Chapter-1
Introduction

1.1 Inflammation

Inflammation is a biological defense mechanism against harmful stimuli controlled by the body's immune system. It is an essential process of the body to maintain homeostasis. However, inflammation-related disorders may develop due to an aberrantly extended host's defensive response when tissue loses homeostasis (Libby 2007). Inflammation is biochemically characterized by a local increase in the concentration of lipid mediators such as prostaglandins, leukotrienes, platelet-activating factors, leukocyte-derived reactive oxygen species, and cytokines (Alharbi, Alenezi et al. 2023). A specific inflammatory mechanism can trigger inflammation but is more likely to manifest with other inflammatory pathways. Inflammation is categorized broadly into acute and chronic forms (Figure 1.1). Acute inflammation is short-term, lasting a few days, and is marked by redness, swelling, pain, and heat, aiming to eliminate the cause of injury and promote healing, like in cuts, wounds, and infections. Chronic inflammation lasts months or years, often resulting from persistent infections, irritants, or autoimmune disorders. It can cause long-term tissue damage and is linked to conditions such as rheumatoid arthritis, asthma, and heart disease (Medzhitov 2008).

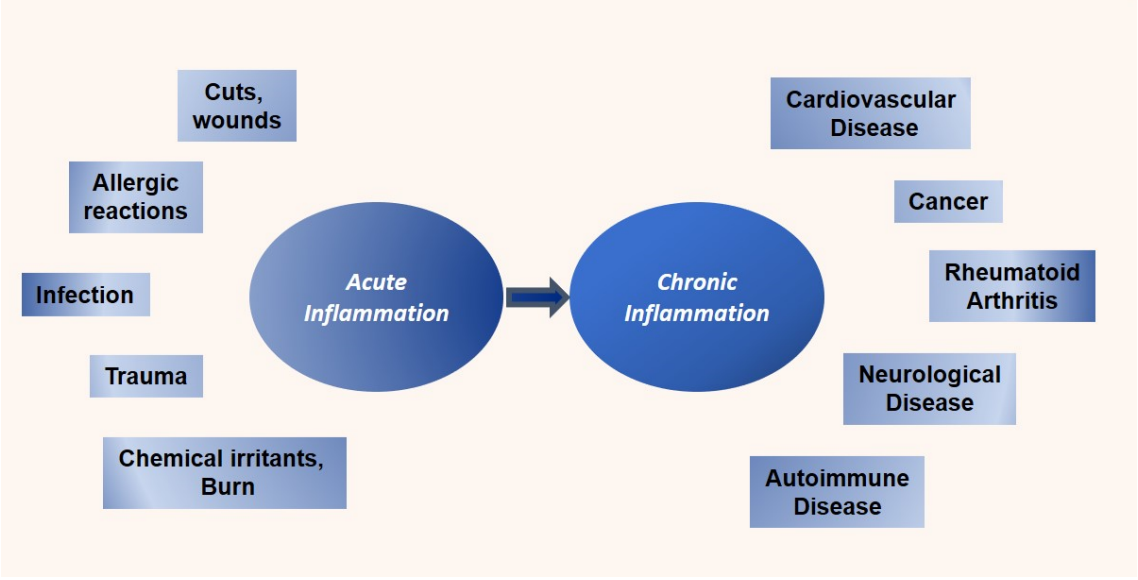

Figure 1. 1. Types of Inflammation

1.2 Anti-inflammatory medications and their limitations

Nonsteroidal anti-inflammatory drugs (NSAIDs) are prevalently included as prototypic medications for the relief therapy of inflammation, pain, and arthritis (Figure 1.2).

