Bioorganic & Medicinal Chemistry Letters 20 (2010) 3760–3763

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of 4-{1-[({1-[4-(trifluoromethyl)benzyl]-1*H*-indol-7-yl}carbonyl)amino]cyclopropyl}benzoic acid (MF-766), a highly potent and selective EP_4 antagonist for treating inflammatory pain

John Colucci [a], Michael Boyd [a], Carl Berthelette [a], Jean-Francois Chiasson [a], Zhaoyin Wang [a], Yves Ducharme [a], Rick Friesen [a], Mark Wrona [b], Jean-Francois Levesque [b], Danielle Denis [c], Marie-Claude Mathieu [c], Rino Stocco [c], Alex G. Therien [c], Patsy Clarke [d], Steve Rowland [d], Daigen Xu [d], Yongxin Han [a,*]

[a] *Department of Medicinal Chemistry, Merck Frosst Canada Ltd, 16711 Trans-Canada Highway, Kirkland, Quebec, Canada H9H 3L1*
[b] *DMPK, Merck Frosst Canada Ltd, 16711 Trans-Canada Highway, Kirkland, Quebec, Canada H9H 3L1*
[c] *In Vitro Sciences, Merck Frosst Canada Ltd, 16711 Trans-Canada Highway, Kirkland, Quebec, Canada H9H 3L1*
[d] *In Vivo Sciences, Merck Frosst Canada Ltd, 16711 Trans-Canada Highway, Kirkland, Quebec, Canada H9H 3L1*

ARTICLE INFO

Article history:
Received 19 January 2010
Revised 14 April 2010
Accepted 16 April 2010
Available online 18 April 2010

Keywords:
EP4 antagonist
Inflammatory pain

ABSTRACT

The discovery of a highly potent and selective EP_4 antagonist **MF-766** is discussed. This *N*-benzyl indole derivative exhibits good pharmacokinetic profile and unprecedented in vivo potency in the rat AIA model.

Arthritis (OA and RA) is a chronic inflammatory condition leading to bone and cartilage destruction. Accumulated evidence in the past decade suggests that PGE_2 plays an important role in the pathogenesis of this disease such that inhibition of PGE_2 production by NSAIDs and more recently COX-2 inhibitors relieves arthritis symptoms.[1] PGE_2 is the ligand of four subtype EP receptors, EP_{1-4}. In a mouse model of collagen-antibody induced arthritis (CAIA), the $EP_4^{-/-}$ mice showed a remarkable resistance in both the incidences and symptom scores (paw swelling/redness, ankylosis) compared to the wild type control, while the $EP_{1-3}^{-/-}$ mice had no effect,[2] suggesting that the effect of PGE_2 in inflammation was mediated predominantly by the EP_4 receptor. Lin et al. demonstrated that EP_4, not EP_{1-3}, contributed to inflammatory pain hypersensitivity in rats.[3] Using highly selective EP_1, EP_3 and EP_4 antagonists, we recently demonstrated that EP_4, not EP_1 or EP_3, was the primary receptor involved in joint inflammation and pain in rodent models of rheumatoid and osteoarthritis,[4] further supporting EP_4 antagonism as a valid strategy for treating inflammatory pain. In addition, it is plausible that a highly selective EP_4 antagonist can be a safer alternative in relieving arthritis symptoms without causing potential cardiovascular side effects observed with NSAIDs and COX-2 inhibitors[5] since it does not interfere with the biosynthesis of important prostanoids such as prostacyclin (PGI_2) and thromboxane A_2 (TxA_2).[6]

We recently reported the discovery of our first generation acylsulfonamide-based EP_4 antagonists **MF-498**[4] and **MF-310**.[7] These compounds, unfortunately, suffered from species dependent CYP 3A mediated hydrolysis of the acylsulfonamide moieties, resulting in highly variable pharmacokinetic profile in preclinical species. The corresponding sulfonamide from **MF-310** also had the potential to accumulate in vivo due to its low clearance rate and excessively long elimination half-life.[8] SAR effort to prevent the acylsulfonamide hydrolysis was unsuccessful, so we turned our attention to non-acylsulfonamide analogs. This effort resulted in the discovery of a highly potent and selective second generation EP_4 antagonist **MF-766** (Fig. 1). We herein describe the SAR leading to **MF-766**, its in vitro potency and selectivity, its pharmacokinetic properties and its in vivo potency profile in the rat adjuvant-induced-arthritis (AIA) model.

