Biochemistry and Therapeutic Accesses for Enzyme Facilitated Inflammation: A Review

Parteek Prasher[*] and Manjeet Singh

Department of Chemistry, University of Petroleum and Energy Study, PO Bidholi via Premnagar, Dehradun, Uttrakhand, India

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ABSTRACT

Inflammation is a defensive measure against the chemical or biological incursions of pathogens, damaged cells or irritants so that the homeostasis in the vascular tissues is maintained. Biochemically, the commencement of the defensive reaction pertains to the production of inflammatory mediators such as chemokines, cytokines, and eicosanoids. Subsequently, the neutrophils rush to the site of infection. Targeting the invading agents, the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) is also an ingredient of the defensive plan of the body. The acute inflammation, symptomized by pain, redness, swelling, heat and loss of function might eventually lead to the development of ailments like asthma, atherosclerosis, arthritis etc.

Keywords: Reactive oxygen species; Reative nitrogen species; Cyclooxygenase; Non-steroidal anti-inflammatory drug

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INTRODUCTION

The metabolites of arachidonic acid produced by the catalytic role of lipoxygenase and cyclooxygenase enzymes are found to be the sole perpetrators of chronic inflammation [1-4]. Arachidonic acid which is a polyunsaturated fatty acid present in the phospholipids (especially phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides) of the membranes of body's cells and is also profusely available in the brain, muscles and liver. The lipoxygenase channel of the arachidonic acid pathway is responsible for the production of leukotrienes whereas the overexpression of COX enzyme, the cyclooxygenase channel of arachidonic acid pathway, results in the production of inflammation causing prostaglandins [5,6]. A brief explanation for the cyclooxygenase catalyzed arachidonic acid metabolism and the structures of inflammation causing prostaglandins is shown in Figure 1.

Cyclooxygenase enzyme has two isoforms, COX-1 and COX-2 both existing as homodimers of which COX-1 is constitutive isoform and it functions as a housekeeping enzyme [7]. It is contained in almost all the tissues under normal conditions with a chief purpose to supply prostaglandin precursors for sustaining the homeostasis [8]. It is also accountable to provide the precursors for thromboxane synthesis to the blood platelets which helps in clot formation [9]. The other inducible isoform COX-2 is localized primarily to the inflammatory cells macrophages, fibroblasts and leukocytes [10]. It is expressed in response to inflammatory and other physiologic stimuli and is implicated in the production of those prostaglandins that arbitrate pain and support the inflammatory course. It is therefore hypothesized that the toxicity associated with the clinically useful NSAIDs is caused by the inhibition of COX-1, whereas the anti-inflammatory properties were caused by the inhibition of inducible COX-2.
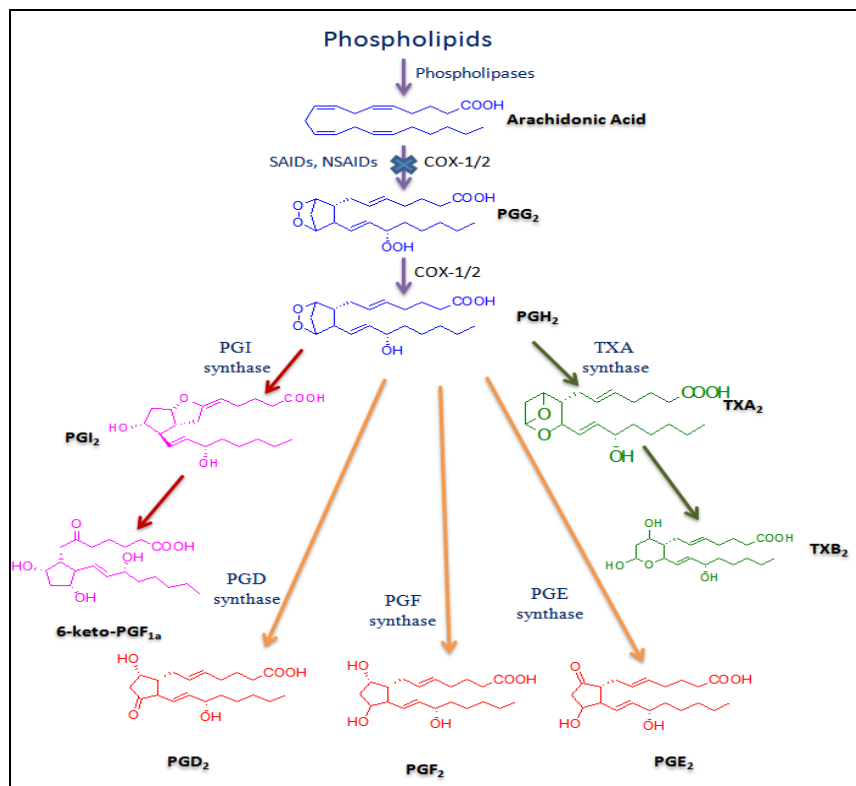
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Figure 1: Arachidonic acid cascade and biosynthesis of prostaglandins

