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Targeting inflammation: multiple innovative ways to reduce prostaglandin E₂

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Abstract

The PGE₂ pathway is important in inflammation-driven diseases and specific targeting of the inducible mPGES-1 is warranted due to the cardiovascular problems associated with the long-term use of COX-2 inhibitors. This review focuses on patents issued on methods of measuring mPGES-1 activity, on drugs targeting mPGES-1 and on other modulators of free extracellular PGE₂ concentration. Perspectives and conclusions regarding the status of these drugs are also presented. Importantly, no selective inhibitors targeting mPGES-1 have been identified and, despite the high number of published patents, none of these drugs have yet made it to clinical trials.

The process of inflammation is complex and leads to a plethora of mediators that activate many signaling pathways. Among the major players involved in this complex process are the prostaglandins (PGs) [1]. Among these bioactive lipids is the PGE₂ [2,3]. PGE₂ is a pivotal PG produced by most mammalian tissues and it regulates multiple biological processes under both normal and pathological conditions [4]. PGE₂ is released at several sites, including blood vessel walls, in response to infection or inflammation [5]. In addition to being a key mediator of inflammation, PGE₂ plays an important role in cellular physiological events such as neuronal functions via prostanoid E receptors (EPs), female reproduction, vascular hypertension, kidney function, gastric mucosal protection, pain hypersensitivity and inflammation. Importantly, PGE₂ has been shown to support tumor growth [4] by inducing angiogenesis [6], modulating tumor-cell apoptosis [7] and suppressing immune surveillance [8]. PGE₂ has also been shown to induce colon

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carcinogenesis in the presence of bile acid, deoxycholic acid in male Sprague-Dawley rats [9], and to enhance azoxymethane-induced colon tumors in mice by increasing cellular proliferation and inhibiting apoptosis [10]. Finally, elevated levels of PGE₂ have been observed in various types of human cancers including colon and pancreatic cancers [11,12]. It has been suggested that increased levels in PGE₂ in the portal venous drainage of colorectal cancers may serve as a predictor of tumor recurrence [13]. Finally, many recent reports also attribute a role for PGE₂ in the process of metastasis [14]. Taking into account the multiple roles of PGE₂, targeting the PGE₂ synthesis pathway is of relevance to several inflammation-driven diseases such as arthritis, uveitis and inflammatory bowel disease to name a few. This review focuses mainly on the inflammation–cancer axis but, also includes patents on compounds that were shown to be effective in other inflammatory related diseases. As such, the background regarding the key proteins involved in the PGE₂ synthesis pathway is mainly related to cancer.

The PGE₂ synthesis pathway

There are three steps in PGE₂ biosynthesis (Figure 1A). First, phospholipase A2 promotes the cleavage of phospholipids into arachidonic acid (AA), which becomes substrate of the COX-1/2 to produce the unstable endoperoxide metabolite PGH₂. PGH₂ is then isomerized into PGE₂ by the PGE₂ synthases (PGES1–3). PGH₂ is also the precursor for several other PG structurally related to PGE₂. This includes PGD₂, PGF_{2α}, PGI₂ and TXA₂ (Figure 1A) [15].

In this review, we focused on the key proteins involved in PGE₂ overall concentration (Figure 1B) and they are: the PGE₂ synthases (terminal steps for PGE₂ synthesis), 15-PG dehydrogenase (15-PGDH) (metabolizes PGE₂ into its inactive metabolite), and the PGE₂ transporters MRP4 and PG transporter [PGT]). Below is a brief background on each of these potential targets for therapeutic intervention.

PGE₂ synthases

Three different genes with PGES activity have been cloned [16]. The first, microsomal PGE₂ synthase-1 (mPGES-1) is a member of the membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) superfamily [17]. In most normal tissues, mPGES-1 expression is low; however, constitutive and copious expression is detected in a limited number of organs including the lungs, kidneys and reproductive organs. Both COX-2 and mPGES-1 are induced by pro-inflammatory cytokines and these enzymes have been shown to cooperate in producing PGE₂ from AA *in vitro* [18]. This suggests that both enzymes are essential for PGE₂ biosynthesis and that inhibition of either is sufficient to inhibit the production of PGE₂ [19,20]. The kinetics of induction of mPGES-1 and COX-2 has been reported to be different [20–22] suggesting a differential regulation of the enzymes. mPGES-1 expression can be specifically induced by liposaccharide (LPS), IL-1β and TNF-α in various cell types with or without induction of COX-2 [23–25]. mPGES-1 shows significant homology with other MAPEG superfamily proteins, including microsomal glutathione-S-transferase (GST)-1-like 1 (MGST-1), 5-lipoxygenase (LOX)-activating protein (FLAP) and leukotriene C₄ synthase (LTC4S). All MAPEG proteins are small proteins of 14–18 kDa and have a similar 3D structure [26]. Recently, an electron

crystallographic structure (3.5 Å) was published, and the coordinates were released over the summer of 2009 [27]. Structural details of the protein and its active site were reviewed in [28]. mPGES-1 requires glutathione as an essential cofactor for its activity [18]. Importantly, human and mouse or rat mPGES-1 differ in critical amino acids, which have been reported to delineate the entrance of the active site of the enzyme [29]. These differences, particularly in the three amino acids Val131, Phe135 and Phe138, which are present in the rat mPGES-1, may prevent inhibitor binding, while Thr131, Leu135 and Ala138, in the human mPGES-1, may preferentially allow for conformational changes of the enzyme and/or targeted compound binding. Studies with mPGES-1 deficient mice have revealed mPGES-1 as a key mediator of inflammation, fever, pain, bone metabolism, angiogenesis and tumorigenesis [19,30–32], thus highlighting this protein as an attractive therapeutic target for the treatment of disorders such as osteo and rheumatoid arthritis, acute or chronic pain and cancer. The biology and the pathways in which mPGES-1 is involved in processes such as fever, pain, arthritis, and cardiovascular diseases have been extensively reviewed by Dallaporta *et al.* in [33]. Two other PGESs have been described and extensively reviewed in [28]. They do not represent a therapeutic target *per se* and to our knowledge there is no patented inhibitor for either mPGES-2 or cPGES.

15-PG dehydrogenase

The overall levels of PGE₂ are regulated by the activity of PGE₂ synthases and the 15-PGDH. 15-PGDH has been found down-regulated in human epithelial tumors [34,35] and the enzyme is lost in many types of cancers [34,36–38]. As mentioned above, 15-PGDH is a PG-degrading enzyme, which catalyzes oxidization of the 15(*S*)-hydroxyl group of PGE₂ to yield an inactive 15-keto PGE₂ [13,39]. Although the 15-keto derivatives are not active in mediating prostanoid receptor-dependent signaling, they have recently been reported to have peroxisome proliferator-activated receptor- γ -ligand activity and therefore could promote antitumor responses [40]. Genetic deletion of 15-PGDH in mice leads to increased levels of PGE₂ in tissues and in blood [41]. Although 15-PGDH may promote certain androgen sensitive prostate cancers [42], Loss of expression of 15-PGDH correlates with tumor formation in other cancers, including pancreatic cancer [38], colorectal cancer [34,43], lung [44] and transitional bladder cancer [45]. Interestingly, NSAIDs have been shown to up-regulate 15-PGDH expression in colorectal and medullary thyroid cancers [34,46]. Importantly, histone deacetylase inhibitors, such as scriptaid, apicidin and oxamflatin were recently demonstrated to restore 15-PGDH expression in A-549 lung cancer cells [47].

PGE₂ transporters

An investigation of the interactions between PGs and members of the MRP family of membrane export pumps, revealed a role for MRP4 in the transport of both PGE₁ and PGE₂. The study suggested that MRP4 can specifically function as a PGE efflux transporter, releasing newly synthesized PGs from cells to the extracellular milieu [48]. The PGT, on the contrary, transports exogenous PGE₂ back into the cytoplasm (Figure 1). Forced over-expression of PGT *in vitro* reduced extracellular levels of PGE₂ and increased intracellular levels of 15-keto PGE₂, the catabolic product of PGE₂. In an analysis of a panel of human cancer cell lines on the expression of MRP4 and other members of the MRP family, MRP4 was not over-expressed in any of the cell lines tested [49]. MRP4 expression was however

shown to be elevated, whereas PGT expression was decreased (although in some cases dramatically increased) in human colorectal cancer specimens compared with expression in normal mucosa.