Non-acylsulfonamide EP_4 antagonists such as **CJ-042,794** (Fig. 1) were reported recently.[9] Our efforts focused on compounds bearing suitable heterocyclic templates in place of the central phenyl core. By screening a number of monocyclic or bicyclic heterocycles, we discovered that the indole core in **1** (Fig. 1) was an excellent template, yielding highly potent and selective

* Corresponding author. Tel.: +1 514 428 3301; fax: +1 514 428 4900.
E-mail address: yongxin_han@merck.com (Y. Han).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.04.065

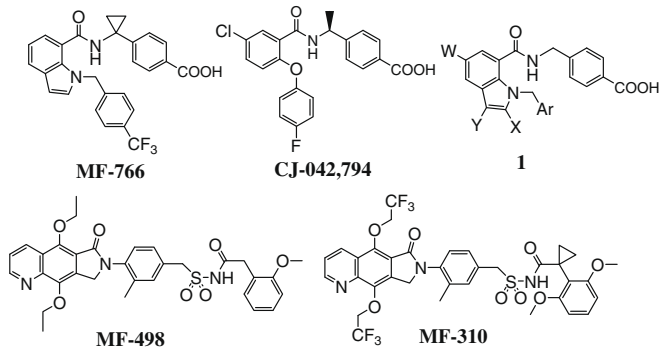
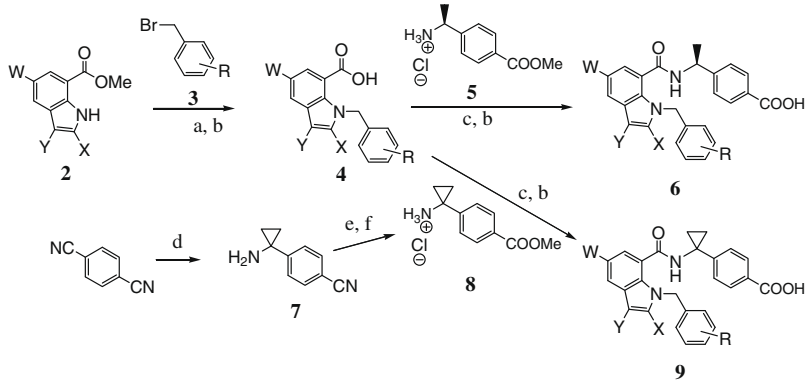


Figure 1. Structures **MF-766**, **CJ-042,794**, **MF-498**, **MF-310** and a suitable indole template **1**.

antagonists such as **MF-766** with vastly improved overall profile compared to previously reported compounds.

Synthesis of these indole analogs was easily accomplished as shown in Scheme 1. Alkylation of commercially available methyl 1*H*-indole-7-carboxylates (**2**) with a variety of benzyl bromides **3** followed by hydrolysis gave the alkylated indole acids **4**. Standard amide coupling with commercially available (*S*)-methyl 4-(1-aminoethyl)benzoate (**5**) or cyclopropyl analog **8** in the presence of HATU and Hunig's base, followed by hydrolysis of the resultant methyl esters, gave the final desired compounds **6** and **9** in excellent overall yield (>35% in four steps). The cyclopropylbenzamine derivatives **8** was prepared by first submitting 1,4-dicyanobenzene to the Szymoniak variation[10] of the Kulinkovich reaction, in which one of the cyano groups was modified to afford the cyclopropylamine derivative **7** (Scheme 1). The other cyano group was then hydrolyzed in boiling 6 N hydrochloric acid and the resultant carboxylic acid was esterified in MeOH in presence of catalytic amount of concentrated HCl to give the methyl ester **8**. The potency and selectivity data of these compounds are summarized in Table 1.

