CHAPTER 1

INTRODUCTION
1.1. Introduction:

Discovery of new chemical entities that combat diseases and disorders to improve the life span of human population has been the primary objective of the medicinal chemists. According to WHO estimates, inflammatory disorders and cancer are the most prevalent diseases that are affecting the world population. The origin for these conditions is mostly attributed to the present day lifestyle of the people and changing environmental conditions. Inflammation, free radical damage and oxidative stress are the major health concerns in recent years and as such they have become the priority subject areas for research currently around the world.\textsuperscript{1}

1.2. Inflammation:

Inflammation is a complex biological response in vascular tissues against the harmful stimuli, such as pathogens, damaged cells, or irritants.\textsuperscript{2} It is a protective mechanism adopted by the organisms to remove the injurious stimuli and to initiate the healing process. Hence, inflammation is considered as an essential survival strategy adopted by the body to ward off major damage. However, inflammation can also lead to a host of diseases, such as hay fever, atherosclerosis, rheumatoid arthritis and even cancer.\textsuperscript{3}

Inflammation can be classified as either \textit{acute} or \textit{chronic}. \textit{Acute inflammation} is the initial response of the body to tissue damage. A cascade of biochemical events propagate and mature into an inflammatory response, which involve wide range of chemical mediators and various cells within the injured tissue to alter local vascular and immune responses. The resident inflammatory cells such as macrophages, mast cells and dendrite cells at the site of injury undergo activation following the onset of tissue damage and release a host of inflammatory mediators including tumor necrosis factor $\alpha$ (TNF$\alpha$), interleukin 1$\beta$ (IL-1$\beta$) and a variety of chemokines.

The cytokines TNF$\alpha$ and IL-1$\beta$ form an important part of the inflammatory response of the body against infection by penetrating into nearby blood vessels and increasing the expression of adhesion factors ICAM-1 and VCAM-1 on endothelial
cells of vascular endothelium. These adhesion molecules allow the attachment of leukocytes (white blood cells) to the endothelium and enable their subsequent transmigration into peripheral tissue.

The leukocytes include granulocytes (neutrophils, basophils and eosinophils) and agranulocytes [lymphocytes (T cells, B cells and natural killer cells), monocytes and macrophages]. The chemokines play a major role by acting as chemo attractant and guiding the inflammatory cells to sites of inflammation (chemotaxis). Monocyte Chemo attractant Protein-1 (MCP-1), Rantes, Eotaxin and IL-8 are some important chemokines. The circulating leukocytes detect a small concentration of chemokines and follow a signal of increasing concentration (concentration gradient) towards the source of chemokines, i.e. site of injury.

The leukocytes so recruited from the lumen of the blood vessel to the site of injury elicit an immune response and make a coordinated effort to resolve injury or damage. NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of genes involved in cellular responses to stimuli through the expression of pro-inflammatory genes including cytokines, chemokines and adhesion molecules. It contributes to the feedback control of inflammation by various mechanisms to affect the magnitude and duration of the inflammatory response.

Tumor necrosis factor-alpha (TNFα) is a central regulator of inflammation and as such TNFα antagonists may be effective in treating inflammatory disorders in which TNFα plays an important pathogenetic role. Acute inflammation is a common inflammatory disorder with incidences that have increased significantly over the past few decades. Prolonged inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. The prolonged inflammation leads to the onset of chronic inflammatory disorders.
Introduction

Chapter -1

Figure 1.1: Inflammation pathways mechanism

In acute inflammation, the cellular phospholipases are activated to break down membrane phospholipids into arachidonic acid, which in turn is metabolized to inflammatory prostaglandins and leukotrienes respectively by the enzymes cyclooxygenase (COX) and lipoxygenase (LOX) as depicted in Figure 1.1. The formations of these inflammatory mediators aggravate the inflammation by causing vasodilation, increased permeability and bronchoconstriction and platelet aggregation. The formation of prostaglandins and leukotrienes from arachidonic acid can be suppressed by inhibiting cyclooxygenase and lipoxygenase respectively.