The second channel of arachidonic acid pathway is mediated by the lipoxygenase enzyme which is an oxidative enzyme carrying non-heme iron cofactor [11]. The iron is present as Fe^{+2} in a distorted octahedral geometry [12] in the active site of the enzyme [13]. It has been discovered through mossbauer spectroscopy and EPR [14] that the non heme iron switches between the two redox states [15] viz. Fe^{+2} and Fe^{+3}. The reactions catalyzed by lipoxygenases involve one electron oxidation by the Fe^{+2}, cofactor present in the active site [16]. During the course of reaction, Fe^{3+} is reduced to Fe^{2+} followed by the oxidation of the fatty acid substrate by hydrogen abstraction from a bis-allylic methylene to give a pentadienyl radical, which is re-arranged to a 1-cis, 3-trans-conjugated diene moiety [17]. Finally, a stereo-specific insertion of oxygen at the pentadienyl radical takes place to form a fatty acid hydroperoxide radical centered on the oxygen atom [18]. This hydroperoxide radical is further reduced to the corresponding anion with a re-oxidation [19] of iron to Fe^{3+} (Figures 2 and 3).
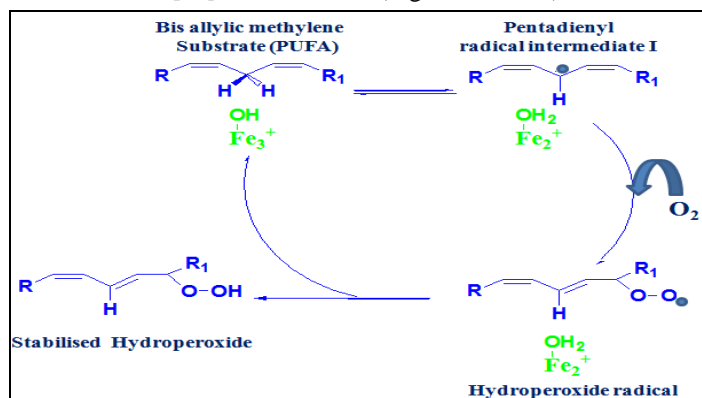

Figure 2: Various oxidation states of iron during the biosynthesis of leukotrienes
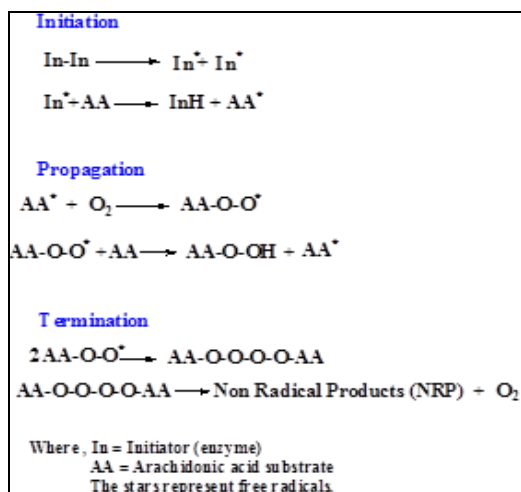
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Figure 3: Generalization of the free radical mechanism for the arachidonic acid metabolism