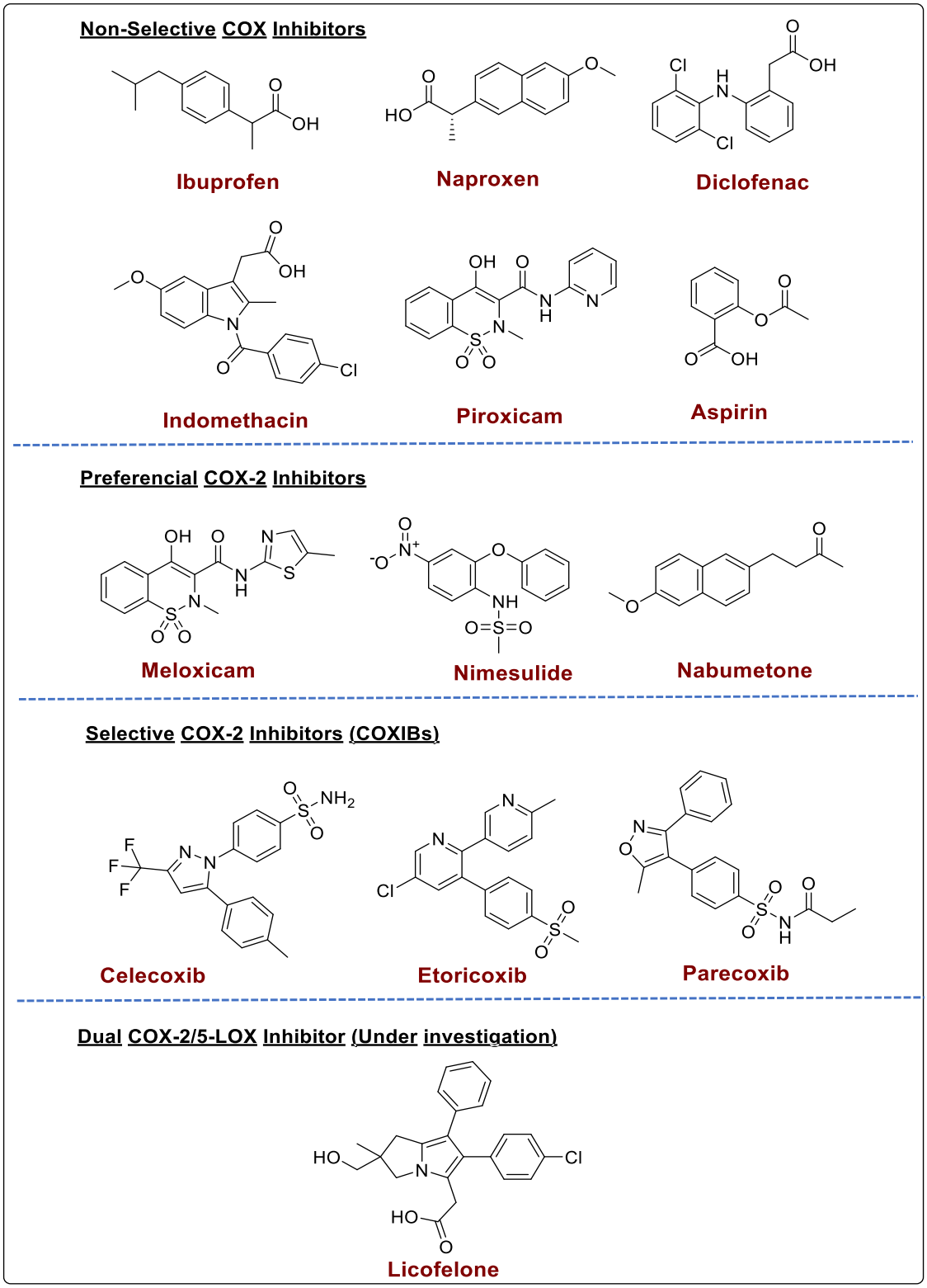

Figure 1. 2. Classification of NSAIDs with examples

NSAIDs exert their effects by inhibiting the cyclooxygenase (COX) enzymes in the arachidonic acid pathway, thereby reducing the production of pro-inflammatory prostaglandins. However, long-term usage remains restricted due to a wide range of health implications that may lead to diseases such as gastrointestinal (GI) ulcers and bleeds, kidney disease, and hepatotoxic effects (Badri, Miladi et al. 2016). Available NSAIDs are insufficient for the management of chronic inflammatory conditions because they do not effectively target complex disease mechanisms and can cause severe side effects. Their inability to address underlying causes of inflammatory disorders highlights the need for more targeted therapeutic options.

1.3 Arachidonic acid pathway

The arachidonic acid (AA) pathway is a key biological process involved in inflammation and immune response. It begins with the release of AA from cell membrane phospholipids, which is then metabolized by two main enzyme pathways: the cyclooxygenase (COX) pathway and the lipoxygenase (LOX) pathway (Figure 1.3). The COX pathway produces prostaglandins and thromboxanes (TXs), which regulate inflammation, pain, and blood clotting whereas the 5-LOX pathway produces leukotrienes, involved in the inflammation of bronchioles and immune responses (Okunishi and Peters-Golden 2011). Both pathways play critical roles in pathological processes, especially in inflammation and various diseases (Charlier and Michaux 2003). Through selective modulation of these pathways, therapies can be tailored to control excessive inflammation, enhance immune function, and prevent disease progression, ultimately improving patient outcomes and quality of life (Wang, Wu et al. 2021).

