

A STUDY ON MOLECULAR TARGETED APPROACHES TO CANCER THERAPY AND THE ROLE OF CHALCONES IN CHEMOPREVENTION

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ABSTRACT

Cancer is a widely spreading disease all over the world and the second most leading cause of mortality worldwide. In the broader sense cancer refers to more than 277 different types of diseases. Scientists have identified different stages of cancers, indicating several gene mutations which are involved in cancer pathogenesis. These gene mutations lead to abnormal cell proliferation. Genetic disorders caused by inheritance or inheritance factors have a pivotal role in the increase of cell growth. With the assistance of technological advances in molecular techniques and bioinformatics extra information can be obtained which can be useful for early diagnosis and proper treatment. In recent years, carcinogenesis mechanisms have been detected by molecular genetic studies. Consumption of fruits, vegetables, spices, cereals and pulses has been associated with lower incidence of cancer and other chronic diseases, but how these dietary agents and their active ingredients minimize these diseases, is not fully understood. Whether it is oranges, hops, water-lily, licorice, wax apple or mulberry, they are all connected by a group of aromatic ketones, called chalcones (1,3-diaryl-2-propen-1-ones). Chalcones are a group of polyphenolic compounds derived from plants which belong to the flavonoids family and own a wide variety of modulatory and cytoprotective functions. They have been linked with anti-bacterial, anti-fungal, anti-inflammatory, anti-oxidant, anti-cancer and anti-diabetic activities. Immunoblot assay showed that chalcone decreased the expression of cyclin B1, cyclin A and Cdc 2 protein, as well as increased the expression of p21 and p27. The current review, however, deals with the role of various chalcones in biologically, pharmacologically, and medicinally important entities.

Keywords: Cancer, Chalcone, Gene Mutations, Biological, Pharmacological Activities, Molecular Targets.

1. INTRODUCTION

In the world, cancer remains a major cause of mortality. Despite great progresses made in understanding the molecular basis of cancer, the progress in cancer detection and treatment,

mortality is still high and there still is not a cure. Great improvements have been made in therapies but still the prevalence of cancer has actually increased. In India alone, approximately 1,665,500 people suffered from cancer, and 585,700 of them died due to this disease by 2019. Therefore, cancer is a solemn problem affecting the health of humans in all societies. Unfortunately, it is a combination of various diseases occurring at the tissue level and this variety is a major challenge for its specific diagnosis, followed by efficacy of treatment. In men, the highest percentages of cancer types occur in the prostate, lung, bronchus, colon, rectum, and urinary bladder respectively. In women, cancer prevalence is highest in the breast, lung, bronchus, colon, rectum, uterine corpus and thyroid, respectively. This data indicates that prostate and breast cancers constitute a major portion of cancer in men and women, respectively. For children, the highest percentage types of cancer disease are related to the brain, lymph nodes, and blood respectively. Cancer occurs due to a series of successive mutations in genes, as a result of these mutations cell functions changes. Chemical compounds play an evident role in gene mutations and formation of cancer cells. For instance, smoking involves several carcinogenic chemical compounds that lead to lung cancer. Environmental chemical substances with carcinogenic properties influence indirectly or directly the cytoplasm and nucleus of cells, and leads to genetic disorders and gene mutations.

Chalcones (trans-1,3-diaryl-2-propen-1-ones) are α,β -unsaturated ketones consisting of two aromatic rings (ring A and B) having diverse array of substituents. The skeleton of chalcone molecule consists of two aromatic rings linked by an aliphatic three-carbon chain. The two rings of chalcone are joined by a highly electrophilic three-carbon α, β -unsaturated carbonyl system that assumes linear or nearly planar structure. They possess conjugated double bonds and a completely delocalized π -electron system on both the aromatic rings.

