Innovations in Pharmaceuticals and Pharmacotherapy

www.innpharmacotherapy.com

eISSN: 2321–323X

Review Article

A review on COX and their inhibitors: Present and future

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Abstract

Cyclooxygenase (COX), officially known as prostaglandin-endoperoxide synthase (PTGS), is an enzyme that is responsible for formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. These prostaglandins are also known as autocoid mediators that affect virtually all known physiological and pathological processes via their reversible interaction with G-protein coupled membrane receptors. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain. The inflammatory molecule PGE2 lowers pain thresholds and the primary goal of oral inhibitors of PGE2 is to reduce pain. This review article provides an overview and an update on the progress achieved in the area of COX inhibitors and their role in health and disease conditions. It also discusses some unresolved issues related to the use of selective COX-2 inhibitors as a safe and promising therapeutic option not only for the treatment of inflammatory states but also for cancer and Alzheimer disease.

Keywords: Cyclooxygenase, prostaglandins, biosynthesis, NSAIDS, inflammation

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1. Introduction

The enzyme cyclooxygenase (COX) is an integral membrane protein found mainly in microsomal membranes, firstly purified in 1976 and on further cloned in 1988 [1]. The enzyme was constitutively expressed in a variety of tissues, including brain, kidney, vascular endothelium and reproductive system [1]. This cyclooxygenase (COX) enzyme or prostaglandin H_2 synthase (PGHS) plays a central role in the synthesis of biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane by subsequently transforming arachidonic acid [2]. Presently, three COX isoenzymes are known: COX-1, COX-2 and COX-3; COX-1 considered as a constitutive enzyme, being found in most mammalian cells whereas COX-2 is an inducible enzyme abundant in activated macrophages and other cells at sites of inflammation. Furthermore, COX-3 is a splice variant of COX-1. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain. Although both enzymes (COX-1 and COX-2) act basically in the same fashion, sometimes, selective inhibition produces difference which results in numerous side-effects.

Role of COX enzyme in Prostaglandin Biosynthesis:

COX is functional enzyme that catalyzes the first two steps namely cyclooxygenation and `peroxidation, in the pathway leading to the formation of prostaglandins and thromboxane from the substrate arachidonic acid. In the first step, COX cyclizes and add two molecules of oxygen (O_2) to arachidonic acid to form the cyclic hydroperoxide endoperoxide PGG_2 and in the second step, PGG_2 subsequent reduce to hydroxy endoperoxide PGH_2 [3-4]. PGH_2 is a highly unstable endoperoxide, which is transformed by a

range of enzymes and nonenzymic mechanisms into the primary prostanoids, PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2, and TXA_2 [5]. Interestingly, the coupling of PGH_2 synthesis to its transformation to prostaglandin and thromboxane by downstream enzymes is intricately orchestrated in a cell specific fashion. That is, any given prostanoid-forming cell tends to form only one of these compounds as its major product. Thus, for example, in brain and mast cells, PGH_2 is converted by cytosolic enzyme PGD synthase to PGD_2 whereas in the uterus PGH_2 can alternatively be converted by PGF synthase to PGF_{2a}. Vascular endothelial cells produce PGI_2 or prostacyclins from PGH_2 by means of the PGI or prostacyclin synthase, and platelets release TXA_2 from the same precursor (PGH_2) as the PGs through the action of the enzyme thromboxane synthase. Both PGI_2 and TXA_2 are rapidly hydrolyzed to the inactive compounds TXB_2 and 6-keto-PGF_{1a}, respectively. Finally, PGE_2 is formed in many cell types by the enzyme PGE synthase [2]. A schematic representation of these pathways is shown in Figure 1.

Classification of COX Isozymes: COX-1, COX-2 and now COX-3:

In 1991, it was disclosed that COX exists in two distinct isozymes, COX-1, which is constitutively expressed; and COX-2, which is inducible [6-8]. COX-1 is expressed in most tissues and described as a "housekeeping" enzyme, which regulates normal cellular processes such as gastric cytoprotection, vascular homeostasis, platelet aggregation, and kidney function and is stimulated by hormones or growth factors. On the other hand, COX-2 is constitutively expressed in the brain, kidney, bone and probably in the female reproductive system, primarily responsible for inflammation but apparently not for gastrointestinal integrity or platelet aggregation.

In terms of molecular biology, COX-1 and COX-2 exhibits similar molecular weight, approximately 70 and 72 kDa, respectively, and having 65% amino acid sequence homology in a single species [9].

COX-1 protein contains a 17 amino acid sequence near its amino terminus that is absent in COX-2. In

