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Inhibitors of the inducible microsomal prostaglandin E_2 synthase (mPGES-1) derived from MK-886

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Abstract—A series of potent and selective inhibitors of the inducible microsomal PGE_2 synthase (mPGES-1) has been developed based on the indole FLAP inhibitor MK-886. Compounds **23** and **30** inhibit mPGES-1 with potencies in the low nanomolar range and with selectivities of at least 100-fold compared to their inhibition of mPGES-2, thromboxane synthase and binding affinity to FLAP. They also block the production of PGE_2 in cell based assays but with a decreased potency and more limited selectivity compared to the enzyme assays.

An inducible form of prostaglandin E_2 (PGE_2) synthase (mPGES-1) has been identified as a member of the family of membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG), which includes certain glutathione transferases and the 5-lipoxygenase activating protein (FLAP).[1,2] Since its discovery in 1999, mPGES-1 has been shown to be inducible in various models of pain and inflammation, where it appears to be the predominant synthase involved in COX-2 mediated PGE_2 production,[3] both at peripheral inflamed sites[4] and in the CNS.[5,6] Furthermore, mice deficient in mPGES-1 show both a reduction in the production of inflammatory PGE_2[7] and a decrease in inflammatory responses in the collagen-induced arthritis model.[8] Thus, mPGES-1 represents a potential target for the development of novel analgesic and anti-inflammatory agents. Herein, we report the identification and in vitro profiles of the first potent and selective inhibitors of mPGES-1.

Only a few compounds have been reported to act as inhibitors of mPGES-1 activity and none of them are selective mPGES-1 inhibitors. Stable analogs of the PGH_2 substrate[9] and the COX-2 inhibitor NS-398[10] inhibit mPGES-1 with a relatively low potency (IC_{50} = 10–20 μM) while several polyunsaturated fatty acids and 15-deoxy-PGJ_2 are more potent inhibitors (IC_{50} = 0.3 μM).[9] Since mPGES-1 is a member of the MAPEG family which includes FLAP, the inhibitory potency of several FLAP inhibitors was analyzed. The potent FLAP inhibitor MK-886 (**1**) (FLAP IC_{50} = 26 nM (n = 2)) functions as a modest inhibitor of human mPGES-1[11] with an IC_{50} of 1.6 μM using our assay conditions (Table 1, entry 1). Using MK-886 as a lead structure,[12] structure–activity relationship (SAR) studies were then undertaken with the aim of identifying more potent and selective inhibitors of mPGES-1 (Tables 1–3).

Replacement of the *N*-*p*-chlorobenzyl substituent with sterically smaller (R^1 = H, Me, allyl, Table 1, entries 2–4) or larger ($R^1 = (CH_2)_3Ph$, Table 1, entry 5) substituents resulted in a 2- to 7-fold loss in potency. There did not appear to be a significant effect on potency by making simple changes to the *p*-chloro substituent (data not shown) so the remaining SAR was conducted with the *N*-*p*-chlorobenzyl group. The importance of the carboxylic acid function on the side chain at C2 was exemplified by the dramatic loss in potency when it was modified, either by esterification ($R^2 = CO_2Me$, Table 1, entry 6) or by replacement with a primary amide ($R^2 = CO_2NH_2$, Table 1, entry 7). The C3 position of the indole was much more tolerant of modification.

Keywords: Prostaglandin synthase; FLAP inhibitors; MK-886; Prostaglandin E2; Inflammation.
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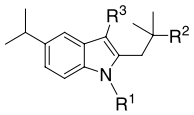
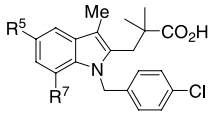
Table 1. Structural modification of MK-886