1.3.1. The Cyclooxygenase (COX) pathway

The COX enzyme is one of the important targets of drug intervention against inflammatory processes. Literature reveals that the COX enzyme exists in three isoforms

(COX-1, COX-2, and COX-3) (Bhardwaj, Kaur et al. 2017). COX-1 is a ubiquitous form produced in normal quiescent conditions. It is a constitutive protein of normal cells, whereas COX-2 is an inducible form expressed in endothelial cells, macrophages, synovial fibroblast, mast cells, chondrocytes, and osteoblasts after the tissue trauma and therefore plays an important role in inflammation (Hawkey 1999). Both isoforms of the COX enzyme share similar cellular expression locations, molecular weight, and amino-acid composition. Also, both isoforms share more than 60% sequence homology, and their 3-D structures are almost superimposable. The key difference between the COX-1 and COX-2 isozyme active site is the exchange of isoleucine (ILeu) amino acid in COX-1 for valine (Val) in COX-2 at positions 523 and 434 (Figure 1.4). The difference in the amino-acid sequence makes the COX-2 substrate-binding site more flexible and approximately 25% larger by creating a distinct secondary-binding pocket (Hla, Bishop-Bailey et al. 1999). NSAIDs typically inhibit the COX-1 enzyme, which plays a role in protecting the gastrointestinal lining. However, due to their acidic nature, NSAIDs can irritate the stomach, triggering H_2 receptors and leading to gastrointestinal issues like ulcers and bleeding (Kuna, Jakab et al. 2019).

Selective COX-2 inhibitors were developed to specifically target the COX-2 enzyme, which is primarily involved in inflammation, while sparing COX-1, thus reducing the gastrointestinal side effects commonly associated with non-selective NSAIDs. Selective COX-2 inhibitors typically display bulkier moieties that interact with the COX-2 specific active site and preclude the molecule from fitting within the relatively smaller COX-1 channel. Although selective COX-2 inhibitors were devoid of GI toxicity, but causes serious cardiovascular disease (CVD) risks, stroke, and cardiac arrest (Mukherjee, Nissen et al. 2001). These adverse effects are attributed to the disruption of the prostacyclin-thromboxane balance, where selective inhibition of COX-2 reduces prostacyclin

production, a vasodilator and inhibitor of platelet aggregation, without affecting thromboxane, a vasoconstrictor and pro-thrombotic agent. This imbalance creates a pro-thrombotic state, increasing the risk of cardiovascular events such as myocardial infarction and stroke, thus limiting the long-term safety and therapeutic viability of COX-2 inhibitors (Mukherjee, Nissen et al. 2001).

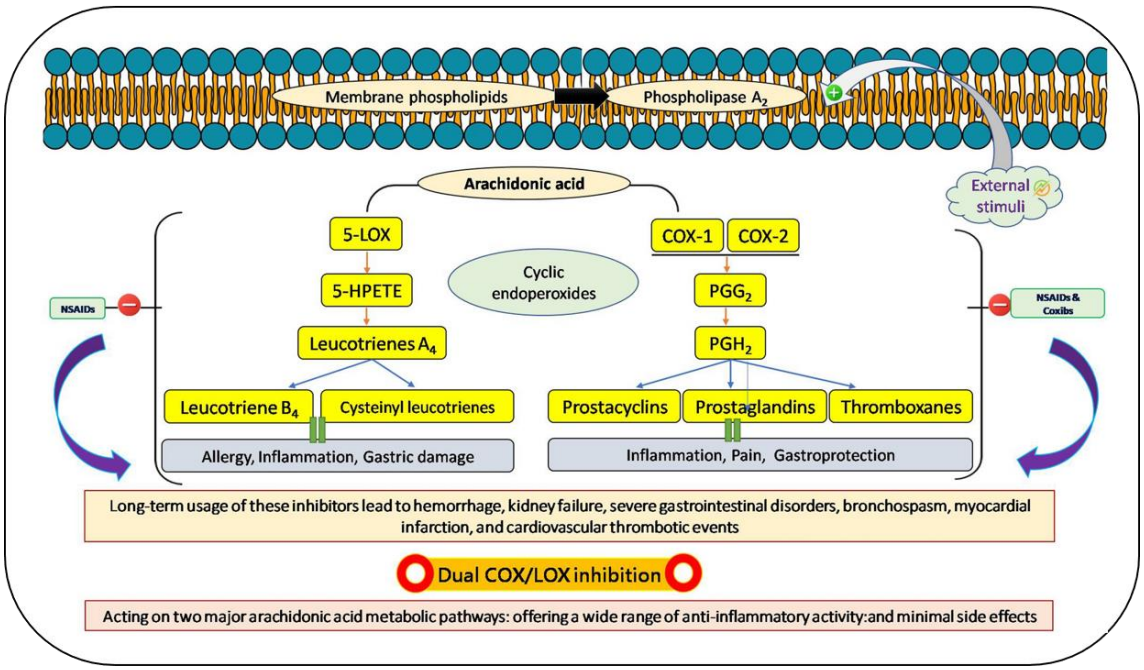

Figure 1. 3. Mechanism of Action of Anti-inflammatory Drugs (Rudrapal, M., et al. (2023)

