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Miniperspective

Microsomal Prostaglandin E_2 Synthase-1 (mPGES-1): A Novel Anti-Inflammatory Therapeutic Target

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Introduction

The use of NSAIDs[a] (nonselective inhibitors of cyclooxygenase (COX-1 and COX-2) and coxibs (selective inhibitors of COX-2) is a mainstay of anti-inflammatory and analgesic therapy. Since coxibs and NSAIDs intervene in the prostaglandin (PG) pathway at the level of PGH_2 synthesis, the question that arises is whether similar or greater, anti-inflammatory and analgesic efficacy might be obtained by selective inhibition of one of the downstream PG synthesizing enzymes (Figure 1). Present thinking is that COX-2 derived PGE_2, acting on one or more of its cellular receptors EP1 through EP4, is the major mediator of inflammatory pain.[1] However, the suggestion that PGI_2 is also a significant contributor in the inflammatory process is supported by preclinical studies using PGI_2 receptor (IP) deficient mice and selective IP antagonists.[2,3] The availability of a pharmacologically active molecule capable of selective inhibition of PGE_2 synthesis may provide the answer to the question of whether this therapeutic approach would exhibit anti-inflammatory and analgesic efficacy equal to that of selective COX-2 inhibitors or NSAIDs.

COX-1 and COX-2 are the enzymes that catalyze the formation of the unstable cyclic peroxide PGH_2 by the bis-oxygenation of arachidonic acid. PGE_2 is synthesized from PGH_2 by the PGE_2 synthase enzymes. The first PGE_2 synthase was identified in 1999 and termed microsomal PGE synthase-1 (mPGES-1).[4] Two other PGE_2 synthases have been cloned: cytosolic PGES (cPGES) and membrane PGES-2 (mPGES-2).[5,6] Both cPGES and mPGES-2 are constitutively expressed enzymes with cPGES coupling through COX-1 and with mPGES-2 coupling through both COX-1 and COX-2.[6,7] The mPGES-1 enzyme, which is inducible by various inflammatory stimuli, is primarily coupled to COX-2.[8] Initial studies from several reports of mPGES-1 null mice define a significant role for this enzyme in mediating pain, inflammation, fever, arthritis, atherosclerosis, stroke, and cancer. A number of excellent reviews have summarized the biology and pharmacology of mPGES-1 and provide a firm rationale for targeting this enzyme in drug development.[9,10] Therefore, the following sections will only briefly outline the rationale behind the advocacy of this particular enzyme as the key player in these therapeutic areas, and the focus of this review will be on summarizing the progress to date in the development of selective mPGES-1 inhibitors and their efficacy in preclinical animal models of inflammation.

Identification of mPGES-1 and Tissue Distribution

mPGES-1 was first identified as an inducible, glutathione (GSH) dependent enzyme during cloning and identification of homologues of 5-lipoxygenase activating protein (FLAP). mPGES-1 was originally termed mGST1-L1 because its closest

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[a] Abbreviations: AA, arachidonic acid; CAIA, collagen antibody induced arthritis; CHO, Chinese hamster ovary; CIA, collagen induced arthritis; COX, cyclooxygenase; coxib, selective inhibitor of COX-2; CSF, cerebrospinal fluid; EIA, enzyme immunoassay; FBS, fetal bovine serum; FLAP, 5-lipoxygenase activating protein; GSH, glutathione; HTS, high throughput screen; IL, interleukin; IP, prostacyclin receptor; JAK, Janus kinase; KI, knock-in; KO, knockout; LDLR, low density lipoprotein receptor; LPS, lipopolysaccharide; LT, leukotriene; MAPEG, membrane associated proteins involved in eicosanoid and glutathione metabolism; mGST, microsomal glutathione transferase; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; PGES, prostaglandin E_2 synthase; PPAR, peroxisome proliferator-activated receptor; QSAR, quantitative structure−activity relationship; TX, thromboxane; WT, wild type.

