Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6816–6820
Bioorganic & Medicinal Chemistry Letters

Substituted phenanthrene imidazoles as potent, selective, and orally active mPGES-1 inhibitors

Bernard Côté,* Louise Boulet, Christine Brideau, David Claveau, Diane Ethier, Richard Frenette, Marc Gagnon, André Giroux, Jocelyne Guay, Sébastien Guiral, Joseph Mancini, Evelyn Martins, Frédéric Massé, Nathalie Méthot, Denis Riendeau, Joel Rubin, Daigen Xu, Hongping Yu, Yves Ducharme and Richard W. Friesen

Merck Frosst Centre for Therapeutic Research, 16711 Trans Canada Hwy, Kirkland, Que., Canada H9H 3L1

Received 21 August 2007; revised 4 October 2007; accepted 8 October 2007
Available online 17 October 2007

Abstract—Phenanthrene imidazole **3** (MF63) has been identified as a novel potent, selective, and orally active mPGES-1 inhibitor. This new series was developed by lead optimization of a hit from an internal HTS campaign. Compound **3** is significantly more potent than the previously reported indole carboxylic acid **1** with an A549 whole cell IC_{50} of 0.42 μM (50% FBS) and a human whole blood IC_{50} of 1.3 μM. It exhibited a significant analgesic effect in a guinea pig hyperalgesia model when orally dosed at 30 and 100 mg/kg.

Prostaglandin E_2 (PGE_2) is widely recognized as a key mediator in fever, pain, and inflammatory response.[1] The biosynthesis of PGE_2 involves the release of arachidonic acid (AA) from membrane glycerophospholipids followed by several enzymatic transformations (Fig. 1). First, AA is converted to PGH_2 by the cyclooxygenases (COX-1/COX-2) and then, this intermediate prostanoid is further metabolized by downstream specific synthases to PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$, and thromboxane A_2 (TXA_2). Non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors (coxibs) intervene in this prostaglandin pathway at the level of PGH synthase. These drugs exert their anti-inflammatory and analgesic properties mainly through PGE_2 and PGI_2 suppression.[2] As a potential way to improve the current therapeutic window of NSAIDs and coxibs, we investigated a new mechanism for selective PGE_2 modulation through downstream PGE synthase inhibition. To this end, the inducible microsomal PGE synthase-1 (mPGES-1) represents a potential novel target for drug development in the treatment of inflammatory diseases.[3] Supporting this hypothesis are the observations that mice deficient in mPGES-1 are protected from fever[4]

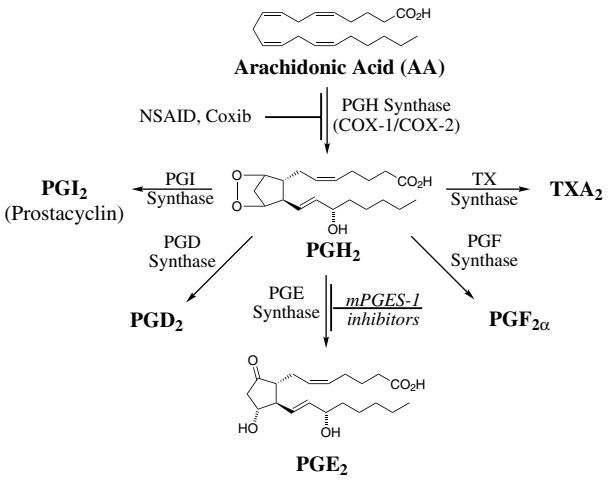


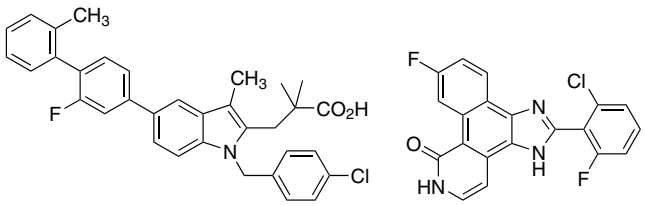
Figure 1. The prostaglandin pathway.