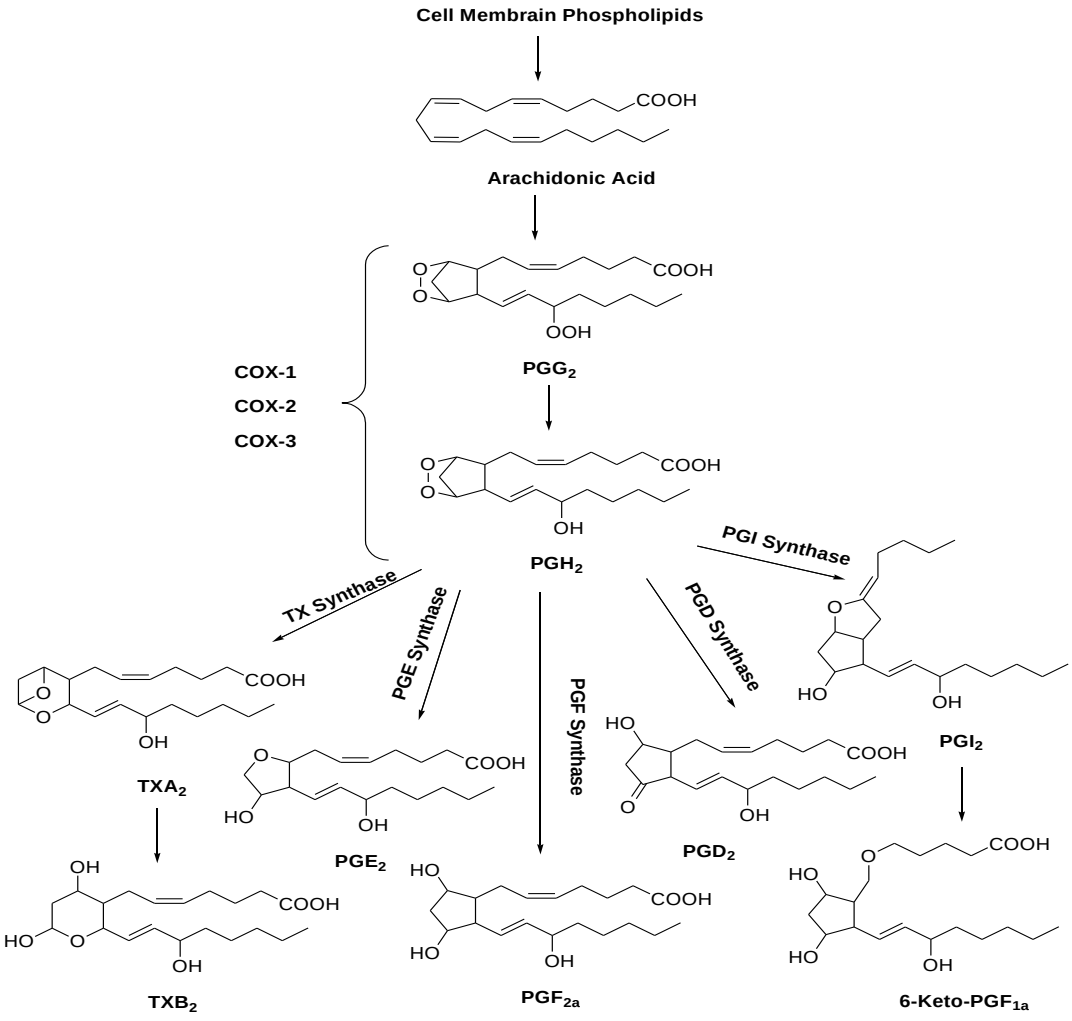


Figure 1. Enzymatic pathway of prostaglandin (PG) formation from arachidonic acid.

contrast, COX-2 contains 18 amino acid sequences near its carboxyl terminus that is not present in COX-1 [10-12]. Thus, the two isoforms of COX are almost identical in structure but have important differences in substrate and inhibitor selectivity and in their intracellular locations [10-12]. The confocal fluorescence imaging microscopy and histofluorescence staining techniques reveal that COX-1 and COX-2 are located in the endoplasmic reticulum and nuclear envelope where as COX-2 is more highly concentrated in the nuclear envelope [9]. Besides this, COX isoforms catalyze identical reactions and exhibit the same kinetic constants for the conversion of arachidonic acid to prostanoids. There are, however, two structural differences between the two isozymes that have important pharmacological and biological consequences. First the cyclooxygenase active site of COX-2 is larger and more accommodating than that of COX-1 (Table 1) and secondly the distribution of enzyme (Table 2).

Table 1. Difference between COX-1 and COX-2 [13]

Parameter	COX-1	COX-2
Gene size	22kb	83kb
Chromosome	9	1
mRNA regulation	Constitutional	Constitutional and Inducible
Molecular weight	70kDa	72kDa
Substance specificity	AA, γ-linolenic acid	AA, γ-linolenic acid, α- linolenic acid
Amino acid	599	604
Active site	Smaller	Larger

Prostaglandins made by COX-2 are also important in ovulation and in the birth process. However, the inducing stimuli include pro-inflammatory cytokines and growth factors, implying a potential role for COX-2 in both inflammation and control of cell growth. Thus, discovery of COX-2 provides possibility for designing new drugs which can reduce inflammation without removing the protective PGs in the stomach and kidney made by COX-1.

Table 2. COX-1 and COX-2 enzyme distribution [13-14]

Location	COX-1	COX-2
Brain	-	+
Breast Cancer	-	+
Chondrocyte	+	+
Endothelial cell	+	-
GI Track	+	-
Lungs	-	+
Liver	+	-
Platelets	+	-
Renal medulla	+	-
Renal Cortex	-	+
Synovial tissue	-	+
Spleen	+	-

Apart from this, Both COX-1 and COX-2 Recently, the third isoform of COX enzyme was identified and named as COX-3 as a splice variant of COX-1, which retains intron one and have a frameshift mutation due to this sometimes preferbly named as COX-1b or COX-1 variant (COX-1v) [15]. This isoform is most abundantly present in cerebral cortex and heart [15]. Literature survey also reveals that COX-3 was selectively inhibited by paracetamol, phenacetin, antipyrine, dipyrone, and some other NSAIDs in rodents [15]. Finally, the discovery of the frame-shift mechanism has made it highly unlikely that COX-3 plays a role in inflammation and fever in humans.

