Inhibitors of Microsomal Prostaglandin E_2 Synthase-1 Enzyme as Emerging Anti-Inflammatory Candidates

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Abstract: Cyclooxygenases (COX-1 and COX-2) catalyze the conversion of arachidonic acid (AA) into PGH_2 that is further metabolized by terminal prostaglandin (PG) synthases into biologically active PGs, for example, prostaglandin E_2 (PGE_2), prostacyclin I_2 (PGI_2), thromboxane A_2 (TXA_2), prostaglandin D_2 (PGD_2), and prostaglandin F_2 alpha ($PGF_{2\alpha}$). Among them, PGE_2 is a widely distributed PG in the human body, and an important mediator of inflammatory processes. The successful modulation of this PG provides a beneficial strategy for the potential anti-inflammatory therapy. For instance, nonsteroidal anti-inflammatory agents (NSAIDs), both classical nonselective (*c*NSAIDs) and the selective COX-2 inhibitors (coxibs) attenuate the generation of PGH_2 from AA that in turn reduces the synthesis of PGE_2 and modifies the inflammatory conditions. However, the long-term use of these agents causes severe side effects due to the nonselective inhibition of other PGs, such as PGI_2 and TXA_2, etc. Microsomal prostaglandin E_2 synthase-1 (mPGES-1), a downstream PG synthase, specifically catalyzes the biosynthesis of COX-2-derived PGE_2 from PGH_2, and describes itself as a valuable therapeutic target for the treatment of acute and chronic inflammatory disease conditions. Therefore, the small molecule inhibitors of mPGES-1 would serve as a beneficial anti-inflammatory therapy, with reduced side effects that are usually associated with the nonselective inhibition of PG biosynthesis. Med. Res. Rev., 00, No. 0, 1–31, 2014

Key words: atherosclerosis; cancer; cyclooxygenase-2 (COX-2); microsomal prostaglandin E_2 synthase-1 (mPGES-1); prostaglandin E_2; rheumatoid arthritis; osteoarthritis

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1. INTRODUCTION

Prostaglandins (PGs) are a class of potent biological lipid mediators, which are found ubiquitously in animal tissues, and mediate diverse physiological and pathological processes. PGE_2, the most abundant PG in humans,[1–3] has been reported to mediate several biological phenomena such as homeostasis,[2,4] inflammation,[5] pain,[5] tumorigenesis, female reproduction, gastric mucosal protection, neuronal, and kidney functions.[6] The biological functions of PGE_2 are primarily mediated through its binding with one or more of its four receptors EP1–4. For instance, inflammatory pain and cardiac homeostasis are mediated through interaction of PGE_2 with EP1 subtype,[7] inflammatory responses through EP2 and EP4 subtypes,[8–11] while anti-inflammatory responses through interaction with EP3 subtype.[12,13]

PGE_2 also acts as a principal mediator in various inflammatory disease conditions, for example, rheumatoid arthritis, and the inhibition of its synthesis by nonsteroidal anti-inflammatory agents (NSAIDs) has been viewed as an important anti-inflammatory therapy since long time.[14] Though NSAIDS are clinically successful as an anti-inflammatory therapy, the nonselective inhibition of other PGs caused by them also leads to several side effects such as gastrointestinal complications (upper gastrointestinal bleeding, gastric ulceration, life-threatening perforations of stomach and duodenum), renal complications (acute renal failure, hypertension, and electrolyte imbalance), cardiovascular complications (coronary heart disease), and reduced platelet aggregation.[15–22] During PG biosynthesis, the release of arachidonic acid (AA) is stimulated through phospholipase A_2 (PLA_2) and phospholipase C (PLC).[23–26] PLA_2 hydrolyzes the membrane phospholipids, preferentially phosphatidylcholine and phosphatidylethanolamine, which serves as the main source of AA. On the other hand, PLC hydrolyzes phosphatidylinositol and phosphatidylinositol phosphates to form diacyl glycerol (DAG), which is thereafter metabolized by DAG lipase to release endocannabinoid 2-arachidonoyl glycerol (2-AG), an important precursor for the release of AA.[26–28] Recent findings also suggested that the metabolism of endocannabinoids 2-AG and anandamide (*N*-arachidonoylethanolamine) by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase,[29–32] respectively, leads to the release of AA (Fig. 1). This evidence is experimentally supported with an MAGL expression gene knockout, and MAGL inhibitor (JZL184) treated mice, in which reduction of the brain AA level, as well as neuroprotection in the animal model of parkinsonism was observed.[32]

PGH_2, itself not a significant inflammatory mediator, serves as a common substrate for other specific downstream synthases to produce various stable PGs such as PGI_2, TXA_2, PGD_2, $PGF_{2\alpha}$, and PGE_2.[33,34] *c*NSAIDs inhibit cyclooxygenases (COX-1 and COX-2), and block the biosynthesis of PGH_2, which serves as a common precursor for PGE_2 as well as several other PGs, leading to severe side effects (gastrointestinal, renal, and hypertension) due to nonselective inhibition of PGs. Although coxibs are superior to *c*NSAIDs in above-mentioned aspects, they exhibit cardiovascular side effects (myocardial infarction, stroke, and heart attack), and consequently some of them have been withdrawn from the market due to their undesired effects.[35] Hence, there is a strong need to develop safer alternatives to *c*NSAIDs and coxibs, which specifically inhibit the production of PGE_2, and are free from side effects associated with existing NSAIDs. Researchers looking for means to block the synthesis of PGE_2 without affecting the levels of PGH_2 have found that mPGES-1, an inducible enzyme that functions downstream to COX-2 for the production of PGE_2, is an attractive target to achieve these goals.[36,37] Recently, some excellent reviews have described specific aspects of mPGES-1, and this review is an effort to integrate detailed information of molecular structure, regulation, therapeutic significance, and small molecule inhibitors of mPGES-1 for the researchers involved in the same field.[38–42]

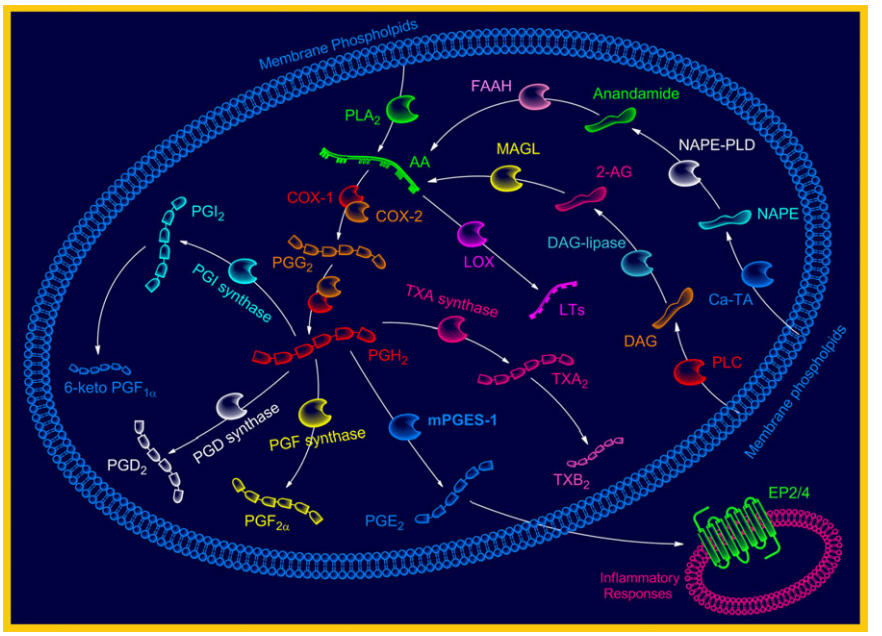

Figure 1. Role of mPGES-1 in arachidonic acid cascade: PLA_2, phospholipase A_2; PG, prostaglandin; TX, thromboxane; LTs, leukotrienes; EP, E prostanoid receptor; DAG, diacyl glycerol; 2-AG, 2-arachidonoyl glycerol; MAGL, monoacylglycerol lipase; FAAH, fatty acid amide hydrolase.