As shown in Table 1, the *m*-Cl-benzyl analog **6a** exhibited good EP_4 affinity (K_i = 2.7 nM) and functional activity (IC_{50} = 5.9 nM), but was poorly selective against the DP_1 receptor with a K_i of 16 nM. Introduction of a methyl group at the 2-position (**6b**) was detrimental to the EP_4 binding affinity (K_i = 56 nM). Incorporation of substituents such as Cl (**6c**, K_i = 20 nM) and Me (**6d**, K_i = 39.5 nM) at the 3-position was also detrimental to EP_4 binding affinity. Introduction of a larger group such as COMe at this position resulted in an inactive compound (not shown). The 5-position of the indole core, on the other hand, tolerated small groups such as Cl (**6e**, K_i = 0.81 nM, IC_{50} = 1.5 nM) and F (**6f**, K_i = 2.4 nM, IC_{50} = 3.3 nM). These two compounds also displayed marginal DP_1 selectivity but lose selectivity against the EP_2 receptor (EP_2 K_i = 110 and 504 nM, respectively). Benzyl groups bearing small lipophilic moieties such as Cl or CF_3 are preferred. The *m*-CF_3



Scheme 1. Reagents and conditions: (a) NaH, DMF; (b) LiOH, MeOH; (c) HATU, Hunig's base, DMF; (d) $Ti(O^iPr)_4$, EtMgBr, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt; (e) 6 N HCl, reflux; (f) MeOH, cat. concd HCl, reflux.

Table 1
Binding affinity, selectivity and functional potency of compounds **6** and **9**

Compd	X, Y, W	R	Binding affinity[a] (nM)[b]			EP_4 Functional IC_{50}[d] (nM)
			EP_4	DP_1	Others[c]	
6a	H, H, H	*m*-Cl	2.7	16	⩾2500	5.90
6b	Me, H, H	*m*-Cl	56.0	450	nd[e]	nd
6c	H, Cl, H	*m*-Cl	20.1	14	⩾930	nd
6d	H, Me, H	*m*-Cl	39.5	1205	nd	nd
6e	H, H, Cl	*m*-Cl	0.81	63	⩾110	1.50
6f	H, H, F	*m*-Cl	2.40	404	⩾504	3.30
6g	H, H, H	*m*-CF_3	4.80	885	⩾785	11.8
6h	H, H, H	3-Cl-5-OCF_3	0.38	49	⩾260	1.40
6i	H, H, H	*p*-CF_3	0.62	1389	⩾580	2.70
6j	H, H, Cl	*p*-CF_3	0.44	209	⩾100	1.12
9a (MF-766)	H, H, H	*p*-CF_3	0.23	1648	>6000	1.30
9b	H, H, Cl	*p*-CF_3	0.31	561	⩾1291	1.26

[a] For details on the binding assay for all PG receptors, see Ref. 4 and references cited therein.
[b] Values are means from at least two independent experiments.
[c] Other PG receptors refers to EP_1, EP_2, EP_3, DP_2, IP, TP and FP.
[d] The EP_4 functional assay measured the inhibition of PGE_2-induced cAMP accumulation in HEK 293 cells, for details, see Ref. 4.
[e] nd: not done.

analog (**6g**, K_i = 4.8 nM, IC_{50} = 11.8 nM), for example, is only slightly less potent than the *m*-Cl derivative **6a** but is substantially more selective against the DP_1 receptor (K_i = 885 nM). The 3-Cl-5-OCF_3 analog **6h** shows excellent EP_4 affinity (K_i = 0.38 nM) and functional potency (IC_{50} = 1.4 nM) but is again less selective against DP_1 (K_i = 49 nM). Moving the CF_3 group from the *meta*-position in **6g** to the *para*-position (**6i**) resulted in a significant improvement in EP_4 binding affinity (K_i = 0.62 nM), functional potency (IC_{50} = 2.7 nM) and selectivity against DP_1 (K_i = 1389 nM), suggesting that the *para*-substitution was superior for both affinity and selectivity. The corresponding 5-Cl analog **6j** had similar affinity (K_i = 0.44 nM) and functional potency (IC_{50} = 1.12 nM) but was again significantly less selective against DP_1 (K_i = 209 nM). Finally, replacing the chiral (*S*)-Methyl 4-(1-aminoethyl)benzoate in **6i** with the achiral 4-(1-aminocyclopropyl)benzoate group gave the title compound **MF-766** (**9a**) with further improved affinity, functional potency and selectivity. The corresponding 5-Cl analog **9b** had similar affinity and functional potency but was less selective. The full in vitro profile of **MF-766** is summarized in Table 2.