Furthermore, PGT expression was attenuated in premalignant adenomas in *Apc*^{Min/+} mice [50]. High expression of MRP4 was identified as a prognostic marker of poor prognosis in neuroblastoma. High expression of the MRP4 gene, but not of any other MRP gene family members, was significantly linked to poor outcome [51]. Furthermore, MRP4 expression was detected in metastatic but not primary prostate cancer in human tissues [52], and presence of tumor-derived factors was shown to up-regulate MRP4 as well as COX-2, mPGES-1 and PGE₂ expression, and reduce expression of 15-PGDH and PGT in bone marrow cells from naive BALB/c mice [53]. A cisplatin (DDP)-resistant cell line (SGC7901/DDP) derived from a gastric cancer cell line (SGC7901) was established by a step-wise increase in DDP treatment. Over a 21.9-fold increase in resistance to DDP was observed compared with the parental cell line. siRNA reversed this resistance and decreased MRP4 expression; thus, suggesting a role for MRP4 in conferring DDP resistance in a SGC7901 gastric cancer cell line [54]. A preliminary study performed at the Changhai Hospital in Shanghai (China), showed a relationship between MRP4 expression and sensitivity to preoperative radiation treatment in patients with advanced rectal cancer. A total of 95 patients received radiation therapy between January 2000 and January 2009, and MRP4 expression was detected in paraffin-embedded specimens by immunohistochemistry. Patients with low expression of MRP4 showed a significantly higher response rate (66.7%) than that of patients with high MRP4 expression (29.1%) ($P < 0.05$) [55]. They later showed that down-regulation of MRP4 expression in HCT-116 colorectal carcinoma cell line *in vitro*, enhanced radiosensitivity [56]. Interestingly, MRP4 also has been reported to decrease DNA damage from environmental carcinogens in human bronchoalveolar H-358 cells. Treatment of H-358 cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin or (-)-benzo[a]pyrene-7,8-dihydro-,7,8-diol (B[a]P-7,8-dihydrodiol), the proximate carcinogen of B[a]P, increased MRP4 expression. Upregulation of MRP4 was shown to increase cellular efflux of (-)-B[a]P-7,8-dihydrodiol, hence diminishing DNA-adduct formation [57]. In a study where middle frontal gyrus brain tissue from patients diagnosed with Alzheimer's disease and that of aged-matched control brains were examined for PGT expression pattern, PGT levels were found to be significantly lower in Alzheimer's disease compared with age-matched control brain homogenates [58]. On the contrary, PGT was found to be over-expressed in epithelial cells of patients with ulcerative colitis and rats with dextran sodium sulfate-induced colitis. PGT were over-expressed in epithelial cells at the colonic mucosal folds, and EPR4 throughout the mucosa, compared with healthy controls in which EPR4 were localized on apical plasma membrane of epithelial cells at the tip of mucosal folds, and PGT were least expressed on epithelial cells [59].

Prostanoid receptors

The effects of PGE₂ are exerted through its binding to a group of G-protein-coupled receptors. These EPR responding to PGE₂ are designated EPR1, EPR2, EPR3 and EPR4, and differ in tissue localization as well as signal transduction [60,61]. The role of each EPR subtype in both physiological and pathological responses have been determined through

studies using knock-out mice deficient in each EPR subtype and summarized extensively [60]. This review focuses on modulation of free extracellular PGE₂ concentration and hence the EPRs and their signaling will not be further detailed.

Patents on the structure of mPGES-1 & on methods for mPGES-1 activity assays

Recombinant mPGES-1 or purified mPGES-1 can be used in cell-free biochemical assays for the screening of mPGES-1 inhibitors. Table 1 [101–116] summarizes the patents describing the cloning of human mPGES-1 and the methods using purified or recombinant protein to measure enzyme activity of mPGES-1. In 2000, Jakobsson *et al.* patented the sequencing and cloning of human mPGES-1 as well as the method to assay for the enzyme activity [16] (Table 1; method 1 [101,102]). mPGES-1 was discovered as a member of a protein superfamily consisting of the MAPEG family. Previous designations of the protein were PIG12, TP53I12 and MGST1-L1.

NovaSAID filed a patent in 2011 on the method and use of homology model building for mPGES-1, which could be used in drug design (Table 1; method 2, [103]). While another group from the University of Kentucky have a US patent as well on the modeling of mPGES-1 3D structures (Table 1; method 3, [104,105]) based on work published, which detailed a novel computational structural model of the enzyme along with a 3D quantitative structure–activity relationship (SAR) analysis [62,63]. Earlier, the SAR studies of Riendeau *et al.* at Merck Frosst (Canada) revealed that modification of the parent compound at the C5 position (Figure 2C; compound A), initially discovered using a high throughput screen (HTS) screen, had the greatest impact on potency. An additional substitution of the biphenyl group yielded the most potent inhibitor in this series (Figure 2C, Compound C, IC₅₀ = 3 nM) [64]. Using molecular docking of the compounds shown in Figure 2C and the crystal coordinates, we show that there seems to be strong evidence suggesting that if the drug molecule candidate is designed to occupy both the substrate and the cofactor glutathione (not shown) binding sites, it will have higher potency against the target mPGES-1 activity [Meuillet, Zhang, Unpublished Data]. Indeed, a recent study by He and Lai demonstrated that a dual-site binding mode improves the potency of mPGES-1 inhibitor, such as the compounds shown in Figure 2 [65]. Clearly, future rational design and optimization of mPGES-1 inhibitors should be carried out based on this binding mechanism.

Several patents issued by Karolinska Innovations AB describe the methods to assay mPGES-1 activity (Table 1, methods 5 and 6) [107–109], following protein purification, which can be obtained as described in patent [106] (Table 1, Method 4) [26]. Most importantly was a method to develop a technique assaying PGE synthase with PGH₂ as a substrate, a molecule which is very labile and decomposes non-enzymatically into a mixture of PGE₂ and PGD₂ [66]. Finally, when measuring PGE synthase fast reaction, it is important to separate the remaining unreacted PGH₂ from the newly formed PGE₂ in order not to interfere with the results. Thus, a stop solution containing FeCl₂ that converts any remaining PGH₂ into 12-hydroxyheptadecatrienoic acid, was applied by scientists at the Karolinska Institutet (SE) to efficiently measure the activity of the enzyme. Moreover, reverse-phase HPLC following immediate extraction of PGE₂ produced by the enzyme appeared to be the

best way to measure the activity as well. Finally, a facilitated approach to evaluate the potency of compounds targeting mPGES-1 was recently published by a group from Sweden (some belonging to NovaSAID AB) [67]. The method described in this paper uses an assay with thiobarbituric acid (TBA)-based detection and a kinetic-based approach using glutathione and substrate PGH₂, confirming the above mentioned literature about the dual binding sites. Scientists at Pfizer developed the method to assay for mPGES-1 activity using TBA. It is a detection assay that does not produce malondialdehyde or 12-hydroxyheptadecatrienoic acid and uses 2-TBA as the detection method (Method 8) [112,113].

Aventis Pharmaceuticals, Inc. further developed cellular assays to screen for inhibitors of mPGES-1 using a flowchart based on A-549 lung cancer cells treated with either TNF α or IL-1 β in the presence or the absence of compounds at one concentration (single point screen) (Table 1; Method 7) [110,111]. The procedure describes the use of enzyme immunoassay-PGE₂ and enzyme immunoassay-PGF_{1 α} kits to account for COX-1 and/or COX-2 inhibition. The same company also developed an assay using fluorescent PGs and a fluorescent polarization detection method (Table 1, Method 9) [114,115]. In 2006, Garrett Fitzgerald (University of Kentucky) filed a patent on the use of mPGES-1 inhibitors as a treatment for inflammation, which limits cardiovascular risk (Table 1, Method 10) [116]. This invention disclosure was based on the fact that mPGES-1 deletion is as effective as traditional NSAIDs in treating pain and inflammation in animal models without modifying PGH₂ derived metabolite profiles [68–71].

The following part of the review is organized into three sections that summarize patents issued on PGE₂ lowering agents that act via: direct enzymatic inhibition; modulation of enzymatic expression; and reduction of free extracellular PGE₂ concentration (Figure 1B).

True inhibitors of mPGES-1

An extensive review on the development of mPGES-1 inhibitors was recently published by our group and it covered the challenges regarding inhibitor design and selectivity for this novel therapeutic target [28]. This section of the Patent Review analysis comprehensively summarizes inhibitor structures that were patented as well as *in vitro* and *in vivo* activities of such compounds inhibiting mPGES-1. Other inhibitors have been described in the literature, for example, endogenous lipids [21,72] and fatty acids, such as arachidonic acid, docosahexaenoic acid, eicosapentaenoic acid and palmitic acid [72]. In addition, PGH₂ analogs, as well as natural products including curcumin from turmeric [73], epigallocatechingallate from green tea [74], garcinol from the fruit rind of *Guttiferae* species [75], myrtucommulone from myrtle [76], arzanol from *Helichrysum italicum* [77], and the acylphloroglucinol hyperforin from St. John's wort [78]. All of these inhibit mPGES-1 without affecting COX-2 activity *in vitro*, although some of them inhibit COX-1 to some extent. These compounds and their activity on mPGES-1 were summarized in the recent review by Chang and Meuillet [28]. Below is a summary of compounds targeting mPGES-1 that were developed and patented since the discovery of the enzyme in the late nineties.