2. PROSTAGLANDIN E_2 SYNTHASES (PGESs)

PGESs, also known as terminal synthases, catalyze the biosynthesis of active PGE_2 from COX-1- and COX-2-derived PGH_2, which is the final step of PGE_2 biosynthesis. Three types of PGESs have been discovered so far, which are referred to as microsomal PGES-1 (mPGES-1), microsomal PGES-2 (mPGES-2), and cytosolic PGES (cPGES).[1,43] Among them, cPGES and mPGES-2 are expressed constitutively, while mPGES-1 is an inducible enzyme.[6] The cell expression studies demonstrate that cPGES is coupled with COX-1, mPGES-2 with both COX-1 and COX-2, whereas mPGES-1 is specifically coupled with COX-2.[32,44] The expression of mPGES-1 is induced by various inflammatory stimuli, such as interleukin-1-beta (IL-1β).[45] The induction of mPGES-1 by inflammatory stimuli and its role in the production of COX-2-derived PGE_2 makes it an alternative target to COX-2 for the management of inflammatory conditions.[36]

3. MICROSOMAL PROSTAGLANDIN E_2 SYNTHASE-1 (mPGES-1)

mPGES-1 is a member of a membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) family, and its expression gene (*PTGES*) is localized to chromosome 9q34.3 in humans.[46] Earlier, PGH_2 to PGE_2 isomerase activity proteins were partially purified from bovine and sheep vesicular glands by Ogino and Moonen research groups in 1977 and 1982, respectively.[47,48] Thereafter, Ogorochi and Meyer research groups purified enzymes from the cytosol of human brain and *Ascaridia galli*, respectively, and identified them to be glutathione S-transferases (GSH).[49,50] The occurrence of mPGES-1 activity was first observed by Watanabe and co-workers in 1997, who reported that the activity of microsomal fraction of identified protein was glutathione dependent, and specific for the production of PGE_2.[45] This

microsomal protein fraction was characterized by Jakobsson and his co-workers in 1999 as an inducible and glutathione-dependent enzyme with molecular weight of 15–16 kDa.[51] They expressed human microsomal glutathione transferase-1 like-1 (MGST1-L1), the member of MAPEG superfamily, in *Escherichia coli* and observed that membrane but not the cytosolic protein fraction possesses glutathione-dependent PGE synthase activity (0.25 μmol/min/mg), consequently the membrane protein fraction (i.e., synthase) was termed as mPGES-1.[6,51]

The experimental studies utilizing PTGES knockout mice have clearly demonstrated the resistance of knockout phenotype to develop various pathological conditions related to inflammation, for example, pain, fever, rheumatoid arthritis, atherosclerosis, cancer, and stroke, and proves the central role of this enzyme in inflammatory processes.[6,52]

A. Tissue Distribution

Low but constitutive expression of mPGES-1 is observed to be ubiquitous, which is highly upregulated in response to various inflammatory stimuli.[53,54] Primarily, the expression of mPGES-1 is observed in accessory genital organs while in nongenitals, its high activity is described in kidneys.[47] mPGES-1 immunoreactivity is detected in the epithelium of human seminal vesicles, and in the epithelial lining of the ampula of vas deferens using immunohistological analysis.[55] The immunohistochemical staining with mouse monoclonal anti-human mPGES-1 antibody revealed mPGES-1 localization in spermatogonia, sertoli cells, spermatocytes of testis, and epithelial cells of epididymis.[56] Moreover, the expression of mPGES-1 was also observed to be species specific. For instance, Western blotting revealed the intense expression of mPGES-1 in the seminal vesicles, moderate in testis, and weak in the vas deferens of the male monkey as compared to rats, rabbit, and pigs. In rat and rabbit, the intense expression was observed in vas deferens, while in pigs this was highly expressed in the testis.[56] In the female macaque, weak expression of mPGES-1 was detected in oviduct.[57] mPGES-1 mRNA expression was also observed in bovine endometrial cells, determining the importance of mPGES-1 in preovulatory follicle before ovulation.[58] Another study for analyzing the menstrual cycle of the rhesus monkey exhibited intense expression of mPGES-1 in the glandular epithelium, and moreover the coupling of mPGES-1 with COX-2 was hypothesized to be important for the formation of receptive endometrium.[59] The expression and the role of mPGES-1 has also been checked in mouse ovary during sexual maturation, and luteal development using in situ hybridization. In this analysis, mPGES-1 expression was observed to be localized in the granulosa cells, and moreover the mPGES-1-dependent biosynthesis of PGE_2 was exhibited to be important for follicular development, ovulation, and luteal formation.[60] In situ hybridization of rabbit tissues have been reported to show high expression of mPGES-1 with COX-1 in cortical-collecting ducts and renal medullary collecting ducts, while weak expression in macula densa and medullary interstitial cells.[61] On the other hand, in situ hybridization, immunostaining, and immuohistochemistry of mouse tissue has also confirmed the co-expression of mPGES-1 with COX-1 in distal convoluted tubule, cortical-collecting ducts, and medullary-collecting ducts, while co-expression with COX-2 was observed in macula densa and medullary interstitial cells.[61] Experimental analysis of the rat models described the LPS-induced expression of mPGES-1 mRNA in lung, brain, heart, stomach, and spleen, which is also supported by the results of Western blot analysis in the same tissues.[62]

B. Structure of mPGES-1

1. Monomer Topology

The monomer structure of mPGES-1 starts with a 10 amino acid long and highly flexible N-terminal (Met1-Ser10) chain located on the luminal side of the membrane, subsequently

followed by a 31 amino acid residues long transmembrane helix (TH1; Pro11-Lys41), and a cytoplasmic loop (Lys42-Arg60). This cytoplasmic loop of monomer structure is highly variable in other MAPEG family members, thus is unlikely to be crucial for their catalytic functions. Monomer structure then continues with second transmembrane helix, that is, TH2 (Ser61-Ser90), which contains a highly conserved amino acid residue Pro81 (proline at position 81). This amino acid is conserved in all members of MAPEG family, and the shifting of its position between mPGES1/mGST1 and leukotriene C4 synthase/5-lipoxygenase-activating protein (LTC4S/FLAP) gives rise to different helical arrangements. A short loop (Phe91-Asn95) on the lumenal side connects TH2 to TH3 (Pro96-Gly119), and another four residue loop (Lys120-Ala123) connects TH3 to TH4 (Pro124-His151), which are less exposed to the cytoplasmic side of the membrane than TH1 and 2.[63]

2. Three-Dimensional Structure

mPGES-1 exists as a transmembrane homotrimer consisting of 152 amino acid residues long chain.[63] The secondary structure of this protein is predominantly composed of α-helical region (74%) comprising 113 amino acid residues.[63] The crystal structure of mPGES-1 in complex with tripeptide gamma-L-glutamyl-L-cysteinyl-glycine, that is, glutathione (GSH) molecule was determined by Jogerschold and co-workers at a resolution of 3.5 Å using electron crystallography, which reveals that the arrangement of mPGES-1 trimer subunits is similar to that present in other structurally similar members of MAPEG family,[63] for example, FLAP,[64] mGST1,[65] and leukotriene C_4 synthase (LTC_4S).[66] The exhaustive analysis of the crystallographic structure of mPGES-1 (Fig. 2A and B) reveals that the inner core of trimer consisting of three TH2 domains narrows down toward the luminal side by aromatic side chains of amino acids, which results in almost complete closure of the core. The amino acid residues responsible for this narrowing down of the inner core include Tyr80, Phe84, Phe87, and Phe91. On the other hand, cytoplasmic side of this core is wide open attributing a "funnel like" shape of the core. The analysis of GSH binding exhibits that it binds in "U"-shaped geometry (Fig. 2C) within the binding site/cleft composed of three distinct regions of two monomers, that is, Tyr28-Arg38 of one monomer, Arg70-Glu77 and Arg110-His113 of another monomer.[63]

Zhan and co-workers have reported structural and functional characteristics of human mPGES-1 by constructing a computational three-dimensional (3D) structural model of human mPGES-1 trimer using combined homology modeling, molecular docking, and molecular dynamics simulation analyses that provided a 3D structure of the substrate-binding domain (SBD).[67–70] The study proposed five key amino acid residues, that is, Gln36, Arg110, Thr114, Tyr130, and Gln134, which together are essential for substrate binding and catalytic functioning. The computational and experimental results have described that Tyr130 is highly important for binding of PGH_2 and catalytic functioning. Other two amino acid residues Arg110 and Thr114 are responsible for interaction with the carboxyl tail of PGH_2 molecule, whereas Gln36 and Gln134 are important for binding affinity of PGH_2.[68,70] Prague and co-workers utilized backbone amide H/D exchange mass spectroscopy to map the binding sites of different types of mPGES-1 inhibitors, and exhibited the location of specific inhibitor binding sites including GSH-binding site and a hydrophobic cleft thought to accommodate PGH_2 molecule. In their analysis, they also suggested the fundamental differences in the binding mode of GSH molecule to mPGES-1 and mGST-1. In case of mPGES-1, GSH molecule acts as a cofactor and binds in "U"-shaped geometry while in mGST-1, GSH acts as a substrate and attain "C" shape geometry.[71]

Pawelzik and co-workers,[72] on the other hand, described the inefficiency of some human mPGES-1 inhibitors in rat and mouse orthologues. In their experiments, through site-directed mutagenesis in combination with the available crystal structure of mPGES-1 (3DWW),[63] they