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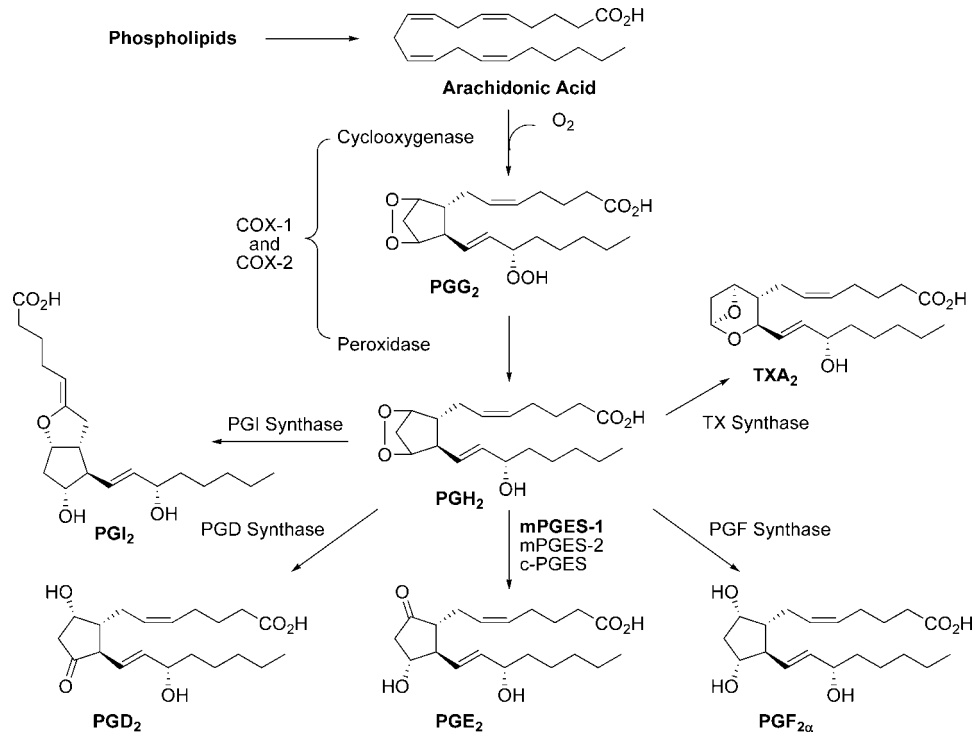


Figure 1. Prostaglandin synthesis pathway.

homologue is mGST-1 with 38% sequence identity.[4] This enzyme belongs to the extended family of membrane associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) gene families, which includes LTC_4 synthase and FLAP.

The constitutive expression of mPGES-1 in various tissues has been demonstrated to be ubiquitous, but to a low level, and is significantly up-regulated in response to various inflammatory stimuli. Rat studies have shown LPS induced expression of mPGES-1 at the RNA level in lung, brain, heart, testes, spleen, and seminal vesicles.[11] Protein levels as measured by Western blot also demonstrate constitutive levels in lung, spleen, kidney, and stomach in mice.[12] A number of preclinical in vivo models of inflammation, pain, and fever have been studied, and the induction of mPGES-1 has been demonstrated in these various models.[13–15] For example, increased expression of mPGES-1 has been observed in the rat adjuvant induced arthritis model and up-regulation of mPGES-1 is observed in rat brain upon treatment with IL-1β. The expression of mPGES-1 is up-regulated in the synovial lining of joints of patients with rheumatoid arthritis and the level of expression correlated with the level of disease severity.[8,16] Studies with experimental animals have suggested that PGE_2 may promote tumorogenesis.[17] In paired analyses of human colorectal cancer samples from both tumor and normal adjacent tissue, mPGES-1 was overexpressed in 83% of the tumor samples.[18] Studies have also described increased expression of mPGES-1 in various cancers including breast and non-small-cell lung cancer.[19,20]

mPGES-1 Knockouts and Roles in Atherosclerosis, Stroke, Inflammation, Pain, and Fever

One of the postulated themes in plaque formation suggests that PGI_2 provides a protective role during clot formation as observed in the PGI_2 receptor null mice.[21] The LDLR (−/−) mouse model was used to assess atherosclerotic plaque formation, and mPGES-1 levels were up-regulated in the vasculature of these mice during atherogenesis.[22] Stimulation of vascular smooth muscle cells and foam macrophages from mPGES-1 (−/−) mice with LPS results in an increase in PGI_2 production. The combination of mPGES-1 (−/−) and LDLR (−/−) mice results in a significant decrease in development of atherosclerotic lesions when these mice are placed on a high fat diet over a 3−6 month paradigm.[22]

Inflammation has also been implicated in ischemic stroke.[23] Stroke can be described as an occlusion of major arterial vessels resulting in brain ischemia and damage to the corresponding affected area. A recent report of rats undergoing ischemic stroke through middle cerebral artery occlusion demonstrated expression of both COX-2 and mPGES-1 in the corresponding ipsilateral cortex.[24] Inducing ischemic stroke in mPGES-1 null mice resulted in a significant reduction in the infarct size and volume, a result that may be explained by a decreased apoptosis in the region of the effected occlusion.[24]

Numerous studies using mPGES-1 deficient mice demonstrate that mPGES-1 inhibition is effective in providing symptomatic relief in models of inflammation, pain, and fever and support the hypothesis that selective inhibition of mPGES-1 might be a viable and effective therapeutic approach for the treatment of diseases involving inflammatory pain and arthritis. The initial knockout (KO) data in 2002 establishing a link between mPGES-1 and inflammation were demonstrated during the study of LPS induced PGE_2 production in peritoneal macrophages.[25] Wild type (WT) mouse macrophages produced greater than a 30-fold increase in PGE_2 following LPS challenge, while no significant change above baseline unchallenged macrophages was detected in the mPGES-1 null mice. One year later, the role of mPGES-1 in experimental collagen induced arthritis (CIA) was reported.[26] In this mouse CIA model, type II collagen injected subcutaneously was used to induce peripheral paw inflammation and joint damage, and 60% of the WT mice progressed to a significant CIA phenotype. The mPGES-1 null mice (DBA1/Lac J) were resistant to the development of arthritis in this model with only 10% of the mice progressing to CIA. The severity of the disease in the affected mice was also