and chronic inflammation,[5] and are not predisposed to thrombogenesis or hypertension.[6] Herein we report the identification of the first orally active mPGES-1 inhibitor in a model of hyperalgesia.[7]

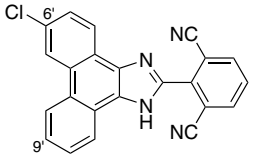
We recently reported that the indole carboxylic acid **1** is a potent and selective mPGES-1 inhibitor (Fig. 2).[8] Despite its high intrinsic inhibitory potency (IC_{50} = 0.003 μM) on

Keywords: mPGES-1; PGE_2; Inflammation; Prostanoid.
* Corresponding author. Tel.: +1 514 428 2683; fax: +1 514 428 8670; e-mail: bernard_cote@merck.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.033



1 **2a**



3 (MF63)

Figure 2. mPGES-1 inhibitors.

the recombinant human mPGES-1 enzyme,[9] a 2000-fold shift was observed with **1** for PGE_2 inhibition in the IL-1β stimulated A549 whole cell assay[10] in the presence of 50% fetal bovine serum (FBS, see Table 1).

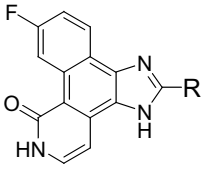
This poor whole cell potency precluded the use of this compound for in vivo proof-of-concept studies in models of inflammation. Moreover, efforts to improve the cellular activity of analogs of indole carboxylic acid **1** were unsuccessful and a search for a new chemical class of inhibitors was undertaken. An HTS campaign was conducted[11] using the enzyme assay and among several hits, the JAK kinase[12] inhibitor azaphenanthrenone **2a** was identified as a potent lead structure (mPGES-1 IC_{50} = 0.14 μM). Further testing of analogs of **2a** from our sample collection revealed a strong preference for an *ortho*-substituted phenyl ring at the R position (Table 1).[13] It was also found that the active analogs tend to be substantially less shifted in the A549 whole cell assay (50% FBS) when compared to indole carboxylic acid **1**.

SAR studies and lead optimization were conducted with the initial objective of dialing out the JAK kinase activity of **2** while maintaining the mPGES-1 potency of this new class of inhibitors. Internal data from the JAK kinase program revealed that the pyridone moiety was essential for good potency. The azaphenanthrenone template was therefore replaced by the phenanthrene ring system through the simple assemblage of phenanthrenequinone and 2-chloro-6-fluoro benzaldehyde to produce phenanthrene imidazole **4** (Scheme 1).[14] As presented in Table 2, compound **4** is intrinsically four times more potent than the lead compound **2a**, maintains good cellular activity, and is still less shifted than the acid **1** in presence of proteins. When $PGF_{2\alpha}$ inhibition was measured in the A549 whole cell assay (2% and 50% FBS), the phenanthrene imidazole **4** was found to be at least 10-fold selective for the inhibition of PGE_2.

Using the phenanthrene imidazole template as a reference (Table 3, compounds **4** and **5**), we then investigated the minimal structural requirements for mPGES-1 activity. In general, alteration of the phenanthrene motif for compounds **6**, **7**, **9**,[15] **10**, and **11** resulted in a significant decrease in inhibitory activity. Substitution of the imidazole (**12**) or replacement by other heterocycles (**13**–**15**) was also detrimental for mPGES-1 inhibition.