Entry	Compound	R^1	R^2	R^3	mPGES-1 Inhibition IC_{50}, μM[a]
1	**1** (MK-886)	CH_2(4-Cl–Ph)	CO_2H	S^{t}Bu	1.6 (515)
2	**2**	H	CO_2H	S^{t}Bu	>10 (1)
3	**3**	Me	CO_2H	S^{t}Bu	>10 (3)
4	**4**	CH_2(CH=CH_2)	CO_2H	S^{t}Bu	6.7 (5)
5	**5**	$(CH_2)_3$Ph	CO_2H	S^{t}Bu	3.2 (2)
6	**6**	CH_2(4-Cl–Ph)	CO_2Me	S^{t}Bu	7.2 (3)
7	**7**	CH_2(4-Cl–Ph)	CO_2NH_2	S^{t}Bu	>10 (1)
8	**8**	CH_2(4-Cl–Ph)	CO_2H	Ph	6.4 (3)
9	**9**	CH_2(4-Cl–Ph)	CO_2H	OPh	0.65 (2)
10	**10**	CH_2(4-Cl–Ph)	CO_2H	CH_2(4-^{t}Bu–Ph)	0.29 (3)
11	**11**	CH_2(4-Cl–Ph)	CO_2H	CO(2-Me–Ph)	0.90 (3)
12	**12**	CH_2(4-Cl–Ph)	CO_2H	$COCH_2S^tBu$	0.26 (2)
13	**13**	CH_2(4-Cl–Ph)	CO_2H	$COCH_2{}^tBu$	0.25 (2)
14	**14**	CH_2(4-Cl–Ph)	CO_2H	Me	1.1 (3)

[a] Mean of two or more experiments, *n* values given in parentheses.

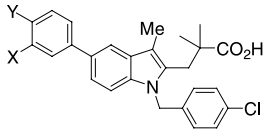
Table 2. SAR at C5 and C7



Entry	Compound	R^5	R^7	mPGES-1 Inhibition IC_{50}, μM[a]
1	**14**	^{i}Pr	H	1.1 (3)
2	**15**	H	^{i}Pr	4.3 (2)
3	**16**	H	H	3.2 (2)
4	**17**	F	H	2.6 (3)
5	**18**	^{t}Bu	H	0.33 (2)
6	**19**	Ph	H	0.60 (5)

[a] Mean of two or more experiments, *n* values given in parentheses.

Table 3. SAR of 5-phenyl indoles



Entry	Compound	X	Y	mPGES-1 Inhibition IC_{50}, μM[a]
1	**19**	H	H	0.60 (5)
2	**20**	Ph	H	0.16 (3)
3	**21**	H	Ph	0.016 (6)
4	**22**	Cl	Ph	0.022 (2)
5	**23**	F	Ph	0.007 (9)
6	**24**	F	pyrz[b]	0.032 (2)
7	**25**	F	pyr[b]	0.012 (2)
8	**26**	F	2-MeO–Ph	0.005 (2)
9	**27**	F	2-Cl–Ph	0.004 (2)
10	**28**	F	2-F–Ph	0.008 (2)
11	**29**	F	2-MeCO–Ph	0.006 (2)
12	**30**	F	2-Me–Ph	0.003 (8)
13	**31**	F	3-Me–Ph	0.033 (2)
14	**32**	F	4-Me–Ph	0.031 (2)

[a] Mean of two or more experiments, *n* values given in parentheses.
[b] pyrz = 5-(1,3-pyrazinyl); pyr = 3-pyridinyl.

While direct attachment of a phenyl moiety (R^3 = Ph, Table 1, entry 8) resulted in a 4-fold loss in potency, insertion of an oxygen atom (R^3 = OPh, Table 1, entry 9) or CH_2 moiety (R^3 = CH_2(4-^{t}BuPh), Table 1, entry 10) between the indole core and the aromatic group gave more potent inhibitors. A variety of C3 acyl substituents were also tolerated (Table 1, entries 11–13), giving inhibitors 2- to 6-fold more potent than MK-886. Although not optimum in terms of increasing potency relative to the acyl substituents, the simple introduction of a methyl group at C3 (Table 1, entry 14) provided **14**, a 1 μM inhibitor of mPGES-1. This C3 substituent was used for further SAR studies (Table 2) since it was found that modification at C5 had the greatest impact on potency.