Benefits of COX in Health and Disease:

The most important targets of COX enzyme are to synthesize inflammatory mediators called prostaglandins and thromboxanes. COX-1 provides PGs in the stomach and intestine to maintain the integrity of the mucosal epithelium and its inhibition leads to gastric damage, hemorrhage and ulceration. On the other hand, COX-2 inhibitors may not only be anti-inflammatory but may also be active in colon cancer and Alzheimer's disease. A list of COX mediated along with their effect on various tissue/organs is reported in Table 3.

Table 3. Biological effects of cyclooxygenase products [2]

Tissue/Organ	Mediators	Effects
Central Nervous System	PGE_2 PGD_2 PGE_2, PGI_2	Fever Sleep Pain
Cardiovascular System	TXA_2, PGI_2 TXA_2 PGE_2, PGI_2 TXA_2, PGF_2a PGE_2, PGI_2	Thrombosis, Platelet Aggregation Vascular Permeability Arterial Vasodilation Venous vasoconstriction Patency of the Fetal Ductus Arteriosus
Respiratory System	PGE_2 PGF_2a, TXA_2	Bronchodilation Bronchoconstriction
Renal System	PGE_2, PGI_2 PGE_2, PGI_2 PGE_2	Regulation Renal Blood Flow and Glomerular Filtration Rate Renin Release Inhibition Hydroosmotic Effect of ADH
Gastrointestinal System	PGE_2, PGI_2	Cytoprotection
Immune System	PGE_2, PGI_2	Inhibition T and B lymphocyte activation and proliferation
Reproductive system	PGE_2, PGF_2a	*Female* Uterine Contraction, Oxytocic Action *Male* Fertility

Anti-inflammatory activity:

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. It is a dynamic process and can be classified as either acute or chronic. Acute inflammation is the initial response and is characterized by the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. Chronic inflammation concerns a progressive change in the type of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissue [16]. The pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL) - 1β, and vascular endothelial growth factor (VEGF) plays a central role in inflammation [17]. In mammalian cells, eicosanoid biosynthesis is usually initiated by the activation of phospholipase A_2 and the release of arachidonic acid from membrane phospholipids in response to the interaction of a stimulus with a receptor on the cell surface. Long term inflammation leads to development of diseases such as rhematoid arthritis, multiple sclerosis, inflammatory bowel disease, psoriasis and chronic asthma etc.

Non-steroidal anti-inflammatory drugs (NSAIDs):

The history of analgesic and anti-inflammatory substance started with the use of decocted salicylate containing plants by ancient Greek and Roman physicians. In light of the growing number of applications in written human history salicylates containing plants extracts can be traced throughout to treat inflammation, pain and fever. Over the past 140 years, diverse classes of compounds have been introduced having analgesic, anti-inflammatory and antipyretic properties and these are colectively termed as non steroidal anti-inflammatory drugs (NSAIDs). One hundred and fifty years ago, Felix Hoffmann, a chemist with the German company Bayer was first isolates acetylated salicylic acid called Aspirin [18-19]. Aspirin had ability to block production of cyclooxygenase (COX) enzymes COX-1 and COX-2, responsible for the synthesis of inflammatory mediators known as prostaglandins and thromboxanes. Thus, it makes aspirin as one of the most widely used medications in the world, with an estimated 40,000 tonnes of it being consumed each year [20]. However, aspirin differs mostly from other NSAIDs in their mechanism of action by inhibiting the COX-1 variant than the COX-2 variant of the enzyme in irreversible manner [21]. In 1971, Vane, Ferreira et al. along with Smith and Willis were first to observe and report that NSAIDs reduce or prevent the production of PGs by direct inhibition of COX enzymes [22-24]. The discovery of the COX-2 isozyme and its characterization plays role in inflammation which fostered the development of a new class of compounds that selectively inhibit COX-2, without affecting the COX-1 dependent PG biosynthesis necessary for physiological functions [25-28]. At present, NSAIDs are among the most widely prescribed class of pharmaceutical agents worldwide, having broad clinical utility in treating pain, fever and inflammation [29-30].

Selective COX-2 Inhibitors Coxib's:

As previously described, the rationale behind the development of selective COX-2 inhibitors was the concept that selective inhibition of COX-2 isoenzyme may induce anti-inflammatory and analgesic effects comparable to nonselective COX inhibitors, with considerably less damage to the gastric mucosa. The first selective COX-2 inhibitor Coxib's approved by Food and Drug Administration (FDA) in 1999 was celecoxib, which was followed by introduction of rofecoxib, valdecoxib, parecoxib, aceclofenac and etoricoxib [31]. The clinical trial had proven that both celecoxib (Celebrex) and rofecoxib (Vioxx) provide significant relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis and in alleviating pain following dental extraction, while reducing the incidence of gastrointestinal ulcers and erosions seen with standard NSAID therapy [32-36]. Moreover, these eagerly awaited highly selective COX-2 inhibitors are of great interest because they may represent an alternative therapeutic option for the treatment of inflammation in diseases, such as in cirrhosis with ascites, in which renal function is critically dependent on PGs [37-38]. A second-generation of selective COX-2 inhibitors (i.e., valdecoxib and etoricoxib) with a higher COX-1 to COX-2 selectivity ratio than celecoxib and rofecoxib is currently under evaluation in patients with osteoarthritis and rheumatoid arthritis. However, valdecoxib has also been approved for the treatment of dysmenorrhea. Furthermore, the analgesic efficacy and tolerability of parecoxib, an injectable prodrug of valdecoxib, is currently being tested in postoperative laparotomy and orthopedic knee surgery patients [39-40]. These drugs have at least 200-300 fold selectivity for inhibition of COX-2 over COX-1. The Structure of currently available NSAIDs and COXIBs are provided in Figure 2.