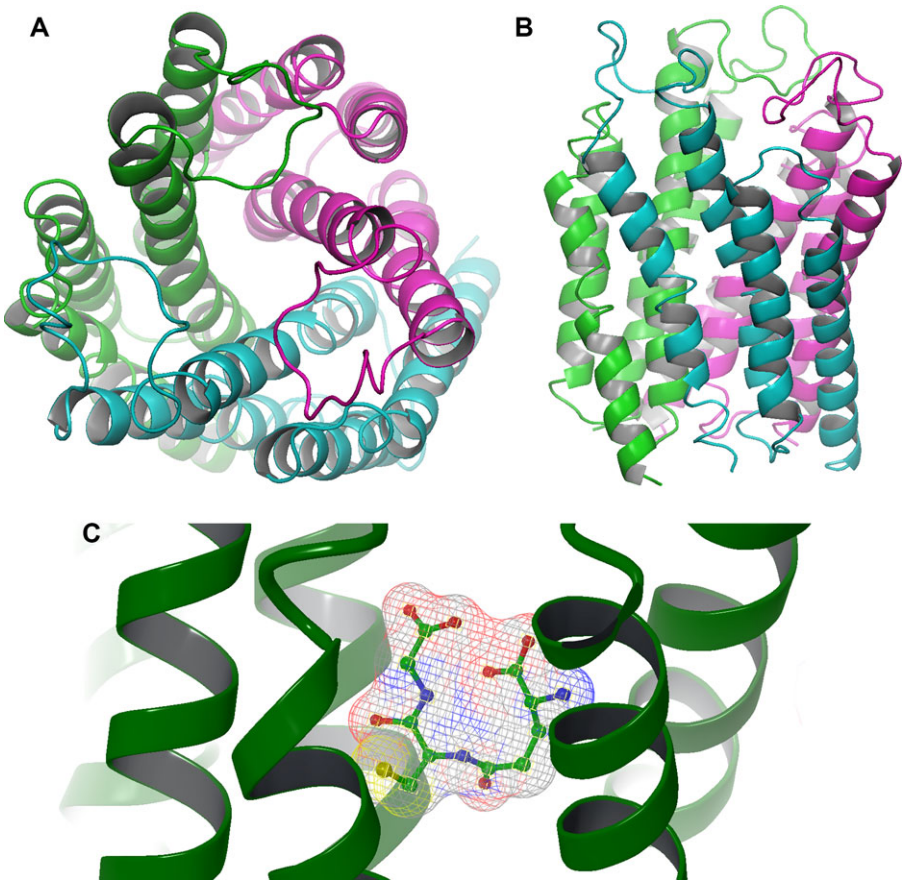

Figure 2. Structures of mPGES-1 trimer: top view (A); side view (B); and U-shaped geometry of glutathione molecule (C). Chains of trimer are colored differently.

identified the key residues Thr131, Leu135, and Ala138 in human mPGES-1, lining the active site between TM1 and TM4 of two adjacent monomers. These amino acid residues play a crucial role as gatekeepers for the active site of human mPGES-1, and regulates the access of inhibitor molecules coming from the lipid bilayer of the membrane while the same amino acid residues (Val131, Phe135, and Phe138) present at the respective positions in rat orthologue prevent inhibitor access to the active site resulting the interspecies differences of mPGES-1 inhibitor binding.[72]

C. Mechanism of Action

The mechanism of action behind the catalytic action of mPGES-1 has been hypothesized based on its experimental 3D structure determined in its inactive form or closed conformation (Fig. 3A).[63] The closed state of trimer is hypothesized to be in rapid equilibrium with its open state, which pulls the equilibrium in favor of product formation when substrate comes into the proximity of mPGES-1. In this way, the glutathione molecule wrapped inside the resting enzyme is protected from unwanted reactions such as oxidation. PGH_2, the substrate of mPGES-1, is produced by cyclooxygenases on the luminal side of the endoplasmic reticulum, and diffuses through the membrane to the active site of mPGES-1 on the cytoplasmic side.[63] In this process,

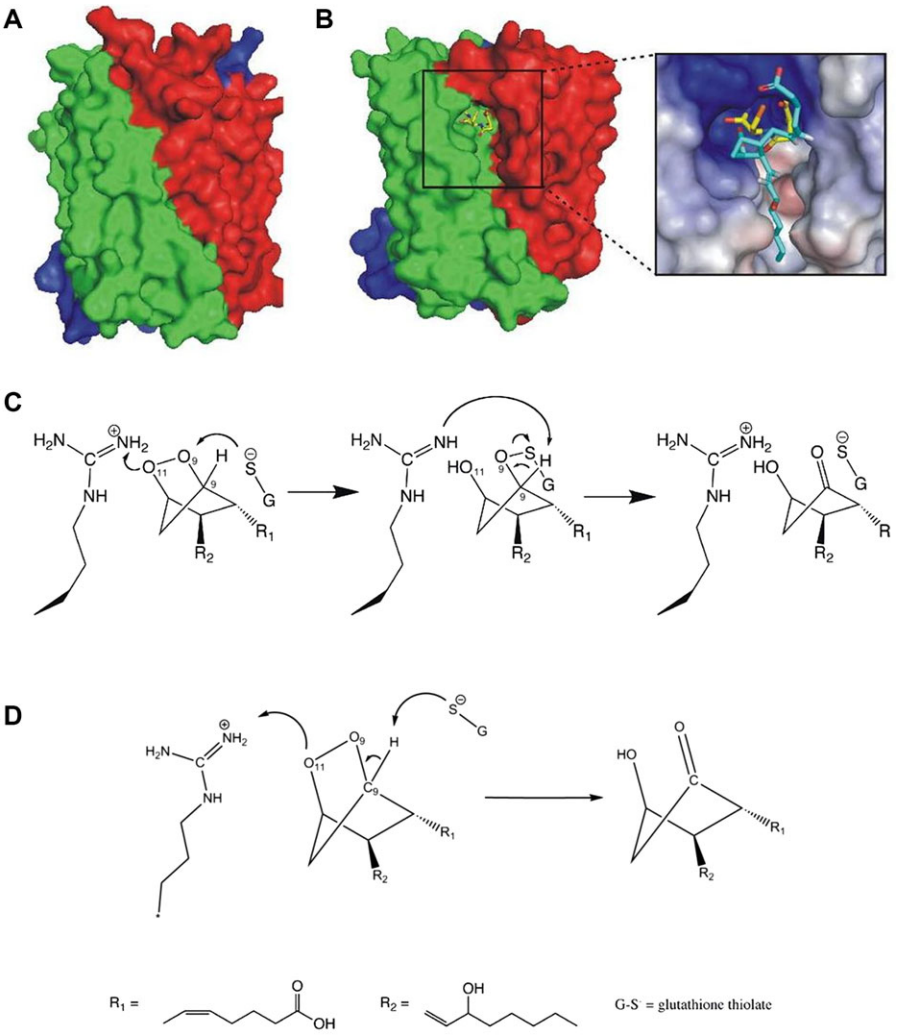

Figure 3. Crystal structure of mPGES-1 in closed state (A); open conformation of trimer based on homology modeling from the structure of LTC4S (PDB entry 2UUH) exposes the GSH molecules (B); chemical mechanisms for the catalysis of PGH_2 to PGE_2 (C and D).[63]

dynamic opening and closing of the active site is supposed to be involved, since there is no alternative way for PGH_2 to diffuse inside and access the glutathione molecule. In case of structurally similar protein leukotriene C4 synthase (LTC4S), a "V"-shaped opening is present between TH1 of one monomer and TH4 of neighboring monomer (Fig. 3B), which may also be possible in case of mPGES-1 through dynamic opening.[63,66,73,74]

On a molecular level, two mechanisms have been proposed for the conversion of PGH_2 to PGE_2 in the cavity of mPGES-2,[75] and the one involving the attack of a thiol on the O_9 of the endoperoxide bridge in a glutathione peroxidase like mechanism is also supported in mPGES-1 reaction by Jegerschold et al.[63] In the analysis, they supported the attack of glutathione molecule stabilized by Arg126 on O_9 of the endoperoxide bridge of PGH_2 molecule (Fig. 3C).[63,75] For chemical conversion of a substrate into a product, proper environment of acceptor and donor candidates is required in the active site of the enzyme. In mPGES-1, this environment is provided by three appropriate candidates, that is, one arginine and two

tyrosine amino acid residues located in the nearby region of glutathione molecule. Following the attack of the thiol group on single oxygen atom O_9 of the endoperoxide bridge of PGH_2, Arg126 residue stabilizes the evolved oxyanion (O_{11}) by donating it a proton, and form a hydroxyl group during the opening of the endoperoxide bridge of PGH_2 molecule. Finally, the Arg126 residue extracts back its proton from carbon (C_9) atom forming the carbonyl group when a bond between oxygen and sulfur is broken, consequently regenerating the glutathione molecule.[76] On the other hand, the second proposed mechanism for the conversion of PGH_2 to PGE_2 is reported only in mPGES-2 active site,[75] but we hypothesized its correlation in the mPGES-1 pocket also. The deprotonated "S" of glutathione may abstract the hydrogen atom of C_9 position leading to cleavage of O_9-O_{11} bond by acid catalysis with Arg126 residue (NH^{2+}).