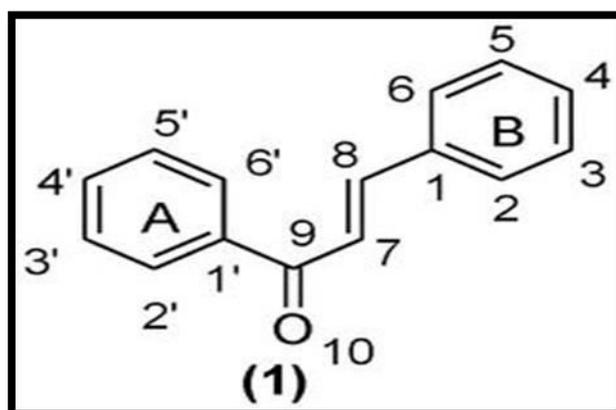


Figure 1: Structure of Chalcone

Chalcones, named so by Tambor and Kostanecki, are commonly known by different names such as benzylideneacetophenone, β -phenylacrylophenone, phenyl styryl ketone and α -phenyl- β -benzoylethylene, etc. and constitute the central core of biologically active heterocyclic compounds. Chalcones constitute good source for a variety of novel heterocycles of good pharmaceutical profile and high therapeutic potential. Chalcones themselves are identified as interesting entities associated with several biological activities. The structural modifications of the chalcone rings have led to a high degree of

diversity that has proven useful for the development of new medicinal agents, and thus chalcones have become an object of continued interest in both academia and industry. The chalcones are well documented for a broad spectrum of biological activities including anti-microbial, anti-cancer, cytotoxic, anti-oxidative, anti-inflammatory, anti-viral and others. Currently, chalcone derivatives have been widely used for the treatment of cardiovascular diseases, stomach cancer, viral disorders. Also used as cosmetic formulation ingredients and food additives.