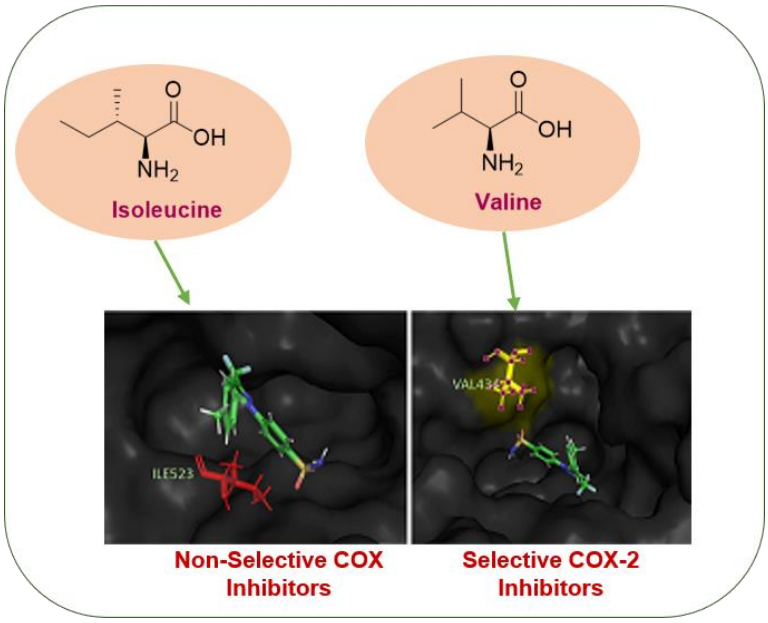

Figure 1. 4. Structural difference between COX-1 and COX-2 isozymes

1.3.2 The Lipooxygenase (LOX) pathway

The LOX is a family of iron-containing enzymes that catalyze the oxygenation of polyunsaturated fatty acids (PUFAs) to hydroperoxides (Figure 1.3). It converts AA into 5-hydroxperoxyeicosatetraenoic acid; which is then converted into Leukotriene A4 and metabolized into LTB4, LTC4, LTD4, and LTE4. The LOX pathway is parallel to the COX pathway in AA metabolism, where it utilizes more AA on COX inhibition and enhances the production of leukotrienes which is a major class of AA derivatives synthesized by the 5-lipoxygenase (5-LOX) enzyme and plays a pivotal role in mediating the inflammatory response (Gedawy, Kassab et al. 2020). The 5-lipoxygenase activating protein (FLAP) activates the liberated AA to trigger the 5-LOX enzyme and prominently shunts LT synthesis which mediates inflammatory reactions by promoting bronchoconstriction, and edema indicating adverse effects in pathological illnesses like CVD, asthma, inflammatory bowel disease, and psoriasis (Poeckel and Funk 2010). Dual COX-2/5-LOX inhibition is a proclaiming strategy that focuses on retaining the fundamental activity of NSAIDs, culminating in safer and efficacious anti-inflammatory drugs.

1.3.3. Lipoxins

Lipoxins, LXA_4, and LXB_4 (Lipoxygenase interaction products LXs) are yet another group of lipid mediators formed during AA metabolism as a part of the multicellular host response to inflammation. Lipoxins are endogenous anti-inflammatory, pro-resolving molecules that play a vital role in reducing excessive tissue injury and chronic inflammation. It regulates components of both the innate and adaptive immune systems including neutrophils, macrophages, T-cells, and B-cells. Lipoxins are synthesized not only through the 5-LOX pathway but also by the action of 12-LOX and 15-LOX. Therefore, dual 5-LOX/COX inhibitors block both prostaglandins and leukotrienes

pathway but do not affect the lipoxin (LXA_4 and LXB_4) formation (Chandrasekharan and Sharma-Walia 2015).