Upon inflammatory stimulation, cytosolic phospholipase A2-α (cPLA2α) releases arachidonic acid from the membrane lipids to initiate the leukotrienes biosynthesis [20]. The biosynthesis of leukotrienes is regulated by the activity of the enzyme 5-lipoxygenase [21] which catalyzes the oxidative conversion of arachidonic acid to 5-HPETE and subsequently to leukotriene A4 (LTA4) [22]. LTA4 is hydrolyzed to form dihydroxy acid leukotriene LTB4. LTA4 is also converted to cysteinyl leukotriene LTC4 by addition of a glutathione group by LTC4 synthase (Figure 4). Conversion of LTC4 by γ-glutamyl transferase results in the synthesis of LTD4 with the subsequent release of glutamic acid. Further, dipeptidase (DiP) breaks the amide bond in LTD4 to give LTE4 which is finally excreted in urine [23].
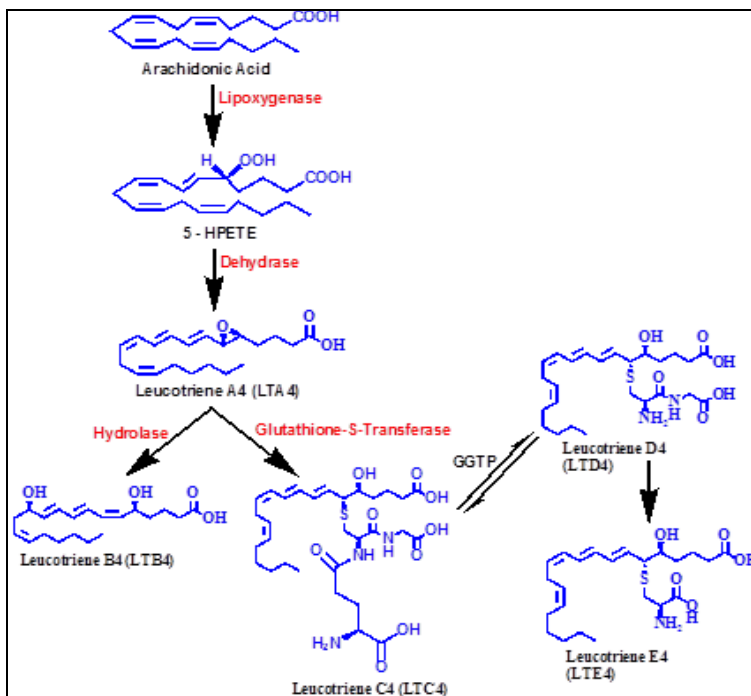

Figure 4: Arachidonic acid cascade and biosynthesis of leukotrienes

Overexpression of Leukotrienes and Prostaglandins: Acute to Chronic Inflammation

Leukotrienes and Prostaglandins are culprits for the enzyme mediated inflammation in the humans. The preliminary symptoms of acute inflammation are symptomised by swelling, redness, loss of function and heat of the affected area (Figure 5). The situation might get chronic characterized by several diseased conditions in humans such as

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asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, neurodegeneration and cancer (Figure 6).
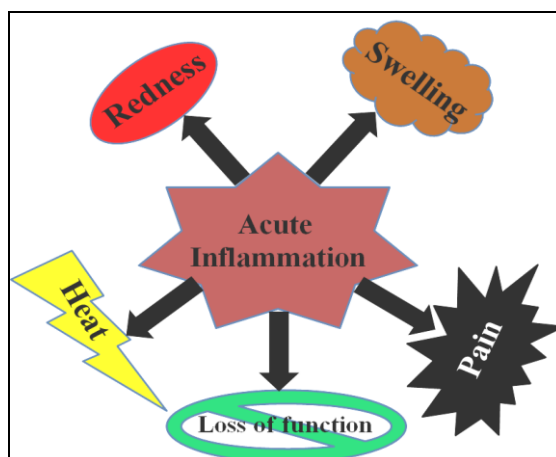

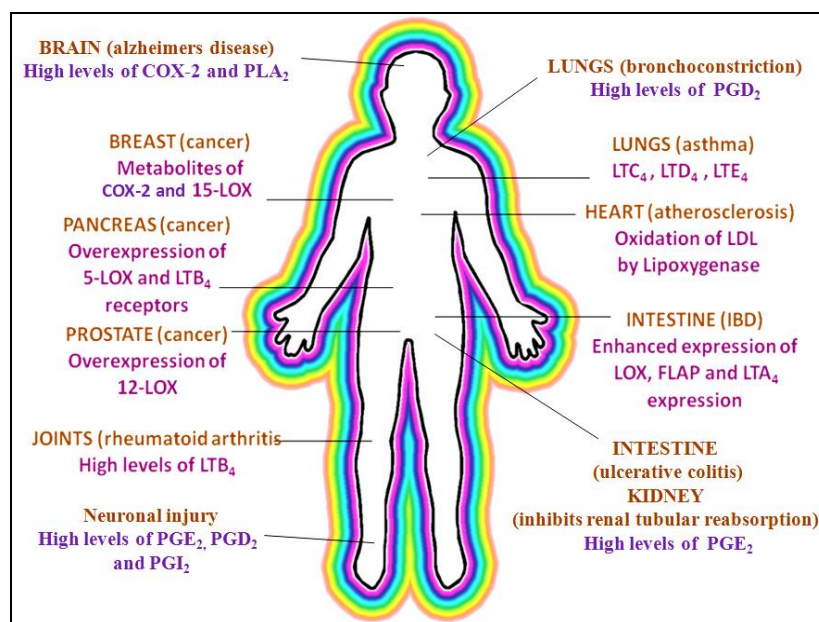**Figure 5: Consequences of enzyme mediated acute inflammation**

Figure 6: Consequences of enzyme mediated chronic inflammation

Asthma

Leukotrienes are the regulators of smooth muscle contraction during bronchoconstriction. But a lofty level of LTC4, LTD4 and LTE4 impedes the lung tissues.