Indole derivatives

Indole derivatives as inhibitors of mPGES-1 were first patented by Biolipox AB (Sweden). A series of patents and patent applications (Table 2; [117–125]) from 2005–2010 based on the indole scaffold (**1**) (Figure 3) were filed, some as inhibitors of mPGES-1 alone, and others as inhibitors of mPGES-1 and other members of the MAPEG family. These derivatives were shown to inhibit mPGES-1 with an IC_{50} value of 62 nM (**2**) as measured by a cell-free assay; however, these compounds have not been tested in a cell-based assay nor *in vivo* in an animal model. As previously summarized [28], a series of indole carboxylic acid compounds based on the indole FLAP inhibitor MK-886 has been developed by Merck Frosst [64], but, to our knowledge, not patented (Figure 2; compounds A–C). Some of these compounds show promising *in vitro* inhibition and selectivity towards mPGES-1. However, when tested in cell-based assays, the compounds suffered from high protein binding and a shift in potency, making these compounds ineffective in reducing LPS-stimulated PGE_2 production in human whole blood and thereby not suitable for *in vivo* testing. Similar problems or fear thereof may be one reason as to why the indole derivatives developed by Biolipox AB, some of which are also indole carboxylic acids (e.g., **2**), never made it into further testing. A.C.R.A.F.S.P.A. (Italy) patented a series of indole derivatives as mPGES-1 inhibitors as well, and more specifically a series of 5-benzoylamino-indoles substituted in position 2 (**3**), most commonly with an aryl group. However, it should be mentioned that a nitrogen atom has been introduced into the benzene ring of the indole in some of these compounds, classifying these particular compounds as pyrrolopyridines. Two of the compounds (**4**, **5**) (both indoles) have been tested *in vivo*, showing a dose-dependent antinociceptive effect in an acetic acid-induced stretching assay of inflammatory pain in mice (52 and 57.5% inhibition at 10 mg/kg, respectively) (Table 2, [126,127]). The human pulmonary adenocarcinoma cell line A-549 is particularly sensitive to stimulation by pro-inflammatory cytokines, such as IL-1 β and TNF- α , and is active in producing PGE_2 and $PGF_{2\alpha}$ in response to this stimulation. The compounds reduced PGE_2 production in IL-1 β stimulated A-549 cells compared with stimulated nontreated control (stimulated cells treated with the reference compound indomethacin was defined as 100% inhibition), with EC_{50} values of 2 and 1.6 μ M, respectively. None of the compounds significantly inhibited $PGF_{2\alpha}$ production. Nor did they significantly inhibit PGE_2 production in U-937 cells, a human lymphoma cell line preferentially expressing cPGES, indicating selectivity among the isoforms of PGES.

Benzoxazoles, benzoimidazoles & imidazopyridines

Scientists at Biolipox AB introduced an oxygen atom into the pyrrol ring of the indole structure and patented a series of benzoxazol derivatives (**6**) (Figure 4) as inhibitors of the MAPEG family (Table 2, [128,129]). The most potent of these molecules (**7**) inhibits mPGES-1 with a modest IC_{50} value of 0.13 μ M, as measured by a cell-free assay, and no cell-based or *in vivo* data have been published for these compounds. Another compound patented as an inhibitor of mPGES-1 containing a benzoxazol group, is the piperidine-carboxamide developed by Pfizer (**28**), which will be discussed later in this review. Boehringer Ingelheim International GmbH (Germany) also patented a series of benzazole derivatives (**8**) (Table 2, [130]), including a benzoxazole compound. However, this series

mainly consists of benzimidazoles, in which a second nitrogen atom has been introduced into the indole scaffold as an alternative to the oxygen. Compound **9**, the most potent in this series, inhibits mPGES-1 with an IC₅₀ value of 2.9 nM as measured by a cell-free assay; however, the efficacy of PGE₂ reduction in cells was not determined. A number of companies have patented compound series based on the benzoimidazole scaffold, as selective inhibitors of mPGES-1 or as dual inhibitors of the same and other members of the MAPEG family, among them, Crystalgenomics (South Korea) (**10**). For example, compound **11** inhibits mPGES-1 with an IC₅₀ value of 0.29 μM, decreases IL-1β stimulated production of PGE₂ in A-549 cells with an EC₅₀ value of 0.13 μM, and shows selectivity against PGF_{2α} through a simultaneous increase in PGF_{2α} production (Table 2, [131]). Scaffold **12** represents a group of benzoimidazoles patented by Boehringer Ingelheim, and **13** is one of the compounds in this series that were able to inhibit mPGES-1 in a cell-free assay with an IC₅₀ value of 1 nM thiobarbituric acid [132]. Boehringer Ingelheim introduced a carboxamid substituted in position 5 of the imidazole scaffold, resulting in a series of benzoimidazole-5-carboxamides (**14**) (Table 2, [133,134]). A nitrogen atom was also introduced into the benzene ring of the benzoimidazole scaffold, creating a group of imidazopyridine-6-carboxamides (**16 & 17**; Table 2) [135,136]. Both of these compound series have IC₅₀ values against mPGES-1 of less than 10 μM as determined by a cell-free assay. Test results have been published using only one concentration (10 μM), and **15** and **18** are two of the compounds that were shown to completely inhibit mPGES-1 at this concentration (Table 2) [133–136]. The piperidinyl benzoimidazole group (**19**) patented by NovaSAID AB (Sweden), directly connects the imidazole group to a piperidine ring in position 2. In a cell-free assay, compound **20** was able to inhibit mPGES-1 with an IC₅₀ value of 64 nM and also it decreased PGE₂ production in A-549 cells stimulated by IL-1β and TNF-α with an EC₅₀ value of 0.13 μM (Table 2) [137]. Furthermore, **20** as an anti-inflammatory was tested in rats in a Carrageenan-induced paw edema assay and a Complete Freund's Adjuvant (CFA)-induced arthritis assay (3–30 mg/kg). The compound was able to reduce paw swelling by 22, 34 and 39% at 10, 30 and 100 mg/kg, respectively.

Phenanthreneimidazoles

Scientists at Merck Frosst Ltd. Canada have disclosed (Table 2 [138–144]) a group of phenanthreneimidazoles, based on the structure of **21** (Figure 5). Development of this series of compounds, as well as their *in vitro* and *in vivo* activity testing have been previously summarized by Chang and Meuillet [28]. Briefly, the JAK inhibitor azaphenanthrenone was discovered as a hit from a HTS campaign using an mPGES-1 cell-free assay. Through further optimization, the phenanthrene imidazole MF-63 (**22**) was then identified as a potent selective mPGES-1 inhibitor. MF-63 was able to suppress PGE₂ synthesis in preclinical animal models without causing gastrointestinal toxicity and PGI₂ reduction, and demonstrated desirable pharmacokinetic properties in guinea pigs. However, a short half-life in rats and rhesus monkeys lead to further SAR studies aiming favorable pharmacokinetic profiles while improving the potency of mPGES-1. Through these studies, two other phenanthreneimidazoles (**23 & 24**) were identified as selective mPGES-1 inhibitors. No detailed biological data are reported in the patented literature on these compounds, but has been published elsewhere [28]. MF-63 has an IC₅₀ of 1.3 nM against the human mPGES-1 enzyme, and is highly selective (>1000-fold) over both human mPGES-2 and thromboxane

synthase. Furthermore, it inhibits PGE₂ production in A-549 cells (EC₅₀ = 0.42 μM) in the presence of 50% fetal bovine serum (FBS), and in LPS-stimulated human whole blood (EC₅₀ = 1.3 μM), without simultaneous inhibition of TXB₂ (EC₅₀ > 40 μM) [28]. However, MF-63 does not inhibit mouse or rat mPGES-1 in the cell-free assay, due to a difference in amino acid residues at the active site [29]. The anti-inflammatory property of MF-63 has, therefore, been tested in guinea pigs and knock-in mice expressing human mPGES-1, instead of the well-established rat models for inflammatory pain (ED₅₀ = 100 mg/kg in guinea pig hyperalgesia model).

Both **23** and **24** are superior to MF-63 when tested in human whole blood, A-549 cells with 50% FBS, and *in vivo*. They exhibit EC₅₀ values of 0.2 and 0.14 μM, and 0.02 and 0.01 μM, in human whole blood and A-549 lung cancer cells, respectively. Compound **23**, which has a tertiary alcohol substituted alkyne at the 9' position of the phenanthrene backbone, proved the most promising of the chlorophenanthrene imidazole series. It demonstrates analgesic activity in the LPS-induced hyperalgesia guinea pig model (ED₅₀ = 30 mg/kg) after oral administration. Pharmacokinetic studies in rat, however, indicated potential problems with an excessive long half-life and low metabolism in human, leading to the further development of compound **24** (also known as MK-7285). MK-7285 shows excellent activity in cell-free and cell-based assays without concomitant reduction of other prostanoids, as well as a good bioavailability (68%) and a promising pharmacokinetic profile in rat and human hepatocytes. Interestingly, it also exhibits a greater efficacy *in vivo* compared with compound **23** and MF-63 in the LPS-induced hyperalgesia guinea pig model (ED₅₀ = 14 mg/kg) [27]. These compound series are also included in a patent filed on the usage of said compounds in treating or preventing a neoplasia in a human patient, through the inhibition of mPGES-1 (Table 2) [144].