The search for inhibitors of these enzymes thus forms the basis for development of new anti-inflammatory agents. In the enzyme family of cyclooxygenases, inhibition of cyclooxygenase-2 (COX-2) is more desirable. However, recent studies revealed that selective inhibition of COX-2 does reduce
inflammation, but causes side effects, particularly those leading to cardiovascular complications. On the contrary, the inhibition of the 5-lipoxygenase (5-LOX) enzyme of the alternative pathway of inflammation not only reduces inflammation but also improves cardiac health by reducing risk of atherosclerosis. The alternative pathway mediated by 5-LOX has thus become an important target for the development of new anti-inflammatory drugs.

1.2.1. Inflammatory drug compounds:

Hence the compounds that can modulate the activity or expression of inflammatory enzymes, cytokines and chemokines have the potential to be useful as anti-inflammatory drugs. The anti-inflammatory drugs currently in use may be of synthetic or natural or biological origin. Some of important anti-inflammatory drug compounds 1.01 to 1.33 of synthetic origin are summarized in Figure 1.2.
Introduction

Chapter - 1

Flubiprofen (1.10)

Oxaprozin (1.11)

Loxoprofen (1.12)

Indomethacin (1.13)

Tolmetin (1.14)

Sulindac (1.15)

Etodolac (1.16)

Ketorolac (1.17)

Diclofenac (1.18)

Nabumetone (1.19)
Introduction

Chapter -1

Meloxicam (1.21)

Meloxicam (1.21)

Lornoxicam (1.23)

Lornoxicam (1.23)

Mefenamic acid (1.25)

Mefenamic acid (1.25)

Flufenamic acid (1.27)

Flufenamic acid (1.27)

Celecoxib (1.28)

Celecoxib (1.28)

Rofecoxib (1.29)

Rofecoxib (1.29)
1.2.2. Anti-inflammatory agents of natural origin:

Because of the undesired toxic effects associated with steroidal and non-steroidal anti-inflammatory drug (NSAID) medications, there has been a sustained urge for natural compounds, such as dietary supplements and herbal remedies, which have been used for centuries to reduce pain and inflammation. Many of these natural compounds also work by inhibiting the inflammatory pathways in a similar manner as NSAIDs. In addition to the COX and LOX pathways, many natural compounds act by modulating the inflammatory pathways. Herbal medications are becoming increasingly popular because of relatively fewer side effects associated with them and their traditional use. The following is the summary of key anti-inflammatory agents of natural origin.

Omega-3 polyunsaturated fatty acids are some of the very important natural anti-inflammatory agents known. With the discovery that vascular inflammation is the underlying cause of coronary artery disease, fish and fish oil supplements are now recommended by the American Heart Association for the prevention of heart
diseases. The active ingredients in fish oil are eicosapentaenoic acid (EPA, 1.35) and docosahexaenoic acid (DHA, 1.36).

Curcuminoids (1.37 to 1.39) is a naturally occurring yellow pigment derived from turmeric (Curcuma longa), a flowering plant of the ginger family. Curcumin has long been used in both Ayurvedic and Chinese medicines as an anti-inflammatory agent. Curcumin has also been suggested as a treatment for colitis, chronic neurodegenerative diseases, arthritis, and cancer. Curcumin is known to inhibit inflammation by suppressing NF-κB and restricting various activators of NF-κB.

Green tea has long been recognized to have cardiovascular and cancer preventative characteristics due to its anti-oxidant properties. Its use in the treatment of arthritic disease as an anti-inflammatory agent has been recognized more recently. The constituents of green tea are polyphenolic compounds called catechins and epigallocatechin-3-gallate (EGCG), EGCD is the most abundant catechin in green tea. Epigallocatechin-3-gallate (1.40) suppresses IL-1β and attenuates activation of the transcription factor NF-κB. Green tea also inhibits the aggrecanases, which is known to degrade cartilage.

