. Findings of RT-PCR showed that compound A7 was efficient at inhibiting the gene expression of epidermal growth factor, whereas compound A32 did not provide the desired results. So, the compound A7 will be the target of our future investigations. The efficiency of the compound can be further assessed using an *invivo* model.

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