Hammarberg et al.[77] identifies Arg126 as potential catalytic residue on the basis of GSH-bound 3D homology model of mPGES-1 derived from experimental 3D structures of MAPEG members. The site-directed mutations of Arg126 with alanine and glutamine amino acid residues result in the abrupt loss of mPGSES-1 catalytic activity, but exhibits novel glutathione-dependent reductase activity, which allows the conversion of PGH_2 to $PGF_{2\alpha}$.

D. Physiological Significance

PGE_2, the major product of AA pathway has long been viewed from its physiological and pathological aspects. Since mPGES-1 is specifically linked to the synthesis of COX-2-derived PGE_2, the role of mPGES-1 has been explored in both normal physiological as well as in pathological conditions.

1. Synaptic Transmission, Long-Term Plasticity, and Memory

PGE_2, synthesized by postsynaptically localized COX-2, helps in hippocampal synaptic transmission, and long-term plasticity acting via presynaptic EP_2 receptors. The inhibition of COX-2-derived PGE_2 reduces the long-term potentiation (LTP), which could be reversed only by the addition of PGE_2, not with others such as PGD_2 or $PGF_{2\alpha}$. Supporting to this evidence, the experimental mice deficient of PGE_2/EP2 receptors showed altered long-term synaptic plasticity suggesting the important role of PGE_2/EP2 signaling for maintaining the long-term synaptic plasticity.[78,79] The extensive studies described that PGE_2 regulates the synaptic activity mediated via cAMP-PKA (cyclic adenosine monophosphate protein kinase A) and PKC (protein kinase C) pathways.[80] Additionally, the contribution of PGE_2 in the memory and cognition has also been described using in vivo experiments where the administration of a COX-2 inhibitor leads to the impairment of memory acquisition, memory consolidation, passive avoidance memory, and spatial memory retention.[79]

2. Renal Sodium and Water Transport

PGE_2, the major PG produced in the kidneys, is a natriuretic (excrete sodium through urine) and diuretic, thus regulates hypertension.[81] It promotes the excretion of salt and water through a combination of various mechanisms including inhibition of renal tubular transport, enhancement of renal hemodynamics, and reduction of vasopressin-dependent osmotic water flow.[82] Moreover, the exhaustive analyses also explained that mPGES-1-derived PGE_2 maintains the blood pressure acting through NO/cGMP (nitric oxide/cyclic guanosine monophosphate) pathway.[81]

Additionally, the beneficial physiological role of mPGES-1 in maintaining the transport of renal sodium and water has also been exhibited through aldose escape mechanism. Since aldosterone is a sodium-retaining hormone that reduces renal sodium excretion and stimulates sodium appetite, the adaptive regulatory mechanisms of the body get stimulated to override

the sodium-retaining action of this steroid, the phenomenon termed as aldosterone escape. The aldosterone escape is supposed to be appearing through various natriuretic factors including the release of PGE_2, kinins, and nitric oxide.[82] It has also been reported that the infusion of aldosterone for 14 days in a normal mice did not produce significant differences in the renal sodium-water transport, but the 14 day infusion of aldosterone in PTGES knockout mice leads to enhanced sodium and water balance, persistent reduction of hematocrit, hypernatremia, and body weight gain, all evidence of fluid retention. This analysis clearly demonstrated the role of mPGES-1 in maintaining the normal diuresis through maintaining the renal sodium and water excretion.[82] This evidence is also supported in the acute water loading experiment, in which mPGES-1 was exhibited to promote water excretion.[83]

3. Reproductive System Functions

PGE_2 has been demonstrated to exert a regulatory role in female reproductive functions such as ovulation, embryo implantation, and formation of corpus luteum.[61,84–86] The induced expression of COX-2 and enhanced levels of PGs in the granulosa cells, prior to ovulation, has been observed in several species and similar pattern is also observed in bovine granulosa cells of ovarian follicles in vivo after treatment with human chorionic gonadotropin (hCG).[61] Interestingly, the levels of mPGES-2 and cPGES are not altered after hCG administration or onset of estrus indicating the significance of mPGES-1 for the enhancement of PGE_2 levels in follicles that is responsible for ovulation.[85] PGE_2 has also been demonstrated to play an important role in the implantation of embryo and formation of corpus luteum that is formed after ovulation, and helps in maintaining early pregnancy through secretion of progesterone, thus mPGES-1 would also play a significant role in such condition.[6,86]

E. Therapeutic Significance

A number of experiments using in vivo and PTGES knockout animals have been carried out to evaluate the therapeutic potential of mPGES-1 in various pathological conditions such as inflammation, pain, fever, angiogenesis, tumorigenesis, rheumatoid arthritis, and clearly demonstrated the benefits of mPGES-1 inhibition in such conditions.[36,87–89] Besides, exhibiting potential therapeutic significance, targeting of this enzyme also offers good safety profiling as compared to inhibition of existing molecular targets of inflammation such as COX-1 and COX-2.

1. Therapeutic Efficacy of mPGES-1

1.1. Fever, pain and anorexia

Bacterial wall lipopolysaccharide (LPS) injection, which causes fever in wild-type mice, fail to elicit febrile response as well as PGE_2 synthesis in PTGES knockout mice, while this response is intact in knockout animals with centrally administered PGE_2.[90] PTGES knockout mice also exhibited 40% reduction in acetic acid induced pain response as compared to normal mice treated with piroxicam.[36,89] PTGES knockout mice do not display anorectic behavior and reduced food intake in response to the dose of injected IL-1β, which causes such behavior in wild-type mice.[91,92] These experiments demonstrated that the mPGES-1 inhibition can be useful for treating pain, fever, and anorexia.

1.2. Rheumatoid arthritis and osteoarthritis

COX-2-derived PGE_2 is a key mediator of inflammation and pain in rheumatoid arthritis[93] and osteoarthritis.[94] Elevated levels of mPGES-1 have been reported in synovial linings of

joints in patients suffering with rheumatoid arthritis, and these elevated levels of mPGES-1 expression have also been observed to be correlated with the severity of disease condition. In PTGES knockout animals, the levels of PGE_2, incidences, and severity of joint damage has been observed to be absent in collagen and carrageenan-induced rheumatoid arthritis models as compared to wild-type phenotypes.[6] The elevated levels of mPGES-1 in the cartilages and chondrocytes of patients suffering from osteoarthritis have also been observed, consequently making this enzyme a potential target for counterbalancing these pathological conditions.[95,96]

1.3. Atherosclerosis

The elevated levels of mPGES-1 and COX-2 have been observed in atherosclerotic plaque of normal mice,[97] whereas significantly reduced atherosclerotic lesions have been observed in PTGES knockout animals.[98] The studies utilizing PTGES and low-density lipid receptor knockout mice, in combination, have reported significant improvement of the atherosclerotic condition of the knockout animals when kept on high fat diet over a period of 3–6 months.[6,98]

1.4. Cancer

PGE_2 is a well-reported promoter of tumor growth, immune suppression, angiogenesis, and apoptosis inhibition.[99–101] The elevated levels of PGE_2 have been demonstrated in a variety of human tumors, and the people who take NSAIDs, especially the low dose of aspirin, which is a *c*NSAID, on the regular basis are observed to be less prone to colorectal and other types of cancers.[102] On the other hand, the potential benefits of the coxibs in cancerous conditions are obsolete due to severe cardiovascular hazards associated with their use.[103]

mPGES-1 was found to be overexpressed in 80% of cancer cells in nonsmall cell lung cancer.[104] Similar overexpression of mPGES-1 have also been reported in several other cancerous conditions including helicobacter infections,[105] esophageal,[106] breast,[107] gastric,[108–111] colorectal,[112,113] liver,[114,115] pancreatic,[116] brain,[117,118] kidney,[119] thyroid,[120] penis,[121] larynx,[122] cervix,[123] and ovary cancers.[124]