The main cause of asthma is due to the up-regulation of these mediators. Cysteinyl leukotrienes contribute to the plasma leakage from post-capillary venules in respiratory tissues, which can lead to inflammatory edema [24,25]. Apart from that, a higher level of prostaglandin PGD_2 protects lower airways of the lungs from bronchoconstriction [26]. Hence, with some cadence in the production of pro-inflammatory leukotrienes and prostaglandins, a prospective anti-asthma rehabilitation could be developed.

Atherosclerosis

Lipoxygenases participate in the oxidation of low density lipoproteins (LDLs) present in the macrophages to form foam cells [26]. These cells develop plaques of atheroma and their accretion in the arteries leads to atherosclerosis. An increase in the cysteinyl LTE4 levels in urine and LTB4 in the atheroma were observed in patients with

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atherosclerosis. Hence, the reticence of lipoxygenase activity may endow with a treatment strategy against this inflammatory disease.

Rheumatoid Arthritis

High level of LTB4 in the synovial fluid of rheumatoid arthritis patients has been reported [27]. This leukotriene is produced principally by neutrophils which are most bountiful leukocytes in rheumatoid joints [28]. The inflammatory responses were reduced in mice with the deficiency of 5-LOX and leukotriene A4 hydrolase enzyme [29]. This specifies the probability of development of a curable remedy against rheumatoid arthritis by the inhibition of lipoxygenase activity.

Inflammatory Bowel Disease

Patients with inflammatory bowel disease (IBD) confirm several fold enhancements of 5-lipoxygenase, FLAP and LTA4 hydrolase expression in the mucosa of colon and the rectum dialysates. The urinary excretion of LTE4 significantly increased in patients with IBD [30]. Such information evidently indicate the roles of leucotrienes in IBD and motivates the researchers to find possibilities for curing IBD by targeting lipoxygenase.

Cancer

The leucotrienes and prostaglandins are allied with some carcinogenic processes such as tumor cell proliferation, differentiation, and apoptosis [31]. Overexpression of platelet 12- lipoxygenase (p12-LOX) has been pragmatic in human prostate cancer cells [32]. The role of 5-LOX metabolites have been reported in the development of breast cancer by promoting the invasion of tumor cells into the lymphatic vessels and the formation of lymph node metastasis [33]. Increased expression of the 5-LOX enzyme and the LTB4 receptors were observed in pancreatic cancer. In addition, 5-LOX expression levels were suggested as indicator for early neoplastic lesions [34]. Albeit, the cyclooxygenases are reported to aggravate mammary cancer due to eminent PGE2 tumour yields isolated from the breast tissues [35]. The prostaglandins such as PGE1, PGF2, PGI2 and TXA2 are formed by human mammary cancers [36]. These studies visibly indicate that the increased lipoxygenase and cyclooxygenase activity is associated with the development of cancer and this enzyme could be the probable target for cancer treatment as well.

Targetting Lipoxygenases and cyClooxygenases for the Treatment of Inflammatory Diseases

The menace of enzyme mediated inflammation has been successfully countered with the development of several strategies to put a check over the over expression of the culprit enzymes. Anti inflammatory medication even though clouded by the fog of limitations certainly is picking up the pace with the clinical needs. By designing target specific effectively functioning chemical entities and core motifs the modulation in the lipoxygenase and cyclooxygenase channel of arachidonic acid pathway forms the basis of anti-inflammatory therapy. Several synthetic molecules as well as isolated natural products have been tested for the inhibition of these enzymes. Δ9-Tetrahydrocannabinol (Δ9-THC), extracted from cannabis, inhibits 15-lipoxygenase with IC_{50} of 2.42 μM [37]. Another compound with iron binding properties; 4-(2-oxopentadeca-4-yne)phenyl propanoic acid (OPP) shows a mixed type of inhibition towards 12-lipoxygenase with an appreciable Ki and Ki' values 0.2 μM and 4.5 μM respectively [38]. The natural product curcumin, is a modulator of arachidonic acid metabolism, acting through the 5-LOX pathway [39]. It also restrains the expression and activity of COX-2 in the gastrointestinal cell lines viz. colon, esophagus and small intestine [40]. Apart from that several phenolics and flavonoids bear a promising stance towards the COX-2 inhibition [41]. The broad spectrum strategies for the inhibition of the enzymes lipoxygenase and cyclooxygenase are discussed below.