Having recognized the importance of the *ortho*-substituent of the biaryl system, we next oriented our SAR investigation toward the optimization of this critical portion of the molecule (Table 4). The phenyl ring was

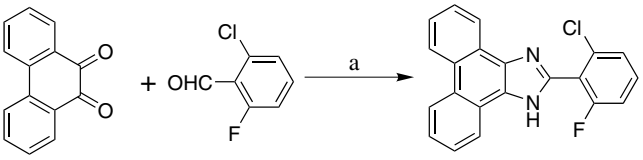
Table 1. mPGES-1 inhibition IC_{50} for azaphenanthrenones **2**



2

Compound	R	IC_{50}[c] (μM)	
		mPGES-1[a] inhibition	A549, 50% FBS[b] PGE_2
1		0.003	5.8
2a	2Cl, 6F-phenyl	0.14	1.6
2b	H	>10	—
2c	*n*-Propyl	>10	—
2d	*tert*-Butyl	>10	—
2e	*c*-Pentyl	>10	—
2f	Phenyl	>10	—
2g	2Cl-phenyl	0.53	0.56
2h	3Cl-phenyl	2.5	5.3
2i	4Cl-phenyl	0.41	0.82
2j	4MeO-phenyl	>10	—
2k	2Br-phenyl	0.33	0.65
2l	$2CF_3$-phenyl	0.56	5.1
2m	2,6-Di-Cl-phenyl	0.10	9.8

[a] For assay conditions, see Ref. 9.
[b] For assay conditions, see Ref. 10.
[c] Values are means of at least two experiments.



4

Scheme 1. Reagents and condition: (a) NH_4OAc, AcOH, 100 °C.

Table 2. Comparative profile of in vitro activity and selectivity for inhibitors **1**, **2a**, and **4**

Compound	mPGES-1 IC_{50} (μM)	A549 whole cell, IC_{50}[a](μM)			
		2% FBS		50% FBS	
		PGE_2	$PGF_{2\alpha}$	PGE_2	$PGF_{2\alpha}$
1	0.003	0.27	2.4	5.8	>50
2a	0.14	0.21	1.1	1.6	19
4	0.036	0.35	5.2	2.6	>30

[a] Values are means of at least two experiments.

Table 3. Structural modifications of the phenanthrene imidazole motif

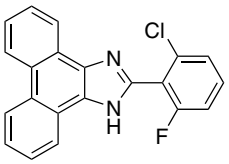
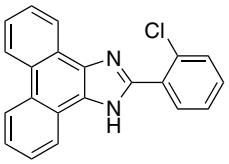
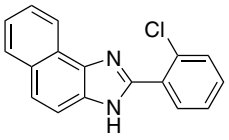
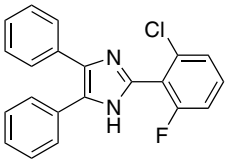
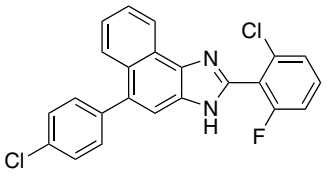
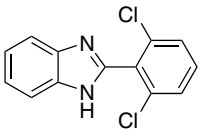
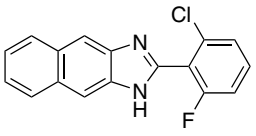
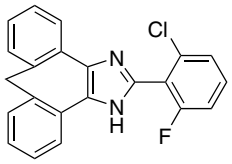
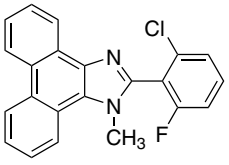
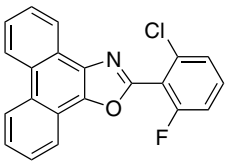
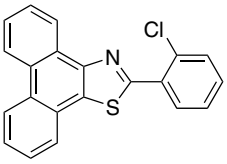
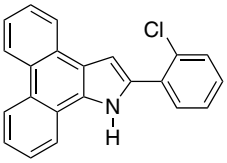
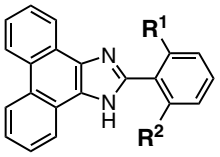
Compound	Structure	mPGES-1 inhibition IC_{50}[a] (μM)
4		0.036
5		0.087
6		6.6
7		2.0
8		0.073
9		>10
10		8.0
11		1.3
12		>10
13		0.70

Table 3 (*continued*)

Compound	Structure	mPGES-1 inhibition IC_{50}[a] (μM)
14		>10
15		0.71

[a] Values are means of at least two experiments.