Phenylimidazoles & biarylimidazoles

A patent application from IRM LLC (Table 2) [145] describes a series of 174 phenylimidazole derivatives (Figure 6; **25**), with 159 of them (including the three most potent compounds, **26–28** being biarylimidazoles, a scaffold that resides within the structure of MF-63. Compounds **26–28** all inhibit human mPGES-1 with IC₅₀ values of 1 nM, with no further biological data disclosed. Interestingly, however, Merck Frosst also identified (but not patented) a group of biarylimidazoles as mPGES-1 inhibitors [79], previously summarized in [28]. In a HTS screening, scientists at Merck Frosst found a moderately potent mPGES-1 inhibitor with low molecular weight and structural simplicity, suitable for further SAR studies in search for more potent molecular structures. The 2-, 4- and 5-imidazole groups as well as the imidazole core in the biarylimidazole backbone were explored by SAR. These studies led to the discovery of a highly potent inhibitor of the mPGES-1 enzyme, with a remarkable resemblance to compounds **26** and **27**. Merck's lead compound differs from **26** only by having a bromine substituent in the imidazole ring in place of the nitrile group, and from **27** only by the substitution of a pyrazine for the pyridine ring in the backbone structure. It demonstrates an IC₅₀ value of 1 nM in a cell-free assay, and an EC₅₀ value of 13 nM and 0.16 μM in the presence of 2% and 50% FBS, respectively. The compound also inhibits LPS-induced PGE₂ formation in human whole blood with an EC₅₀ value of 1.6 μM, and reveals great bioavailability (127%), as well as a satisfactory

half-life (4.8 h) in rats. However, the *in vivo* efficacy of this compound has yet to be determined [79]. The patent application from IRM was filed in April 2010, and the work by Merck was received by the publishing journal in August the same year.

Pyrrolopyridines & thienopyrroles

Five pyrrolopyridines, 38 thienopyrroles and their inhibitory capacities against human mPGES-1 for some of the exemplified derivatives have been described in the patent applications (Table 2) [146], disclosed by Biolipox AB (Figure 7; **29 & 30**). The compounds display modest potency against mPGES-1, the most effective compounds showing IC₅₀ values of 0.75 and 0.39 μ M (Table 2; **31 & 32**) [147], for the pyrrolopyridines and the thienopyrroles, respectively. No further biological data has been reported.

Piperidines

As previously mentioned, scientists at Pfizer have developed a piperidine-carboxamide compound (Figure 8; **33**) containing a benzoxazole group, as an mPGES-1 inhibitor (Table 2) [148]. The efficiency of **33** to inhibit mPGES-1 was tested by a cell-free assay and in a beagle whole blood assay, and an IC₅₀ value of 90 nM and an IC₉₀ value close to 1 μ M were reported. The piperidine derivatives (based on **34**) exemplified by Convergence Pharmaceuticals Ltd (UK) (Table 2) [149], were screened by a coupled mPGES-1 assay. Compounds were determined to be mPGES-1 inhibitors when activity was observed in the mPGES-1 coupled assay but not in the COX-2 or 15-PGDH secondary assays (IC₅₀ > 100 μ M). Compound **35** is one of the ten suggested inhibitors, which all exhibited an IC₅₀ value of 2.5 μ M or less.

Low molecular weight derivatives

Two patent applications (Table 2, [150,151]) from NovaSAID AB display two series of mPGES-1 inhibitors of low molecular weight. One of these series presents 2,5-disubstituted thiophene derivatives (Figure 9; **36**) and the other 1,3,4-trisubstituted 2-isimidazole-5-one derivatives (Table 2; **37**) [150]. In **36**, the substituent in position 2 always contains an aromatic hydrocarbon structure; for example, an indole, benzene, benzoimidazole or carbazole. Compound **37** showed an unimpressive inhibition of mPGES-1 (IC₅₀ = 4.19 μ M) in a cell-free assay; however, it exhibited a more promising activity in cell-based assays and *in vivo*. It decreased PGE₂ production in IL-1 β and TNF- α stimulated fibroblasts (74% inhibition at 0.1 μ M) and A-549 cells (EC₅₀ of 0.25 μ M). Furthermore, **37** demonstrated anti-inflammatory properties in a CFA-induced arthritis assay at 50 mg/kg, as well as in a LPS air pouch model of acute inflammation in rat (57% reduction in inducible PGE₂ production at 75 mg/kg) (Table 2) [150]. Compound **39** demonstrated a superior inhibitory capacity of mPGES-1 as measured by cell-free assay (IC₅₀ = 0.32 μ M), but a poorer inhibition of PGE₂ production in cells (60% inhibition at 0.1 μ M in fibroblasts, EC₅₀ = 0.75 μ M in A-549 cells), compared with **37**. However, **39** also was antiinflammatory in the CFA-induced arthritis assay at 50 mg/kg, and demonstrated a 65% reduction in inducible PGE₂ production in the LPS air pouch model of acute inflammation at 75 mg/kg, slightly higher than that for **37** (Table 2) [151].

Substituted benzene derivatives

In line with the patents mentioned above [150,151], NovaSAID AB also published a patent application (Table 2) [152] on 1,3-disubstituted benzene derivatives (Figure 10; **40**). The compounds of this series displayed IC₅₀ values against mPGES-1 of 0.3–5.8 µM, and compound **41** has been tested in cells and *in vivo*. It inhibited PGE₂ production in fibroblasts by 57, 73, and 98% at 0.1, 1 and 10 µM, respectively, and in A-549 cells with an EC₅₀ value of 2.96 µM (both IL-1β and TNF-α-stimulated). It showed antiinflammatory properties in the CFA-induced arthritis assay at 100 mg/kg, and in the LPS air pouch model (35% reduction at 75 mg/kg). A patent application from Boehringer Ingelheim (Table 2) [153] describes another group of substituted benzene derivatives, more specifically a group of aminocarbonylphenyl-aminomethyl-benzamide derivatives (**42**). The most potent of these compounds, **43**, demonstrated a promising IC₅₀ value of 1 nM in a cell-free assay. No further biological data has been reported for this group of compounds.

3-aminocarbozoles

A group of 3-aminocarbozoles (Figure 11; **44**) was exemplified by A.C.R.A.F.S.P.A. in Table 2 [154], the most promising compound being **45**. It decreased PGE₂ production in IL-1β-stimulated A-549 cells (EC₅₀ = 2.9 µM), with selectivity against PGF_{2α} (EC₅₀ > 100 µM). Furthermore, it proved anti-nociceptive activity in an acetic acid induced writhing assay of inflammatory pain in mice; 21, 35 and 74% inhibition at 0.1, 1 and 10 mg/kg, respectively. Compound **45** was also found to have good metabolic stability (81%), high *in vitro* absorption (Caco-2 cell test) and good *in vivo* absorption and bioavailability (cassette method).

Sulfonamides

A number of patent applications have been published on sulfonamides (Table 2) [155–163] by Biolipox, Boehringer Ingelheim, AstraZeneca and NovaSAID, all but patent number [163] being disulfonamides. None of these compounds have been tested *in vivo*, and most of them only in cell-free assays. First to be presented were the naphthalene disulfonamides (Figure 12; Table 2; **46**) from Biolipox, a group of compounds with modest inhibition of mPGES-1, the most potent being **47** (IC₅₀ = 1.1 µM). Next, the disulfonamides exemplified by Boehringer Ingelheim, which are either poly-substituted benzenes (**50**) or polycyclic hydrocarbons (**48**), with, for example, an indole, benzoimidazole, benzoxazole, quinolone or a benzopiperidine, in their backbone. Compounds **49** and **51** are the most potent out of these series, with an IC₅₀ value of 67 and 680 nM, respectively. AstraZeneca generated related bis-(sulfonylamino) derivatives based on scaffolds **52** and **54**, with a higher potency towards mPGES-1, as represented in (Table 2) [158–162]. These compounds are generally di- or tri-substituted benzenes, with a few exceptions having additional substitutions. They differ from the disulfonamides presented by Boehringer Ingelheim in that the two sulfonamide substituents are positioned 1,2 instead of 1,3 in the benzene, and by one of the sulfonamide substituents generally being a primary sulfonamide. The two most effective compounds, in these series as well as among all exemplified sulfonamides, are **53** and **55**, with IC₅₀ values of 3.2 and 22 nM as measured by cell-free assay. Finally, NovaSAID AB has demonstrated a group of benzoindol-2-one derivatives (**56**), substituted by at least one sulfonamide, the

most potent compound being the disulfonamide **57** ($IC_{50} = 1 \mu M$). Compound **57** also decreased PGE_2 production in IL-1 β and TNF- α stimulated A-549 cells ($EC_{50} = 7.6 \mu M$) and synovial fibroblasts (Table 2) [163].