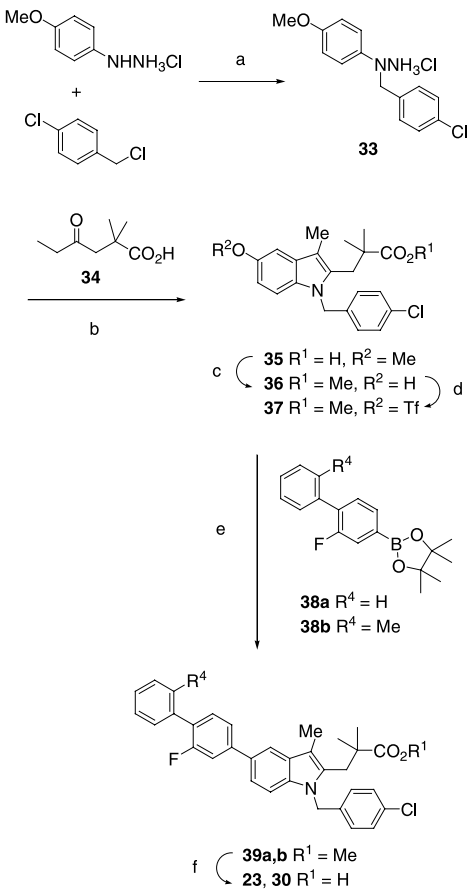
The relocation of the isopropyl group from C5 to C7 (Table 2, entry 2) or its removal (Table 2, entry 3) resulted in a 3- to 4-fold loss in potency compared to **14**. While introduction of a fluorine atom at C5 (Table 2, entry 4) was not advantageous, sterically larger substituents (Table 2, entries 5 and 6) led to a moderate potency enhancement.

One of the most interesting avenues of SAR that was pursued, and that lead to a dramatic increase in inhibitor potency, derived from functionalization of the C5 phenyl moiety of inhibitor **19** (Table 3). In particular, the biphenyls **20** (Table 3, entry 2) and **21** (Table 3, entry 3) exhibited potency enhancements of approximately 4- and 40-fold, respectively, compared to **19**. Extensive SAR around the biphenyl moiety of **21** provided a number of potent inhibitors. The key modification appeared to involve appropriate ortho substitution to the biphenyl bond. For example, introduction of a fluorine atom at this position provided **23** (Table 3, entry 5), a 7 nM inhibitor of mPGES-1.

Although the terminal phenyl group could be replaced with heterocycles (Table 3, entries 6 and 7) with only

moderate loss in potency, additional ortho substitution to the biphenyl bond on the terminal phenyl group yielded the best inhibitors in this series (Table 3, entries 8–12). The effect of this double ortho substitution is exemplified in the last three entries in Table 3. While the ortho methyl group in **30** (Table 3, entry 12) provided the most potent inhibitor in the series, 3 nM, the corresponding meta and para substituted analogs (Table 3, entries 13 and 14) were 10-fold less potent. Thus, the biaryl indole **30** is the optimized compound in this series in terms of in vitro potency and is a 500-fold more potent inhibitor of mPGES-1 than MK-886.

The syntheses of **23** and **30** (Scheme 1) exemplify the general route that was used to prepare the biaryl indoles described in Table 3. The methoxy indole **35** was prepared in 54% by the Fisher indole coupling of *N*-4-chlorobenzyl-*N*-4-methoxyphenyl hydrazine **33** and 2,2-dimethyl-4-oxohexanoic acid **34**.[12] Demethylation, followed by triflation converted **35** into the triflate **37**. The biaryl boronates **38a**,**b** were prepared by sequential palladium-catalyzed coupling reactions of commercially available 4-bromo-2-fluoro-1-iodobenzene with phenylboronic acid or 2-tolylboronic acid and then bis(pinacolato)diboron.[13] Suzuki–Miyaura palladium-catalyzed cross-coupling[14] of **37** and **38a**,**b** provided the indoles **39a**,**b**. Saponification yielded the desired indole inhibitors **23** and **30**.



Scheme 1. Reagents and conditions: (a) Et_3N, PhMe, reflux, 15 h; HCl (52%); (b) ^tBuOH, reflux, 15 h (54%); (c) BBr_3, CH_2Cl_2, −60 to 0 °C; MeOH (100%); (d) Tf_2O, Et_3N, CH_2Cl_2, 0 °C (81%); (e) for **38a**: $PdCl_2$(dppf) (cat.), aq Na_2CO_3, DMF, 80 °C, 2 h (40%); for **38b**: $PdOAc_2$ (cat.), PPh_3 (cat.), aq Na_2CO_3, DME, reflux, 2 h (99%); (f) LiOH, THF, MeOH, 60 °C, 4 h (82–97%).