Table 4. SAR at the *ortho*-position of the biaryl system



16

Compound	R^1	R^2	IC_{50} (μM)		
			mPGES-1	A549 whole cell assay[a] 50% FBS	
				PGE_2	$PGF_{2\alpha}$
5	Cl	H	0.087	1.0	6.4
16a	Br	H	0.051	1.6	17.1
16b	CN	H	0.075	3.0	26.7
4	Cl	F	0.036	2.2	>30
16c	Cl	Cl	0.047	1.1	>50
16d	CN	Cl	0.012	0.76	>30
16e	CN	CN	0.009	0.33	>30
16f	Br	Br	0.045	2.7	>30
16g	Br	F	0.019	1.6	>30
16h	CN	Br	0.017	0.57	>30
16i	CN	F	0.021	1.1	>30

[a] Values are means of at least two experiments.

mono- and bis-substituted with several groups such as F, Cl, Br, and CN. We first observed that, although potent, mono-substituted analogs **5**, **16a**, and **16b** were generally less selective in the cellular assay. Among several bis-substituted analogs, the optimal pattern was obtained with $R^1 = R^2 = CN$ (**16e**) where the intrinsic and the cellular activity as well as the selectivity (PGE_2/$PGF_{2\alpha}$) were significantly improved.

After optimizing the *ortho*-substituents of the biaryl system, we next studied the effect of substitution on the phenanthrene core of the molecule. In vitro potency could be favorably modulated by the simple incorporation of a chloro substituent at the 6′ position as exemplified with the inhibitor **3** (Fig. 2). Profile comparison is

Table 5. Comparative data on potency and selectivity of mPGES-1 inhibitors **1** and **3**

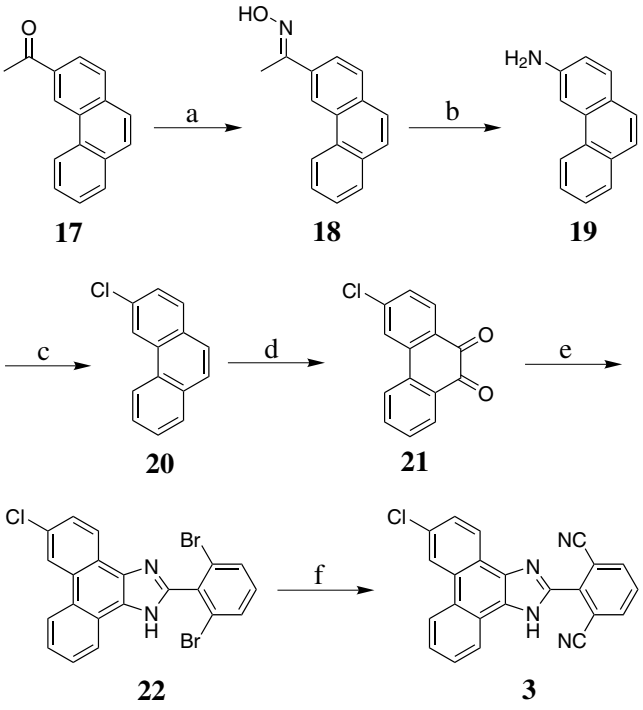
Enzyme or cell assay	IC_{50}[a] (μM)	
	1	**3**
Human mPGES-1	0.003	0.001
Rat mPGES-1	0.13	>30
Guinea pig mPGES-1	N/A	0.0009
Human mPGES-2	>1	>30
TX synthase	0.95	3.0
A549 cells, PGE_2, 50% FBS	5.8	0.42
A549 cells, $PGF_{2\alpha}$, 50% FBS	>50	>50
Human whole blood, PGE_2	>40	1.3
Human whole blood, TXB_2	>40	>40
Human JAK2	N/A	0.1
Human JAK3	N/A	>10