2. BIOLOGICAL ACTIVITIES OF CHALCONES

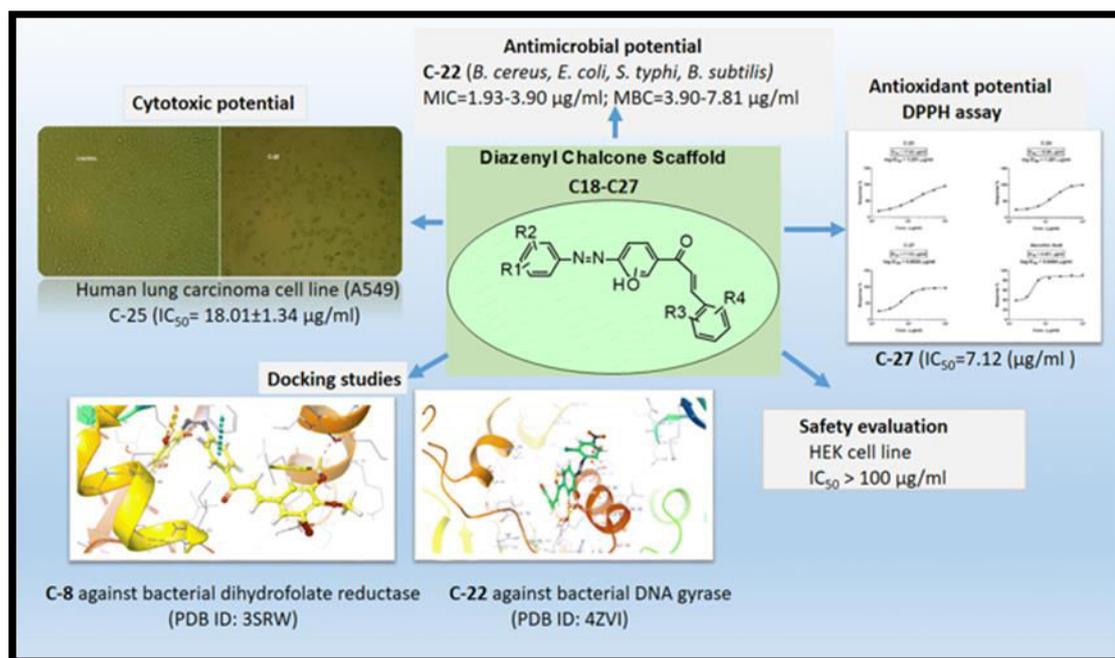


Figure 2: Biological Activities of Chalcones

I. ANTI-MICROBIAL CHALCONES

Anti-microbial agents are the agents used to treat infectious diseases caused by different types of microbes such as bacteria and fungi. These drugs are commonly used for research purposes and efforts are put by the scientific community to search for newer anti-microbial agents due to anti-microbial resistance (AMR) shown by the microbes. Gene transfer, phenotypic change, selective pressure and mutation are some of the causes behind AMR. Drug resistance is commonly developed by all types of microbes including bacteria, fungi, parasites, viruses, when the microbe does not respond to a drug that previously treated them effectively. This AMR can lead to several issues including difficulty in longer stay of the microbes in the host, controlling the disease, spreading at a higher risk, and increase in mortality rates.

Globally, Infectious diseases are one of the common problems that has been encountered. Though several marketed drugs are available on a commercial scale, the search for new drug molecules has become essential for the treatment of various infectious diseases.

Consequently, the search for new anti-microbial agents becomes essential. The recent updates in the search of chalcones as an attempt to develop anti-microbial agents is the point of discussion.

Methoxy-4'-amino chalcones showed good *in vitro* anti-microbial activities against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. A molecular docking study also supported the observed results showing good results.

The quinoxaliny chalcones synthesized by the Claisen-Schmidt condensation were found to be good anti-microbial agents. The anti-microbial studies were carried out against *Staphylococcus aureus* and *Escherichia coli*.

Talniya and Sood documented the synthesis and anti-bacterial activity of chalcones against *Bacillus subtilis* bacteria and *Aspergillus niger* fungi by disk diffusion method. The chalcones possessing o-chloro, p-chloro, and p-hydroxyl substituents showed remarkable anti-microbial activity against the screened microbes.

II. ANTI-CANCER CHALCONES

Cancer therapy is a complicated process as the drugs used on target human cells, even though cells that have undergone genetic changes and are dividing at a fast and uncontrolled rate. However, only a few anti-cancer drugs are able to discriminate between normal tissue cells and cancer cells to a large extent. Thus, there has been a constant need to develop a synergistic or an alternative anticancer drugs with minimal side effects. This part of the present chapter highlights recent developments and significance in chalcones used as anti-cancer agents:

The design, synthesis, and anti-tumor potential of chalcones were studied against human breast adenocarcinoma MCF-7 cells in a concentration-dependent manner. They triggered significant changes in biochemical/molecular parameters, cell morphology and revealed the nature of apoptosis inductor of the labelled compounds and their application as an optimistic alternatives for the treatment of neoplasia, especially in terms of drug resistance development.

Apoptosis is the principle phenomenon, which affects many diseases, such as Alzheimer's disease and cancer. Chalcones can induce apoptosis of human lung, hepatic cancer cells and also cause hindrance in cancer cell migration and invasion.

The bis-chalcone derivatives were studied for their ability to inhibit growth inhibitory activity against MCF-7, caco-2 human cancer cell lines and xanthine oxidase *in vitro*. The bis-chalcone with fluoro group at the 2nd or 2, 5th position of B-ring was found to be a potent inhibitor of the enzyme possessing IC₅₀ values in the low micromolar range. The activities of the compounds were found to be around seven times higher than the standard allopurinol.

Some novel Pt(IV) complexes of chalcone analogs were synthesized and evaluated for anti-proliferative activity by using MTT assay. The *in vitro* evaluation revealed that all Pt(IV) complexes showed good activity against the three human cancer cells.

Chalcones were studied for anti-proliferative activities against the human cervical (HeLa), ovarian (Caov-3), TRAIL-resistant breast (MCF-7, MDA-MB-231), T-lymphoblastoid (CEM-SS), lung (A549), liver (HepG2), colorectal (HT-29), nasopharyngeal (CNE-1), and erythromyoblastoid (K-562) cancer cells by Mai.