1.5. Neuronal diseases

Several experimental analyses have demonstrated the significant role of PGE_2 in various neurological disorders including Alzheimer's disease (AD), amyotrophic lateral sclerosis, Creutzfeldt–Jakob disease, ischemic stroke, and HIV-associated dementia. Increased levels of PGE_2 have been demonstrated in the cerebrospinal fluid of the patients suffering from these diseases, and NSAIDs, especially the coxibs, have been reported to exert neuroprotection in such disease conditions. The significant contribution of PGE_2 in neuronal disorders has been well established in animal models of ischemic brain injury, multiple sclerosis, Parkinson's, and AD.[125,126] Interestingly, the NSAIDs described varying outcomes, including beneficial, neutral, and detrimental, in the clinical trials of patients with mild-to-moderate Alzheimer condition.[127,128] For instance, diclofenac[129] and indomethacin[130,131] showed beneficial effects in 6–12 month study, while celecoxib,[132,133] rofecoxib,[127,128] naproxen,[127] ibuprofen,[134] and nimusulide[135] showed neutral to detrimental effects in 3–18 months study. Usually, the chronic intake of NSAIDs is beneficial in the early stage of AD process but no longer effective once Aβ deposition starts, and then their use may even be detrimental because of the inhibition of activated microglia, which on long term mediates the clearance of Aβ deposits.[129] Since mPGES-1 is linked to COX-2 downstream pathway, the inhibition of this downstream PGE_2 synthase would be beneficial in reversing the above-mentioned neuronal pathological conditions.[136]

Similar beneficial effects have also been observed in the animal model of stroke, where postischemic PGE_2 production in the cortex was absent, and other markers of injury such as the infarction, edema, apoptotic cell death, and caspase-3 activation were all reduced in PTGES knockout mice models as compared to those in wild-type mice.[137–139]

2. Safety Potential of mPGES-1 over NSAIDs

COX-1 is expressed constitutively under normal conditions in most of the cells, especially in gastric mucosa and kidneys, whereas COX-2 is inducible in the inflammatory conditions.[140] *c*NSAIDs, which inhibit both COX-1 and COX-2, lead to severe side effects including reduced platelet aggregation, gastric irritation, renal problems, and hypertension, etc.[15–17] Usually, *c*NSAIDs are acidic in nature, thus can directly irritate and break the gastric mucus barrier. Moreover, they also inhibit the production of cytoprotective PGE_2 (acts though EP1), which in turn contributes to gastric ulceration. On the other hand, the selective inhibition of COX-2 with coxibs significantly reduces the level of PGE_2 along with PGI_2, and the reduced levels of PGI_2 contributes to enhanced fibrosis leading to cardiovascular diseases upon their chronic administration.[141] For instance, rofecoxib (VIOXX®) and valdecoxib (BEXTRA®), two selective COX-2 inhibitors, have been withdrawn from the market in 2004 and 2005, respectively, due to cardiovascular side effects (myocardial infarction), and similar cardiovascular hazards have also been reported with continued use of other coxibs such as celecoxib (CELEBREX®).[142–144] Therefore, it is an obvious need to target specific COX-2 downstream candidates, which affects only the levels of PGE_2 without affecting other PGs, and for this purpose mPGES-1 would serve well. Since mPGES-1 specifically catalyzes the production of COX-2-derived PGE_2, without affecting the levels of PGI_2, the cardiovascular problem of existing coxibs would be surpassed.

Cheng and co-workers reported the decreased expression of PGE_2 in PTGES knockout mice, augmenting PGI_2 expression, without affecting thromboxane biosynthesis in vivo. The deletion of PTGES was also observed to be devoid of altering the blood pressure.[95,144] Moreover, the preclinical studies of mPGES-1 inhibitors favor the cardiovascular profile, however due to their limited cardiovascular system (CVS) hazards per se, in the patients already in heart problems, their use should be judged.[40] In contrast to the natriuresis potential of mPGES-1, its inhibition has been studied to ameliorate the progression of renal disorders associated with inflammation.[145]

F. Methods of Evaluating mPGES-1 Inhibitors

A number of assay procedures have been reported to measure the biological activity of molecules against mPGES-1. The basic principle of these assays is the quantitative measurement of reaction product PGE_2 formed from substrate PGH_2 through the action of mPGES-1. The assay procedures are complicated due to instability of substrate PGH_2, which decomposes to PGD_2 and PGE_2 spontaneously. Moreover, the half life ($t_{1/2}$) of reaction product PGE_2 at pH 7.0 and temperature 20°C is only 10 min.[146] Due to these reasons, short reaction times and low temperatures are required for assay sensitivity.

For the experimental determination of mPGES-1 activity, mPGES-1 is needed to be expressed and that can be done in A549, HeLa, HEK293, RAW 264.7 (murine macrophages), *Pichia pastoris* (KM71H strain), and Sf9 insect cells.[37,77,123,147–150]

Ouellet et al. described the baculovirus expression, stabilization, and purification of mPGES-1, in which the sequence containing an open-reading frame of mPGES-1 is digested with *Eco*RI/*Not*I from vector pT7T3D, and subsequently subcloned into the *Eco*RI/*Not*I multiple cloning site of pFastBac vector.[150] The initial bacmid is then transfected into Sf9 cells in serum-free Sf-900 media to generate the initial recombinant baculovirus, and thereafter Sf9

insect cells are cultured in Graces Insect Media with 10% fetal bovine serum, fungizone, and 50 μg/mL gentamicin. An mPGES-1 baculovirus stock is then used to infect 1.2×10^6 cells/mL of Sf9 cells, and the time period for the optimal expression of mPGES-1 is observed. These cells are then harvested at various time periods by centrifugation at $300 \times g$ for 5 min. The cell pellets (70 g, representing 30% of a 20 L bioreactor of Sf9 cells, 96 h postinfection) are then resuspended in five volumes of 15 mM Tris-HCl, pH 8.0/0.25 M sucrose/0.1 mM EDTA/1 mM glutathione, and subsequently sonicated at 4°C (4 × 30 s). The disrupted cells by sonication are centrifuged at $5000 \times g$ for 10 min, and thereafter the supernatant is further centrifuged at $100{,}000 \times g$ for 90 min. The membrane pellet obtained by centrifugation is then resuspended in buffer **A** (10 mM potassium phosphate (pH 7.0), 10% glycerol, 0.1 mM EDTA, 1 mM glutathione, and complete protease inhibitor cocktail). The microsomal fraction of mPGES-1 (544 mg protein at 7 mg/mL) in buffer **A** is solubilized by adding 1% w/v of diheptanoylphosphatidylcholine with stirring for 60 min at 4°C. This mixture is then centrifuged at $100{,}000 \times g$ for 90 min, and corresponding supernatant is filtered through 0.22 μm filter. The solubilized enzyme is finally applied to a hydroxyapatite column (20 × 80 mm, Macroprep ceramic hydroxyapatite type 1), pre-equilibrated with buffer **B** (buffer **A** plus 1% w/v octylglucoside), for the determination of purity level.[150]

Generally, the required amounts of mPGES-1 are preincubated with inhibitor molecule prior to initiating the reaction by addition of substrate PGH_2. The reaction is allowed to proceed for a period of 0.5–1 min at 4°C, after which it is stopped by adding stop solution. Thereafter, reaction product PGE_2 is separated and measured by different analytical techniques.[43]

Thoren and co-workers have reported a quantitative method based on reverse-phase high-performance liquid chromatography (RP-HPLC) with UV detection for the measurement of mPGES-1 activity.[151] In this procedure, the enzyme is diluted in potassium buffer (0.1 M, pH 7.4) containing 2.5 mM reduced GSH. Afterwards PGH_2 (4 μL) dissolved in acetone (0.28 mM) is added into Eppendorf tube and kept on CO_2 ice (−78°C). Prior to incubation, substrate and samples are transferred onto wet ice for temperature equilibration. The reaction is then started by adding the required amount of substrate and terminated by adding stop solution (25 mM $FeCl_2$, 50 mM citric acid, and 2.7 μM 11β-PGE_2). Product PGE_2 is immediately separated on solid-phase extraction using C18 columns with acetonitrile. Finally, PGE_2 is analyzed on RP-HPLC (water, acetonitrile, trifluoroacetic acid; 72:28:0.007) combined with UV detection at 195 nm.

Koeberle and co-workers have also reported the modification in the assay protocol reported by Thoren and co-workers.[152] In this modified protocol, the enzyme is diluted with potassium phosphate buffer (0.1 M, pH 7.4) containing 2.5 mM glutathione (100 μL) and test compound is added. The formation of PGE_2 is initiated after 15 min with the addition of PGH_2 (20 μM). The reaction is terminated after 1 min at 4°C with the addition of stop solution consisting of 40 mM $FeCl_2$, 80 mM citric acid, and 10 μM 11β-PGE_2. The formed PGE_2 is separated by solid-phase extraction on reverse-phase C18 column using acetonitrile as an eluent and analyzed by RP-HPLC (30% aqueous acetonitrile and 0.007% trifluoroacetic acid) with UV detection at 195 nm. For the purpose 11β-PGE_2 is used as an internal standard to quantify the PGE_2 formation by integrating the area under the curve.