The Boswellia species are trees distributed in India, Ethiopia, Somalia and Arabian Peninsula, and they produce a gum resin called olibanum, popularly known in the western world as frankincense. This resin possesses anti-inflammatory, anti-arthritic and analgesic properties. Boswellic acids are known to be the active constituents of Boswellia species and 3-O-acetyl-11-keto-β-boswellic acid (AKBA, 1.41) was found to be most potent among the six natural boswellic acid congeners. Boswellic acids inhibit the leukotriene biosynthesis in neutrophilic granulocytes by inhibiting 5-LOX. Boswellia gum resin and its extracts also demonstrated significant therapeutic improvements in human clinical trials confirming the in vivo anti-inflammatory effects.

Resveratrol (1.42) is a plant-based polyphenol molecule found in various plant sources, most prominently in Japanese Knot weed or Polygonum cuspidatum and skins of red wine grapes. The anti-inflammatory properties of resveratrol were
demonstrated in in vitro and experimental animal models with paw edema. Resveratrol is a potent inhibitor of COX and LOX enzymes and it is also a specific inhibitor of TNFα and IL-1β-induced NF-κB activation.

_Capsicum annum_ is a small spreading shrub which is grown throughout the world for its food usage. It is one of the most potent anti-inflammatory plant and numerous biological properties exhibited by _C. annum_ are attributed to the chemical entity called capsaicin (1.43). Capsaicin produces highly selective regional anesthesia by causing degeneration of capsaicin-sensitive nociceptive nerve endings which can produce significant and long-lasting increases in nociceptive thresholds. It also inhibits NF-κB, thus producing an anti-inflammatory effect.

_Zingiber officinale_ (Ginger) is the most widely used condiment with long history of medicinal use dating back to 2500 years. It is a supplement of choice for treating nausea, sickness and inflammation. The major active constituents of Ginger are gingerols (1.44 to 1.46), especially 6-gingerol (1.44). Ginger is an anti-oxidant and also an inhibitor of COX-2 inflammatory enzyme.

The natural anti-inflammatory agents including those described above are summarized in Table 1.1 and few important molecule structures are summarized in Figure 1.3.
Introduction

Chapter -1

Curcumin-I (1.37)

Curcumin-II (1.38)

Curcumin-III (1.39)

Epigallocatechin-3-gallate (1.40)

3-acetyl-11-keto-β-boswellic acid (1.41)

Resveratrol (1.42)

Capsaicin (1.43)

6-Gingerol (1.44)

8-Gingerol (1.45)
**Figure 1.3**: Structures of some major natural anti-inflammatory compounds (1.34-1.55).
Introduction

<table>
<thead>
<tr>
<th>S. No</th>
<th>Plant name</th>
<th>Active Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fish oil</td>
<td>Omega-3-fatty acids (1.34-1.36)</td>
</tr>
<tr>
<td>2</td>
<td>Curcuma longa</td>
<td>Curcuminoids (1.37-1.39)</td>
</tr>
<tr>
<td>3</td>
<td>Green tea</td>
<td>Epigallocatechin-3-gallate (1.40)</td>
</tr>
<tr>
<td>4</td>
<td>Boswellia serrata</td>
<td>3-O-acetyl-11-keto-β-boswellic acid (1.41)</td>
</tr>
<tr>
<td>5</td>
<td>Polygonum cuspidatum</td>
<td>Resveratrol (1.42)</td>
</tr>
<tr>
<td>6</td>
<td>Capsicum annum</td>
<td>Capsaicin (1.43)</td>
</tr>
<tr>
<td>7</td>
<td>Zingiber officinale</td>
<td>Gingerols (1.44-1.46)</td>
</tr>
<tr>
<td>8</td>
<td>White willow</td>
<td>Salicin (1.47)</td>
</tr>
<tr>
<td>9</td>
<td>Piper nigrum</td>
<td>Piperine (1.48)</td>
</tr>
<tr>
<td>10</td>
<td>Sphaeranthus indicus</td>
<td>7-Hydroxyfrullanolide (1.49)</td>
</tr>
<tr>
<td>11</td>
<td>Bone oil</td>
<td>Lutidine (1.50)</td>
</tr>
<tr>
<td>12</td>
<td>Citrus</td>
<td>Quercetin (1.51), Rutin (1.52), quercetrin (1.53)</td>
</tr>
<tr>
<td>13</td>
<td>Garcinia mangostina</td>
<td>- mangostin (1.54), γ - mangostin (1.55)</td>
</tr>
</tbody>
</table>