1.4 Dual COX-2/5-LOX Inhibition: A promising approach for effective inflammation management

The need for dual COX-2/5-LOX inhibition arises from the recognition of the complex interplay between COX-2/5-LOX pathways in mediating inflammation and pain. By targeting both enzymes, researchers aim to develop more effective treatments for various inflammatory diseases while minimizing side effects. Dual COX-2 and 5-LOX inhibition can be effective in managing situations where inflammation is associated with allergic airway inflammation (AAI) and asthma (Sokolowska, Rovati et al. 2021) and acquired prominence in various ailments such as cancer (Che, Chen et al. 2016) and psychological illnesses, including dementia and Alzheimer's (Deshmukh and Sharma 2013). As research continues, dual inhibitors may play a crucial role in the future of inflammatory disease management.

The significance of the dual COX-2/5-LOX inhibition is following:

1. **Comprehensive Control of Inflammation**

   Dual inhibitors can offer a more thorough blocking of the inflammatory cascade by concurrently inhibiting COX-2 and 5-LOX, which lowers the levels of prostaglandins and leukotrienes in chronic conditions including arthritis, asthma, and some types of cancer. This broad-spectrum inhibition may be well utilized in the management of pain, inflammation, and immunological responses (Leone, Ottani et al. 2007).

2. **Broad Spectrum of Action**

   Dual inhibition synergistically manages pain and inflammation, potentially allowing for lower doses of each inhibitor and reducing the risk of side effects associated with higher doses of single inhibitors. This approach may be especially beneficial in

disorders with overlapping inflammatory pathways, such as rheumatoid arthritis, osteoarthritis, and certain forms of cancer (Charlier and Michaux 2003).

3. **Reduced side-effects**

   Dual COX-2/5-LOX inhibitors offer anti-inflammatory efficacy without disproportionately increasing the cardiovascular and gastrointestinal risks associated with selective COX-2 inhibitors or traditional NSAIDs. By targeting both pathways in arachidonic acid metabolism, these inhibitors prevent the shunting of arachidonic acid towards the 5-LOX pathway, which can result in excess leukotriene production and associated cardiovascular risks. Additionally, they avoid the gastrointestinal toxicity linked to COX-1 inhibition. This dual inhibition approach provides a more balanced suppression of prostaglandins and leukotrienes, reducing inflammation while minimizing adverse cardiovascular and gastrointestinal side effects (Mukhopadhyay, Shukla et al. 2023).

4. **Treatment of Complex Conditions**

   Multifactorial diseases such as asthma, cardiovascular diseases, and cancer involve multiple inflammatory mediators. Dual COX-2/5-LOX inhibition offers a more effective approach for addressing these complex pathologies compared to targeting a single pathway. In cancer therapy, dual inhibitors are gaining attention as both COX-2 and 5-LOX pathways contribute in tumor progression and metastasis. The dual COX-2/5-LOX inhibition strategy is a model for halting cancer growth and spread (Mittal, Sharma et al. 2022).

5. **Improved Safety Profile**

   Dual COX-2/5-LOX inhibitors may reduce the risks of thrombosis, hypertension, and vascular inflammation by providing balanced regulation of eicosanoids. Selective COX-2 inhibition can increase thrombotic risk by disrupting the balance between pro-

thrombotic thromboxane and anti-thrombotic prostacyclin. By inhibiting 5-LOX, dual inhibitors prevent excess leukotriene production, which contributes to vasoconstriction and inflammation. This balanced inhibition helps in maintaining vascular homeostasis, reducing the risk of cardiovascular complications, and making dual inhibitors potentially safer for long-term use (Manju, Ethiraj et al. 2018).

1.5 Unmet Need and Research Opportunity: Lack of Approved Dual COX-2/5-LOX Inhibitors

The absence of approved dual COX-2/5-LOX inhibitors highlights a significant unmet need in treating various inflammatory diseases. While both pathways play critical roles in conditions such as arthritis, cardiovascular diseases, asthma, and cancer, existing therapies often target only one pathway, leading to compensatory mechanisms that can exacerbate disease. Despite the compelling need, progress in developing dual inhibitors has been limited, creating substantial research opportunities (Figure 1.5). This gap presents avenues for developing new therapies that effectively inhibit both COX-2 and 5-LOX, conducting mechanistic studies to explore their impact on inflammation, and expanding clinical applications to various inflammatory conditions. The lack of FDA-approved dual COX-2/5-LOX inhibitors represents both an unmet clinical need for research and innovation in anti-inflammatory therapies and exposes a crucial therapeutic gap with the possibility of the development of novel, safer medications for inflammatory conditions. The dual action may contribute to increased effectiveness while reducing adverse effects, such as the cardiovascular risks associated with selective COX-2 inhibitors and the gastrointestinal toxicity of non-selective NSAIDs. The development of such compounds provides an intriguing opportunity to address the therapeutic gap and offer safer and more efficient ways to treat chronic illnesses that are connected to inflammation. This breakthrough has the potential to considerably benefit people

suffering from chronic inflammatory disorders such as arthritis and asthma, improving their quality of life (Aliabadi, Khanniri et al. 2023).