Ouellet and co-workers have developed the mPGES-1 activity assay using 96-well plates and radiolabeled or nonradioactive PGH_2 as a substrate.[150] They have reported two buffer systems, that is, one for radiolabeled PGH_2 and another for nonradioactive PGH_2, and either can be selected for the analysis as both show similar effects. The buffer systems for radiolabeled PGH_2 assay is composed of 100 mM sodium phosphate, pH 7.0, 0.1 mM EDTA, and 2.5 mM GSH, whereas the composition for nonradioactive PGH_2 assay includes 10 mM sodium phosphate, pH 7.0, 0.1 mM EDTA, and 2.5 mM GSH for nonradioactive PGH_2 assay. The amount of mPGES-1 required to convert approximately 50% of the substrate is added to

the precooled buffer and reaction is initiated by adding 6 μM [^{3}H] PGH_2 or 20 μM PGH_2, thereafter at set time interval, the reaction is quenched by adding stopping solution, that is, 20 μL of 2 mg/mL $SnCl_2$ for radiolabeled PGH_2 assay or 20 μL of 0.4 mg/mL $SnCl_2$ along with 10 μL of 2% trifluoroacetic acid for nonradioactive PGH_2 assay. $SnCl_2$ helps to convert unreacted PGH_2 into $PGF_{2\alpha}$ otherwise it interferes with the analysis. PGE_2 is then separated on C18 columns using RP-HPLC and water:acetonitrile:acetic acid (60:40:0.1) or water:acetonitrile:trifluoroacetic acid (63:37:0.002) solvent systems as mobile phases for radiolabeled and nonradiolabeled PGH_2, respectively, and thereafter detected by UV (206 nm) or radiometric detectors.

G. Small Molecules as mPGES-1 Inhibitors

The development of mPGES-1 inhibitors started with the testing of known inhibitors of FLAP, a protein belonging to the same family as of mPGES-1, for their capacity to inhibit mPGES-1. Among the known FLAP inhibitors, an indole carboxylic acid derivative MK-886 (cpd. 1; Table SI) is the first potent inhibitor of mPGES-1 reported in the literature. Various other inhibitors were then developed on the basis of SAR of this molecule. At present, various research groups have developed several inhibitors including indole carboxylic acid (MK-886 derivatives), phenanthrene imidazole, imidazole, trisubstituted urea, biarylimidazole, imidazoquinoline, and benzoxazole derivatives. Stable analogues of PGH_2 substrate (i.e., 15-deoxy-PGJ_2; cpd. 2), and a selective COX-2 inhibitor NS-398 (cpd. 3; Table SI), which were reported earlier, also displayed low potency for mPGES-1, that is, 0.3 μM and 10–20 μM, respectively.[151,153]

1. Indole carboxylic Acid or MK-886 Derivatives

MK-886 (cpd. 1; Table SI) is an indole carboxylic acid derivative, and the inhibitor of FLAP protein. Various other indole carboxylic acid derivatives were developed by making modifications in the parent structure of this first identified mPGES-1 inhibitor.[154] In the structure of MK-886, position R_1 is substituted with *para*-chloro benzyl, R_2 with carboxylic acid group, R_3 with *tert*-butyl sulfane, and R_4 with *iso*-propyl moiety (Fig. 4A). The researchers of the Merck Frosst Center have developed various analogues of MK-886, which exhibited good mPGES-1 inhibitory activity. Position R_2 is optimal for carboxylic acid group, and any modification of this acid group result in the downfall of the biological activity (cpd. 4 and 5). Position R_1 can be substituted with hydrophobic groups to retain the activity, and modification of this group by small hydrophobic group reduces the biological potential of the molecules (cpd. 6 and 7), emphasizing the contribution of this group for proper fitting of molecules into the active site of mPGES-1 through hydrophobic interactions. Position R_3 can be substituted with hydrophobic groups such as alkyl or aryl, however the presence of small alkyl groups such as methyl improves potency of molecules as compared to MK-886 (cpd. 8). Direct attachment of the aryl group (phenyl) to indole nucleus results in a loss of potency (cpd. 9), whereas insertion of oxygen, acyl or CH_2 between indole, and aryl ring results in potent molecules (cpd. 10, 11, and 12). Position R_4 is optimal for large hydrophobic groups, and when substituted by hydrophobic substituents including *tert*-butyl (cpd. 13) and phenyl ring (cpd. 14) results in highly potent molecules as compared to MK-886. The phenyl ring present at the same position can further be substituted, since it enhances the potency of molecules dramatically (cpd. 15–17). The favorable effect of substituting hydrophobic/steric groups at position R_1 and R_4 of indole ring have also been confirmed by AbdulHameed et al. on the basis of their Three dimensional-Quantitative structure activity relationship (3D-QSAR) analysis using CoMFA (comparative molecular field analysis) and CoMSIA (comparative molecular similarity indices analysis) approaches.[155] They have also reported that *para*-chlorophenyl group present at position R_1 is helpful for the tight

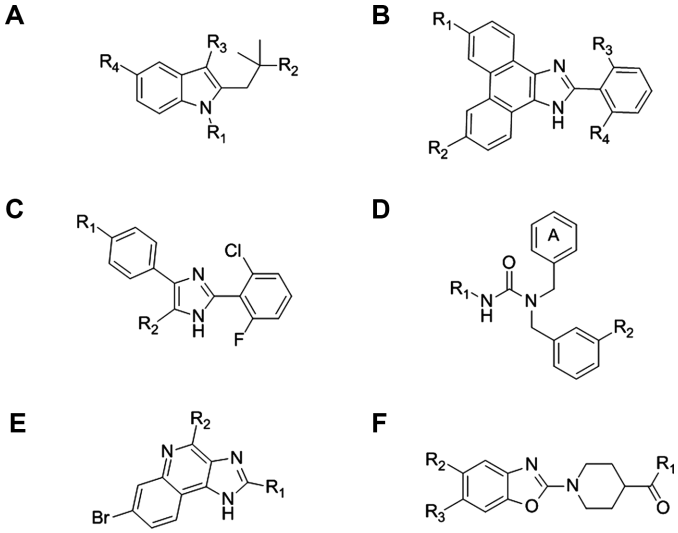

Figure 4. General structures of different chemical classes of mPGES-1 inhibitors: indole carboxylic or MK-886 (A); phenanthrene imidazole (B); biarylimidazole (C); trisubstituted urea (D); imidazoquinolines (E); and benzoxazole derivatives (F).

binding of molecules through its accommodation into the cleft composed of Tyr130, Tyr80, and Phe84 amino acid residues, and through strong interactions with these amino acids. Although, indole carboxylic acid derivatives exhibited remarkable inhibition of mPGES-1 in vitro, they show large shifting in the potency caused by plasma protein binding in the whole cell activity assay, thus precluded the in vivo testing in animal models of inflammation.

2. Phenanthrene Imidazole Derivatives

The failure to improve the cellular activity of indole analogues led to efforts aimed at the identification of new mPGES-1 inhibitors using high-throughput screening (HTS) with enzyme assay. These experiments explored a JAK (Janus kinase) inhibitor, that is, azaphenanthrenone indole derivative (cpd. 18; Table SI), showing moderate mPGES-1 inhibitory activity, which provided a new lead compound for inhibitor development. Subsequently, it was found out that the modification of this scaffold to phenanthrene imidazole resulted in high potency analogues.[156–160] The general structure of phenanthrene imidazole derivatives is mentioned in Fig. 4B. Position R_1 can be substituted with halogen moieties, and bromine has been observed to be superior as compared to chlorine (cpd. 19, 20). Position R_1 and R_2 can simultaneously be substituted with similar groups, without significantly affecting the biological potential of molecules, and this results in almost equipotent molecules as the monosubstituted ones at position R_1 (cpd. 20, 21, 22). Interestingly, the presence of heteroatoms at position R_2 enhances the whole cell activity of molecules (cpd. 23, 24). Contrary to this, the simultaneous presence of heteroatoms at positions R_1 and R_2 drastically decreases the biological potential of a molecule (cpd. 25). The most potent molecules of this series, which also exhibit good whole cell activity bears *tert*-alcohol substituted alkynes at position R_2 (cpd. 26, 27). The most selective molecule of the series (cpd. 26) show good in vivo activity in LPS-induced hyperalgesia guinea pig model but it suffers from the limitation of the long half life (20 hr) and slow absorption rate. Among different derivatives, MF63 (cpd. 28) is the first reported potent phenanthrene imidazole derivative to demonstrate the mPGES-1 inhibition potency in whole human blood assay. Introduction of ethyl group at position R_1 results in reduced half life (7 hr) of phenanthrene imidazole derivatives (cpd. 29).

The substitution of ethyl group with 3-trifluorobutyl and cyclopropylethyl ether at position R_1, and the saturation of triple bond at position R_2 have been reported to improve the biological potential in addition to enhancing the metabolism, and reducing half lives, for example, 3.3 hr and 2.3 hr for compound 30 and 31, respectively.