FLAP Inhibiton

The compound MK-886 is an effective anti-inflammatory agent which reduces the leucotrienes biosynthesis [42] by binding to a 1800 KDa protein, better known as Five Lox Activating Protein (FLAP) [42,43]. For the catalytic activity of lipoxygenase, FLAP should be bound to the substrate [44] but in the presence of MK-886, this becomes limiting [45]. MK-0591, BAY-X-1005 (Figure 7) are the other compounds of this class with inhibitory concentration in nm range [45,46]. But the FLAP inhibition therapy is of less significance [47] as there is a competitive binding for FLAP in presence of arachidonic acid [48].
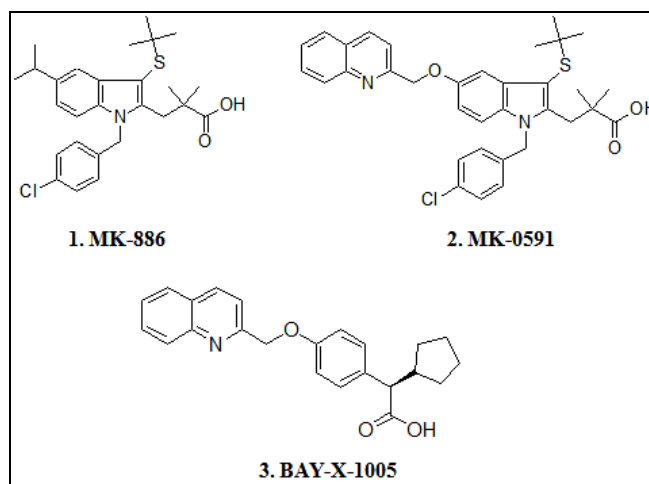
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Figure 7: Structures of some known FLAP inhibitors

Iron Chelation

The activity of lipoxygenase is closely related to the oxidation states of the cofactor [49]. Inhibition of 5-LOX can be achieved by replacing one of the ligands of octahedral Fe^{2+} to create a new complex. Molecules with iron-chelating functionalities such as hydroxamic acid or N-hydroxyurea are potent inhibitors for 5-LOX [50]. Zileuton (Figure 8) is one of the 5-LOX iron chelator inhibitors that is commercially available for the treatment of asthma [51,52]. Despite its effectiveness, zileuton is not the first choice therapy due to its side effects such as nausea and idiosyncratic effects on the liver [53]. Further development of this class of inhibitors led to the identification of atreleuton, which inhibits LTB4 and cys-LTE4 production and has a potency that is about 5 fold enhanced in comparison to zileuton [54]. Atreleuton, which has entered clinical trials for atherosclerosis and cardiovascular diseases [55], is one of the leading 5-LOX inhibitors in clinical development [56]. These studies suggest that the development of iron-chelator inhibitors for lipoxygenases could be an appreciable initiative for the development of anti-inflammatory therapy.
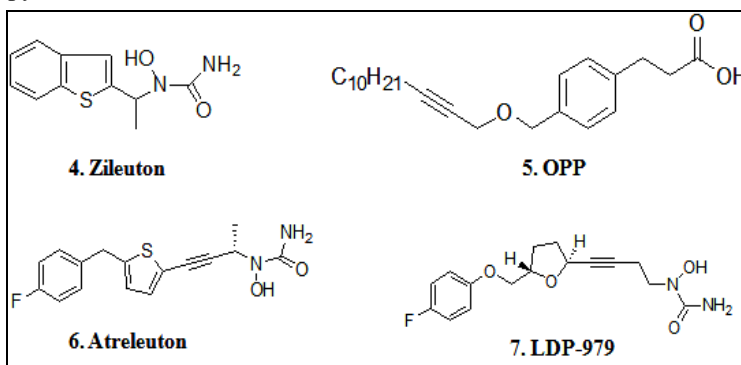