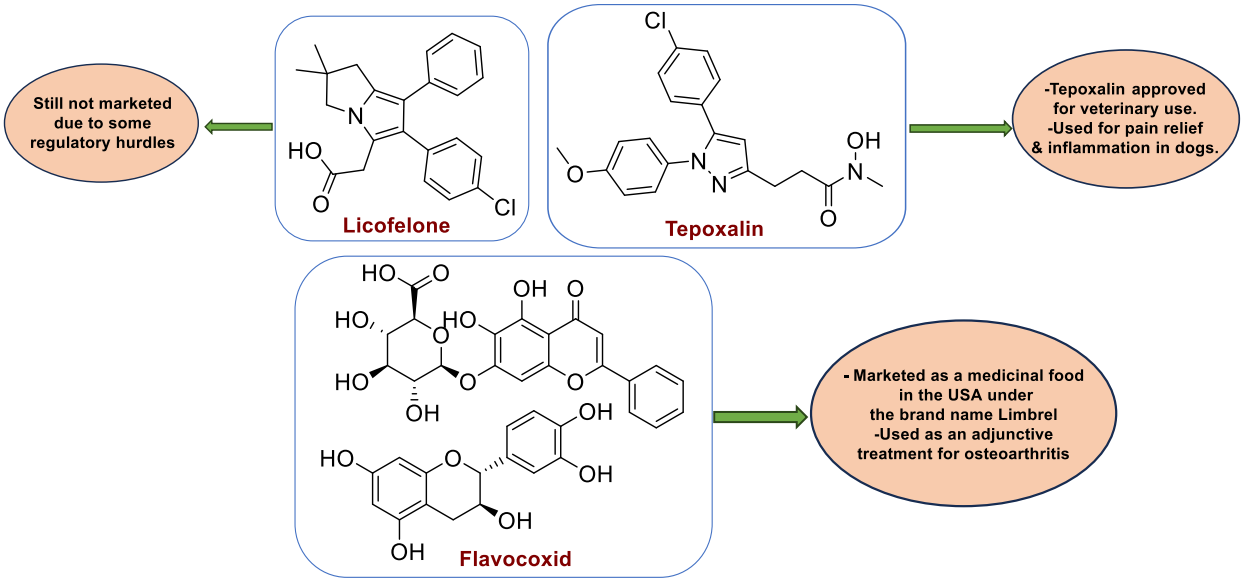

Figure 1. 5. Some examples of dual COX-2/5-LOX inhibitors

1.6 Role of Dual COX-2/5-LOX Inhibition in Various Diseases

Dual COX-2/5-LOX inhibitors provide an extensive approach in addressing disorders in which inflammation is a major factor by targeting dual pathways. This dual action manages complicated situations more efficiently than a single-pathway inhibition.

1.6.1. Role of Dual COX-2/5-LOX Inhibition in Cardiovascular Diseases

Cardiovascular diseases are among the leading causes of morbidity and fatalities worldwide (Martinez and White 2018). The development of successful therapeutic approaches should be a major objective to address this worldwide health issue. The potential significance of dual inhibition of COX-2/5-LOX has gathered significant interest in this domain. Dual COX-2/5-LOX inhibition aims to manage cardiovascular diseases by targeting the inflammatory processes associated with various cardiovascular illnesses (Du, Du et al. 2023). Dual inhibition is an emerging method to target inflammatory and thrombotic pathways in CVDs, potentially improving the condition of

patients with disorders like atherosclerosis, myocardial infarction, and ischaemic heart disease (Libby 2012). This strategy provides benefits in various ways:

- **Prevention of Thrombosis**: Dual inhibition helps to maintain a balance between thromboxane A2 (TXA2) and prostacyclin (PGI2). By reducing leukotriene production, dual COX-2/5-LOX inhibitors minimize vascular inflammation, diminish thrombus formation, and reduce the chances of thrombotic events (de Gaetano, Donati et al. 2003).
- **Regulation of Blood Pressure**: Dual inhibition preserves the synthesis of vasodilatory prostaglandins while simultaneously reducing the vasoconstrictive effects of leukotrienes. This helps in regulating blood pressure and preventing hypertension, which is critical for cardiovascular health(Leone, Ottani et al. 2007).
- **Reduction of Vascular Inflammation**: By targeting both COX-2 and 5-LOX pathways, dual inhibitors decrease the production of pro-inflammatory mediators. This reduction mitigates endothelial dysfunction and slows the progression of atherosclerosis, contributing to improved vascular health (Bitto, Minutoli et al. 2012).
- **Enhanced Treatment of Comorbid Conditions**: Patients with cardiovascular diseases often have comorbid inflammatory conditions. Dual COX-2/5-LOX inhibitors address multiple inflammatory pathways, improving treatment efficacy and patient outcomes across various overlapping conditions (Pillai, Burnett et al. 2010).