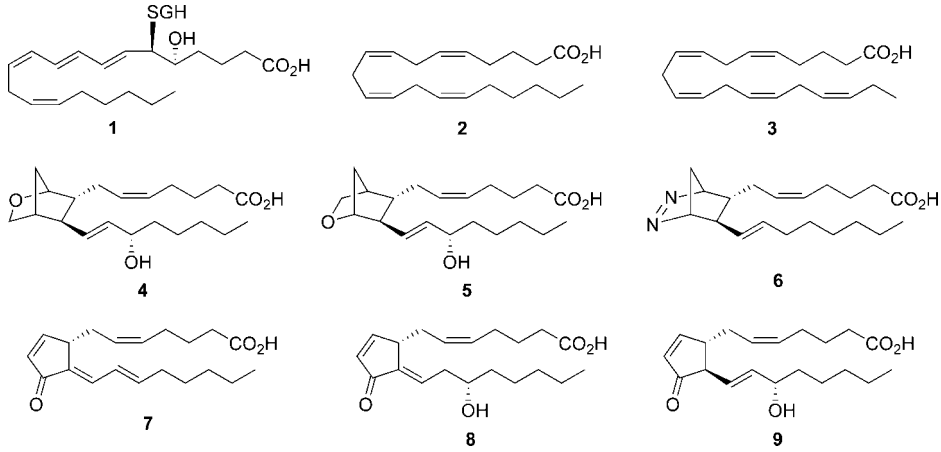


Figure 2. Fatty acid and PGH_2 analogue mPGES-1 inhibitors.

significantly diminished in the mPGES-1 KO mice. The observation of clinical end points such as cartilage architecture, cellularity, proteoglycan staining, hyperplasia, and inflammation of the synovium resulted in scores of 0−1 (minimal) in the mPGES-1 KO mice, while the WT mice had scores approaching 4 (severe). In this same study, an acute acetic acid writhing test was performed. Intraperitoneal injection of this noxious agent resulted in increased writhing over a 20 min period in the WT mice, while the mPGES-1 null mice had a 40% reduction in this pain response, similar to what was observed with WT mice treated with the NSAID piroxicam. A second independent report of mPGES-1 null mice utilized the modified acetic acid writhing test, and a 64% and 47% reduction in the writhing reaction was observed in the null mice in the presence or absence of concomitant LPS injection, respectively.[27] These data confirm a significant role for mPGES-1 in pain hypersensitivity. This latter report also analyzed the role of mPGES-1 in a collagen antibody induced arthritis (CAIA) model and demonstrated significant reduction of >40% in peripheral signs of clinical inflammation. The CAIA differs from the CIA model in that the CAIA model surpasses the need for immune cell activation prior to development of peripheral inflammation. These data suggest that mPGES-1 deletion may provide greater efficacy in a combined immune modulation and inflammation paradigm.

The role of mPGES-1 in pyresis has also been investigated. Analysis of brain sections using in situ hybridization demonstrated that IL-1β injection, which can stimulate pyresis in rats, results in an initial peak of COX-2 RNA after 1 h and declining by 3 h.[28] The mPGES-1 RNA displayed a later onset of induction, with detection at 1 h, a peak at 3 h, and a decrease to baseline by 5 h. LPS can be utilized to initiate fever in mice, and experiments in WT mice demonstrate a significant febrile response lasting up to 6 h. This response was completely ablated in the mPGES-1 null mice compared to the WT animals.[28] The corresponding PGE_2 levels in the CSF were also significantly decreased in mPGES-1 null mice compared to WT animals. These data confirm the role of mPGES-1 in PGE_2 production in the CNS and in pyresis. The central production of PGE_2 and its role in peripheral inflammation are also observed in the carrageenan induced paw edema model.[14] Analyzing the levels of PGE_2 in the brain and spinal cord during development of peripheral inflammation results in an induction of mPGES-1 that precedes the spike in spinal PGE_2 levels and, to a lesser extent, the increase in cortical PGE_2 levels.

Therefore, there are multiple avenues for the therapeutic intervention of mPGES-1. The development of robust assays and preclinical models has led to the development of selective inhibitors for novel therapeutic development. The remainder of this review will focus on these compounds and their preclinical potential in models of pain and inflammation.