Table 1.1: Natural anti-inflammatory compounds and their source

1.2.3. Role of free radicals in inflammation:

The Reactive Oxygen Species (ROS) are other important mediators of inflammatory response. The common types of ROS include the hydroxyl radical (OH), the superoxide radical (O₂) and the lipid peroxyl radical (LOO•). Nitric oxide radical (NO•) is other important free radical in Reactive Nitrogen Species (RNO). The ROS are produced by several means, which include, 1) in mitochondria as bi-product of respiration, 2) at the endoplasmatic reticulum by cytochrome 450, 3) in the cytoplasm by Xanthine oxidase and 4) at the plasma membrane by NADPH oxidase and phospholipids.

Reactive oxygen species play important biological roles in cell signaling, a process termed redox signaling. The excess ROS are neutralized by cellular antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase. To maintain proper cellular homeostasis, a balance must be struck between reactive
Introduction

Chapter -1

oxygen species production and their consumption. In a compromised state associated with increased production of reactive oxidizing species or a significantly decreased effectiveness of anti-oxidant defenses, the cellular homeostasis is disturbed leading to a condition called oxidative stress.

Extensive research studies performed during the past two decades suggests that continued oxidative stress can lead to a disease condition called chronic inflammation. Chronic inflammation is a prolonged pathological condition characterized by mononuclear immune cell infiltration, tissue destruction and fibrosis. ROS act as a molecular trigger of the mechanism of inflammation. Oxidative stress mediates the activation of nuclear factors NF-κB and AP-1, which in turn induce the transcription of the genes promoting cytokine production. The release of these cytokines results in enhancement of the inflammatory response. The free radicals generated in the blood stream mainly from circulating neutrophils activate endothelial cells triggering induction of adhesion molecules and generating cytokines and other inflammatory mediators.25

1.3. Cancer: Etiology and treatments

The human body is made up of many types of cells, which grow and divide in a controlled way to produce more cells as and when they are needed to keep the body healthy, especially to replenish the old or damaged cells. Sometimes this orderly process goes haywire and the cells undergo mutations leading to uncontrolled cell growth and division. The tissue mass created from such an abnormal growth is called cancer. The abnormal cells are termed cancer cells, malignant cells, or tumor cells. The abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may randomly occur through mutations via errors in DNA replication, or are inherited.

Tumors are broadly grouped into two types. Tumors that are not cancerous and do not spread to other parts of the body are called benign tumors. Malignant tumors are cancerous and their cells can break free, enter bloodstream and spread to other parts of the body through a process called metastasis. Cancer can be further
categorized into several groups. 1) Carcinomas are cancer that begins in the skin or in tissues that line or cover internal organs, for example adenocarcinoma, squamous cell carcinoma etc. Sarcoma is a cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. 2) Leukemias are cancer that starts in blood-forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood. 3) Lymphoma and myeloma are cancers that begin in the cells of the immune system. 4) Central nervous system cancers are cancers that begin in the tissues of the brain and spinal cord. There are more than 100 different types of cancers and most of them are named after the organ in which they start, for example colon cancer.

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008. Deaths from cancer continue to rise and it is projected to cross 11 million by 2030. Lung cancer is the most common cancer contributing nearly 13% to the total number of new cases. Breast cancer is the second most common cancer with 1.4 million new cases in 2008. Colorectal cancer is the third most common cancer followed by cancers of stomach, prostate, liver, cervix uteri, oesophagus, bladder, non-hodgkin lymphoma and leukemia. Skin cancer is the most commonly diagnosed cancer among men and women. Over one million cases are diagnosed each year. The recent statistics is summarized in Figure 1.4.