The inhibitors **23** and **30** were further characterized in various enzyme assays (Table 4) and were found to be at least 100-fold less potent inhibitors of recombinant human mPGES-2[15] ($IC_{50} > 1$ μM) and TXA_2 synthase (IC_{50}s = 0.95–1.4 μM). Interestingly, both inhibitors showed a similar high degree of selectivity when tested in the FLAP binding assay ($IC_{50} > 1$ μM), being approximately 40-fold less potent than the original lead MK-886 ($IC_{50} = 26$ nM) in the same assay. The inhibitors were also tested for their effects on prostanoid formation in human A549 epithelial lung carcinoma cells and primary synoviocytes from rheumatoid arthritis patients, cells which have been reported to express mPGES-1.[10,16,17] When incubated with the cells at low serum concentration (2% FBS), **23** and **30** inhibited the production of PGE_2 by IL-1β-stimulated human synoviocytes with IC_{50}s of 0.61 and 0.25 μM, respectively. Similar data were obtained for the inhibition of PGE_2 using IL-1β-stimulated A549 cells (IC_{50}s of 0.3–0.5 μM) (Table 4). In A549 cells, **23** and **30** were 4- to 8-fold less potent at inhibiting the production of $PGF_{2\alpha}$ (IC_{50}s of 2.0 and 2.4 μM, respectively) compared to PGE_2. At intermediate concentrations, a stimulation of $PGF_{2\alpha}$ production was observed, which could be due to shunting of the PGH_2 substrate from the PGE_2 to the $PGF_{2\alpha}$ biosynthetic pathway.

As can be seen by the data summarized in Table 4, this class of inhibitor is also characterized by a shift in potency from enzyme assays at low protein concentrations to whole cell assays in the presence of plasma proteins, presumably due to a high degree of protein binding. For example, the potency of **30** is shifted from an IC_{50} of 3 nM in the enzyme assay to values of 0.27 and 5.8 μM in the A549 assay in the presence of 2% and 50% FBS, respectively. In addition, no inhibitory effects on PGE_2 could be detected in LPS-stimulated human whole blood. Although **23** and **30** are potent and highly selective inhibitors of mPGES-1 activity in enzyme assays, the large shifts in potency caused by the presence of plasma protein, and their limited selectivity in cell-based assays, preclude the use of these compounds for studying the in vivo effect of selective mPGES-1 inhibition in models of inflammation.

The results of the present study demonstrate that the structure of MK-866 can be modified to yield potent in vitro inhibitors of mPGES-1, with selectivity >100-fold compared to FLAP binding affinity and inhibition of both mPGES-2 and thromboxane synthase. However, to provide information regarding the effect of selective mPGES-1 inhibition in vivo, additional studies are required to identify those analogs with improved potency and selectivity in cell-based assays in the presence of plasma protein.

Table 4. Summary of the potency and selectivity of inhibitors **23** and **30** in various in vitro assays

Enzyme or cell assay	Inhibition potency IC_{50}, μM[a]	
	23	**30**
Human mPGES-1	0.007 (9)	0.003 (8)
Human mPGES-2	>1 (3)	>1 (3)
Thromboxane synthase	1.4 (3)	0.95 (3)
FLAP binding	>1 (3)	>1 (3)
Human synoviocytes (PGE_2) (2% FBS)	0.61 (4)	0.25 (3)
A549 cells (PGE_2) (2% FBS)	0.49 (49)	0.27 (3)
A549 cells (PGE_2) (50% FBS)	8.0 (49)	5.8 (3)
A549 cells ($PGF_{2\alpha}$) (2% FBS)	2.0 (49)	2.4 (3)

[a] Mean of three or more experiments, *n* values given in parentheses.