Assays

A number of assays have been developed to measure the activity of mPGES-1 and are based on the quantitative detection of the reaction product PGE_2. Complicating factors in these assays are related to the instability of the PGH_2 substrate, which spontaneously decomposes to PGD_2 and PGE_2 with a $t_{1/2}$ of 10 min at pH 7 and 20 °C.[29] Therefore, short reaction times and low temperatures are required to optimize assay sensitivity. Typical assay conditions use 5−10 μg protein/mL of a microsomal preparation of recombinant human mPGES-1 expressed in CHO cells, 1 μM PGH_2, and an incubation time of 0.5 min.[11] Inhibitors are preincubated with the recombinant enzyme for 15 min before initiating the reaction by the addition of PGH_2. The reaction is terminated with a mixture of 0.1 N HCl and stannous chloride to convert all unreacted PGH_2 to $PGF_{2\alpha}$, and PGE_2 accumulation in the media is measured by EIA (Assay Designs, MI). This assay is amenable to inhibitor screening in a 96-well format.

A secondary assay in mPGES-1 expressing human A549 epithelial lung carcinoma cells has enabled the testing of compounds for their differential inhibition of prostanoid formation.[30] Selectivity of mPGES-1 inhibition in these A549 cells is defined as the ratio of inhibition of $PGE_2/PGF_{2\alpha}$.[31] The production of PGE_2 in the human whole blood is one of the key assays that was used in the development of COX-2 inhibitors and therefore has direct clinical relevance for development of mPGES-1 inhibitors.[32]

An alternative method for inhibiting inducible PGE_2 synthesis is to target the transcriptional activation of mPGES-1. A cell-based screening system, using luciferase activity as readout, has been developed to identify selective down-regulators of mPGES-1 expression.[33]

The interest in mPGES-1 as a therapeutic target is reflected in the increased number of publications, patent applications, and conference presentations that have recently started to appear. The small molecule medicinal chemistry approaches to selectively inhibiting the biosynthesis of PGE_2 can be divided into two main groups: those that target mPGES-1 enzymatic activity and those that target mPGES-1 expression. The former strategy

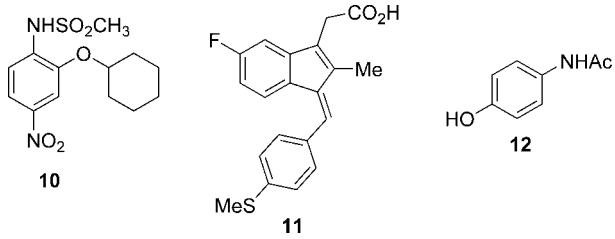


Figure 3. NSAID and coxib mPGES-1 inhibitors.

is much further advanced, and a number of structural classes have been identified.

(a) Small Molecule Inhibitors of mPGES-1 Activity

Fatty Acids and PGH_2 Analogues. A number of fatty acids demonstrate weak mPGES-1 inhibitory activity (Figure 2). mPGES-1, mGST-1, and LTC_4 synthase are members of the MAPEG family of enzymes, and the product of the LTC_4 synthase catalyzed reaction, the cysteinyl leukotriene LTC_4 (**1**), inhibits mPGES-1 with an IC_{50} of 1.2 μM (rat enzyme)[11] to 5 μM (human enzyme).[30] It has been reported that glutathione (GSH) is essential for the activity of these enzymes and that LTC_4 inhibits mGST-1 activity by competing with GSH. Therefore, it is reasonable to postulate that LTC_4 inhibition of mPGES-1 occurs through its GSH moiety as well. The polyunsaturated fatty acids arachidonic acid (**2**, AA) and eicosapentaenoic acid (**3**) are more potent inhibitors than LTC_4 and exhibit IC_{50} of 0.3 μM.[34] The acid moiety in these fatty acids is required for inhibition because AA methyl ester or the methyl fluorophosphonate and trifluoromethyl ketone derivatives of AA are much less active (<20% inhibition at 10 μM).[34] Although **4** (U-44069) and **5** (U-46619), stable analogues of PGH_2, are not inhibitors of mPGES-1 (<50% inhibition at 100 μM), the diazo analogue **6** (U-51605) shows 70% inhibition at 10 μM.[30,34] Of the various PGs tested as potential inhibitors of mPGES-1, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (**7**) was found to be the most potent (IC_{50} = 0.3 μM) while the structurally related PGs $\Delta^{12,14}$-PGJ_2 (**8**) and PGJ_2 (**9**) were more than 2 orders of magnitude less potent inhibitors (IC_{50} = 64 and 55 μM, respectively).[34]

It is interesting to note that, in contrast to the less potent fatty acid inhibitors, the more potent analogues **6** and **7** both lack the polar hydroxyl group at C-15. The fact that PGE_2 itself is not an inhibitor of mPGES-1 (<30% inhibition at 10 μM) suggests that the mPGES-1 catalyzed turnover of PGH_2 is not subject to product inhibition.[34]