As shown, **MF-766** exhibited excellent affinity at the EP_4 receptor (K_i = 0.23 nM), was not significantly shifted in presence of 10% human serum (HS) (K_i = 0.34 nM) and had excellent selectivity against other prostanoid receptors (>7000-fold). It behaved as a full antagonist with an IC_{50} of 1.4 nM (shifted to 1.8 nM in the presence of 10% HS) in the functional assay. In the human whole blood (HWB) assay[9c,11] measuring the blockade of inhibition of TNF_α-induced IP-10 release by the specific EP_4 agonist L-000902688[12], **MF-766** showed excellent activity with an IC_{50} of 9.5 nM. In addition, it was evaluated in the MDS Pharma screen against >170 enzymes and receptors, and was found to have no appreciable activity at concentrations up to 10 μM.

The in vitro metabolic stability of **MF-766** was evaluated from incubations in human, dog and rat hepatocytes. The major metabolite observed in all three species was the glucuronide conjugate of the carboxylic acid. A minor taurine conjugate and several minor oxidative metabolites were also observed in these species. **MF-766** was most stable in human, followed by rat and dog hepatocytes. The acyl glucuronide conjugate was also found to be the only circulating metabolite in rat and dog plasma samples. Additional in vitro incubations were performed in presence of fresh rat and human hepatocytes using [^{3}H]-**MF-766** to assess potential protein labeling due to acyl glucuronide migration or oxidative metabolism, and acceptable levels of covalent binding were obtained (rat: 61 pmol-equiv/mg @ 2 h; human: 50 pmol-equiv/mg @ 2 h). No tritium loss was observed upon HPLC-radiometric detection analysis of the in vitro samples.

The pharmacokinetic profile of **MF-766** was evaluated in SD rats and beagle dogs. The results are summarized in Table 3. As shown, **MF-766** exhibited desirable pharmacokinetic properties with high oral bioavailability (F = 74–86%), low to moderate clearance rate (CL = 3.7–6.3 mL/min/kg) and good elimination half-life ($t_{1/2}$ = 2.6–4.6 h), all of which contributed to the observed high systemic exposures.

The excellent in vitro potency and PK profile of **MF-766** translated into impressive in vivo potency and efficacy. When tested in the rat AIA model,[4,13,14] this compound was found to have unprecedented potency, with an ED_{50} of approximately 0.004 mg/kg/day (Fig. 2). The minimum dose required for maximum inhibition

Table 2
Full in vitro affinity, selectivity and functional potency profile of **MF-766**

Binding affinity K_i (nM)[a]										Functional potency IC_{50} (nM)[b]		
EP_1	EP_2	EP_3	EP_4	EP_4 + 10%HS	DP_1	DP_2	FP	IP	TP	HEK-293 cell	HEK-293 cell +10% HS	HWB
20,000	8000	7400	0.23	0.34	1800	>21,000	6400	>22,000	6700	1.4	1.8	9.5

[a] Average of 3–11 independent experiments.
[b] Average of 4–5 independent experiments.

Table 3
Pharmacokinetic parameters of **MF-766**

Species	Dose iv/po[a] (mg/kg)	Cl_p (mL/min/kg)	Vd (L/kg)	$t_{1/2}$ (h)	F (%)	po AUC (μM∗h)$_{0-24}$
Rat	5/20	3.7	1.0	4.6	86	150
Dog	1/4	6.3	0.8	2.6	74	15

[a] iv: n = 2 (male), free acid in 60% PEG 200 at a dosing volume of 1 mL/kg; po: n = 4 in male rat, n = 2 in male dog, in 60% PEG 200 at a dosing volume of 5 mL/kg.