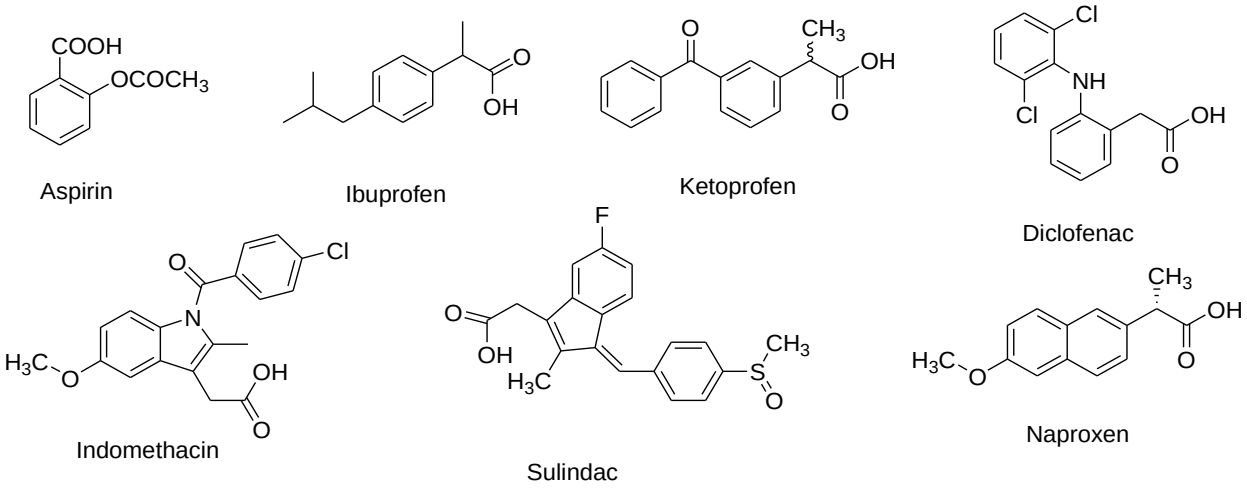
Natural COX Inhibitors:

However, NSAIDs and COXIBs are the most widely prescribed drugs in the world, to treat inflammation, pain and arthritis. But their use is also associated with wide range of toxic effect. In contrast, Botanicals and in particular plant food supplements (PFS) receive great acceptance by European consumers as they can deliver significant health benefits at relatively low costs. In addition, a variety of chemical constituents such as alkamide [41-42], coumarins [43], carotenoid [44], flavonoids [45], steroids [46-47], fatty acids [46-48], stilbenes [49-50], terpenoids [51] etc. are isolated from plant origin which significantly inhibits the expression of the COX gene. A detailed classification of these herbal COX inhibitors is given in Table 4. Apart from this, Seungma-galgeun-tang a folk medicine of China [52] and Shosaikoto a Kampo medicine of Japan [53] were used widely to treat inflammation by inhibiting COX enzyme. Furthermore, prenylated resveratrol derivative 4-(3-methyl-but-1-enyl)-3,5,3′,4′-tetrahydroxystilbene from fungal infected peanuts [54] and caffeic acid phenethyl ester (CAPE) from alcoholic extract of propolis [55] contribute the overall COX inhibitory activity. In search for new biologically active natural products, a number of isolates derived from algae and sponges were evaluated. Of these, palisol and dictyol C demonstrated the most potent inhibition of COX-2 [56]. In addition, Fish oils also found to contain natural inhibitor of COX and have been proposed as a reasonable alternative for the treatment of rheumatoid arthritis [57]. The structures of some active chemical constituents are provided in Figure 3.

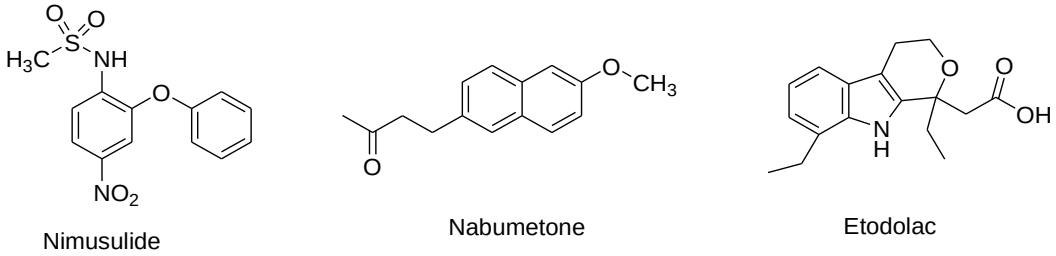
Anti Tumor Activity:

In addition to their widespread benefit in arthritis, COX-2 specific inhibitors are also used to reduce the development of colon cancer in high-risk patient as adenocarcinoma cells in the colon overexpress COX-2. COX is suspected to play a key role in the progression of colon cancer, since PGE_2 levels are increased in tumors. Literature survey reveals that, approximately 85% of adenocarcinomas exhibit a two- to fifty-fold increase in COX-2 expression at both mRNA and protein levels compared with matched, macroscopically normal, colonic mucosa from the same patient [70-73]. Both carcinogens induced (azoxymethane-induced colonic tumors in rats) and genetic (Min mice with multiple intestinal neoplasia) animal models of colon cancer had confirmed the existence of an increased expression of COX-2 in tumors [73-75]. On the contrary, subsequent studies have demonstrated that the increased expression of COX-2 in colon cancer originates mainly from the interstitial cells (i.e., macrophages), whereas little COX-2 expression is found in epithelial cancer cells [76-77]. Moreover, *in vitro* studies of over expression of COX on different cell lines (rat intestinal cell line, gastrocolonic cell lines HT-29 and HCA-7 and human colon cell line Caco-2) revealed that cells over-expressing COX-2 undergo phenotypic changes that could enhance their tumorigenic

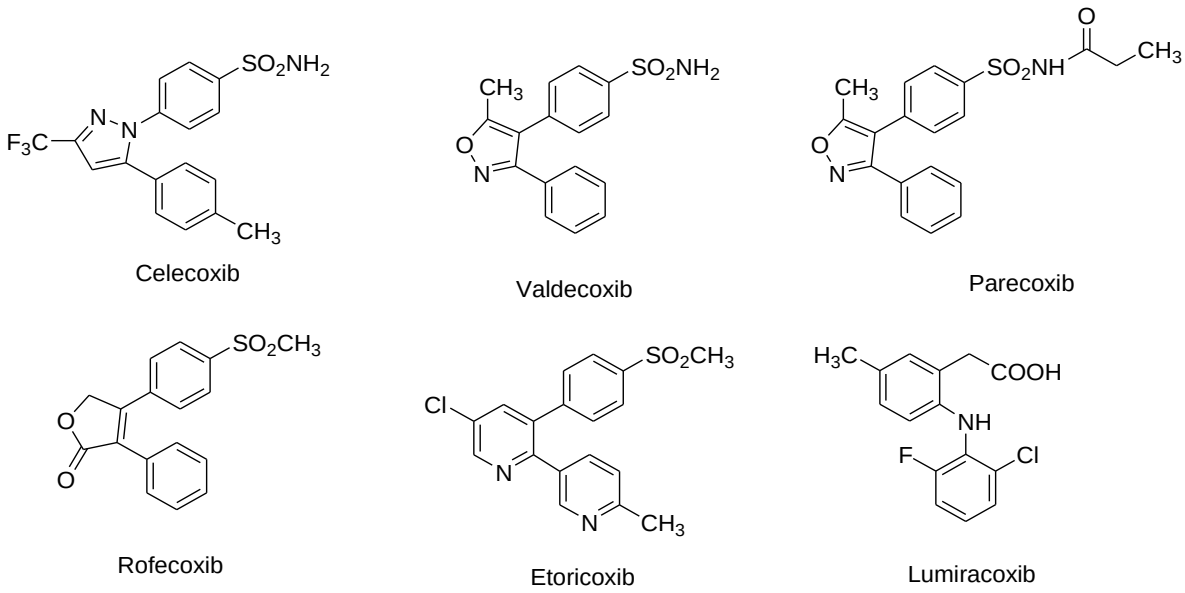
potential, such as exhibition of an increased adhesion to extracellular matrix proteins and resistance to apoptosis [78-79]. However, human gastrocolonic cancer cell line, HT-29, showed increased proliferation in the presence of PGs in the culture medium [80]. A conclusive proof of the role of COX-2 in cell growth is provided by the use of selective COX-2 inhibitors. The effects of the highly selective COX-2 inhibitor, SC-58125, was tested in two different cell lines, only one of which had a high level of COX-2 expression and activity. It was observed that SC-58125 decreased cell growth only in the COX-2 expressing cell line [81].



A) Traditional Non-Steroidal Anti-inflammatory Drugs



B) Preferential COX-2 inhibitors



C) Selective COX-2 inhibitors

Figure 2: Classification of NSAID's and Coxib's according to their selectivity towards COX enzyme.