Pirinixic acid derivatives

The Werz group in Germany (University of Tübingen) published a patent application on a group of pirinixic acid derivatives (Figure 13; Table 2; **58**) [164] only available in original language. However, biological data on these compounds has been published elsewhere in the literature, and previously summarized in [28]. Pirinixic acid itself does not show inhibitory effects on 5-LOX or mPGES-1, but bulky lipophilic α -substitution of the carboxylic acid group results in mPGES-1 and 5-LOX inhibition. The α -(*N*-hexyl)-substituted compound **59** (also named as YS121) exemplified in Table 2, inhibits mPGES-1 ($IC_{50} = 3.4 \mu M$) and 5-LOX ($IC_{50} = 6.5 \mu M$) in respective cell-free assay. YS121 dose-dependently reduced PGE_2 production in IL-1 β -stimulated A-549 cells ($EC_{50} = 12 \mu M$), and in human whole blood ($EC_{50} = 2 \mu M$), in the latter without affecting the levels of other prostanoids (up to $30 \mu M$). The compound was also tested *in vivo*, in a carrageenan-induced rat pleurisy model, where it proved anti-inflammatory. Leukocyte formation and infiltration was remarkably inhibited and pleural levels of PGE_2 and LTB_4 were significantly reduced (36 and 48% inhibition, respectively); albeit, the contribution of mPGES-1 inhibition to the *in vivo* efficacy is uncertain. In addition, further SAR studies based on α -naphthyl substituted pirinixic acid have been conducted, which led to the identification of compounds with higher potency for mPGES-1. However, further studies are needed to evaluate identified lead compounds. For more details on SAR studies performed, as well as the biological activity of these compounds, see previously published review by Chang and Meuillet [28].

Boswellic acid derivatives

The Werz group (University of Tübingen) has also reported a number of natural anti-inflammatory compounds as novel mPGES-1 inhibitors, as summarized in [28]. Out of these, only the boswellic acid derivatives (Figure 13; Table 2; **60**) [165,166] have, to our knowledge, been patented as mPGES-1 inhibitors, as exemplified in patent applications (published in German). Compound **61** is a boswellic acid derivative that inhibited mPGES-1 in a cell-free assay ($IC_{50} = 3 \mu M$), and decreased PGE_2 production in IL-1 β -stimulated A-549 cells ($EC_{50} = 20$ – $30 \mu M$) without affecting other prostanoids derived mainly from COX-2, under these experimental conditions (6-keto $PGF_{1\alpha}$ was not inhibited at $30 \mu M$). Furthermore, **61** had no effect on 6-keto $PGF_{1\alpha}$ and TBX_2 in LPS-stimulated human whole blood at $10 \mu M$ [28]. However, for some of the natural compounds demonstrated, it is not clear whether mPGES-1 inhibition is a main mechanism of PGE_2 reduction *in vivo*.

Other patented potential mPGES-1 inhibitors

There are a number of other patent applications published on compounds that may exhibit activity against mPGES-1 (Table 2, [167–175]). Among these are a group of FLAP inhibitors (**62**–**64**) by Amira Pharmaceuticals, Inc., presented in Figure 14 and Table 2 [167–172]. These compounds have shown activity against FLAP ($IC_{50} = 50 \mu M$) and it has been

suggested that they may also inhibit mPGES-1; albeit, no biological assays have been performed to test this hypothesis. Furthermore, there are two patent applications on compounds claimed to be inhibitors of mPGES-1 (Figure 15; **65–67**), available only in their original language, which will not be evaluated in this review (Table 2) [174,175]. There are also other nonpatented mPGES-1 inhibitors, which are covered in the recently published review [28]. There is a profusion of other patented inhibitors of targets upstream of mPGES-1 in the PGE₂ pathway, such as PLA₂ and COX-2. However, none of these are, to our knowledge, claimed as dual inhibitors of mPGES-1, and, hence, not covered in this review.

Methods for targeting mPGES-1 expression

Schmidt and Rachakonda (FreieUniversity, Berlin; Table 3; Method 1) [176] describe a method that utilizes an *ex vivo* IL-4 gene therapy approach to modulate chronic inflammation, for example, arthritis. A construct of IL-4 under the COX-2 promoter added to canine articular chondrocytes, expressed IL-4 only in the presence of canine IL-1 β and TNF- α . This triggers a negative feedback mechanism that attenuates inflammation and decreases the expression of genes regulated specifically by the COX-2 promoter, which includes *mPGES-1*, *TNF- α* , *inducible NOS*, and *MMP3* and *MMP13*. QRT-PCR demonstrated that this stimulated construct down-regulated pro-inflammatory cytokines such as IL-6, IL-8, TNF α , iNOS, MMP3, MMP13 and mPGES-1 compared with the nontransfected control. In addition, nitrite and PGE₂ assays showed reduced nitrite and PGE₂ levels, respectively confirming the reduced expression of iNOS and mPGES-1. In summary, this report proposes a novel method which uses the ability of IL-4 under the COX-2 promoter in the presence of IL-1 β and TNF α , to down-regulate mPGES-1 expression (Table 3, [176]).

Another method developed by Pfizer, Inc. uses a homologous recombination approach in which the *PGES-2* gene (i.e., the gene encoding mPGES-1) is disrupted as well as methods of treating chronic inflammation (e.g., rheumatoid arthritis) or acute inflammatory pain (e.g., injury-mediated pain) involving administration of a pharmacological agent that inhibits mPGES-1 activity (Table 3; Method 2, [177,178]). Kyrkanides *et al.* at the University of Rochester have developed a method that combines the use of the feline immunodeficiency viral vector with the therapeutic properties of siRNA targeting important inflammatory genes such as mPGES-1 to specifically inhibit mPGES-1 expression (SEQ. ID. NO:75) (Table 3, Method 3, [179,180]). Feline immunodeficiency viral vector (siRNA) vectors were developed for the transfer of the siRNA constructs to joints (knee and tempromandibular joint) in mice suffering from arthritis. NIH3T3 cells were transfected with 200 nM of siRNA and total RNA was collected 60 h later. mPGES-1 mRNA knockdown was assessed by RT-PCR. Western immunoblotting confirmed siRNA knockdown of mPGES-1, cPGES, COX-2 and COX-1 protein.

Pharmacia Corporation designed a series of anti-sense oligonucleotides (8–30 nucleobases in length) using published sequences from GenBank, to target different regions of the human mPGES-1 RNA to inhibit mPGES-1 expression (SEQ ID NO:1 – SEQ ID NO:1802) (Table 3, Method 4, [181,182]). More specifically, chimeric antisense oligonucleotides having

phosphorothioate oligonucleotides with 2'-*O*-methylethyl sugar moieties and 5-methylcytosines were developed. These antisense compounds specifically hybridize with and inhibits the expression of mPGES-1. No biological data was disclosed in these patents, however, each claim to inhibit the expression of mPGES-1 in cells or tissues. Pharmacia also patented a series of antisense compounds for modulating expression of glutamine-fructose-6-phosphate amidotransferase [183], which they claim may also inhibit the expression of mPGES-1.

Methods & compounds targeting free extracellular PGE₂ concentration

Modulators of the PGT, which result in a change in cellular PGE₂ concentration, are detailed in this chapter (Table 4). PGE₂ antibodies as a way to reduce PGE₂ concentration are also referenced, as well as activators of 15-PGDH, which metabolizes PGE₂.