Each type of cancer cell has a unique molecular signature referred to as biomarker, which include genes, protein and other molecular features. A cancer biomarker is indicative of the presence of cancer in the body and it can be a molecule secreted by a tumor or a specific response of the body to the presence of cancer. Hence, these genetic, epigenetic, proteomic, glycomic and imaging biomarkers can be used for cancer diagnosis, prognosis and epidemiology. Ideally, such biomarkers can be assayed in non-invasively collected bio-fluids like blood or serum.
Numerous challenges exist in translating biomarker research into the clinical space. However, a number of gene and protein based biomarkers have already been used in patient care, which include, AFP (Liver Cancer), BCR-ABL (Chronic Myeloid Leukemia), BRCA1 / BRCA2 (Breast/Ovarian Cancer), BRAF V600E (Melanoma/Colorectal Cancer), CA-125 (Ovarian Cancer), CA19.9 (Pancreatic Cancer), CEA (Colorectal Cancer), EGFR (Non-small-cell lung carcinoma), HER-2 (Breast Cancer), KIT (Gastrointestinal stromal tumor), PSA (Prostate Specific Antigen) (Prostate Cancer), S100 (Melanoma) and many others.28-37

Cancer biomarkers, particular those associated with genetic mutations or epigenetic alterations, often offer a quantitative way to determine when individuals are predisposed to particular types of cancers. Notable examples of potentially predictive cancer biomarkers include mutations on genes KRAS, p53, EGFR, erbB2
for colorectal, esophageal, liver, pancreatic cancer; mutations of genes BRCA1 and BRCA2 for breast and ovarian cancer; abnormal methylation of tumor suppressor genes p16, CDKN2B and p14ARF for brain cancer. Hypermethylation of MYOD1, CDH1 and CDH13 for cervical cancer and hypermethylation of p16, p14 and RB1, for oral cancer.\textsuperscript{38}

Owing to the growing cancer incidence across the globe, there is an intense research interest for the discovery and development of new cancer drugs. Many companies (pharmaceutical and biotechnology) are focusing on developing advanced cancer drugs for targeted therapy. Besides, many domestic companies have also increased their focus on R&D capabilities to develop effective cancer treatment for breast, prostate, colon and pancreatic cancers. With significant advancements in newer cancer drugs and enhanced focus on tailored drug delivery in cancer treatment coupled with benefits of molecular biomarkers in drug discovery, the market is set to witness the launch of advanced cancer drugs and entry of new players. Some of important anti-cancer drug molecules (1.56 to 1.76) available in the market are summarized in Figure 1.5.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{cancer_drugs.png}
\caption{Important anti-cancer drug molecules.}
\end{figure}

\textbf{Busulfan (1.56)}

\textbf{Carboplatin (1.57)}

\textbf{Carmustine (1.58)}

\textbf{Chlorambucil (1.59)}

\textbf{Cytarabine (1.60)}

\textbf{Diethylstilbestrol (1.61)}

\textbf{Mitotane (1.62)}
Mitomycin (1.63)  
Mitoxantrone (1.64)  
Etoposide (1.65)  
Paclitaxel (1.66)  
Pentastatin (1.67)  
Pipobroman (1.68)  
Prednisone (1.69)
Introduction

Chapter -1

Plicamycin (1.70)

Procarbazine (1.71)

Streptozocin (1.72)

Tamoxifen (1.73)

Teniposide (1.74)

Vinblastine (1.75)

Vincristine (1.76)

Figure 1.5: Existing anti-cancer therapies in the market
1.4. Oxidative stress, Inflammation and Cancer:

Inflammation is established to be a critical component of cancer progression. The link between inflammation and cancer was known early as 1863. This association is also proven by recent epidemiologic studies. One large study found that women who regularly use aspirin developed fewer cancers than women who did not use aspirin. The scientists at the University of California, San Diego, School of Medicine recently confirmed that chronic inflammation predisposes to some forms of cancer and a molecular link has been established between inflammation and cancer. The study demonstrated that the inactivation of a gene called I-kappa-beta kinase (IKK\(_\beta\)), which is involved in the inflammatory process, can dramatically reduce tumor development in mice with a gastrointestinal form of cancer.\(^{40}\)