Table 4: Herbs with active constituents having COX inhibitory activity

Plant Name	Family	Active constituent	Reference
Agrocybe aegerita	Strophariaceae	Ergosterol and fatty acids (palmitic, stearic, oleic and linoleic acids)	[47]
Aiphanes aculeate Willd.	Arecaceae	Aiphanol and Isorhapontigenin	[49]
Apium graveolens Linn.	Apiaceae	Sedanolide, senkyunolide-N, senkyunolide-J, L-tryptophan and 3-hydroxymethyl-6-methoxy-2,3-dihydro-1H-indol-2-ol.	[58]
Aralia continentalis	Araliaceae	Kaurenoic acid	[51]
Cannabis sativa	Cannabaceae	Canniprene, olivetolic acid	[59]
Ceiba pentandra	Malvaceae	5-hydroxy-7,4',5'-trimethoxyisoflavone 3'-O-beta-D-glucoside, 5,3'-dihydroxy-7,4',5'-trimethoxyisoflavone and flavan-3-ol, (+)-catechin	[60]
Cornus kousa	Cornaceae	Kaempferol 3-O-rhamnoside, myricetin 3-O-rhamnoside, kaempferol 3-O-glucoside, cornin and stenophyllin	[61]
Cymbidium goeringii	Orchidaceae	Gigantol	[62]
Dracaena loureiri	Asparagaceae	4,3',5'-trihydroxystilbene, 4,3'-dihydroxy-5'-methoxystilbene and 4-hydroxy-3',5'-dimethoxy stilbene	[50]
Dystaenia takeshimana	Umbelliferae	Coumarins, β-sitosterol and dacusterol	[43]
Echinacea purpurea (Linn.) Moench	Arecaceae	Undeca-2E,4Z-dien-8,10-diynoic acid isobutylamide, undeca-2Z,4E-dien-8,10-diynoic acid isobutylamide, dodeca-2E,4Z-dien-8,10-diynoic acid isobutylamide, undeca-2E,4Z-dien-8,10-diynoic acid 2-methylbutyl amide, dodeca-2E,4Z-dien-8,10-diynoic acid 2-methylbutylamide, dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide.	[42]
Evodia rutaecarpa	Rutaceae	Evodiamine, rutaecarpine and goshuyuamide II	[63]
Grifola frondosa	Meripilaceae	Ergosterol, ergostra-4,6,8(14),22-tetraen-3-one, 1-oleoyl-2-linoleoyl-3-palmitoyl glycerol and fatty acids (palmitic, oleic, and linoleic acids)	[46]
Houttuynia cordata	Saururaceae	Fatty acids (linolenic, linoleic, oleic, palmitic and stearic acid)	[48]
Hypericum perforatum	Hypericaceae	Hyperforin	[64]
Nigella sativa	Ranunculaceae	Thymoquinone and thymohydroquinone	[65]
Ocimum sanctum Linn.	Lamiaceae	Eugenol, cirsilineol, cirsimaritin, isothymonin, apigenin and rosmarinic acid	[66]
Perilla nankinensis	Labiatae	Luteolin diglucuronide, apigenin diglucuronide, and semi-pure luteolin diglucuronide	[44]
Piper methysticum Forst	Piperaceae	Dihydrokawain, yangonin and flavokawain B	[67-68]
Stereocaulon alpinum Laur.	Stereocaulonaceae	9-cis-octa-decenamide	[41]
Zingiber cassumunar	Zingiberaceae	Phenylbutenoids	[69]