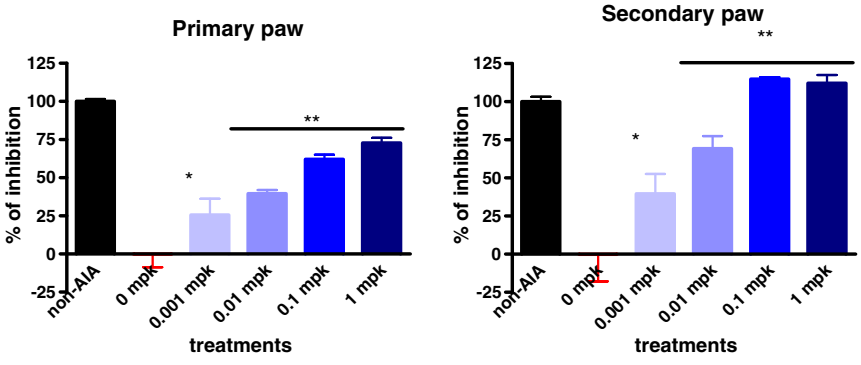


Figure 2. In vivo potency and efficacy of **MF-766** in the rat AIA model measuring inhibition of paw swelling at day 18, n = 3 for the non-AIA group and 6–7 for vehicle/drug treated groups. Arthritis was induced by sub-plantar injection of complete Freund's adjuvant in the primary paw at day 0, compound was dosed once daily in 0.5% methocel starting from day 9. * P <0.005 and ** P <0.001 versus vehicle by one way ANOVA/Dunnett's test.

(ED_{100}) of both the primary and the secondary paw swelling was established at ~0.025 mg/kg/day from a separate experiment, and the corresponding drug levels at 2 h and 24 h post the final dose were 28 and 3.1 nM, respectively. This potency was far superior to that reported for **CJ-042,749** (ED_{80} = 11.5 mg/kg).[9b] As a comparison, the ED_{50}s established for potent COX-2 inhibitors such as refocoxib[13] and MF-tricyclic[14] from separate experiments under similar paradigms were 0.7 and 0.5 mg/kg/day, respectively. Furthermore, we observed that the maximum efficacy of **MF-766** and other highly selective EP_4 antagonists in inhibiting swelling in the primary paw (70%) and secondary paw (100%) in this model were comparable to both COX-2 inhibitors and NSAIDs.

In conclusion, we have described the discovery and preclinical characterization of the highly potent and selective EP_4 antagonist **MF-766**. In the rat AIA model of chronic inflammation, **MF-766** exhibited comparable maximum efficacy to Coxibs and NSAIDs but was >200-fold more potent than potent COX-2 inhibitors like refocoxib and MF-tricyclic. Overall, **MF-766** displayed the desired potency, selectivity and pharmacokinetic profile for further development, and would represent a potential safer alternative for the treatment of pain and inflammation than traditional NSAIDs and Coxibs.