Chalcones were studied to inhibit the proliferation of MDA-MB-231 and MCF-7 by blocking cell cycle progression in the G2/M phase or by inducing apoptosis. Immunoblot assay exhibited that chalcones can significantly decrease the expression of cyclin A, cyclin B1 and Cdc2 protein, and can increase the expression of p53 and p23 in a p27-independent manner, contributing to cell cycle arrest.

III. ANTI-OXIDANT CHALCONES

Anti-oxidants are the compounds that inhibit the oxidation process. These substances can prevent or slow damage to cells caused by free radicals. Oxidation is a chemical reaction that generates free radicals, thereby leading to chain reactions which may damage the cells of organisms and hence responsible for oxidative stress resulting in chronic diseases such as heart diseases, stroke, cancer, arthritis, respiratory diseases, Parkinson's disease, and other inflammatory conditions.

Cao et al. documented a series of 4'-OH-flurbiprofen-chalcone hybrids and evaluated them as prospective multifunctional agents for the management and cure of Alzheimer's disease. Besides, the compounds were reported for good biometal chelating abilities, *in vitro* anti-neuroinflammatory activities, antioxidant activities and MAO inhibitions.

The chalcone derivatives were synthesized by the Claisen-Schmidt condensation with KOH in ethanol at room temperature under sonication conditions and screened for anti-oxidant potential by Polo et al.

IV. ANTI-INFLAMMATORY CHALCONES

Anti-inflammatory drugs are the drugs which are used to reduce pain and inflammation. Generally referred to pain-relieving drugs. These drugs mainly act by inhibiting the cyclooxygenase enzymes that produce prostaglandins namely COX-1 and COX-2. Herein we discuss some of the efforts for the development of chalcone-based heterocycles as effective anti-inflammatory compounds.

α -Substituted 2',3,4,4'-tetramethoxychalcones and were evaluated for their ability to modulate inflammatory responses to influence on nitric oxide synthase, cytokine and heme oxygenase-1-expression levels. Anti-inflammatory activity was correlated with thiol-alkylating activity, such as stronger electrophiles substituted with CF₃, Br, and Cl were found to be more potent than the remaining derivatives.

Pyrazole- and morpholine-containing chalcones were reported for anti-inflammatory activity by Gadhave and Uphade. The anti-inflammatory activity performed by carrageenan-induced rat paw edema method showed good potency of some of the tested compounds as compared with the standard diclofenac drug.

3. REVIEW METHOD

Initially, we searched research papers using keywords such as cancer and molecular process, cancer and treatment and molecular aspects. Subsequently, the papers that matched such word criteria were fully reviewed and their findings duly noted.

I. MOLECULAR TARGETS OF CHALCONES

Genetic changes that lead to oncogene generation and genetic disorders include amplification (N-myc in neuroblastoma), chromosomal translocation (oncogene Abl and gene Bcr in chronic blood cancer), deletion (Erb-B gene in breast cancer), insertion/activation (C-myc in acute blood cancer) and point mutation (Ras gene in colon cancer). Chronic blood cancer is common in the elders due to exchange of genetic material between chromosomes 9 and 22. This condition leads to production of biomarker termed as p115, which has been located in 95% of patients and can expedite a correct diagnosis. The connection of Bcr gene to Abl oncogene results in creation of a new combination of gene that can be translated to protein with kinase activity.

Mutation in the p53 gene leads to the emergence of an unusual protein that has an important role in disturbance of molecular process related to p53. Abnormality of these molecular and biological events results to formation of cancer cells; therefore, the p53 gene has a vital relationship with cancer and it has been reported that p53 abnormality occurs in 60% of cancer cases. Under normal conditions, p53 plays an important role in cell division, cell death, senescence, angiogenesis, differentiation, and DNA metabolism. Moreover, a large number of mutations related to the p53 gene takes place in the DNA-binding position, and the disorder of genes is controlled by p53 for replication. A cooperative behaviour between p53 and CDK1-P2, CDC2 retains the cancer cells in G1 and G2 phases of cell cycle. In fact, p53 is either an inhibitor or a promoter of cancer cells. The anti-cancer action of p53 is active during three routes stimulating, for DNA repairing proteins, induction of apoptosis, and arresting of cell cycle in G1/S phase.