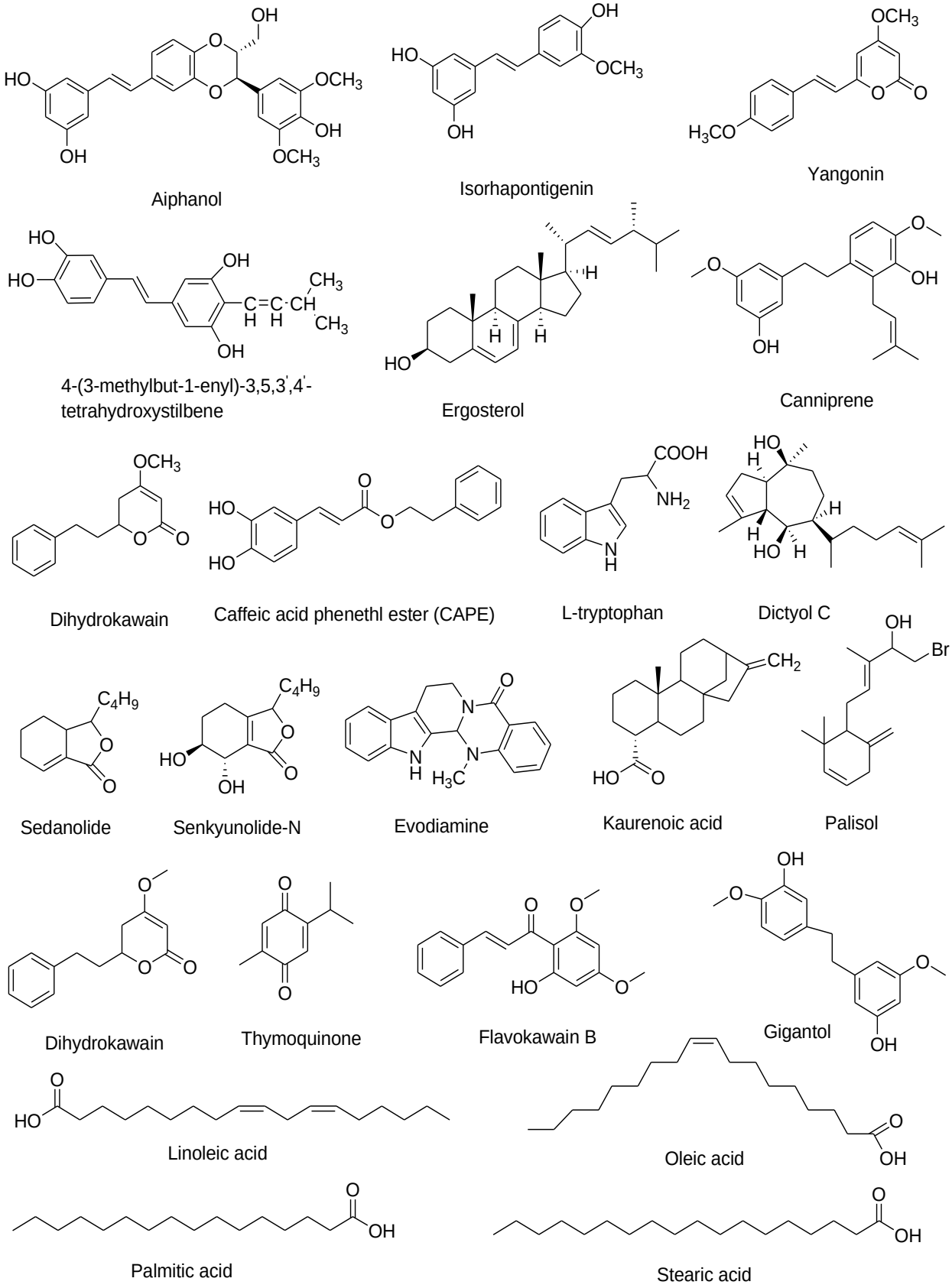


Figure 3: Chemical structure of some natural COX inhibitors

Epidemiological studies also demonstrate the association between regular long-term consumption of NSAIDs, particularly aspirin, reduces incidence of colon cancer [82-84]. Aspirin is also sought as a potentially viable option in the prevention of sporadic colon cancer and neurodegenerative disorders [85-86]. Parallel studies in animal models of colon carcinogenesis proved that aspirin, as well as other traditional NSAIDs, such as piroxicam, indomethacin, sulindac, ibuprofen and ketoprofen, inhibit chemically-induced colon cancer in rats and mice [87]. The mechanism by which NSAIDs reduce the risk of cancer is likely related to the inhibition of COX-2. In addition, some recent studies strongly suggested that COX-2 inhibitors may also be increase the beneficial effects in breast, head and neck, lung, pancreatic and gastric cancers [87-90]. Indeed, celecoxib consistently and dose-dependently inhibits tumor growth and the number of lung metastasis in the syngenic Lewis lung carcinoma model [91]. Interestingly, in animal studies, celecoxib has been shown to potentiate the antitumor activity of conventional chemotherapy and radiation [92-93].

Anti Alzheimer's Activity:

Alzheimer's disease, was first described by German psychiatrist and neuropathologist Alois Alzheimer in 1906 [94] and is the most common form of dementia. Most often, it is diagnosed in people over 65 years of age [95], although the less-prevalent early-onset Alzheimer's can occur much earlier. Research indicates that the disease is associated with plaques and tangles in the brain [96]. In 2006, there were 26.6 million sufferers worldwide. Alzheimer's is predicted to affect 1 in 85 people globally by 2050 [97]. Early symptoms are often mistakenly thought to be 'age-related' concerns, or manifestations of stress [98]. As the disease advances, symptoms can include confusion, irritability, aggression, mood swings, trouble with language, and long-term memory loss. However, five medications are currently used to treat the cognitive problems of AD: four are acetylcholinesterase inhibitors (tacrine, rivastigmine, galantamine and donepezil) and the other (memantine) is an NMDA receptor antagonist [99]. In addition, only donepezil is approved for treatment of advanced AD dementia [100]. Moreover, these drugs are associated with nausea and vomiting as side effect, both of which are linked to cholinergic excess. Other effects include muscle cramps, decreased heart rate (bradycardia), decreased appetite and weight, and increased gastric acid production [101].

Further investigation concludes that Long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) reduces likelihood of developing AD [102]. Various human postmortem studies, in animal models, or *in vitro* investigations also supports that NSAIDs can reduce inflammation related to amyloid plaques [102]. Several case-control studies suggest that NSAID's significantly reduces odds ratio to almost half of the normal risk for Alzheimer's disease in patients those are on anti-inflammatory therapy [103-105]. A report published in April 1997 confirmed the previous findings of an inverse correlation between the severity or incidence of Alzheimer's disease and the ingestion of NSAIDs, with ibuprofen as the most frequently used compound, probably reflecting its availability without prescription [106].

In all these analyses, the mechanisms proposed are essentially anti-inflammatory and reflect the recognition of inflammatory events and components in the Alzheimer's disease lesions [103-104, 107]. Along with the amyloid protein, there are activated microglias, complement fragments, release of cytokines, and other classical signs of inflammation were observed during study at the site of inflammation. A crucial finding is that the protein amyloid is capable of activating the microglia. During the study, although the NSAID would not be expected to modify the abnormal metabolism of amyloid but they reduce the response of microglia to the protein. It was also conclude that, the neuronal damage in Alzheimer's disease may be due more to the inflammatory reaction with the consequent free radical and protease release than to the presence of amyloid per se. Thus, inhibition of inflammation may delay or even abort the loss of neurones consequent on amyloid deposition.

Another study gives evidence that rat microglia, which was stimulated with LPS express COX-2 gives same response as on observe with human microglia [108]. It was also surprising that the total COX-2 content of brain tissue in Alzheimer's disease patients was lower than normal person [109]. This additional explane that in these patients perticularly in late-stage the loss of neurones and their COX-2 outweighed the increased COX-2 in activated microglia. There may also be a detectable increase in the total COX-2

content earlier in the disease process. The lack of a good animal model for Alzheimer's disease has undoubtedly delayed analysis of its causes. A major benefit of the new selective COX-2 inhibitors could be early treatment in asymptomatic, but genetically at risk, subjects, which could result in a delaying or even preventing the clinical disease. Such treatment with the existing NSAIDs with their propensity to cause gastric damage and platelet malfunction has already been shown to have low compliance [110] and would always be difficult to justify in the asymptomatic subjects targeted. Selective COX-2 inhibitors should, however, enable this prophylactic action of decreased PG synthesis to be fully realized with a minimum of side effects.