Inhibitors of PGT

A series of small-molecule inhibitors of PGT has been patented by the Albert Einstein College of Medicine of Yeshiva University (USA). The group identified a new class of PGT inhibitors through screening of a triazine library of 1842 small-molecule compounds, using Madin-Darby canine kidney epithelial (MDCK) cells stably expressing rat PGT. Several effective PGT inhibitors were found. The TGBz scaffold (Figure 16; **68**) was shared among the six compounds with the highest inhibitory activities, the two most potent of these compounds being TGBz T34 (**69**), and T41 (**70**), with K_i values of 3.7 and 6.2 μ M, respectively (Table 4, [184]). Furthermore, the biological effects of these PGT inhibitors on PGE₂ transport were characterized. The discovery of this group of inhibitors allowed the isolation of the efflux process of PGE₂, and aided in demonstrating that PGT is not involved in PGE₂ efflux under normal physiological conditions. Compound T34 was able to rapidly abolish PGT transport activity, suggesting direct inhibition of PGT rather than indirect inhibition via metabolic effects. Compounds T34 and T41 served as lead compounds in a structurally agnostic screen of approximately 2000 small organic molecules. The most effective compound found in this screen, TGBz T26A (**71**), competitively inhibited PGT at a K_i value of 380 nM. T26A increased endogenous PGE₂ in circulating blood of rats, and reduced the degradation of exogenously administered PGE₂. Importantly, the inventors propose a mechanism by which inhibition of PGT and subsequent increased extracellular PGE₂ levels, down-regulates cell-surface expression of the EP4, desensitizing cells to PGE₂. It was shown that specific PGE₂ binding to the EP4 was induced by forced PGT expression in MDCK cells and inhibited by incubation with **70**. Furthermore, chronic oral administration of T26A increased plasma levels of PGE₂, indicating efficiency of the compound when administered orally. Toxicity studies showed that T26A appeared to be well-tolerated in mice. It should be noted that inhibition of PGT also led to suppressed expression (mRNA and protein) as well as function of COX-2 and, thus, the inventors propose the preferred use of said compounds in humans suffering from a disease or disorder at least in part mediated by COX-2 (Table 4, [184]). To our knowledge, there are no patented activators of PGT on the market.

PGE₂ binding antibodies

Abbott Laboratories patented the method and usage of a series of PGE₂-binding antibodies that are wild-type, chimeric, CDR grafted or humanized for detecting and inhibiting PGE₂ activity (Table 4) [185]. *In vitro* analysis using biotinylated PGE₂ ELISA demonstrates that these antibodies bind PGE₂ with very low EC₅₀ values (nanomolar) and neutralize PGE₂-induced calcium efflux in EPR4 assay with low IC₅₀ values. In addition, a fluorescence-activated cell-sorting-based receptor-binding assay and a ³H-labeled PGE₂-binding assay demonstrate these antibodies block PGE₂ from binding to its receptors. *In vivo* efficacy of anti-PGE₂ antibody was shown in a collagen-induced arthritis model to exhibit a lower mean arthritic score than the vehicle control.

Activators of 15-PGDH expression

Scientists at the University of Florida Research Foundation, Inc. developed replication-deficient E1- and E3-deleted adenoviral recombinant vectors encoding the 15-PGDH under the control of the *flt1* promoter (Ad-PGDH) and control adenovirus encoding luciferase gene under the same promoter (control Ad) (Table 4, [186]). *In vivo* studies showed targeted adenovirus-mediated delivery of the *Ad-PGDH* gene into mice with implanted CT-26 colon carcinomas resulted in substantial tumor-growth inhibition by significantly reducing PGE₂ and Th2 cytokines production and promoting *in situ* adenomatous polyposis coli differentiation/maturation. In addition, it showed a reduction in arginase activity and attenuation of STAT6 signaling in CD11b cells. Finally, a long-term survival study demonstrated that a combination of Ad-PGDH and cancer immunotherapy resulted in complete tumor rejection. However, treatment with Ad-PGDH alone showed a significant therapeutic effect promoting tumor eradication and long-term survival in 70% of mice with pre-established tumors. To this end, results suggest that enforced expression of the *15-PGDH* gene at the tumor site may help to remodel the immunosuppressive milieu and promote activation of the local immune system [80].

Finally, Markowitz *et al.* uses a NSAID compound, for instance celecoxib to induce 15-PGDH activity or levels in a model of colon cancer [81]. The observation identifies compounds that induce and/or reactivate 15-PGDH expression (Table 4, [187]). *In vivo* efficacy studies, using AOM-treated 15-PGDH knock-out mice show the chemotherapeutic properties elicited by celecoxib requires the concurrent presence of 15-PGDH, and that 15-PGDH inactivation confers resistance to the antitumor effects of this selective COX-2 inhibitor. More importantly, results show low levels of colonic 15-PGDH are linked with failure of celecoxib colon tumor prevention in humans. Interestingly, this patent also demonstrates an association between β -catenin and 15-PGDH in established colon cancer cell lines via siRNA treatment. A recent related study by Smartt *et al.* shows that β -catenin knockdown upregulates 15-PGDH leading to increased PGE₂ levels in colorectal adenoma and carcinoma cells without affecting COX-2 protein levels [82].

15D-PGJ2 production

Hunter-Fleming Ltd patented the use of a series of compounds to induce 15-deoxy- 12,14-PG J2 (15d-PGJ2) (Table 4, [188]). Wülfert and colleagues hypothesized that agents that tilt the balance of PG production towards PGD₂ and 15d-PGJ2, and away from PGE₂ have

favorable therapeutic effects on a wide variety of inflammation-mediated diseases and disorders associated with activities of COXs and synthesis of PGs, such as cancer. *In vitro* findings in this patent indicate that the antiinflammatory effect of 7 β -hydroxy-EIPA (7 β -hydroxyepiandrosterone) are mediated through an increase in 15d-PGJ2 activity and a concomitant decrease of PGE₂, COX-2, and mPGES-1 levels. *In vivo* studies using a dextran sodium sulfate-induced colitis model and collagen-induced arthritis corroborated similar effects as *in vitro* results with treatment of 7 β -hydroxy-EIPA [83].

Inhibitors of MRP4

The MRP4 has been shown to transport PGE₁ and PGE₂ out of the cytoplasm [48]. To our knowledge, there are currently no patent applications filed on MRP4 inhibitors or the use of such claiming to target PGE₂ transport. What can be found in the patent literature are four patents on the usage of MRP4 inhibitors, one for the treatment of respiratory diseases [189], and three for the treatment and/or prophylaxis of cardiac and/or vascular disorders [190,192]. However, these refer to the inhibition of MRP4 with the aim of inhibiting transport of other small molecules than PGE₂ and hence, are not discussed further in this review. A patent was also filed by Schuetz *et al.* (St. Jude Children's research Hospital, TN, USA) on the function of MRP4 as a drug efflux protein and its role in multidrug resistance, and methods for identifying compounds affecting nucleotide transport in cells or tissues by targeting MRP4 [193]. Another patent describes a method for determining transport activity of transport proteins including MRP4, and the use of it to identify compounds that can modulate such activity [194].

Inhibitors of prostanoid receptors

As mentioned above, there are four EPR known to bind PGE₂. Currently there are roughly 14, 24, 4 and 30 patents and patent applications published on antagonists/ modulators of the EPR1, 2, 3 and 4 receptors, respectively, including usage patents. Although the development of such compounds targeting the EPR could be considered as another way to manage the effects of PGE₂ in cells, this review is focused on direct modulators of free extracellular PGE₂ concentration (as shown in Figure 1B) and thus patents issued on the EPR are not detailed further in this review.

Conclusion & future perspective

To our knowledge, none of these patented compounds has reached clinical trial yet. Although COX-2 inhibitors have been tested or are still tested for a variety of inflammatory related diseases, including cancer; the poor publicity of VIOXX, a COX-2 inhibitor that was retracted from the market for its cardiovascular secondary effects, has tamed down the enthusiasm for targeting this pathway. The identification and better characterization of mPGES-1 has rekindled the interest, mainly from large pharmaceutical companies, to develop selective and potent inhibitors of this pathway. The fact that targeting the terminal enzyme in this cascade, responsible for the synthesis of PGE₂ as shown by knock-out models, should not induce metabolic shunting, has strengthened the fact that mPGES-1 represents a highly attractive therapeutic target for inflammatory-related diseases including cancer. However, compounded by the fact that the enzyme is a trimeric structure and the

difficulties to achieve selectivity between mPGES-1 and other members of the MAPEG family, no true selective potent inhibitor for mPGES-1 has yet reached the market. Interestingly, patents regarding the application of mPGES-1 inhibitors for several disorders continue to appear in the literature. For example, Herlenius *et al.* filed an application regarding the use of mPGES-1 inhibitors for the treatment of breathing disorders such as apnea and sudden infant death syndrome [195]. Puymirat *et al.* (Quebec, Canada) reported the use of and the screening for mPGES-1 inhibitors for the prognosis, treatment, prevention and diagnosis of myotonic dystrophy type 1 (DM1) [196]. The use of mPGES-1 inhibitors also has been suggested in the treatment of patent ductus arteriosus, as a better alternative compared with the currently used COX inhibitors [84].

Clearly, learning more about the biological roles of mPGES-1 in inflammatory-related diseases will continue and should lead to a better knowledge of the pathway and metabolite profile. Pertinently, a recent patent by the University of Laval (Quebec) is based on the importance of the ratio between PGE₂ and PGF_{2α}. More precisely, the inventors claim that overproduction of PGF_{2α} relative to PGE₂ upon the use of a COX-2 inhibitor impairs the feedback mechanism, which exists between the different PGs. The inventors describe the PGF synthase activity of the aldose reductase and have recently published the new implication for such ratio in human biology and strongly suggest PGFS responsible for the production of PGF_{2α} in the human endometrium as a novel therapeutic target for ischemic and inflammatory responses associated with human diseases [85].