3. Biarylimidazole Derivatives

Biarylimidazole derivatives having mPGES-1 inhibitory activity were discovered by HTS using enzyme immunoassay method, where 2,4-disubstituted imidazoles were observed to be potent inhibitors of mPGES-1, for example, compound 32 and 33. The general structure of biarylimidazole derivatives is mentioned in Fig. 4C. Position R_1 can be substituted with hydrophobic groups linked through a linker (–C≡C–), and the incorporation of flexibility to linker results in the reduction of biological activity (cpd. 34–36). Similarly, removal of a linker, that is, direct substitution of hydrophobic groups at position R_1 (cpd. 34 and 37) or incorporation of electronegative atoms in the linker also reduces the biological activity of molecules (cpd. 38, 39). Moreover, position R_1 is also unfavorable for the substitution of hydrophobic groups bearing polar moieties and tend to reduce the activity drastically (cpd. 34, 40, 41). However, the replacement of these polar moieties with small hydrophobic groups significantly improves the activity of molecules (cpd. 41, 42). Position R_2 can be substituted with electron withdrawing groups (cpd. 43, 44) showing a favorable effect on activity, while the presence of other groups at this position reduces the biological activity of molecules (cpd. 45, 46).[161]

Most of the molecules described in this series showed a large shift of activity in the whole cell activity assays due to their high lipophilicity, but molecule 47 of this series showed less shift of potency in the presence of serum proteins as compared to the indole carboxylic acid derivatives.[154] Moreover, the whole cell activity of compound 47 also approaches closer to well-recognized COX-2 inhibitors rofecoxib and etoricoxib.

4. Trisubstituted Urea Derivatives

The research group of Merck Frosst Center identified trisubstituted urea derivatives during in-house HTS campaign, and identified a trisubstituted urea derivative inhibiting mPGES-1 moderately (cpd. 48; Table SI).[162] The general structure of these derivatives is shown in Fig. 4D. Position R_1 of this parent compound can be substituted with small aliphatic/hydrophobic groups, and modifications of these groups with aromatic rings attenuate the biological activity of molecules (cpd. 49–52). Large hydrophobic (phenyl; cpd. 53) or polar groups (amide; cpd. 54) substituted at R_2 position results in moderate mPGES-1 inhibitors whereas incorporation of rigid linker to hydrophobic groups enhances the biological activity of molecules (cpd. 55, 56). Further substitution of these hydrophobic groups with electron-releasing/polar groups reduces the biological activity of molecules (cpd. 57, 58). Ring **A** can preferably be substituted at position 3 as compared to positions 2 and 4 of the ring (cpd. 59–61). The presence of hydrophobic groups attached through restricted linker (–C≡C–) at position 3 of ring **A** significantly enhances the activity of molecules (cpd. 62). Moreover, the biosteric replacement of phenyl group (ring **A**) with 3-pyridyl has also been reported to enhance the potency of the molecule (cpd. 63, 64). The compounds of this series are generally very potent and selective, however display high shift of potency in the whole cell blood assay, thus researchers are working to improve the whole blood cell activity of these derivatives and also for the optimization of their pharmacokinetic properties.

Recently, our research group reported a 3D-QSAR study on urea derivatives, which also described that the hydrophobic groups such as phenyl ring at position R_2 are favorable for the inhibitory activity, and further substitutions of phenyl ring at *meta* and *para* also lead to the improvement in the inhibitory activity. The report also described that hydrophobic substituent

at positions R_1 and ring **A** is important for optimum potency among mPGES-1 inhibitors belonging to urea analogues.[163]

5. Imidazoquinoline Derivatives

Dainippon Sumitomo pharma research group identified an imidazoquinoline molecule (cpd. 65) from their in-house HTS, which described moderate activity for mPGES-1, and further optimized the hit molecule.[164] The general structure of imidazoquinoline derivatives is mentioned in Fig. 4E. Initially modifications were conducted on position R_1 with substituted phenyl rings (cpd. 66–68), and the substitution with 2-chlorophenyl ring resulted in a molecule with good activity in nanomole (nM) range (cpd. 69). Further, the modification of molecule 69 was performed on its R_2 position (cpd. 70–72), and the molecule with the carbonyl oxygen at this position possesses high activity and selectivity (cpd. 73).[165]

6. Benzoxazole Inhibitors

The Pfizer research group has recently described the novel benzoxazole inhibitors of mPGES-1 possessing high potency, selectivity, oral bioavailability, and good pharmacokinetic profile.[166] The general structure of benzoxazole derivatives is shown in Fig. 4F. Earlier, position R_1 of benzoxazole derivatives was targeted to optimize the molecules resulting in good candidates with nM activity (cpd. 74). The stereochemistry of this molecule was very crucial as S,S derivative (cpd. 74) was observed to be 75-folds more potent than its R,R derivative (cpd. 75). This molecule was further explored at position R_2 with different substituents, and chloro substituent (cpd. 75) was observed to be optimal at this position. Subsequently, any change of this substituent was observed to decrease the activity against mPGES-1 (cpd. 76 and 77). Position R_3 of these derivatives is observed to be best suited for hydrophobic, mostly aryl, substituents (cpd. 78–83).

Among these highly active and selective derivatives, compound 82 (PF-4693627) described good in vitro potency, preclinical safety profile, in vivo efficacy, selectivity, and pharmacokinetic properties that encouraged its inventors to forward this molecule for clinical profiling. Although this candidate achieved clinical interest, also described some difficulties as drug molecule due to low solubility (1 μg/mL; pH 6.5) and high susceptibility to oxidation by the CYP P450. For this molecule, chlorophenyl substituent was observed to partially responsible for low solubility, and the simultaneous replacement of this group with methyl (cpd. 78) and trifluoromethyl (cpd. 84) resulted in equipotent molecules with improved solubility, but both molecules possessed high in vivo clearance in rats. Such problems led the inventors to have some backup compounds, till PF-4693627 completes the clinical profiling. During this, they developed two molecules (cpd. 85 and 86) possessing acceptable and excellent in vitro potency and in vivo pharmacokinetic profile.[167]

7. Other Inhibitors of mPGES-1

The efficiency of licofelone molecule (cpd. 87)[147] and its derivatives in blocking mPGES-1 and 5-lipoxygenase (5-LOX) have led to the recognition of arylpyrrolizines as dual inhibitors. The most potent arylpyrrolizine analogue (cpd. 88) showed 1.8 μM inhibitory activity (IC_{50}) for cell-free mPGES-1.[168] In addition, pirinixic acid (cpd. 89) and α-napthylpirinixic acid derivatives (cpd. 90) have also been developed as dual inhibitors of these two enzymes.[152,169] Leukotrienes are important mediators of inflammatory and allergic disorders, consequently the inhibitors of leukotriene biosynthesis are clinically implicated in antiasthmatic therapy; thus the dual inhibitors would be superior over single interference in anti-inflammatory therapy.

Several computational techniques have also been utilized to develop novel mPGES-1 inhibitors. For instance, multistep virtual screening approach has led to the identification of novel

mPGES-1 inhibitors. In this virtual screening protocol, acidic (cpd. 91 and nonacidic (cpd. 92; FR20) mPGES-1 inhibitors have been identified from AsinexGold and AsinexPlatinum commercial databases.[148,170] Bruno and co-workers reported the use of LUDI approach resulting in the designing of γ-hydroxybutenolide analogues (cpd. 93) starting from parent compound petrosaspongiolide (cpd. 94) by replacing its natural sesterterpene scaffold, which inhibit the biosynthesis of PGE_2 via downregulation of mPGES-1 expression.[149,171] The another effort by the same research group for the development of novel compounds downregulating the mPGES-1 expression resulted in failure of downexpression activity, but interestingly produced molecules with mPGES-1 inhibitory activity (cpd. 95 and 96).[172] Nonacidic mPGES-1 inhibitor, that is, quinazolinone analogue has recently been utilized to further explore more mPGES-1 inhibitors.[173] Recently, structure-based database screening has also been reported to explore novel thiazole inhibitors of human mPGES-1 (cpd. 97).[174]

Screening of the drug library with SBD has revealed the moderate mPGES-1 inhibitory activity of oxacillin (cpd. 98), an antibiotic containing β-lactam ring, and a dyphylline molecule (cpd. 99), a bronchodilator bearing methyl xanthine nucleus.[175] Additionally, oxicam derivatives (cpd. 100 {PF9481} and 101) have also been reported as mPGES-1 inhibitors possessing high selectivity against COX-2.[176]