The Chalcone molecule or scaffold has been an obsession among researchers in the 21st century due to its uncomplicated chemistry, simplicity in pattern of synthesis and an extensive variety of propitious biological activities. Several (semi) synthetic and (non-synthetic) natural chalcones have shown anti-cancer activity due to their inhibitory action against various targets namely aromatase, 5 α -reductase, ABCG2/P-gp/BCRP, VEGF, VEGFR-2 kinase, CDC25B, cathepsin-K, 17 β -hydroxysteroid dehydrogenase, proteasome, HDAC/Sirtuin-1, MMP-2/9, JAK/STAT signaling pathways, tubulin, topoisomerase-II, Wnt, NF- κ B, B-Raf and mTOR etc. In this review, a comprehensive study on molecular targets/pathways involved in mechanism of actions (MOAs), carcinogenesis and structure activity relationships (SARs) have been highlighted.

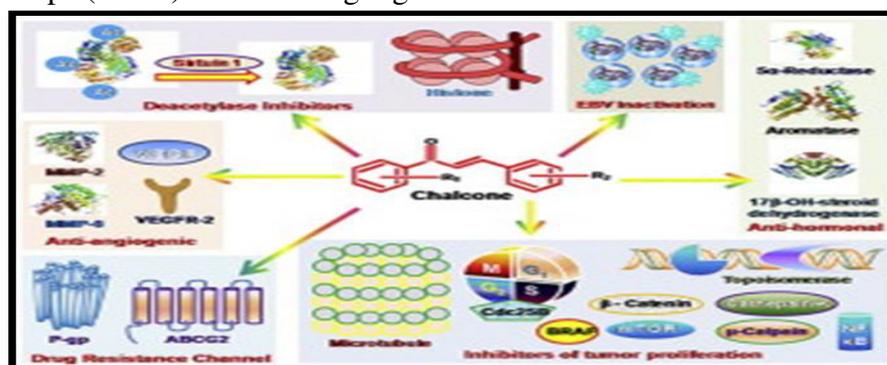


Figure 3: Molecular Targets of Chalcones

Literature on anti-cancer chalcones highlights the employment of three pronged strategies, namely, replacement of aryl rings with heteroaryl scaffolds, molecular hybridization through conjugation with other pharmacologically interesting scaffolds and structural manipulation of both aryl rings for enhancement of anti-cancer properties. Substitutions on both aryl rings (A and B) of the chalcones depend upon their positions in the aryl rings and appear to influence anti-cancer activity by interfering with various biological targets. Likewise, heterocyclic rings either as ring A or as ring B in chalcones can also influence the anti-cancer activity displayed by this class of compounds. Hybrid chalcones are formulated by chemically linking chalcones to other prominent anti-cancer scaffolds such as imidazolones, benzothiazoles, benzodiazepines and have demonstrated synergistic pharmacological activities.

II. ACTION OF CHALCONE ON CELL CYCLE

Chalcones were exploited well for their wide application in pharmacological area. It is reported that chalcones have several advantages such as low risk of mutagenicity and poor interaction with DNA. Some clinically useful anti-cancer drugs have reported genotoxicity due to their interaction with amino groups in nucleic acids. Chalcones are devoid of these side effects due to their structural flexibility. Literature reveals that natural and synthetic chalcones are desirable to elicit cytotoxic and apoptotic activity. For this reason, chalcones have been well documented.