NSAIDs and Coxibs. The cyclooxygenase inhibitors **10** (NS-398) and sulindac sulfide (**11**) (Figure 3) are weak inhibitors of mPGES-1 (IC_{50} = 20 and 80 μM, respectively).[30] While acetaminophen (**12**) does not inhibit mPGES-1 activity in a microsomal preparation up to 1000 μM[11] or in isolated monocytes in the absence of exogenous GSH,[35] LPS-stimulated PG production in human whole blood was inhibited by **12** (IC_{50} = 44 μM for PGE_2 and 94 μM for TXB_2).[35] It was proposed that the reduction of inducible PG synthesis by **12** results from the inhibition of both COX-2 and mPGES-1 activity. Furthermore, the moderately selective inhibition of PGE_2 synthesis was postulated to result from the presence of *N*-acetyl-*p*-benzoquinoneimine (a reactive metabolite generated by oxidation of **12** in whole blood), which reacts with plasma GSH, a cofactor essential for mPGES-1 activity but not that of COX-2.[35]

MK-886 and Indole Analogues. mPGES-1 and FLAP are members of the MAPEG family of enzymes. The FLAP inhibitor **13** (MK-886; IC_{50} for FLAP = 26−100 nM)[11,31] is a moderate inhibitor of both rat (IC_{50} = 3.2 μM)[11] and human (IC_{50} = 1.6 μM)[31] mPGES-1 (Figure 4). The presence of a highly conserved amino acid sequence in both MAPEG enzymes (consensus sequence ERXXXAXXNXXD/E), which was demonstrated to be essential for FLAP binding of **13**, suggests that this region of the mPGES-1 enzyme might also be involved in **13** binding. **13** was able to inhibit mPGES-1 activity in IL-1β-stimulated human chondrocytes (1−10 μM)[36] and in LPS-induced human whole blood (20% inhibition at 100 μM) without affecting TXA_2 synthesis.[35] However, there is some suggestion that the cellular based efficacy of **13** is not due solely to the inhibition of mPGES-1 enzymatic activity because **13** selectively reduced the IL-1β and TNFα-induced production of mPGES-1 protein expression in human gingival fibroblasts (2−4 μM) while not affecting the expression of mPGES-2, cPGES, or COX-2 or the production of PGE_2.[37] Since **13** appears to have diverse mechanisms by which it affects PGE_2 synthesis depending on cell type and also has other activities that could affect observations that link PGE_2 production and mPGES-1 activity/expression inhibition (including PPARα and LT pathway inhibition),[35] one must be very careful about drawing conclusions from studies using **13** regarding the contribution of mPGES-1 activity inhibition to the cellular and tissue biosynthesis of PGE_2.

Researchers at Merck Frosst have carried out SAR studies aimed at improving the potency and selectivity in the series of mPGES-1 inhibitors derived from **13**.[31,38] SAR studies showed that the carboxylic acid moiety at C-2 was preferred compared to the corresponding ester and amide analogues, and the greatest impact on potency enhancement could be realized by the introduction of lipophilic substituents at C-5 of the indole scaffold. Optimized compounds in this series are represented by the biaryl derivatives **14** and **15**.[31] Although these compounds are potent inhibitors of mPGES-1 activity (IC_{50} = 3−7 nM) (no inhibition of mPGES-1 expression was observed (unpublished data)) and are selective with respect to the inhibition of other recombinant PG synthesizing enzymes (for example, IC_{50} for TX synthase and mPGES-2 greater than 1 μM) and FLAP binding (IC_{50} > 1 μM), they are highly protein shifted in cellular based assays (for example, IC_{50} = 6−8 μM for IL-1β-stimulated PGE_2 production in A549 cells containing 50% FBS). In addition, the inhibition selectivity observed against the recombinant PG synthesizing enzymes is eroded in these cells (IC_{50} vs PGE_2/$PGF_{2\alpha}$ synthesis with 2% FBS for **14** and **15** is 0.5/2.0 and 0.3/2.4 μM, respectively). These observations have precluded the use of **14** and **15** in studying the effects of selective mPGES-1 inhibitors in vivo.

Two 3D-QSAR computational studies have provided models that predict a probable binding mode between mPGES-1 and the published molecules in this series.[39,40] Computational methods have also been used to develop a 3D structural model for the substrate-binding domain of mPGES-1 based on the primary amino acid sequence alignment of mPGES-1 with mGST-1, a member of the MAPEG family for which 6 Å resolution 3D data exist.[41] When combined with site-directed mutagenesis studies, these models have helped to locate the PGH_2 binding site in mPGES-1 (Gln36, Arg110, and Thr114 are involved in binding of the acid moiety; Tyr130 is located near the peroxy bridge; Gln134 interacts with the hydroxyl group at C-15). Molecular docking studies using the 3D structural model and the inhibitors from the MK-886 series identified Arg110 as having a close electrostatic interaction with the indole C-2 acid moiety, while Tyr130 and Gln134 were also shown to be key binding residues in the interactions with the indole substituents at N-1 and C-3, respectively.[40] A hydrophobic