Koeberle and co-workers have reported myrtucommulone (cpd. 102), a naturally occurring compound extracted from *Myrtus communis*, as an mPGES-1 inhibitor in an in vitro assay with A549 cells, and in LPS-stimulated human whole blood cell assay. Myrtucommulone significantly inhibit mPGES-1 without affecting the activity of COX-2.[177] In addition, other natural compound, arzanol (cpd. 103) obtained from *Helichrysum italicum* has also been reported to reduce inflammation and PGE_2 levels in carrageenan-induced pleurisy in rats primarily through inhibition of mPGES-1 rather than COX-2.[178]

Other molecules reported to inhibit mPGES-1 include AF3442 (cpd. 104), YS121 (cpd. 105), and pyrrolalkanoic acid (cpd. 106), thienopyrrole (cpd. 107), and naphthalene disulfonamide derivatives (cpd. 108).[179–184]

H. Side effects of the mPGES-1 Inhibitors

Since COX-1 regulates Gastrointestinal (GI), renal, and vascular functions, to overcome the side effects of *c*NSAIDs, the selective inhibition of inducible COX-2 was viewed as a better alternative, which in turn lead to the development of coxibs. Although, coxibs were devoid of side effects associated with the use of *c*NSAIDs, their use contributed to severe cardiovascular side effects. Interestingly, the meta-analyses showed that *c*NSAIDs too have cardiovascular risks, and these side effects are not different in *c*NSAIDs and coxibs, but depends upon the molecules of a particular class. For instance, diclofenac and ibuprofen, two *c*NSAIDs, by far the most marketed drugs have similar cardiovascular complications as of the rofecoxib (a selective COX-2 inhibitor), whereas another *c*NSAID, naproxen, and a coxib, celecoxib, have much lower complications as compared to diclofenac.[20–22,185,186] This does not emphasize that mPGES-1 inhibition would not reduce the side effects of the existing NSAIDs, but it could be molecule dependent, thus exhaustive studies are needed to explain the fact in future.

The inhibition of mPGES-1 would certainly alter the normal physiological processes maintained by it, consequently leading to side effects. As discussed earlier, PGE_2 is a natriuretic and regulates renal sodium and water excretion, thus the inhibition of this enzyme would result in renal sodium and water retention leading to hypertension. This fact has also been observed in PTGES knockout animals, in which elevated blood pressure and hypertension was observed.[84–86]

Since COX-2-derived PGE_2 has a normal physiological role in maintaining the synaptic transmission and long-term plasticity, the inhibition of mPGES-1 is expected to reduce LTP

and impairment of memory. Supporting to this evidence, the experimental mice deficient of PGE_2/EP2 receptors has also been observed to alter the synaptic transmission and long-term synaptic plasticity.[82] Additionally, the contribution of COX-2-derived PGE_2 in the memory and cognition has been well supported by in vivo experiments, and moreover the administration of a COX-2 inhibitor is observed to impair the memory acquisition,[187] memory consolidation,[188] passive avoidance memory,[189] and spatial memory retention.[190] Thus, the inhibitors of mPGES-1 would also impair such conditions.

mPGES-1 promotes angiogenesis, which is crucial for ulcer healing, and moreover its constitutive expression has also been observed in gastric mucosa, thus mPGES-1 inhibitors may delay the ulcer healing process, consequently their use needed to be observed carefully in the patients with gastric ulcers.[191]

The deletion of PTGES in a mouse model has been observed to ameliorate the chronic kidney disorders (CKDs), but simultaneously exacerbates the anemic condition, through reduction of renal erythropoietin synthesis, thus the use of mPGES-1 inhibitors has to be monitored in the treatment of CKD.[145,192]

I. Kinetics of Enzyme Inhibition

A low-resolution crystal structure of mPGES-1 with cofactor molecule GSH has been published, which described the chemical characteristics of the GSH-binding site. Due to lack of reports on the 3D structure of mPGES-1 co-crystallized with inhibitor molecule, chemical environment of the substrate PGH2-binding site remains a mystery. The substrate-binding pattern has been studied by computational chemists utilizing homology modeling, docking, and molecular dynamic simulation approaches, etc. Experimental studies of binding interactions proofs have been explored by Koeberle et al.[181] and recently by He and Lai.[193]

Koeberle et al. investigated the mechanism of mPGES-1 inhibition, the selectivity profile, and the in vivo activity of α-(*n*-hexyl)-substituted pirinixic acid (YS121; cpd. 105). Surface plasmon resonance spectroscopy studies in purified in vitro translated human mPGES-1 conducted by them reveal that YS121 inhibits human mPGES-1 in a reversible and noncompetitive manner ($IC_{50} = 3.4\ \mu M$). In LPS-stimulated human whole blood, they observed the specific inhibition of mPGES-1, without significantly affecting the synthesis of cyclooxygenase COX-2- and the COX-1-derived products. YS121 (1.5 mg/kg i.p.) exhibited anti-inflammatory activity along with reduced pleural levels of not only PGE_2 and leukotriene B_4 but also of 6-keto $PGF_{1\alpha}$ in carrageenan-induced rat pleurisy, which was surprising.

He and Lia recently reported the different binding modes of mPGES-1 inhibitors using molecular docking and competitive binding studies.[193] In this study, they performed inhibitor-cofactor and inhibitor-substrate competition experiments, which revealed that licofelone mainly competes with substrate and is a competitive inhibitor of substrate while molecules MF63 (cpd. 28), 89, and 109 compete with both substrate and cofactor suggesting dual competition ability of these molecules. Moreover, these experiments and docking analysis also suggested equal occupation of substrate and cofactor site by these molecules, consequently this dual-site inhibition showed improved biological activity of molecules (cpd. 28, 89, 105, and 109).

4. FUTURE PERSPECTIVE

mPGES-1 inhibitors selectively attenuate the levels of PGE_2, and are devoid of many side effects associated with the use of NSAIDs. Although existing mPGES-1 inhibitors display good in vitro activity, they describe a poor pharmacokinetics profile due to strong serum binding and poor bioavailability. Therefore, efforts to improve the pharmacokinetics profile of molecules

along with clinical trials to evaluate side effects over long-term use would be needed to start an era of next-generation anti-inflammatory therapy.

For these efforts, licofelone, an anti-inflammatory drug molecule reported to exert biological action through the inhibition of PG and leukotriene biosynthesis pathway, has demonstrated the beneficial potential in phase-III clinical trials results for osteoarthritis (cpd. 87).[194,195] Moreover, in this study licofelone described excellent gastrointestinal tolerability also. Licofelone inhibits COX-1, COX-2, and mPGES-1 at doses (IC_{50} value) of 0.8 μM, >30 μM, and 6 μM, respectively. Interestingly, the dose of licofelone at which the formation of PGE_2 is blocked through the inhibition of mPGES-1 does not affect the activity of COX-2, making this molecule an anti-inflammatory candidate devoid of side effects associated with selective COX-2 inhibition. Recently, published data also suggested the better maintenance of GI benefits of licofelone when taken in combination with aspirin,[196] however, the long-term studies for the evaluation of cardiovascular toxicity of this molecule upon chronic use are needed to establish its safety profile.

5. CONCLUSION

PGE_2, a principal mediator of inflammation is expressed in many cells and tissues throughout the body, and plays an important role in the progression of many disease conditions such as rheumatoid arthritis, osteoarthritis, stroke, cancer, fever, pain, and atherosclerosis. Thus, pharmacological attenuation of this PG offers therapeutic benefits in many disease conditions, where inflammation alleviation is desirable. NSAIDs, both *c*NSAIDs and coxibs, are commonly used clinical anti-inflammatory agents but they suffer from several unavoidable side effects such as gastric ulceration, bleeding in addition to cardiovascular side effects. Therefore, the search for new alternative anti-inflammatory therapy devoid of these side effects has a great potential in improving the life of millions of people around the world. Selective inhibition of mPGES-1, which is an inducible enzyme highly expressed in inflammatory disease conditions, correlates with the production of PGE_2 and emerge as the foremost approach in designing newer generation of anti-inflammatory agents. This enzyme specifically catalyzes the formation of PGE_2 from PGH_2, and it is expected that the selective inhibitors of this enzyme would be safer since they specifically block the synthesis of PGE_2 and not of any other PG. However, this strategy and its benefits are still theoretical, as few molecules have been considered for the clinical evaluation.

6. METHODOLOGY

The literature related to mPGES-1 was surveyed using various search engines and scientific websites including Google Scholar, PubMed, Google, ScienceDirect, Wiley online library, Bentham Science, Taylor and Francis, American Chemical Society, Springer, Royal Society of Chemistry, etc. In addition to this, social websites such as Wikipedia, webpages of pharmaceutical companies, and pharma news were also considered. A search was done with keywords, including mPGES-1, cyclooxygenases, inflammation, PGE_2, cancer, rheumatoid arthritis, atherosclerosis, stroke, AA pathway, NSAIDs, and coxibs, etc.

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