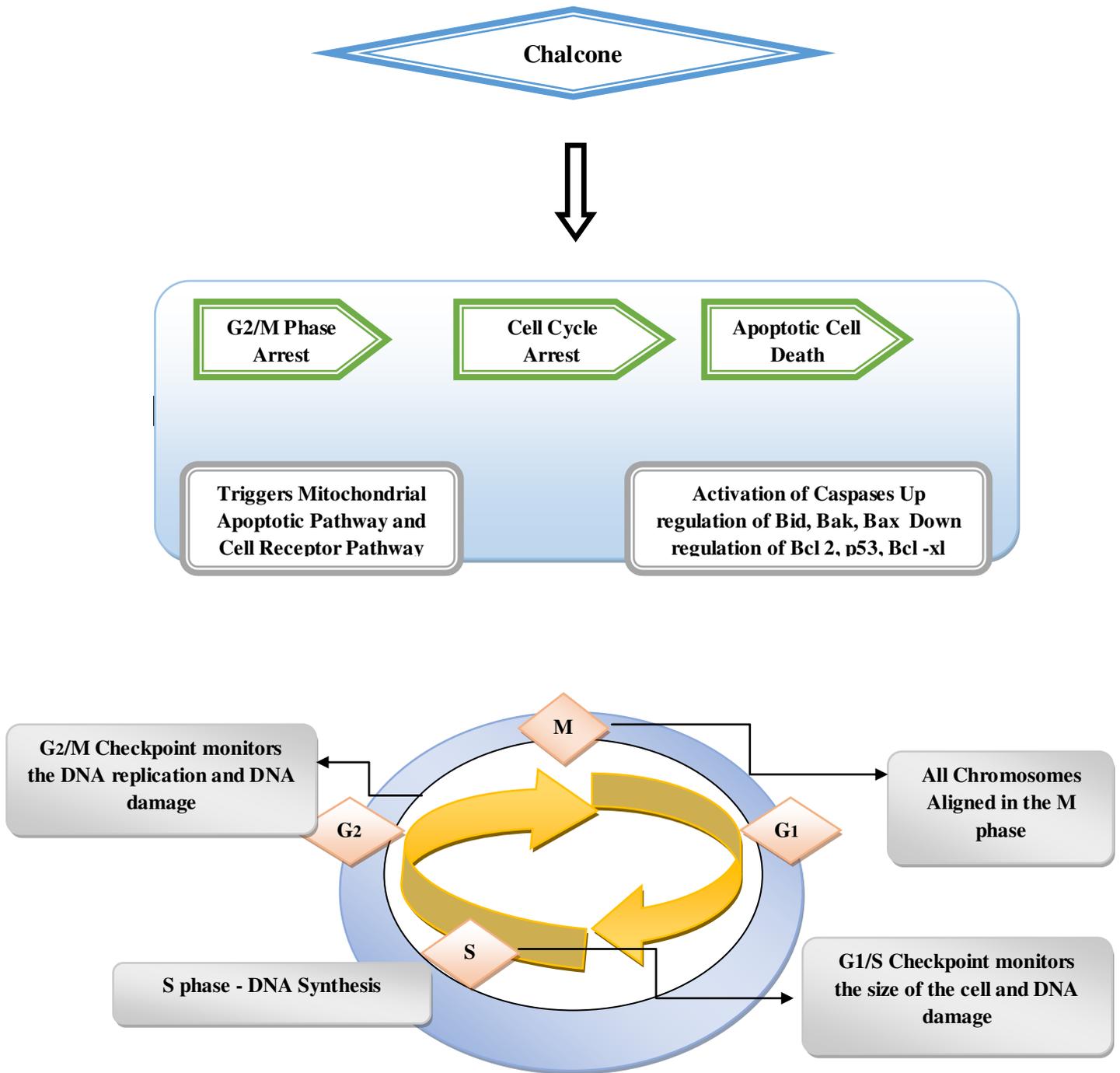


Figure 4: Action of Chalcone on Cell Cycle

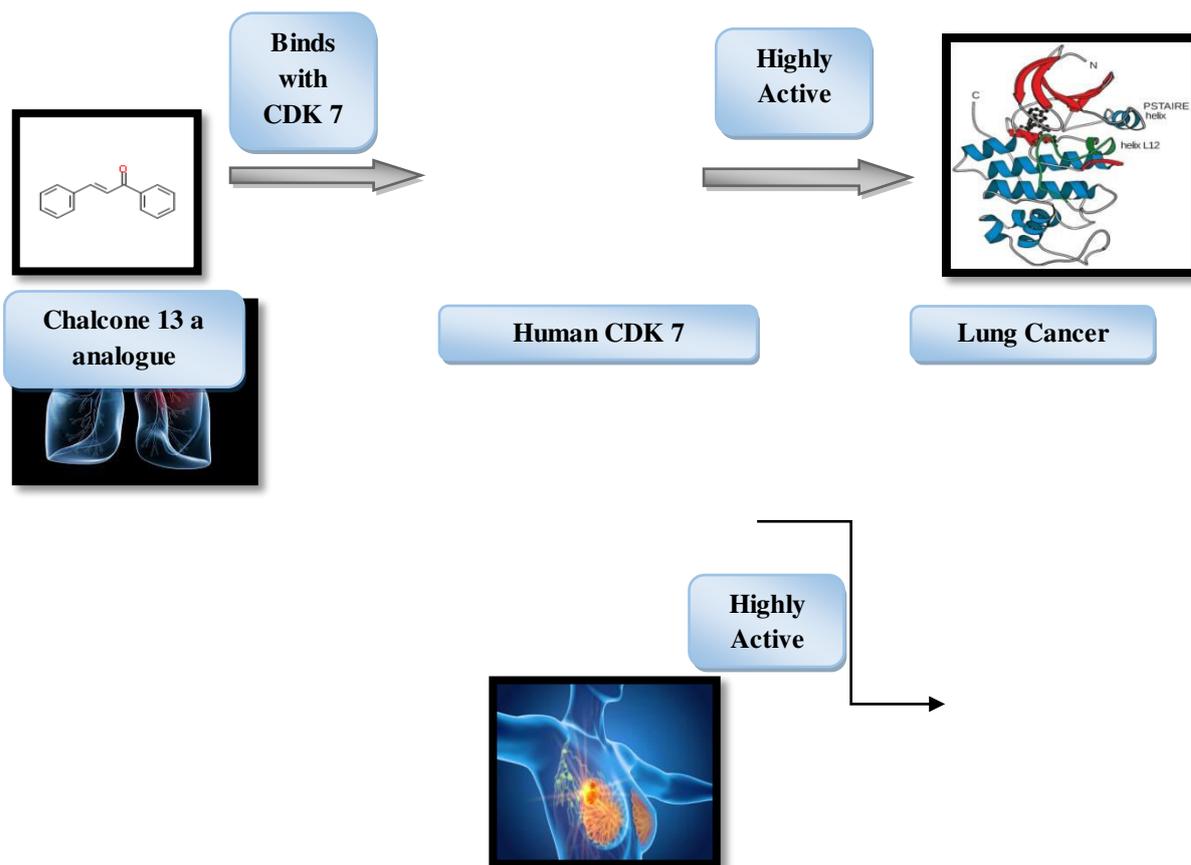


Figure 5: Chalcones Against Lung and Breast Cancer

IV. Chalcone Against Cathepsin B and Cathepsin H Obtained From Goat Liver

Cathepsins have emerged as a potential target for chemoprevention and anti-cancer therapy. Elevated levels of cathepsin B and cathepsin H in a variety of tumors have expressed their contribution towards metastasis and invasion. Molecule 38 has been found to be potent among the synthesized inhibitors, which inhibits cathepsin B and H. The K_i (inhibition constant) value against cathepsin B was 6.18×10^{-8} M and 2.8×10^{-7} M for cathepsin H. Nitrosubstitution is helpful for an effective interaction with cathepsins H and B. Its inhibition to cathepsin H as compared with cathepsin B reveals that cathepsin B's active site is more vulnerable to these compounds when compared with cathepsin H. The results were found to be steady and rational when compared with in silico docking studies.