[a] Values are means of at least two experiments.

presented in Table 5 for the indole carboxylic acid **1** and the phenanthrene imidazole **3**. Both compounds have similar intrinsic mPGES-1 activity with IC_{50}s of 0.003 and 0.001 μM, respectively. They are selective against mPGES-2[16] and thromboxane synthase. Compound **3** is 14-fold more potent than the initially reported inhibitor **1** with an IC_{50} of 0.42 μM in the A549 whole cell assay (50% FBS). Most importantly, the phenanthrene imidazole **3** is the first reported mPGES-1 inhibitor to demonstrate potency in a human whole blood (HWB) assay. When freshly collected blood was stimulated with LPS, compound **3** selectively inhibited the production of PGE_2 with an IC_{50} of 1.3 μM with no concomitant TXB_2 inhibition. It is worth mentioning that this HWB IC_{50} is comparable to the ones of marketed coxibs.[17] With this markedly improved potency and a suitable pharmacokinetic profile,[18] compound **3** may serve as a good tool for in vivo proof-of-concept studies.[19] Gram quantities of this new potent mPGES-1 inhibitor were prepared according to Scheme 2.

The synthesis of **3** starts with commercially available 3-acetylphenanthrene **17**. This methyl ketone was refluxed in ethanol in the presence of hydroxylamine hydrochloride to afford the corresponding oxime **18** in 59% yield. Phenanthreneamine **19** is obtained by a Beckmann rearrangement with polyphosphoric acid at 100 °C followed by amide hydrolysis under acidic conditions in an overall yield of 92%. Conversion of the amine to the aryl chloride **20** was performed with *tert*-butyl nitrite in the presence of copper (II) chloride in 52% yield.[20] Phenanthrene **20** was then treated with chromium (VI) oxide in acetic acid at 100 °C to afford the corresponding quinone **21** in 88% yield. The core of the molecule was assembled using the same transformation described above and phenanthrene imidazole **22** was obtained in 85% yield. Finally, the cyano groups were incorporated by reacting the dibromo precursor **22** with CuCN in DMF at 80 °C to afford the phenanthrene imidazole bisnitrile **3** in 80% yield.

As disclosed in Table 5, the mPGES-1 inhibitor **3** is not active against the rat enzyme which precluded the use of well-established rat inflammatory pain models. This major discrepancy between the human and the rodent enzyme activity was also observed for mice. To demonstrate in vivo efficacy, a LPS-induced hyperalgesia model in guinea pig was therefore developed[7] knowing the ability of compound **3** to inhibit this enzyme (guinea pig IC_{50} = 0.9 nM). When orally dosed at 30 and 100 mg/kg, inhibitor **3** prevented the hyperalgesic response in guinea pig in a dose-dependent manner with a complete blockade of hyperalgesia at 100 mg/kg.[21] This constitutes the first reported example of in vivo efficacy for a selective mPGES-1 inhibitor in a pre-clinical model. A full description of this in vivo pharmacological profile will be published elsewhere.[7]



Scheme 2. Reagents and conditions: (a) $H_2NOHHCl$, EtOH, reflux, 59%, pyridine; (b) PPA, 100 °C, concd HCl, MeOH, reflux, 92%; (c) $CuCl_2$, *t*-BuONO, MeCN, 65 °C, 52%; (d) CrO_3, AcOH, 100 °C, 88%; (e) 2,6-dibromobenzaldehyde, NH_4OAc, AcOH, 100 °C, 85%; (f) CuCN, DMF, 80 °C, 80%.