Figure 8: Structures of some known iron chelator inhibitors

Redox Inhibition

Redox inhibitors have a limiting effect on the expression of lipoxygenase enzyme by acting as antioxidants [57]. Phenidone, BW755C and AA-861 (Figure 9) are well known reducing agents [58,59]. It has been recognized that, apart from the redox potency [60] lipophilicity is also important. Recently, new molecules having a potential for redox inhibition for 5-LOX have been reported. These are a trimer or tetramer of caffeoyl clusters with IC_{50} values of respectively 0.79 μM and 0.66 μM [61]. It is important to mention that redox inhibitors have a low selectivity for 5-LOX inhibition compared to COXs inhibition [62]. Apart from their appreciable potential to inhibit leucotriene biosynthesis, their major drawback is that these interfere with biological redox processes [63]. The formation of methaemoglobin is one of the problems that were reported upon application of redox inhibitors [64].
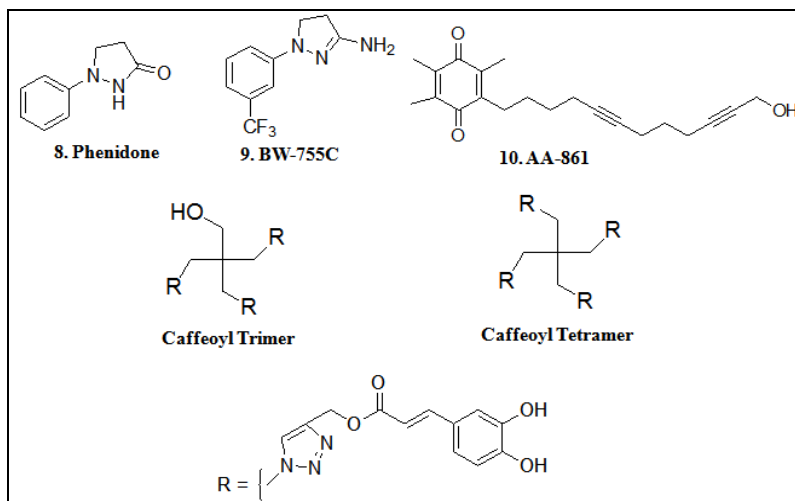
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Figure 9: Structures of some known redox inhibitors

Non-redox Inhibition

Non-redox inhibitors have mode of action different from redox inhibitors, FLAP inhibitors and iron chelation inhibitors. These do not interfere with the oxidation reaction of lipoxygenases neither do they have iron-binding properties [65]. These inhibitors act by competitively binding to the active site of lipoxygenase enzyme [66]. Binding may occur to an allosteric binding site which regulates the activity of the enzyme [67]. The non-redox inhibitor like (methoxyalkyl)thiazole (ICI211965) (Figure 10) selectively inhibits 5-LOX activity, which reduces LTC4 and LTB4 synthesis in animal as well as human blood samples [68]. Although ICI211965 is a highly potent 5-LOX inhibitor [69] from a novel structural class, it has been reported to have a low oral potency [70].
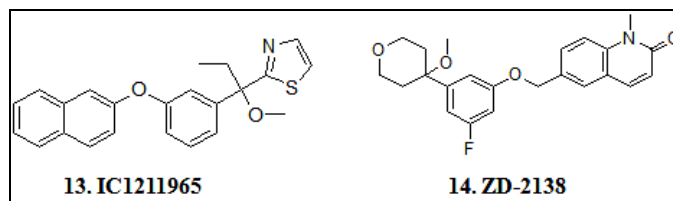

Figure 10: Structures of some known non-redox inhibitors

To cope with this problem, methoxytetrahydropyran compound ZD-2138 shows an improvement in the oral potency compared to ICI211965 for the treatments of arthritis and asthma [71]. Furthermore, ZD-2138 inhibits antigen-induced leukotriene release at the micromolar concentration range [72]. Despite all the positive outcomes of this class of compounds, its use as an anti-arthritis agent has been discouraged due to the poor results obtained from their clinical trials [73].