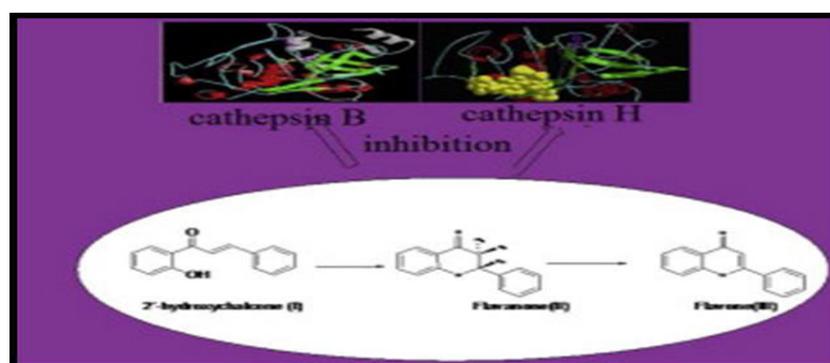


Figure 6: Chalcones Against Cathepsin B and Cathepsin H

V. GENES ASSOCIATED WITH HEREDITARY CANCER BY CANCER TYPE

Types of Cancer	List of Gene Mutations
Breast Cancer	ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NBN, NF1, PMS2, STK11, TP53
Ovarian/Fallopian Tube Cancer	BRCA1, BRCA2, BRIP1, RAD51C, RAD51D, EPCAM, MLH1, MSH2, MSH6, PMS2, STK11
Pancreatic Cancer	BRCA1, BRCA2, EPCAM, MLH1, MSH2, MSH6, PMS2, TP53, STK11
Prostate Cancer	BRCA1, BRCA2, CHEK2
Melanoma	BRCA1, BRCA2, PTEN
Uterine Cancer	EPCAM, MLH1, MSH2, MSH6, PMS2, PTEN, STK11
Colon Cancer	CHEK2, EPCAM, MLH1, MSH2, MSH6, PMS2, PTEN, STK11, TP53
Gastric Cancer	CDH1, STK11

VI. OVERVIEW OF VARIOUS GENES AND THEIR LOCATIONS

Name of Genes	Location
ATM	Chromosome 11
BARD1	Chromosome 2
BRCA1	Chromosome 17
BRCA2	Chromosome 13
CDH1	Cadherin 1
CHEK2	Chromosome 22
PTEN	Chromosome 10
EPCAM	Chromosome 2
MLH1	Chromosome 3
MSH2	Chromosome 2
MSH6	Chromosome 2
PALB2	Chromosome 16
TP53	Chromosome 17
STK11	Chromosome 19

5. CONCLUSION

The architecture of chalcone molecule has been linked with various substitutions, such as amino, hydroxyl, methoxy and a slight alteration of both rings has been found to be productive in fabricating it as a prospective candidate in the anti-cancer therapeutic armamentarium. The literature studies showed magnificent work in cytotoxic activities of chalcones in the different cell death pathways. Inhibitory concentrations in nanomolar range were impressive to show its ability to arrest cell division. Furthermore, besides preclinical study, clinical study of chalcone may yield the development of research in this field. It is expected that this review may give the medicinal chemists a basis to get in touch with the recent updates and will be helpful for enrichment in this field.

ACKNOWLEDGEMENTS

Behind every success there are lots of efforts, but efforts are fruitful due to hands making the passage smoother. I express my deep sense of gratitude for hands, people extended to me during my work.

Firstly, I offer my adoration to God Almighty who created me, gave me the strength and courage to complete my project and gave me the opportunity to thank all those people through whom his Grace was delivered to me.

I pay tribute to My Parents for lifting me up till this phase of life. I thank them for their love, trust, patience, support and bearing all kind of stress to make me what I am.

Last but not least I express my sincere thanks to one and all who gave encouragement and helped me directly or indirectly throughout my educational career.

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