Toxicology:

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs worldwide and represent a mainstay in the therapy of acute and chronic pain. However, their use is frequently associated with a broad spectrum of adverse effects, which are related to the inhibition of prostaglandin (PG) synthesis in tissues where PGs are responsible for physiological homeostasis [111-114]. Since COX-1 derived PGs are presumably involved in housekeeping functions, such as gastrointestinal cytoprotection, and COX-2 derived PGs are implicated in inflammation. In recent years, however, animal data have challenged the initial paradigm, unravelling the constitutive expression of COX-2 in normal tissues, together with new physiologic roles of this isoenzyme, including gastric mucosal defense, renal homeostasis and endothelial PGI_2 production. Therefore, apart from the beneficial anti-inflammatory, antipyretic and analgesic effects of NSAIDs, COX inhibition also results in unwanted side effects, particularly in the gastrointestinal tract [115]. That is, at the concentrations required to inhibit PG biosynthesis at sites of inflammation (COX-2 activity), they also elicit a marked suppression of PG production in the gastrointestinal and renal systems (COX-1 activity) jeopardizing the integrity of the gastric mucosa and renal and platelet function. Gastroduodenal ulceration is the best-characterized serious adverse event of NSAID therapy and is the consequence of inhibiting PGs, which are the most important gastric cytoprotective agents [116]. However, many clinical trials have confirmed the efficacy and relative lack of gastroduodenal toxicity of all of the selective COX-2 inhibitors when compared to nonselective NSAIDs. On the other hand although the incidence of renal side effects in healthy subjects is not significant, adverse renal events are frequent in those patients with impaired effective arterial blood volume in which renal function is critically dependent on PGs, such as decompensated liver cirrhosis [117-118].

Like NSAIDs, Coxib's also becomes the most widely prescribed drugs in the world for arthritis and acute pain. However, on September 30, 2004, the pharmaceutical giant Merck & Company, Inc. announced a voluntary worldwide withdrawal of rofecoxib from the market. The company's decision, was based on data from a new, three-year prospective, randomized, placebo-controlled clinical trial, which showed that after 18 months of use, VIOXX® increased relative risk for confirmed cardiovascular events, such as heart attack and stroke [119]. Although clinical trials gave conflicting results, partly due to the influence of pharmaceutical manufacturers [120], pharmacological evidence seems to support the concept that cardiovascular toxicity of selective COX-2 inhibitors may be a class effect [121]. This has raised serious concerns about the risk of thrombotic events during treatment with coxibs, marking off the therapeutic benefits that could be expected from COX-2 selective inhibition and questioning the need of more selective compounds [122-123]. Several meta-analyses and systematic reviews also indicate that diclofenac has demonstrated the highest cardiovascular risk of any of the nonselective NSAIDs [124].

Future Prospects:

NSAIDs are the most widely prescribed drugs in the world, with an estimated 100 million users of rofecoxib and valdecoxib since the drugs were introduced on the market in 1999. Following the removal of rofecoxib and valdecoxib from the market in 2004 by Merck & Company, Inc, COX-2 inhibitors were rejected almost as enthusiastically and perhaps as irrationally. In addition, the initial use of these drugs should have been restricted to people who suffered gastrointestinal toxicity from NSAIDs. On the other hand, relatively low incidence of cardiovascular events suggests that a large portion of the population can take seletive COX-2 inhibitors safely, especially for short courses of therapy. Approximately 30 million Americans use nonsteroidal anti-inflammatory drugs (NSAIDs) daily for reasons ranging from cardioprotection and everyday aches and pains to more serious complications such as rheumatoid

arthritis, acute gout, and other comorbid conditions [125]. Current evidence suggests that naproxen, a nonselective NSAID, is associated with the lowest risk of cardiovascular events. Therefore, naproxen is the NSAID of choice in patients with high cardiovascular risk [126]. The Prospective Randomized Evaluation of Celecoxib Integrated Safety vs. Ibuprofen or Naproxen (PRECISION) trial is currently under way [127]. It is a randomized, double-blind, parallel-group study comparing the cardiovascular risk associated with celecoxib to that of the two most commonly prescribed nonselective NSAIDs (naproxen and ibuprofen) in patients with either osteoarthritis or rheumatoid arthritis. The primary outcome of the study is the first occurrence of cardiovascular death, nonfatal MI, or nonfatal stroke. Cardiovascular, GI, and renal side effects as well as symptomatic relief will be assessed in the study. The trial is set to be completed in September 2015 and may provide additional evidence for the use of NSAIDs in patients with cardiovascular risk [127]. Moreover, various synthetic approaches based on chemical modification of NSAIDs have been undertaken with the aim of improving their safety profiles [128-129].

In addition, selective COX-2 inhibitors may also used in the detection and/or treatment of cancer [130-132] and neurodegenerative disease like Alzheimer [102]. As noted above, COX-2 expression is elevated in a large number of malignancies, therefore some recent studies suggested that selective COX-2 inhibitors show the beneficial effects in breast, head and neck, lung, pancreatic and gastric cancers [87-90]. Furthermore, the relevance of COX-2 in the progression of Alzheimer disease is also currently being discussed. Prostaglandins are important for the inducing uterine contraction during labour, therefore selective COX-2 inhibitors was shown to be useful in delaying the premature labor without any side effect. Moreover, the perspective of enzymology and protein biochemistry, the study of the COX enzymes may be considered as a mature field. There are few enzymes of lipid biochemistry for which there is such a wealth of structural and functional information. As it is very well evident from the literature that COX inhibitors has got tremendous potential, thus appropriate modification of the molecules to attenuate their toxicity and better economic as well as therapeutic utilization can be of great benefit particularly in various treatments/therapies [133].

Acknowledgment

The authors extend their heartful thanks to Punjab Technical University, Kapurthala, Punjab, India for valuable suggestions and moral support.

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