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References and notes

1. Jakobsson, P. J.; Thoren, S.; Morgenstern, R.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 7220.
2. Murakami, M.; Kudo, I. *Prog. Lipid Res.* **2004**, *43*, 3.
3. 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.
4. Claveau, D.; Sirinyan, M.; Guay, J.; Gordon, R.; Chan, C. C.; Bureau, Y.; Riendeau, D.; Mancini, J. A. *J. Immunol.* **2003**, *170*, 4738.
5. Inoue, W.; Matsumura, K.; Yamagata, K.; Takemiya, T.; Shiraki, T.; Kobayashi, S. *Neurosci. Res.* **2002**, *44*, 51.
6. Engblom, D.; Ek, M.; Andersson, I. M.; Saha, S.; Dahlstrom, M.; Jakobsson, P. J.; Ericsson-Dahlstrand, A.; Blomqvist, A. *J. Comp. Neurol.* **2002**, *452*, 205.
7. Uematsu, S.; Matsumoto, M.; Takeda, K.; Akira, S. *J. Immunol.* **2002**, *168*, 5811.
8. Trebino, C. E.; Stock, J. L.; Gibbons, C. P.; Naiman, B. M.; Wachtmann, T. S.; Umland, J. P.; Pandher, K.; Lapointe, J. M.; Saha, S.; Roach, M. L.; Carter, D.; Thomas, N. A.; Durtschi, B. A.; McNeish, J. D.; Hambor, J. E.; Jakobsson, P. J.; Carty, T. J.; Perez, J. R.; Audoly, L. P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9044.
9. Quraishi, O.; Mancini, J. A.; Riendeau, D. *Biochem. Pharmacol.* **2002**, *63*, 1183.
10. Thoren, S.; Jakobsson, P. J. *Eur. J. Biochem.* **2000**, *267*, 6428. A549 cells were seeded in 96-well dishes at a density of 25,000 cells/well and incubated for 20 h in RPMI 1640 medium supplemented with 2% fetal bovine serum (FBS). The cells were then incubated with 10 ng/mL IL-1β for 24 h in the presence of a vehicle or inhibitor from a 100-fold concentrated DMSO stock solution in a medium containing 2% or 50% FBS. Levels of PGE_2 and $PGF_{2\alpha}$ in the cell-free medium were measured by EIA (Assay Designs, MI). IC_{50} values were derived from eight point titrations.
11. Mancini, J. A.; Blood, K.; Guay, J.; Gordon, R.; Claveau, D.; Chan, C. C.; Riendeau, D. *J. Biol. Chem.* **2001**, *276*, 4469. The activity of mPGES-1 was tested as described in this reference using 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. The reaction was terminated with 0.1 N HCl and stannous chloride. PGE_2 accumulation in the media was measured by EIA (Assay Designs, MI). Inhibitors were pre-incubated with the recombinant enzyme for 15 min before initiating the reaction. IC_{50} values were derived from eight point titrations.
12. (a) Gillard, J. W.; Morton, H. E.; Fortin, R.; Guindon, Y. U.S. Patent 5,081,138, 1992; (b) Hutchinson, J. H.; Riendeau, D.; Brideau, C.; Chan, C.; Falgueyret, J.-P.; Guay, J.; Jones, T. R.; Lépine, C.; Macdonald, D.; McFarlane, C. S.; Piechuta, H.; Scheigetz, J.; Tagari, P.; Thérien, M.; Girard, Y. *J. Med. Chem.* **1994**, *37*, 1153.
13. Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508.
14. (a) Suzuki, A. *J. Organomet. Chem.* **2002**, *653*, 83; (b) Miyaura, N. *Top. Curr. Chem.* **2002**, *219*, 5.
15. Human mPGES-2 (AA88-377) was expressed in bacteria and activity was assayed as described in Tanikawa, N.; Ohmiya, Y.; Ohkubo, H.; Hashimoto, K.; Kangawa, K.; Kojima, M.; Ito, S.; Watanabe, K. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 884.
16. Stichtenoth, D. O.; Thoren, S.; Bian, H.; Peters-Golden, M.; Jakobsson, P. J.; Crofford, L. J. *J. Immunol.* **2001**, *167*, 469.
17. Kojima, F.; Naraba, H.; Sasaki, Y.; Okamoto, R.; Koshino, T.; Kawai, S. *J. Rheumatol.* **2002**, *29*, 1836.