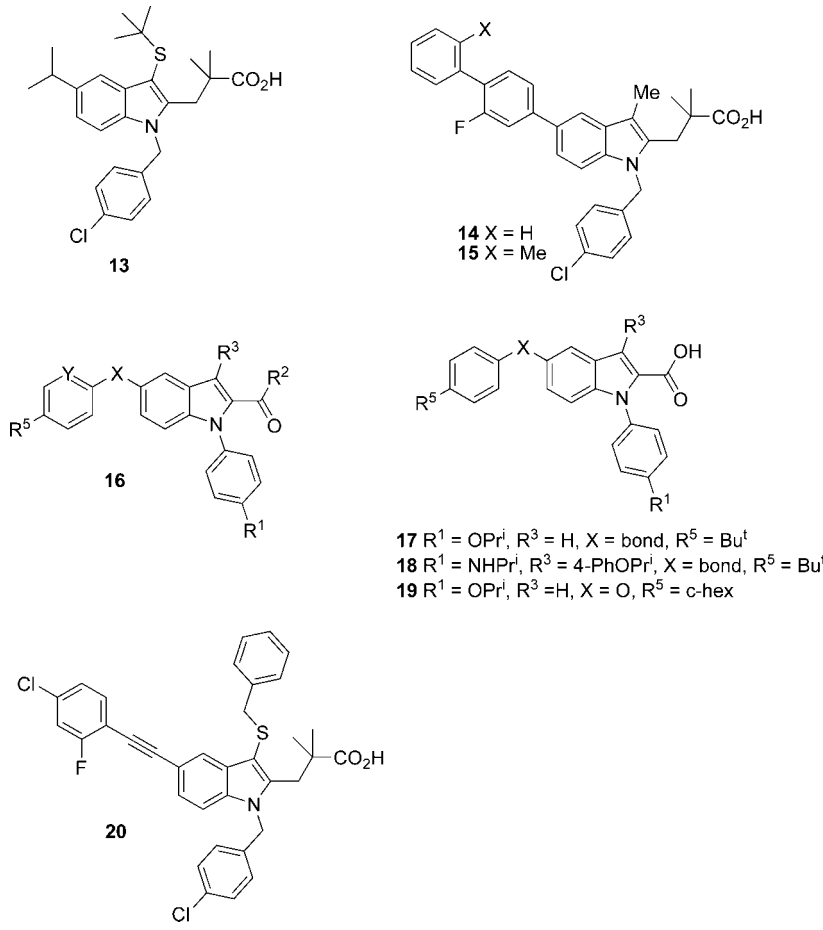


Figure 4. MK-886 and indole mPGES-1 inhibitors.

pocket, defined by Val37 and Val128, provides points of interaction with the lipophilic substituents at C-5. Although the structural insights from these 3D-QSAR analyses and molecular docking studies provide a rationalization of the experimentally observed inhibitory potencies in this series, they have not been used to prospectively design new inhibitors.

The group at Biolipox has published a series of patents claiming inhibitors of mPGES-1 that are based on the indole scaffold **16** (Y = CH, N).[42–50] The C-2 acid or amide moiety is a conserved substructure, and the most potent compounds disclosed are the indolyl acids **17**,[43] **18**,[45] and **19**[46] (IC_{50} = 62−75 nM). No cellular based or in vivo data have been reported for these inhibitors. However, an indole acid of similar inhibitory potency (**20**, IC_{50} = 60 nM) is shifted >100-fold in an LPS-induced A549 cell assay in the presence of 50% FBS (IC_{50} for inhibition of $PGE_2/PGF_{2\alpha}$ synthesis = 16/50 μM).[38] Thus far, it appears that potent and selective inhibitors of mPGES-1 can be derived from the lead **13** but that these lipophilic carboxylic acids are highly protein shifted in cellular based assays and therefore not useful for in vivo applications. Further investigation will be required to determine whether this limitation can be overcome in this structural series.

Phenanthrene Imidazoles. By use of an HTS assay based on a 30-s mPGES-1 mediated conversion of PGH_2 to PGE_2 and detection by enzyme immunoassay (EIA),[51] the JAK kinase inhibitor **21** (Figure 5) was identified as a lead structure amenable for further SAR studies.[52] Removal of the pyridone moiety, which is essential for kinase inhibition, and manipulation of the requisite ortho substituents on the imidazole-bonded phenyl moiety led to the discovery of the phenanthrene imidazole **22** (MF63, Table 1).[52,53] **22** is a potent inhibitor of

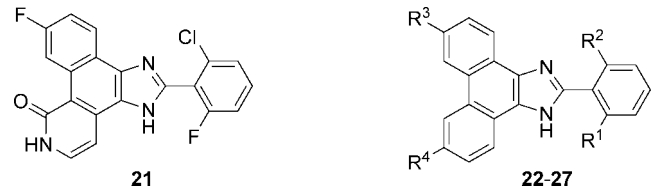


Figure 5. Phenanthrene imidazole mPGES-1 inhibitors.