Leukotriene Antagonists

A new class of compounds, known as leukotriene receptor antagonists, has been developed which have quite an applaudable properties against the leukotriene biosynthesis. Pranlukast, Zafirlukast and Montelukast (Figure 11) are the most popular leukotriene receptor antagonists known for their effectiveness in the treatment of asthma [74,75]. The mode of action of these drugs involve the blocking of the binding of leukotriene D4 and also LTC4 and LTE4 to the cysLTR1 in the lungs and bronchial tubes [76] which resulted in the reduction of airway constriction, and mucus accumulation in the lungs. It has also been reported that Montelukast suppresses the leukotriene biosynthesis by selective inhibition of 5-LOX and gives no effect on the other enzymes involved in the leukotrienes biosynthesis pathway such as LTA4 hydrolase and LTC4 synthase. Despite its efficacy against asthma, the safe use of montelukast is still under question as they cause liver related problems [77].
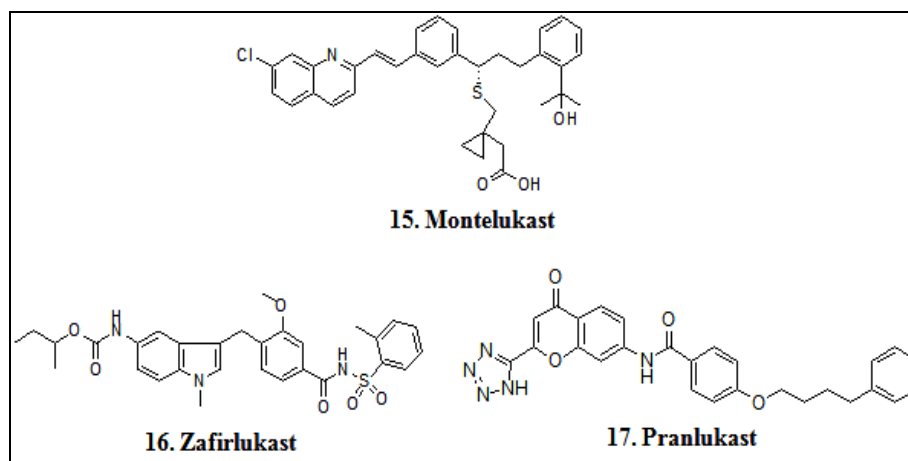
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Figure 11: Structures of some known leukotriene antagonists

Non Selective COX-1/COX-2 Inhibition

These drugs inhibit the expression of both the isoforms of cyclooxygenase enzyme COX-1 and COX-2 which is substantiated by several side effects. Indomethacin, a non-selective inhibitor of cyclooxygenase enzyme is reported to inhibit prostaglandins biosynthesis in the uterus by blocking the calcium channels [78]. However, it being non specific inhibitor restrains the making of prostaglandins in stomach and intestine. It impinges on the mucus lining of the gastrointestinal tract eventually causing the peptic ulcers. Similarly, ibuprofen as naproxen work by reversibly restraining the expression of both the isoforms of cyclooxygenase COX-1 and COX-2 [79]. Sulindac is efficient in the treatment of preterm labor and its role in the treatment of Alzheimer's disease is currently under investigation. Diclofenac is commonly used to cure moderate postoperative or post-traumatic algesia [80]. Piroxicam is a non-selective COX inhibitor NSAID carrying both the analgesic and antipyretic properties. Ketoprofen is considered a very efficient NSAID in relieving moderate to severe algesia and maintaining a good functional status and general condition over that of ibuprofen and diclofenac [81] (Figure 12).
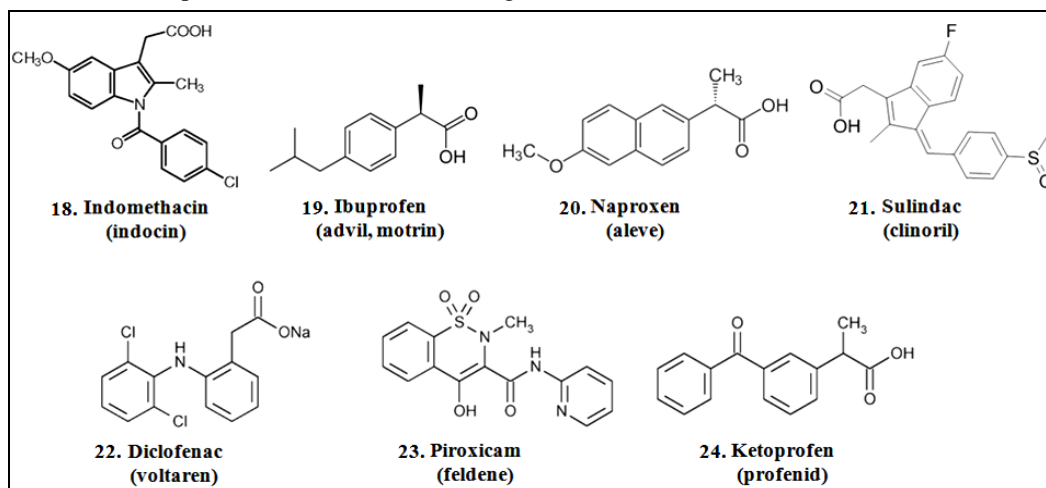