Finally, targeted therapies have been speculated to lead to fewer side effects but dual inhibitors of mPGES-1/5-LOX may represent a different approach to reduce inflammation. Similarly, targeting MRP4 or PGT represent novel avenues of research but do not present selectivity and may result in metabolic profile changes with dramatic physiological consequences.

Taking into account these new reports in the literature, the overall field of inflammation, as well as the identification of the target to treat inflammation, appears to be a moving target and an open-ended problem that may be resolved by drug combinations and/or a much in-depth knowledge of the PG metabolism. It is also hoped that within 10 year, this field will take advantage of the metabolomic technology to pinpoint arachidonic and PG metabolism and their influence in inflammation-driven diseases. Only then, with this kind of knowledge, drugs targeting mPGES-1 or related enzymes involved in PGE₂ metabolism will safely enter clinical trials with predictive biomarkers and a chance of success.

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Key terms

15-PGDH

Enzyme that converts PGE₂ into keto-PGE₂, the inactive metabolite for PGE₂. The enzyme is often inactive in cancer.

MRP4	Member of the family of membrane export pumps that transports prostaglandins and releases newly synthesized prostaglandins from cells to the extracellular milieu.
Prostaglandin transporter	Transports exogenous PGE ₂ back into the cytoplasm.
mPGES-1	Terminal inducible synthase responsible for the production of PGE ₂ .
COX-2	Rate-limiting key enzyme in the lipid biosynthetic pathway that converts arachidonic acid into PGH ₂ , the substrate of mPGES-1 to yield PGE ₂ .

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Executive summary

In summary, after its discovery in the late 1990s, and at the point this review is written:

- mPGES-1 has been validated as a novel and attractive therapeutic target for inflammation-related diseases such as arthritis, pain, fever and cancer.
- There are several ways one can modulate PGE₂ concentration: direct inhibitors of the enzyme responsible for PGE₂ production; inhibitors of the transporters for PGE₂; activators of 15-PGDH; and antibodies binding and sequestering free PGE₂. Several compounds have been identified as inhibitors of mPGES-1 or inhibitors of mPGES-1 expression. Selectivity of compounds targeting mPGES-1 remains to be achieved.
- Other inhibitors targeting PGE₂ concentration such as the prostaglandin transporter and MRP4 inhibitors; 15-PGDH activators or PGE₂ antibodies have been identified and are being developed for the treatment of inflammatory-related diseases.
- A high number of patents on mPGES-1 inhibitors have been filed, yet none of the compounds have been tested in a clinical trial.

Figure 1. Pathway to increase PGE₂

(A) The prostaglandin E₂ synthesis pathway. PGE₂ is synthesized in three steps. First, PLA₂ isoforms promotes the cleavage of AA from PLs. Then, AA is converted to the unstable intermediate PGH₂ by the COXs. In the final step, terminal PGESs isomerize PGH₂ into PGE₂. Other structurally related prostaglandins, such as PGD₂, PGF_{2α}, PGI₂ and TXA₂, are all formed from the common precursor PGH₂ by specialized prostaglandin synthases. 15-PGDH degrades PGE₂ to the inactive metabolite 15-keto PGE₂. MRP4 is a prostaglandin efflux transporter, releasing newly synthesized PGE₂ from cells. Extracellular PGE₂ is free

to bind the prostaglandin E receptors 1, 2, 3 and 4 (EP_{R1-4}), inducing a complex intracellular response leading to increased inflammation and tumor growth. The PGT transports exogenous PGE₂ back in to the cytoplasm. Red symbols indicate targets of the PGE₂ pathway covered in this review and effecting the free extracellular concentration of PGE₂. Green symbols indicate therapeutic approaches for decreasing free extracellular PGE₂: (i) reduced PGE₂ production through direct inhibition or modulated expression of PGES; (ii) inhibition of PGE₂ activity by direct targeting of PGE₂; (iii) increased PGE₂ degradation via induction of 15-PGDH; (iv) reduced release of PGE₂ from the cytoplasm by inhibition of MRP4; (v) enhanced re-uptake of PGE₂ through induction of PGT; and (vi) reduced sensitivity to free extracellular PGE₂ due to down-regulation of EP_{R4} receptor through inhibition of PGT. **(B)** This review will focus on patents for mPGES-1 inhibitors and modulators of mPGES-1 expression (3: section titled 'True inhibitors of mPGES-1'; 4: section titled 'Methods for targeting mPGES-1'), direct inhibitors of PGE₂ activity (5: section titled 'Methods and compounds targeting free extracellular PGE₂ concentration), stimulators of 15-PGDH, and modulators of PGE₂ transporters (section 5). 15-PGDH: Prostaglandin dehydrogenase; AA: Arachidonic acid; COX: Cyclooxygenase; PGD₂: Prostaglandin D₂; PGES: PGE₂ synthases; PGF_{2α}: Prostaglandin F_{2α}; PGH₂: Prostaglandin endoperoxide; PGI₂: Prostaglandin I₂; PGT: Prostaglandin transporter PL: Phospholipid; PLA₂: Phospholipase A₂; TXA₂: Thromboxane-A₂.

Figure 2. Known mPGES-1 inhibitors docked into the crystal structure of mPGES (PDB: 3DWW)

(A & B) The protein is colored according to its chains. The inhibitors are colored as: compound A = blue; compound B = yellow; and compound C = orange. **(A)** represents the crystal structure of mPGES-1 with docked compounds A–C; **(B)**, represents a close up view of the active site of mPGES-1. The hydrogen bonding interactions of compound C with mPGES-1 is depicted in red. The inhibitor occupies both substrate and cofactor binding pockets (critical residues shown in lines). The biphenyl group (orange) extended to the

center of the trimeric structure. (C) Table summarizes the structures of compounds A–C; their docking scores and their *in vitro* activity (IC₅₀, mM).

Figure 3. Indole scaffolds[†] and representative lead compounds[‡] patented as mPGES-1 inhibitors

Figure 4. Benzoxazole, benzimidazole and imidazopyridine scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 5. Phenanthrene scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 6. Phenylimidazole and biarylimidazole scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

29[†]

6-chloro-1-(4-isopropoxyphenyl)-
4-(4-trifluoromethylphenyl)
pyrrolo[3,2-c]pyridine-
2-carboxylic acid

30[‡] **31[†]**

4-(3-chlorobenzyl)-2,6-*bis*-(4-
trifluoromethoxyphenyl)thieno(3,2-
b)pyrrole-5-carboxylic acid

32[‡]

Figure 7. Pyrrolopyridine and thienopyrrole scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 8. Piperidine scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 9. Low molecular weight-derivative scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 10. Substituted benzene derivative scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 11. 3-aminocarbazole scaffold[†] and selected lead compound[‡] patented as mPGES-1 inhibitors

Figure 12. Sulfonamide scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 13. Acid-derivative scaffolds[†] and selected lead compounds[‡] patented as mPGES-1 inhibitors

Figure 14. FLAP inhibitors patented as mPGES-1 inhibitors

Figure 15. Other compounds patented as mPGES-1 inhibitors

Figure 16. Tirazine scaffolds[†] and selected lead compounds[†] patented as prostaglandin transporter inhibitors

Table 1

Patents on structure, assay and methods for mPGES-1 activity measurements.

Method	Assignee	Patent number	Claims	Ref.
1	Karolinska innovations AB	WO0028022 A1	Human mPGES-1 cloning, expression and activity assay	[101]
		US6395502 B1		[102]
2	Novasaid AB	WO0092800 A1	Protein structure and method of using protein structure (modeling for inhibitors)	[103]
3	University of Kentucky	US0177521 A1	Modeling of mPGES-1 structure	[104]
		US0288844A1		[105]
4	Jakobsson <i>et al.</i>	US0157084 A1	Methods for preparing purified PGES	[106]
5	Karolinska innovations AB	US7192733 B2	Methods and means for modulating PGES activity, reverse phase HPLC measurement of PGE ₂	[107]
		US7645601 B2		[108]
6	Karolinska innovations AB	WO0024059 A2	Method to identify mPGES-1 inhibitors	[109]
7	Aventis pharma, Inc.	WO0012259 A1	Cellular assay for mPGES-1, cytokine induction, method for mPGES-1 inhibitor screen, methods for determining selectivity, toxicity and potency of mPGES-1 inhibitors (cell-based assay)	[110]
		US7132256 B2		[111]
8	Pfizer, Inc.	WO0067766 A1	<i>In vitro</i> cell-free enzyme activity assays using FeCl ₂ and TBA	[112]
		US0152148 A1		[113]
9	Aventis pharma, Inc.	WO0016223 A2	PGES and PGDS activity	[114]
		US0082021 A1		[115]
10	Fitzgerald	US7608516B2	Targeting mPGES-1 as a treatment to avoid cardiovascular risks	[116]

PGDS: Prostaglandin D Synthase; PGES: Prostaglandin E synthase; TBA: Thiobarbituric acid.