References and notes

1. Flower, R. J. *Nat. Rev. Drug Disc.* **2003**, *2*, 179.
2. McCoy, J. M.; Wicks, J. R.; Audoly, L. P. *J. Clin. Invest.* **2002**, *110*, 651.
3. Lin, C.-R.; Amaya, F.; Barrett, L.; Wang, H.; Takada, J.; Samad, T. A.; Woolf, C. J. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 1096.
4. Clark, P.; Rowland, S. E.; Denis, D.; Mathieu, M.-C.; Stocco, R.; Poirier, H.; Burch, J.; Han, Y.; Audoly, L.; Therien, A. G.; Xu, D. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 425.
5. (a) Solomon, S. D.; McMurray, J. J. V.; Pfeiffer, M. A.; Wittes, J.; Fowler, R.; Finn, P.; Anderson, W. F.; Zauber, A.; Hawk, E.; Bertagnolli, M. *N. Eng. J. Med.* **2005**, *352*, 1071; (b) Nussmeier, N. A.; Whelton, A. A.; Brown, M. T.; Langford, R. M.; Hoeft, A.; Parlow, J. L.; Boyce, S. W.; Verbug, K. M. *N. Eng. J. Med.* **2005**, *352*, 1081; (c) Bresalier, R. S.; Sandler, R. S.; Quan, H.; Bolognese, J. A.; Oxenius, B.; Horgan, K.; Lines, C.; Ridell, R.; Morton, D.; Lanas, A.; Konstam, M. A.; Baron, J. A. *N. Eng. J. Med.* **2005**, *352*, 1092.
6. (a) McAdam, B. F.; Catella-Lawson, F.; Mardini, I. A.; Kapoor, S.; Lawson, J. A.; FitzGerald, G. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 272; (b) FitzGerald, G. A.; Patrono, C. *N. Eng. J. Med.* **2001**, *345*, 433; (c) Funk, C. D.; FitzGerald, G. A. *J. Cardiovasc. Pharmacol.* **2007**, *50*, 470.
7. Burch, J. D.; Belley, M.; Fortin, R.; Deschênes, D.; Girard, M.; Colucci, J.; Farand, J.; Therien, A. G.; Mathieu, M.-C.; Denis, D.; Vigneault, E.; Lévesque, J.-F.; Gagné, S.; Wrona, M.; Xu, D.; Clark, P.; Rowland, S.; Han, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2048.
8. Unpublished results: the elimination half-life of the corresponding sulfonamide M1 from **MF-310** in rat and dog was estimated to be >24 h. When **MF-310** was dosed orally at 20 mg/kg/day for 5 days in rats, M1 levels at 24 h increased from 1.4 μM after the first dose to 4.6 μM after the last dose. When it was dosed in dogs at 4 mg/kg/day for two weeks, M1 levels at 24 h increased from 1.3 μM after the first dose to 13.7 μM after the last dose.
9. (a) Takeuchi, K.; Tanaka, A.; Kato, S.; Aihara, E.; Amagase, K. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 903; (b) Murase, A.; Okumura, T.; Sakakibara, A.; Tonai-Kachi, H.; Nakao, K.; Takada, J. *Eur. J. Pharmacol.* **2008**, *580*, 116; (c) Murase, A.; Taniguchi, Y.; Tonai-Kachi, H.; Nakao, K.; Takada, J. *Life Sci.* **2008**, *82*, 226.
10. Bertus, P.; Szymoniak, J. *J. Org. Chem.* **2003**, *68*, 7133.
11. Mathieu, M.-C.; Lord-Dufour, S.; Bernier, V.; Boie, Y.; Burch, J. D.; Clark, P.; Denis, D.; Han, Y.; Mortimer, J. R.; Therien, A. G. *Eur. J. Immunol.* **2008**, *38*, 1900.
12. Young, R. N.; Billot, X.; Han, Y.; Slipetz, D. A.; Chauret, N.; Belley, M.; Metters, K.; Mathieu, M. C.; Greig, G. M.; Denis, D.; Girard, M. *Heterocycles* **2004**, *64*, 437.
13. Chan, C.-C.; Boyce, S.; Brideau, C.; Charleson, S.; Cromlish, W.; Ethier, D.; Evans, J.; Ford-Hutchinson, A. W.; Forrest, M. J.; Gauthier, J. Y.; Gordon, R.; Gresser, M.; Guay, J.; Kargman, S.; Kennedy, B.; Leblanc, Y.; Léger, S.; Mancini, J.; O'Neill, G. P.; Ouellet, M.; Patrick, D.; Percival, M. D.; Perrier, H.; Prasit, P.; Rodger, I.; Tagari, P.; Therien, M.; Vickers, P.; Visco, D.; Wang, Z.; Webb, J.; Wong, E.; Xu, L.-J.; Young, R. N.; Zamboni, R.; Riendeau, D. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 551.
14. Rowland, S. E.; Clark, P.; Gordon, R.; Mullen, A. K.; Guay, J.; Dufresne, C.; Brideau, C.; Côté, B.; Ducharme, Y.; Mancini, J.; Chan, C.-C.; Audoly, L.; Xu, D. *Eur. J. Pharmacol.* **2007**, *560*, 216.