Chronic inflammation unleashes a plethora of agents, such as cytokines, prostaglandins, chemotactic factors, reactive oxygen and nitrogen species (which cause the mutations in neighboring cells), as well as changes in gene expression favoring the activation of oncogenes and down-regulation of tumor suppression genes. Many cancers arise from sites of infection, chronic irritation and inflammation.\(^{41}\)

ROS also contribute to cancer initiation and promotion. The reactive species produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic. ROS may also suppress apoptosis and promote proliferation, invasiveness and metastasis.

Nuclear factor kappa B (NF-\(\kappa\)B) acts as a master switch to turn on inflammation in response to tissue damage. It is considered as a key modulator in driving inflammation to cancer. In epithelial cells, NF-\(\kappa\)B promotes the development of cancer not through inflammation, but through inhibition of a cell-killing process called apoptosis. In myeloid cells, NF-\(\kappa\)B causes the expression of pro-inflammatory molecules that stimulate the division of genetically altered epithelial cells and thereby increase tumor size. It is now obvious that the development of cancers from inflammation is driven by a micro environment in the inflamed area involving inflammatory cells, cytokines, chemokines and inflammatory enzyme mediators.
The TNFα for example is a central regulator of inflammation. It induces NF-κB, induce DNA damage, promote angiogenesis and tumor growth by angiogenic factors, thymidine phosphorylase and matrix metalloproteinases (MMPs).

It is evident that inflammation is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. In addition, tumor cells co-opt some of the signaling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. These insights are fostering new anti-inflammatory therapeutic approaches to cancer drug development.42

Overall, the observations to date suggest that oxidative stress, chronic inflammation, and cancer are closely linked. As such, the drugs that target lipoxygenases, leukotrienes and other inflammatory mediators may thus have a potential to become the emerging therapies for inflammatory diseases, cancer and cardiovascular events.43-46

Nature is a rich source of anti-inflammatory and anti-cancer compounds. A significant percentage of anti-cancer drugs in the market are of natural origin. Hence, the author was interested to investigate some molecules of natural origin for developing alternative synthetic processes, developing analogs for SAR studies, and testing the compounds for anti-inflammatory and anti-cancer activities. The summary of the work performed in this regard as part of Ph. D program is described in the following chapters (Chapter 2 – Chapter 6).
1.5. References:


2. Inflammation Research Perspectives, Editors: Giocomo T. Romano.


crohn disease with *Boswellia serrata* extract H 15. *Z. Gastroenterol.*, 2001, 39, 
11 - 17.

22. Setty AR and Sigal LH; Herbal medications commonly used in the practice of 
rheumatology: Mechanisms of action, efficacy, and side effects. *Semin. Arthritis 

23. Shao Y, Ho CT, Chin CK, Badmaev V, Ma W and Huang MT; Inhibitory 
activity of boswellic acids from *Boswellia serrata* against human leukemia HL-60 


25. Daniel Closa and Emma Foleh-Pay; Oxygen free radicals and the systemic 


27. Mishra Alok and Verma Mukesh; Cancer biomarkers: Are we ready for the 

28. Rhea Jeanne and Ross JM; Cancer biomarkers: Surviving the journey from 
bench to bedside. Medical laboratory observer. March 2011, Retrieved 26 April 
2013.

29. Behne Tara and Copur MS; Biomarkers for hepatocellular carcinoma. 

M, Pezzuolo D, Camisa R, Savi M, Neri TM and Ardizzoni A; BRCA 
mutations, molecular markers, and clinical variables in early-onset breast 
280 - 292.


40. Sue Pondrom; UCSD medical researchers are first to demonstrate molecular link between inflammation and cancer, inactivation of pro-inflammatory gene dramatically reduces tumor development. August 5, 2004.


