CHAPTER-1

REVIEW OF LITERATURE

According to a recent survey of the World Health Organization (WHO), cancer causes around 13% of the total deaths worldwide and if it continues rising, it is estimated to cause 13.1 million deaths in 2030.\(^1\) Moreover, the reason for about 15% of the total cancer deaths has been reported\(^2\) to be associated with chronic inflammation. Hence, an obvious way out to minimize cancer deaths is to put control over the inflammatory conditions.

The pioneering work of Sune K. Bergström, Bengt I. Samuelsson and John R. Vane for discovering prostaglandins and related biological substances, which led them to win Nobel Prize in Physiology/Medicine 1982, enabled the scientific community to make extensive exploration of the working of arachidonic acid (AA) pathway and solving several issues related to inflammatory diseases like rheumatoid arthritis and asthma. Dr. J. R. Vane discovered the mechanism of aspirin\(^3\) which was used as a former Non-steroidal anti-inflammatory drug (NSAID) for relieving pain, inflammation and fever by slowing down the production of prostaglandins associated with them. He also discovered prostacyclins\(^4-6\) that relax blood vessels and work for heart and blood vessel diseases and finally led to the development of cyclooxygenase-2 (COX-2) inhibitors. This work further inspired the exploration of arachidonic acid metabolism\(^7\) leading to the production of various prostanoids including prostaglandins, prostacyclins, thromboxanes and leukotrienes.

AA is a polyunsaturated fatty acid present in various phospholipids like phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositolides of body cell membranes. It is found abundantly in brain, liver and muscles. Besides the regulation of various signalling enzymes,\(^8\) it is a key intermediate in inflammatory processes.\(^9,10\) AA generated for signalling purposes appears to be derived by the action of a phosphatidylcholine specific cytosolic phospholipase A2 (cPLA\(_2\)),\(^11\) whereas inflammatory AA is generated by the action of a low molecular weight secretory PLA\(_2\) (sPLA\(_2\)).\(^12\) It is metabolized generally by three types of enzymes viz. cyclooxygenases, lipoxygenases and cytochrome P\(_{450}\) but the fate of the substrate is preferentially decided by cyclooxygenases and lipoxygenase enzymes (Chart 1, 2). Cyclooxygenase has two isomeric forms viz. cyclooxygenase-1 (COX-1) and COX-2.\(^13\) Another isoform cyclooxygenase-3 (COX-3)\(^14,15\) has also been discovered but is not much explored. Out of COX-1 and COX-2, the former is involved in the desired conversion of AA to homeostatic prostaglandins as an innate – immunity mechanism of the body and is known as the housekeeping enzyme while the later
one is involved in the production of pathophysiological prostaglandins which leads to the augmentation of severe inflammation.

**Chart 1. Metabolites of arachidonic acid through cyclooxygenase pathway**

On entering cyclooxygenase metabolic pathway, AA is first converted to the immediate substrate for various prostaglandin, prostacyclin and thromboxane synthases. This involves a two step conversion\(^1^6\) of AA to PGH\(_2\) via the formation of PGG\(_2\). The first step involves the addition of two oxygens into the AA molecule forming the bicyclic peroxide intermediate,
PGG₂. In the second step, the intermediate PGG₂ diffuses to the site where peroxidation takes place and gets converted to PGH₂ which then gets metabolized by prostaglandin synthases, prostacyclin synthases and thromboxane synthases to various prostaglandins, prostacyclins and thromboxanes, respectively (Chart 1).