1.6.2. Role of Dual COX-2/5-LOX Inhibition in Cancer

Dual COX-2/5-LOX inhibition is emerging as a promising therapeutic strategy in cancer management, primarily because both pathways are significantly involved in tumorigenesis, tumor progression, and metastasis. The interplay between these two enzymatic pathways contributes to the complex biology of cancer and enhance treatment efficacy (Goossens, Pommery et al. 2007). Elevated levels of COX-2 and 5-LOX has

been noticed in multiple tumor forms, including colorectal, breast, lung, and prostate cancers, wherein it is linked with the aggressive and drug-resistant characteristics of cancers as following:

- **Reduction of Tumor Growth**: Elevated levels of COX-2 and 5-LOX are frequently observed in various types of cancer, where they facilitate tumor cell proliferation and survival. Prostaglandins are produced through COX-2 activity and leukotrienes synthesized via the 5-LOX pathway known to promote cell growth, inhibit apoptosis, and enhance cell migration. By simultaneously inhibiting both pathways, dual COX-2/5-LOX inhibitors can effectively reduce the production of these growth-promoting mediators, thereby slowing tumor growth and potentially leading to tumor regression (Ye, Wu et al. 2005).
- **Inhibition of Metastasis**: The metastatic spread of cancer cells to distant sites is a significant challenge in cancer treatment. Both COX-2 and 5-LOX contribute to the metastatic process by facilitating tumor cell invasion and promoting angiogenesis, which is the formation of new blood vessels to supply nutrients and oxygen to tumors. Dual inhibition disrupts these processes by decreasing the levels of inflammatory mediators that facilitate cancer cell motility and angiogenesis. As a result, dual COX-2/5-LOX inhibitors can significantly reduce the metastatic potential of tumors, potentially improving patient outcomes and survival rates (Che, Chen et al. 2016).
- **Modulation of the Tumor Microenvironment**: The tumor microenvironment plays a crucial role in supporting tumor growth and progression. It is often characterized by a pro-inflammatory milieu that fosters cancer cell survival and proliferation. By targeting both COX-2 and 5-LOX pathways, dual inhibitors can alter this inflammatory landscape. They reduce the levels of cytokines and chemokines that promote inflammation, creating a less favorable environment for tumor growth. This

modulation can also enhance the effectiveness of other cancer treatments, such as chemotherapy and immunotherapy, by making the tumor cells more susceptible to these therapies (Shen, Wang et al. 2017).

- **Improved Pain Management**: Cancer-related pain is a significant concern for many patients and is often driven by inflammatory processes mediated by COX-2 and leukotrienes. Dual COX-2/5-LOX inhibitors address both pain pathways, providing better analgesic effects than traditional treatments (Singh, Prasher et al. 2015). This dual action not only alleviates pain more effectively but also reduces the gastrointestinal side effects commonly associated with non-steroidal anti-inflammatory drugs (NSAIDs), leading to improved quality of life for patients undergoing cancer treatment (Singh, Prasher et al. 2015).
- **Potential in Chemoprevention**: Emerging research suggests that dual COX-2/5-LOX inhibitors may play a role in cancer chemoprevention, particularly in high-risk populations for certain cancers, such as colorectal cancer. By targeting the inflammatory pathways involved in tumor initiation and progression, these inhibitors could potentially reduce the risk of developing malignancies in individuals predisposed to cancer (Mohammed, Janakiram et al. 2011).
- **Synergistic Effects with Other Therapies**: Combining dual COX-2/5-LOX inhibitors with other anticancer agents may enhance therapeutic outcomes. Their ability to modulate inflammatory pathways can improve the effectiveness of conventional chemotherapy and immunotherapy. This synergy may result from increased tumor cell sensitivity to treatments and reduced resistance mechanisms, ultimately leading to better overall patient responses (Rudrapal, Eltayeb et al. 2023).