Figure 12: Non selective COX-1/COX-2 inhibitors and their brand names

COX-2 Selective Inhibition (COXIBS)

The side effects associated with the non selective NSAIDs prompted the development of selective COX-2 inhibitors. These drugs function by selectively inhibiting the COX-2 isoform while retaining the normal functioning of the constitutive isoform COX-1. A number of COX-2 selective inhibitors are available in the market under different brand names (Figure 13).
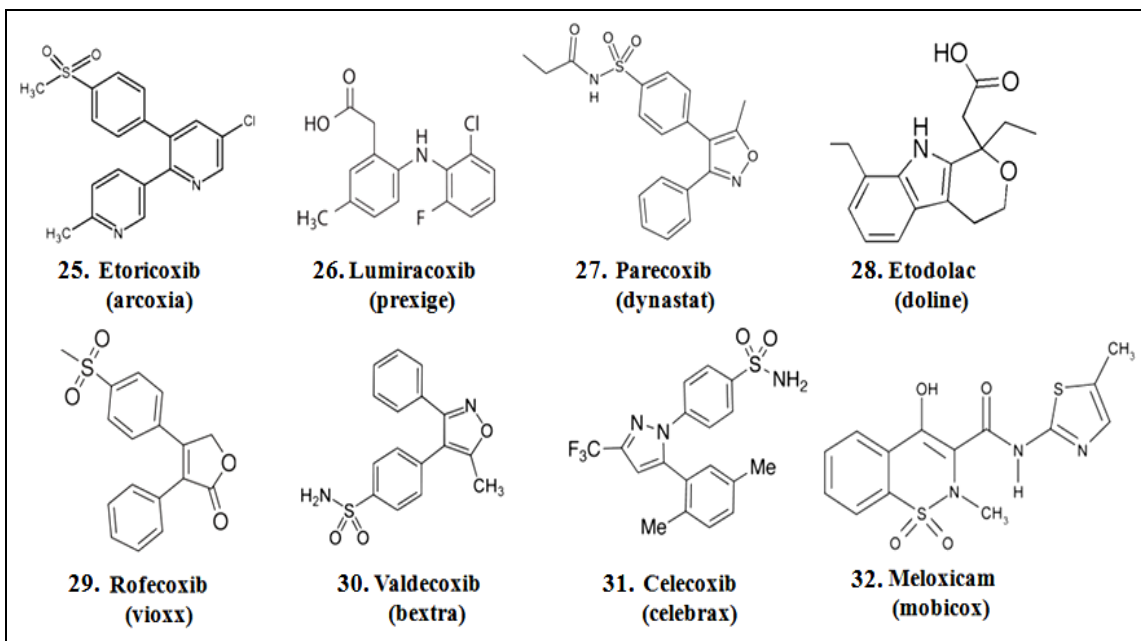
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Figure 13: COX-2 selective inhibitors and their brand names

Etoricoxib is a selective COX-2 inhibitor approved for alleviating chronic pain in the patients diagnosed with osteoarthritis and rheumatoid arthritis [82]. It is also associated with a fewer gastrointestinal adverse effects than the other conventionally used non-steroidal anti-inflammatory drugs (NSAIDs) [83]. Celecoxibs [84] and valdecoxib [85] also exhibit the similar effects. The prolonged use of COXIBS is known to be associated with several heart related problems [86] owing to which some of them are already withdrawn from the market [87]. Hence there is a need to seek the alternate steadfast strategies to develop the novel, resilient and efficacious drugs against inflammation with minimal level of side effects.

Dual Inhibition of COX-1/COX-2 and 5-LOX

The practice of polypharmacology is gaining momentum. In this approach a drug hits multiple targets [88]. This cost effective strategy thus can overcome the side effects spawned by the COXIBS and NSAIDS. A number of compounds [89,90] that inhibit COX-2 and 5-LOX have been reported as prospective representatives for the treatment of arthritis. Licofelone is one such anti-arthritis drug [91,92]. These dual inhibitors also have been employed to treat inflammation [93], pain [94] and cancers [95] (Figure 14).
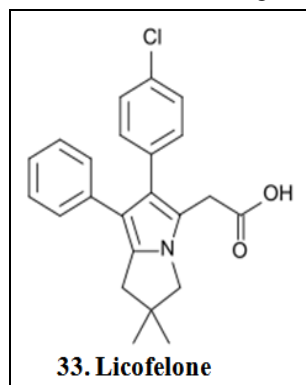

Figure 14: Dual COX/LOX inhibitor

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