Lipoxygenases are a family of enzymes which catalyze the oxygenation of AA, each lipoxygenase forming a distinct hydroperoxy-eicosatetraenoic acid (HPETE). HPETEs may undergo a series of metabolic transformations - what is referred to as a lipoxygenase pathway (Chart 2).¹⁷,¹⁸ Hence, AA metabolism results in the formation of (a) prostaglandins and prostacyclins which control the contraction and relaxation of smooth muscle tissues, (b) thromboxanes which acts as vasoconstrictor and potent hypertensive agents, and facilitates platelet aggregation, and (c) leukotrienes (LTs) which trigger contractions in smooth muscles lining the trachea, act as chemotactic agents while their over-production is a major cause of inflammation in asthma and allergic rhinitis. Basically, inflammation is an innate – immunity
response of the body against harmful stimuli which is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.\textsuperscript{19} It does not need to be suppressed but when the inflammation goes chronic, it can lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gall bladder carcinoma).\textsuperscript{20} In 1863, Rudolf Virchow indicated that cancers tend to occur at the site of chronic inflammation.\textsuperscript{21} Amongst many other factors responsible for initiation and propagation of cancer, it has now been well established after around 150 years of Virchow’s hypothesis, that inflammatory enzymes are over-expressed in cancer cells and a higher concentration of inflammatory metabolites are found in many cases of cancerous cells.\textsuperscript{22-25} This confluence of cancer and inflammation has opened another front to fight against cancer where the inflammatory enzymes could be made the target. Before discussing the design, synthesis and evaluation of the compounds undertaken in the present work, a brief review of the anti-inflammatory and anti-cancer drugs of clinical significance is provided.

Non-Steroidal Anti-inflammatory drugs (NSAIDs)

It is an important class of drugs that have been used as effective analgesics, antipyretics and as anti-inflammatory agents. NSAIDs\textsuperscript{26-28} inhibit the activity of cyclooxygenases, thereby affecting the production of prostaglandins and thromboxanes. These inhibit the activities of COX-1 and COX-2, hence also lead to gastrointestinal side-effects associated with the inhibition of COX-1. Based on their difference in the mechanism of action, NSAIDs have been broadly classified into various categories including;

1.1. Salicylates

The drugs are used for the treatment of arthritis pain and inflammation and have been divided into two categories including the acetylated and non-acetylated salicylates. One of the prominent drug, aspirin (acetyl salicylic acid, 1),\textsuperscript{29,30} which was first discovered from the bark of willow tree in 1763 and was first synthesized in the year 1897 relates to the acetylated subset of the salicylates. Quite lately after its consistent use as a common analgesic and anti-inflammatory agent, its mechanism of action came into limelight. It was later found that aspirin inhibits the COX-1 variant (50% inhibitory concentration, IC\textsubscript{50} 1.67 µM) more than the COX-2 variant (IC\textsubscript{50} 278 µM). Aspirin suppresses the production of prostaglandins and thromboxanes by irreversibly inhibiting cyclooxygenases. Other drugs\textsuperscript{31} including trilisate (2), salsalate (3) and trolamine salicylate (4) also came into use as anti-inflammatory agents (Chart 3).
1.2. Acetic acid derivatives

This category incorporates the most common anti-inflammatory drugs including indomethacin (5) which was discovered in 1963 and was approved in the U. S. by Food and Drug Administration (FDA) in 1965. Indomethacin inhibits COX-2 with an IC$_{50}$ 970 nM$^{30}$. Since, it also inhibits COX-1, therefore it is associated with gastrointestinal side-effects like causing peptic ulcers. Another important drug belonging to this class includes diclofenac (6) which was first discovered in 1973 and is one of the most widely used drugs for pain, fever and swelling. Diclofenac sodium exhibits an IC$_{50}$ 60 and 200 nM for ovine COX-1 and COX-2 respectively$^{32}$ while it shows IC$_{50}$ 0.9-2.7 µM for human COX-1 and 1.5-20 µM for human COX-2$^{33}$ and hence, a non-selective inhibitor of cyclooxygenases. This category also includes other drugs (Chart 4) like sulindac (7), ketorolac (8), etodolac (9), aceclofenac (10) and tolmetin (11). A number of reports on pyridizinone derivatives are available.$^{34-36}$ Abouzid et al. have also reported pyridazinone derivatives (12) as anti-inflammatory agents in 2008$^{37}$ followed by a recent report in 2012.$^{38}$
1.3. Propionic acid derivatives