1.6.3. Role of Dual COX-2/5-LOX Inhibition in Asthma and Chronic Obstructive Pulmonary Disease (COPD)

Both COX-2 and 5-LOX pathways are implicated in the inflammatory processes of asthma and COPD. Increased production of leukotrienes through 5-LOX contributes to bronchoconstriction and airway hyperresponsiveness, while COX-2-derived prostaglandins can exacerbate inflammation. By inhibiting both pathways, dual inhibitors can reduce airway inflammation, improve lung function, and alleviate symptoms in patients with these respiratory diseases (Orafaie, Mousavian et al. 2020). Dual inhibition of COX-2 and 5-LOX indicates possibilities for more efficiently minimizing the inflammatory reaction than targeting both pathways separately. Dual COX-2/5-LOX inhibitors, such as licofelone, provide a more comprehensive strategy for reducing respiratory inflammation and bronchoconstriction since they limit both prostaglandin and leukotriene formation (Martel-Pelletier, Lajeunesse et al. 2003). Preclinical investigations with dual inhibitors showed reductions in bronchoconstriction and inflammatory markers, which are hallmarks of asthma and COPD (Wechsler 2018).

- **Reduction in Pro-inflammatory Mediators**: Dual inhibitors efficiently reduce prostaglandin and leukotriene production by targeting both COX-2 and 5-LOX, minimizing inflammatory cell recruitment and cytokine generation. This approach reduces airway inflammation and improves chronic respiratory symptoms (Mittal, Sharma et al. 2024).
- **Enhanced Bronchodilation**: Inhibiting leukotriene production addresses bronchoconstriction, which is crucial in asthma and COPD. Dual inhibition mitigates smooth muscle contraction and airway constriction, alleviating respiratory symptoms. Studies indicate that dual COX-2/5-LOX inhibition may be particularly advantageous

for patients with severe disease phenotypes who do not respond favourably to conventional therapies (Smith, DeWitt et al. 2000).

1.6.4. Role of Dual COX-2/5-LOX Inhibition in Rheumatoid Arthritis and Osteoarthritis

Inflammatory joint diseases like rheumatoid arthritis (RA) and osteoarthritis (OA) are characterized by chronic inflammation, pain, and joint damage. COX-2 and 5-LOX contribute to the inflammatory cascade in these conditions. Dual inhibitors can provide effective pain relief, reduce joint inflammation, and slow disease progression by targeting multiple inflammatory mediators (Manju, Ethiraj et al. 2018).

1.6.5. Role of Dual COX-2/5-LOX Inhibition in Metabolic Syndrome and Diabetes:

Inflammatory processes are central to the pathogenesis of metabolic syndrome and type 2 diabetes. Increased levels of pro-inflammatory mediators can lead to insulin resistance and impaired glucose metabolism. By inhibiting COX-2 and 5-LOX, dual inhibitors can reduce systemic inflammation, improve insulin sensitivity, and potentially prevent the onset of diabetes (Mittal, Sharma et al. 2022).

1.6.6. Role of Dual COX-2/5-LOX Inhibition in Neurodegenerative Diseases:

Conditions like Alzheimer's disease and Parkinson's disease involve neuroinflammation, which contributes to neuronal damage and disease progression. Both COX-2 and 5-LOX are upregulated in neuroinflammatory processes. Dual inhibition may help to mitigate neuroinflammation, reduce oxidative stress, and protect neuronal function to offer potential therapeutic benefits in these conditions (Kumar, Behl et al. 2020).

1.6.7. Role of Dual COX-2/5-LOX Inhibition in Chronic Pain Conditions:

In conditions such as fibromyalgia and neuropathic pain, chronic inflammation contributes to persistent pain. Dual COX-2/5-LOX inhibitors can target multiple

inflammatory pathways involved in pain signalling, potentially providing more effective pain relief with fewer side effects compared to traditional analgesics (Mittal, Sharma et al. 2022).

Dual inhibition of COX-2/5-LOX has great potential to transform the development of novel anti-inflammatory medications. This approach allows for comprehensive management of comorbid conditions and offers potential in chemoprevention for diseases like cancer and metabolic disorders. Furthermore, dual inhibitors may synergistically enhance existing treatments, leading to better patient responses and reduced resistance. In summary, dual COX-2/5-LOX inhibition represents a promising avenue for innovative therapies aimed at improving outcomes in chronic inflammatory conditions. This approach could mitigate adverse effects associated with long-term NSAID use, such as gastrointestinal and cardiovascular risks, while enhancing therapeutic outcomes in diseases like rheumatoid arthritis, asthma, and inflammatory bowel disease. Thus, dual COX-2/5-LOX inhibition is an intimidating choice for developing anti-inflammatory drugs that are safer and more effective (Du, Du et al. 2023).