human mPGES-1 (IC_{50} = 1 nM) and is >1000-fold selective vs other PG synthases. **22** retains its potency and selectivity in cell-based assays (IC_{50} = 0.42 or >50 μM for inhibition of PGE_2 or $PGF_{2\alpha}$ in A549 cells with 50% FBS) and a LPS-stimulated human whole blood assay where it inhibits PGE_2 production with an IC_{50} = 1.3 μM with no concomitant TXB_2 synthesis inhibition (IC_{50} > 40 μM). Unfortunately, **22** is inactive against rat mPGES-1 (IC_{50} > 30 μM), precluding its use in well-established rat inflammation and pain models. Since there have been no studies that have described the structural differences between the enzymes in these two species, the reason for this discrepancy is unknown. However, **22** is active on the guinea pig mPGES-1 enzyme (IC_{50} = 0.9 nM) and, upon oral dosing in this species, exhibited dose-dependent (3−100 mg/kg, ED_{50} ≈ 30 mg/kg) prevention of LPS-induced hyperalgesia equivalent in magnitude to that of diclofenac, a nonselective COX inhibitor.[52,54] Moreover, **22** caused a selective and dose-dependent inhibition of PGE_2 synthesis over that of PGI_2 in the spinal cord of these animals, demonstrating for the first time the link between selective inhibition of inducible PGE_2 and analgesic efficacy in an in vivo preclinical model.[54] The efficacy

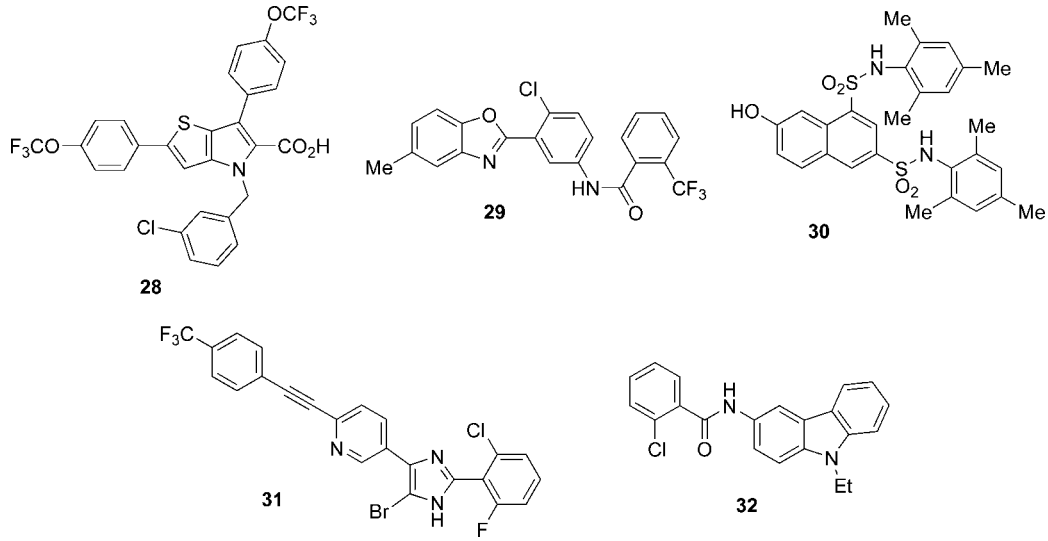


Figure 6. Other mPGES-1 inhibitors.

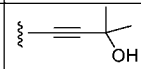
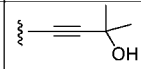
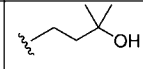
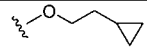
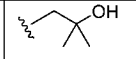
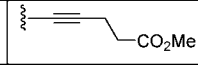
of **22** was also demonstrated in guinea pig models of LPS-induced pyresis and sodium idodoacetate-induced osteoarthritis-like pain.[54]

Since this molecule was not active in the rat or mouse, a humanized knock-in (KI) mouse was generated in which the murine mPGES-1 gene was replaced by a human mPGES-1 minigene.[54] **22** was as efficacious as a selective COX-2 inhibitor in preventing LPS-induced hyperalgesia when dosed orally at 100 mg/kg in the KI mice but, as expected, was inactive in wild-type mice. In an LPS-induced air pouch model, selective inhibition of PGE_2 synthesis over that of PGI_2 was observed in **22** treated KI mice at the peripheral inflammation site. Similarly, when dosed at 100 mg/kg in unchallenged KI mice, PGE_2 production in the stomach was inhibited by 80% while PGI_2 synthesis was not affected. Although **22** suppresses stomach PGE_2 production, it did not cause gastric lesions different from that of vehicle in contrast to the NSAID indomethacin (10 mg/kg).[54] The efficacy of **22** in preclinical models of pain and inflammation is further support for the notion that this mechanism may be a valid target for clinical intervention in these indications.