One of the core medicines declared by the WHO incorporates the drug Ibuprofen (13) which was derived from propionic acid in 1960s. Along with analgesic, antipyretic and anti-inflammatory effects, it also show antiplatelet and vasodilation effects as well and works by inhibiting both COX-1 and COX-2 isoforms of cyclooxygenases. It exhibits an IC$_{50}$ 223 µM against COX-2. Other clinically used members of the family include naproxen (14), ketoprofen (15), oxaprazin (16) and flurbiprofen (17) are shown in Chart 5. Various reports have been published based upon the derivatization of clinically used drugs and their bioevaluation as anti-inflammatory agents.
1.4. Enolic acid derivatives

It incorporates the oxicam class of anti-inflammatory drugs.\textsuperscript{42,43} Piroxacam\textsuperscript{44} (18) is one of the representative members of this category. It shows both analgesic and anti-pyretic effects and is a non-selective NSAID exhibiting an IC\textsubscript{50} 0.6 µM for COX-2. Meloxicam\textsuperscript{45-48} (19), another member of this class has been found to show lesser side-effects than piroxicam with an IC\textsubscript{50} 1.9 nM for COX-2. The sister drugs include tenoxicam (20) and lornoxicam (21) shown in Chart 6. Isoxicam\textsuperscript{49} (22) was also used clinically but its marketing was banned because of its fatal skin reactions. Several piroxicam derivatives\textsuperscript{50-52} were developed in order to reduce their gastrointestinal side-effects and prodrugs like ampiroxicam, droxicam and cinnoxicam have been marketed. The prodrugs were found to be stable under gastric conditions.

\begin{center}
\begin{tikzpicture}
\node (18) at (-2,0) {18; Piroxicam (feldene)};
\node (19) at (2,0) {19; Meloxicam (mobic)};
\node (20) at (-2,-2) {20; Tenoxicam (tilcotil)};
\node (21) at (2,-2) {21; Lornoxicam (loricam)};
\node (22) at (0,-4) {22; Isoxicam (maxicam)};
\end{tikzpicture}
\end{center}

\begin{center}
\text{IC}_{50} \text{ (COX-2) 0.6 µM for Piroxicam} \quad \text{IC}_{50} \text{ (COX-2) 1.9 nM for Meloxicam} \quad \text{IC}_{50} \text{ (COX-2) 0.32 µM for Tenoxicam} \quad \text{IC}_{50} \text{ (COX-2) 5 nM for Lornoxicam} \quad \text{IC}_{50} \text{ (COX-2) 8 nM for Isoxicam}.
\end{center}

Chart 6. Oxicam NSAIDs

1.5. Fenamic acid derivatives

Fenamates are a class of NSAIDs family having a common structure of N-arylanthranilic acid\textsuperscript{53} in their molecules. The fenamates are differentiated by their aryl substituents as shown in Chart 7. One of the members, meclofenamic acid (23) was approved by the FDA in 1980. It is a non-selective NSAID and is used for the treatment of arthritis and pain. It works by inhibiting prostaglandin production. Other members\textsuperscript{54} include flufenamic acid (24), tolfenamic acid (25) and mefenamic acid (26). Fenamates are variously derivatized\textsuperscript{55-57} with benzofurans, triazoles, etc. in order to improve their efficiency and to lower the risk of side-effects.
1.6. Selective COX-2 inhibitors (COXIBs)

Unlike non-selective NSAIDs, the COXIB class\textsuperscript{58} of COX-2 inhibitors slow down the activity of COX-2 only. Very first examples of this category include DUP697\textsuperscript{59} (27; Chart 8) and NS398\textsuperscript{60} (28). They acted as the building blocks for the discovery of selective COX-2 inhibitors. Celecoxib\textsuperscript{61,62} (29) and Rofecoxib\textsuperscript{63} (30) were launched as selective COX-2 inhibitors in 1998 and 1999 respectively. Both the drugs did not produce the ulcerogenic effects like the other NSAIDs. Later, valdecoxib\textsuperscript{64} (31) was also introduced to be used as a selective COX-2 inhibitor but because of the increased side effects associated with rofecoxib and valdecoxib, causing heart attack and strokes (as a result of activation of 5-LOX pathway), they were withdrawn from the market.\textsuperscript{65,66} After the exclusion of rofecoxib and valdecoxib, celecoxib is the only drug which is available in the market as a selective inhibitor of COX-2. Celecoxib exhibits an IC\textsubscript{50} 40 nM for COX-2 as compared to an IC\textsubscript{50} 15 μM for COX-1 thus having a 375-fold selectivity for COX-2 recombinant enzyme assays.\textsuperscript{67} Etoricoxib (32) and Parecoxib (33) are the other selective COX-2 inhibitors used in around 80 countries all over the world but have not still been approved by FDA. Tilmacoxib (JTE-522)\textsuperscript{68} (34) has also been reported as COX-2 selective inhibitor and is used against osteoarthritis and rheumatoid arthritis.
1.7. Sulphonanilides and Dual COX/LOX inhibitors