Table 2

Patented inhibitors of mPGES-1 and their biological activities.

Compound	Assignee	Patent number	IC ₅₀ (μM)	EC ₅₀ (μM)	Ref.
1[†],2[‡]	Biolipox AB	WO005415 A1	0.062	ND	[117]
		WO0077365 A1			[118]
		WO0077367 A1			[119]
		WO0077364 A1			[120]
		WO0123675 A1			[121]
10[†],11[‡]	Boehringer Ingelheim International, GmbH	WO0123674 A1			[122]
		WO0009924 A2			[123]
		WO0077366 A1			[124]
		WO0123673 A1 [‡]			[125]
3[†]	A.C.R.A.F.S.P.A.		ND		
4[‡]	A.C.R.A.F.S.P.A.	WO006663 A1 [‡]		2	[126]
5[‡]	A.C.R.A.F.S.P.A.	WO0083436 A1 [‡]		1.6	[127]
6[†],7[‡]	Biolipox AB	WO0042816 A1	0.13	ND	[128]
		WO0071944 A1 [‡]			[129]
8[†],9[‡]	Boehringer Ingelheim International, GmbH	WO0084218 A1 [‡]	0.0029	ND	[130]
10[†],11[‡]	Crystalgenomics, Inc.	WO0099832 A2 [‡]	0.29	0.13	[131]
12[†],13[‡]	Boehringer Ingelheim International, GmbH	WO0100249 A1 [‡]	0.001	ND	[132]
14[†],15[‡]	Boehringer Ingelheim International, GmbH	WO0034796 A1 [‡]	100% inhibition	ND	[133]
		WO0034797 A1 [‡]	at 10 μM		[134]
16[†],17[†],18[‡]	Boehringer Ingelheim International GmbH	WO0034799 A1	100% inhibition	ND	[135]
		WO0034798 A1 [‡]	at 10 μM		[136]

Compound	Assignee	Patent number	IC ₅₀ (μM)	EC ₅₀ (μM)	Ref.
19 [†] ,20 [‡]	Novasaid AB	WO0023812 A1 [‡]	0.064	0.13	[137]
21 [†] ,22–24 [‡]	Merck Frosst Canada Ltd.	WO0059610 A1	ND	ND	[138]
		WO0059611 A1			[139]
		WO0095753 A1			[140]
		WO0134434 A1			[141]
		US7442716 B2 [‡]			[142]
		US7943649 B2 [‡]			[143]
		US0192158 A1 [‡]			[144]
22 [‡]	Merck Frosst Canada Ltd.	US7442716 B2 [‡]	0.001	0.42 (A-549)	[142,143]
		US7943649 B2 [‡]		1.3 (HWB)	[144]
		US0192158 A1 [‡]			
23 [‡]	Merck Frosst Canada Ltd.	US7442716 B2 [‡]	0.001	0.02 (A-549)	[142]
		WO063466 A1 [‡]		0.2 (HWB)	[143]
		US0192158 A1 [‡]			[144]
24 [‡]	Merck Frosst Canada Ltd.	US7442716 B2 [‡]	0.0009	0.01 (A-549)	[142]
		US7943649 B2 [‡]		0.14 (HWB)	[143]
		WO0124589 A1 [‡]			[144]
25 [†] ,26–28 [‡]	IRM LLC	WO0127152 A2 [‡]	0.001	NDR (A-549)	[145]
29 [†] ,30 [‡]	Biolipox AB	WO0077401 A1 [‡]	0.75	ND	[146]
31 [†] ,32 [‡]	Biolipox AB	WO0077412 A1 [‡]	0.39	ND	[147]
33 [‡]	Pfizer, Inc	WO0077313 A1 [‡]	0.09	NDR (Beagle WB)	[148]
34 [†] ,35 [‡]	Convergence Pharmaceuticals Ltd	WO0131975 A1 [‡]	2.5 COX-2 and 15-PGDH >100	ND	[149]
36 [†] ,37 [‡]	Novasaid AB	WO0098282 A1 [‡]	4.19	0.25 (A-549)	[150]

Compound	Assignee	Patent number	IC ₅₀ (μM)	EC ₅₀ (μM)	Ref.
38[†],39[‡]	Novasaid AB	WO0130242 A1 [‡]	0.32	0.75 (A-549) 60% inhibition at 0.1 (FB)	[151]
40[†],41[‡]	Novasaid AB	WO0103778 A1 [‡]	0.3–5.8	2.96 (A-549) 57% inhibition at 0.1 73% at 1, 98% at 10 (FB)	[152]
42[†],43[‡]	Boehringer Ingelheim International GmbH	WO0048004 A1 [‡]	0.001	ND	[153]
44[†],45[‡]	A.C.R.A.F. S.P.A.	WO0138376 A1 [‡]	ND	2.9 (A-549) PGF2α > 100	[154]
46[†],47[‡]	Biolipox AB	WO0042817 A1 [‡]	1.1	ND	[155]
48[†],49[‡]	Boehringer Ingelheim International GmbH	WO0129276 A1 [‡]	0.067	ND	[156]
50[†],51[‡]	Boehringer Ingelheim International GmbH	WO0129288 A2 [‡]	0.068	ND	[157]
52[†],53[‡]	AstraZeneca	WO0064250 A1 [‡]	0.0032	NDR (HWB)	[158]
		WO0064251 A1 [‡]			[159]
		WO0132016 A1			[160]
54[†],55[‡]	AstraZeneca	WO0082347 A1 [‡]	0.022	NDR (HWB)	[161]
		US0331321 A1 [‡]			[162]
56[†],57[‡]	Novasaid AB	WO0016081 A2 [‡]	1	7.6 (A-549)	[163]
58[†],59[‡]	Medeon Pharmaceuticals GmbH	WO0117985 A1 [‡]	3.4	12 (A-549) 2 (HWB)	[164]
60[†],61[‡]	University Eberhard KARLS & University of Tübingen	WO0058514 A1 [‡]	3	20–30 (A-549)	[165]
		WO0117987 A2 [‡]		NI of 6-keto PGF1α at 30 μM NI of 6-keto	[166]

Compound	Assignee	Patent number	IC ₅₀ (μM)	EC ₅₀ (μM)	Ref.
62 [‡]	Amira Pharmaceuticals, Inc.			PGF _{1α} and TXB ₂ at 10μM (HWB)	
		US0219206 A1	ND	ND	[167]
		US0225285 A1			[168]
		WO0137609 A1			[169]
63 [‡]	Amira Pharmaceuticals, Inc.	WO0141011 A1			[170]
64 [‡]	Amira Pharmaceuticals, Inc.	US0244128 A1	ND	ND	[171]
		WO0137805 A1			[172]
65 [‡]	University of Tübingen	WO0045700 A2	ND	ND	[173]
66 [‡]	Dainippon Sumitomo Pharma Co. Ltd	WO0146696 A1	ND	ND	[174]
		JP143829A	ND	ND	[175]

[‡] Scaffold.

[‡] Lead compound or patent were lead compound is found.

Beagle WB: Beagle whole blood; EC₅₀ Determined by *in vitro* cell-based assays measuring the production of PGE₂ in A549 cells (A549), human whole blood or fibroblasts; FB: Fibroblast; HWB: Human whole blood; IC₅₀ Determined by *in vitro* cell-free assays measuring the conversion of PGH₂ to PGE₂ by human mPGES-1; ND: Not determined; NDR: Tested but no data revealed; NI: No inhibition.

Table 3

Patents on methods for targeting mPGES-1 expression.

Method	Assignee	Patent number	Ref.
1	Freie University Berlin	WO0030650 A1	[176]
2	Pfizer Products, Inc	WO0045136 A1	[177]
		US0106085 A1	[178]
3	University of Rochester	WO0079068 A2	[179]
		US0286233A1	[180]
4	Pharmacia Corporation	WO0028458 A2	[181]
4	Gierse, James K.	US0132063 A1	[182]
4	Broschat, Kay O.	US0102412 A1	[183]

Table 4Other inhibitors that modulate PGE₂ concentration.

Compound	Assignee	Patent number	Target	Ref.
68–71	Albert Einstein College of Medicine, Yeshiva University	WO0136638 A2	Prostaglandin Transporter	[184]
	Abbott Laboratories	WO0006059 A1	PGE ₂ antibodies	[185]
	University of Florida Research Foundation, Inc.	WO0124044 A2	15-PGDH activators	[186]
	Markowitz, Sanford D.	US0284989 A1	15-PGDH activators	[187]
	Hunter-Fleming Ltd	WO0065408 A1	15D-PGJ ₂ production	[188]