Several other patent applications in the phenanthrene imidazole series have appeared from the Merck Frosst group,[55–57] and many of the compounds described (represented by **23**−**27** (Table 1)) inhibit human mPGES-1 with low nanomolar to subnanomolar IC_{50} values when tested using the EIA assay described above. The selectivity over other PG synthesizing enzymes and the in vivo activity of these compounds have not been described.

Other Inhibitors. A number of other mPGES-1 inhibitors have been reported in the patent literature or at major conferences (Figure 6). Thienopyrrole,[58] benzoxazole,[59] and naphthalene disulfonamide[60] inhibitors, represented by **28** (IC_{50} = 390 nM), **29** (IC_{50} = 1.3 μM), and **30** (IC_{50} = 1.1 μM), respectively, have been described by Biolipox although selectivity and efficacy data have not been disclosed. The biarylimidazole **31**[61] from Merck Frosst is a potent mPGES-1 inhibitor (IC_{50} = 0.8 nM). The 3-benzamidocarbazole **32** has been reported to selectively inhibit PGE_2 production (IC_{50} = 2 μM) compared to that of $PGF_{2\alpha}$ (IC_{50} = 79 μM) in IL-1β-stimulated human A549 cells.[62] Carbazole **32** is also efficacious in vivo (30 mg/kg po) in a murine model of acetic acid-induced pain. However, it is not reported whether these observations are due to selective inhibition of mPGES-1 or to another mechanism of selective PGE_2 synthesis inhibition (see below). Nevertheless, the structures of these latter inhibitors suggest that there may

Table 1. Phenanthrene Imidazole Inhibitors (**22**−**27**) of Human mPGES-1

Compound	R^1	R^2	R^3	R^4	IC_{50} (nM)	ref
22	CN	CN	Cl	H	1.0	52,53
23	CN	CN	Cl		0.9	53
24	CN	CN	Br		0.9	53
25	CN	CN	Cl		0.7	55
26	CN	CN			0.9	55
27	Cl	F	Br		1.7	57

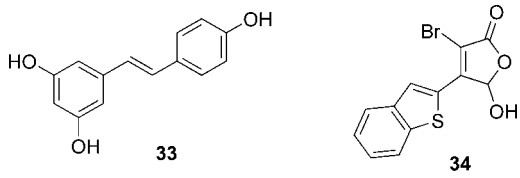


Figure 7. Inhibitors of mPGES-1 expression.

be other non-carboxylic acid containing scaffolds that are viable alternatives to the phenanthrenes described above.

(b) Small Molecule Inhibitors of Expression

Several compounds in addition to **13** (see above) have been shown to selectively inhibit mPGES-1 expression (Figure 7). In addition to its ability to inhibit LPS-induced free radical formation and the peroxidase activity of COX-1, resveratrol (**33**) dose-dependently (1−10 μM) reduced the LPS-induced production of PGE_2 and mPGES-1 (mRNA and protein) in primary rat microglial cells without affecting the expression of COX-2. Compound **34** was identified from a series of γ-hydroxybutenolides as a moderate inhibitor (IC_{50} = 1.8 μM) of LPS-induced PGE_2 production in RAW264.7 cells, a mouse macrophage cell line.[33] The LPS-stimulated PGE_2 inhibition correlated with the concomitant dose-dependent decrease in mPGES-1 protein expression in both RAW264.7 and THP-1 cells while not affecting COX-2 expression. This selective inhibition was recapitulated in vivo in a zymosan-stimulated mouse air pouch inflammation model. Although the mechanism of inhibition of expression has not been elucidated, **33** and **34** provide evidence that the expression of mPGES-1 and COX-2 can be independently modulated and suggest, at least in these settings, that cytokine induced PGE_2 production can be inhibited by selective down-regulation of mPGES-1.

The availability of a pharmacologically active molecule capable of selective inhibition of mPGES-1 and suitable for clinical development will ultimately provide the answer to the question of whether this therapeutic approach will exhibit clinical anti-inflammatory and analgesic efficacy equal to that of selective COX-2 inhibitors or NSAIDs. On the basis of the progress reported to date, it appears that it is only a matter of time before such a molecule is identified.

Biographies

Richard W. Friesen obtained his Ph.D. in Synthetic Organic Chemistry from the University of British Columbia under the supervision of Prof. Edward Piers in 1988. He was a NSERC postdoctoral fellow with Prof. Samuel Danishefsky at Yale University and then an assistant Professor in the Chemistry Department at the University of Toronto. In 1992, he joined the Merck Frosst Centre for Therapeutic Research as a Research Fellow and has led medicinal chemistry projects in a number of therapeutic areas, including those with enzyme and receptor targets in the arachidonic acid cascades leading to leukotrienes and prostaglandins. He presently holds the position of Executive Director and is the head of the Medicinal Chemistry Department at Merck Frosst.

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