Nimesulide\textsuperscript{69} (35; Chart 9) is a preferential inhibitor of COX-2. It exhibits 10-50 folds more potency for COX-2 than COX-1 showing superior gastrointestinal safety than other conventional NSAIDs. Its major side-effects include hepatotoxicity.

Licofelone\textsuperscript{70} (36; Chart 9) is the first dual inhibitor of both COX and 5-LOX. It shows a very few gastrointestinal side-effects as shown by conventional NSAIDs. Nimesulide derivatives\textsuperscript{71} have also been reported as dual inhibitors of COX/5-LOX. Zhi-Shu Huang \textit{et al.}\textsuperscript{72} have reported structurally modified derivatives of nimesulide with the incorporation of 5-LOX inhibitory pharmacophore and evaluated for potent dual COX/5-LOX inhibition. Various other dual COX/5-LOX inhibitors can also be found in the literature\textsuperscript{73a-d}.
Even though, a number of drugs are available in the market which have anti-inflammatory potential but almost all of them are associated with unavoidable side-effects. Therefore, the development of safe and effective remedial measures of chronic inflammation is continuously attracting the attention of scientific community.

**Dihydrofolate reductase (DHFR) as the target for cancer**

Besides the role of COX-2 in the process of initiation and propagation of cancer, DHFR mediated metabolism of folate is another critical cellular target which need to be controlled for the remedial measures of cancer. DHFR is involved in folate metabolic pathway\(^74\) for biosynthesis of thymidine (precursor for DNA replication) where it is responsible for regulating the amount of tetrahydrofolate in the cell (**Chart 10**). Tetrahydrofolate and its derivatives are essential for purine and thymidylate synthesis, which are important for cell proliferation and growth. Being responsible for generating raw materials of DNA replication, inhibition of DHFR forms the basis for treatment of various infectious diseases and is the target of antibacterial drugs like trimethoprim\(^75,76\) as well as anticancer drugs—methotrexate\(^77-79\) and pemetrexate.\(^80,81\)
Methotrexate\(^{77-79}\) (37; Chart 11) is an antifolate drug that inhibits folic acid metabolism by blocking the enzyme, DHFR. Consequently, the formation of vital raw materials for DNA, RNA is hindered and cell proliferation is minimized. It is being used as an anti-cancer drug since its discovery in 1950s. Methotrexate analogs\(^{80a,b}\) are synthesized by various groups to refine its activity. In order to produce potent new leads for anticancer drugs, quinazoline analogs\(^{81a,b}\) have been synthesized to resemble methotrexate structure and fitted with functional groups to enhance inhibition of mammalian DHFR activity.

Pemetrexed\(^{82,83}\) (38) is another antifolate introduced in 2004 and inhibits two more important enzymes \(\text{viz.,}\) thymidylate synthase and glycinamide ribonucleotide formyltransferase (GARFT) along with DHFR. Its use is indicated for the treatment of non-small cell lung cancer. Pralatrexate (39) is one of the recent anticancer drug discoveries and is being used for the treatment of a more diverse kind of blood cancer. The drug was approved by FDA in 2009.\(^{84}\) Trimethoprim (40) is another anti-folate that is used as a bacteriostatic drug. It is among the WHO’s most essential drugs\(^{85}\) needed for basic health.

Chart 10. Role of DHFR in generating raw materials during DNA synthesis

1.8. Present status of DHFR inhibitors as anticancer agents

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1.9. REFERENCES


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