In summary, phenanthrene imidazole **3** (MF63) has been identified as a new potent, selective, and orally active mPGES-1 inhibitor. This new series was developed by lead optimization of an internal HTS hit. Compound **3** shows in vitro superiority over the previously reported indole carboxylic acid **1**, both in the A549 whole cell assay (50% FBS, IC_{50} = 0.42 μM) and in the human whole blood assay (IC_{50} = 1.3 μM). Finally, in a guinea pig hyperalgesia model, inhibitor **3** demonstrated a significant analgesic effect.

References and notes

1. Funk, C. D. *Science* **2001**, *294*, 1871.
2. (a) Portanova, J. P. et al. *J. Exp. Med.* **1996**, *184*, 883; (b) Pulichino, A. M. et al. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1043; (c) Murata, T. et al. *Nature* **1997**, *388*, 678.

3. (a) Jakobsson, P. J.; Thoren, S.; Morgenstern, R.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7220; (b) Murakami, M.; Naraba, H.; Tanioka, T.; Semmyo, N.; Nakatani, Y.; Kojima, F.; Ikeda, T.; Fueki, M.; Ueno, A.; Oh, S.; Kudo, I. *J. Biol. Chem.* **2000**, *275*, 32783; (c) Murakami, M.; Kudo, I. *Prog. Lipid Res.* **2004**, *43*, 3.
4. Engblom, D. *Nat. Neurosci.* **2003**, *6*, 1137.
5. Trebino, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9044.
6. Cheng, Y.; Wang, M.; Yu, Y.; Lawson, J.; Funk, C. D.; FitzGerald, G. A. *J. Clin. Invest.* **2006**, *116*, 1391.
7. Xu, Daigen, et al., submitted for publication.
8. Riendeau, D. et al. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3352.
9. Mancini, J. A.; Blood, K.; Guay, J.; Gordon, R.; Claveau, D.; Chan, C. C.; Riendeau, D. *J. Biol. Chem.* **2001**, *276*, 4469.
10. Thoren, S.; Jakobsson, P. *Eur. J. Biochem.* **2000**, *267*, 6428.
11. Massé, F.; Guiral, S.; Fortin, L. J.; Cauchon, E.; Ethier, D.; Guay, J.; Brideau, C. *J. Biomol. Screen.* **2005**, *106*, 599.
12. Thompson, J. E. et al. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1219, For compound **2a**, JAK2 $IC_{50} = 0.2$ nM; JAK3 $IC_{50} = 0.6$ nM..
13. SAR of the 4-substituted analogs such as **2i** showed that although potent, these inhibitors were not selective for PGE_2 inhibition.
14. Compound **4** was assayed against JAK kinases: JAK2 $IC_{50} = 0.9$ μM; JAK3 $IC_{50} > 10$ μM.
15. For a rigorous SAR analysis, compound **9** should be compared with **16c**.
16. Tanikawa, N.; Ohmiya, Y.; Ohkubo, H.; Hashimoto, K.; Kangawa, K.; Kojima, M.; Ito, S.; Watanabe, K. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 884.
17. Etoricoxib HWB $IC_{50} = 1.1$ μM (PGE_2) Riendeau, D. et al. *J. Pharmacol. Exp. Ther.* **2001**, *296*, 558.
18. Compound **3** is bioavailable in rat ($F = 68\%$) with a C_{Max} of 12 μM when dosed orally at 20 mg/kg.
19. Although some residual JAK2 activity was measured, compound **3** is still 100× more active against mPGES-1. Since this inhibitor is also shifted >1000× in the whole blood assay, we believe that the JAK2 residual activity should not interfere in the in vivo model.
20. Eirin, A.; Fernandez, F.; Gonzalez, C.; Lopez, C. *Org. Prep. Proc. Int.* **1992**, *245*, 509.
21. At 30 mg/kg, a 50% blockade of the hyperalgesia was measured relative to vehicle treated animal and the no-LPS (saline) group.