Bioorganic & Medicinal Chemistry Letters 20 (2010) 2639–2643

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of novel *N*-acylsulfonamide analogs as potent and selective EP3 receptor antagonists

Masaki Asada *, Tetsuo Obitsu, Atsushi Kinoshita, Yoshihiko Nakai, Toshihiko Nagase, Isamu Sugimoto, Motoyuki Tanaka, Hiroya Takizawa, Ken Yoshikawa, Kazutoyo Sato, Masami Narita, Shuichi Ohuchida, Hisao Nakai, Masaaki Toda

Minase Research Institute, Ono Pharmaceutical Co., Ltd, Shimamoto, Mishima, Osaka 618-8585, Japan

ARTICLE INFO

Article history:
Received 29 October 2009
Revised 8 February 2010
Accepted 9 February 2010
Available online 13 February 2010

Keywords:
Prostaglandin
EP3 receptor
Antagonist
N-Acylsulfonamide
N-Acyl (3,4-difluorobenzene)sulfonamide

ABSTRACT

A series of novel *N*-acylsulfonamide analogs were synthesized and evaluated for their binding affinity and antagonist activity for the EP3 receptor subtype. Representative compounds were also evaluated for their inhibitory effect on PGE_2-induced uterine contraction in pregnant rats. Among those tested, a series of *N*-acylbenzenesulfonamide analogs were found to be more potent than the corresponding carboxylic acid analogs in both the in vitro and in vivo evaluations. The structure activity relationships (SAR) are also discussed.

It is well known that prostaglandin E_2 (PGE_2) has diverse biological activities mediated by four receptor subtypes, namely EP1-4 receptors. Recent studies using knockout mice have suggested that the EP3 receptor is involved in a large number of pharmacological actions of PGE_2 including hyperalgesia,[1] pyrexia,[2] uterine contraction,[3] gastric acid secretion,[4] platelet aggregation,[5] and thrombosis.[6] Thus, identification of a potent and selective EP3 receptor antagonist may prove to be an attractive starting point from which to design clinically useful drugs. In one of our preceding papers,[7] we reported the discovery of the carboxylic acid analog **1** as a new chemical lead of a selective EP3 receptor antagonist.

An *N*-acylsulfonamide group, a functionality often found in various drugs, is a well known bioisostere of a carboxylic acid because of its similar acidity. A few EP3 receptor ligands possessing the *N*-acylsulfonamide group are illustrated in Figure 1. Sulprostone (**2**)[8] is a well-known EP3 (and EP1) receptor agonist possessing an *N*-acylmethanesulfonamide group. In addition, researchers at Merck have reported EP3 receptor antagonists with *N*-acylbenzenesulfonamide groups, exemplified by **3**.[9] The aforementioned information prompted us to replace the carboxylic acid of our initial lead **1** with various *N*-acylsulfonamide groups which were expected to be able to obtain more potent affinity for the EP3 receptor, as illustrated in Figure 2. Recently, researchers at de CODE have also

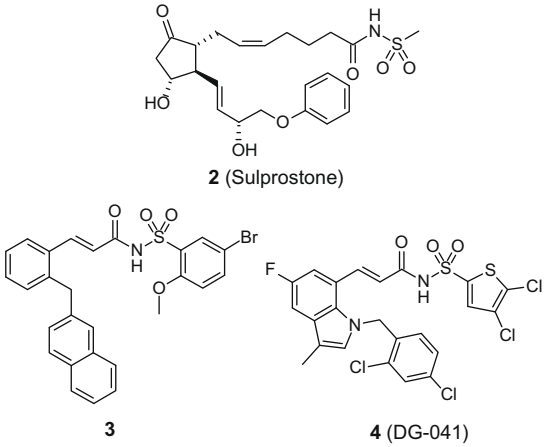


Figure 1. *N*-Acylsulfonamide containing EP3 receptor ligands.

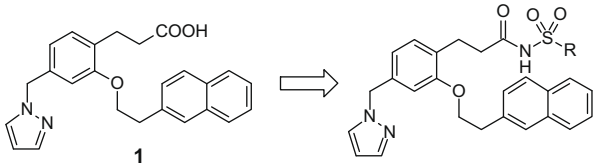


Figure 2. *N*-Acylsulfonamide analogs as a novel EP3 receptor antagonist.

* Corresponding author. Tel.: +81 75 961 1151; fax: +81 75 962 9314.
E-mail address: m.asada@ono.co.jp (M. Asada).

0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.02.034

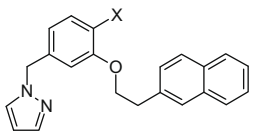
disclosed several series of novel EP3 receptor antagonists possessing *N*-acylsulfonamide groups.[10] Among them, DG-041 (**4**)[11] has been advanced into clinical trials as a therapeutic agent for peripheral occupied arterial disease (PAOD). In this paper, we report the identification of a series of novel *N*-acylsulfonamide analogs as highly potent and selective EP3 receptor antagonists.

As shown in Table 1, several *N*-acylsulfonamide analogs, which were designed based on **1**, were synthesized and evaluated for their in vitro activities. The binding affinity and the antagonist activity were evaluated according to the same protocol as described in our previous papers.[7,12] All antagonist activities were evaluated in the presence of 1% bovine serum albumin (BSA). Replacement of the carboxylic acid of **1** with an *N*-acylmethanesulfonamide and an *N*-acylbenzenesulfonamide afforded **5** and **6**, respectively. Compound **5** showed equipotent binding affinity relative to **1** with reduced antagonist activity, while compound **6** showed increased potency with regard to both the binding affinity and antagonist activity. The *N*-benzenesulfonyl moiety of **6** was found to be a more optimized sub-structure for binding to the EP3 receptor. However, compound **6** showed unexpectedly weak antagonist activity compared with its potent binding affinity relative to the carboxylic acid analog **1** because of its presumed increase in protein interaction.[13] Replacement of the methylene moiety adjacent to the carbonyl group of **6** with NH afforded **7** which exhibited 30-fold less potent binding affinity and 5.9-fold less potent antagonist activity relative to **6**. The reverse *N*-acylsulfonamide analog **8** showed 280-fold less potent binding affinity and less than 70-fold weaker antagonist activity relative to **6**.

Further effort to optimize the *N*-acylsulfonamide moiety was continued as shown in Table 2. Replacement of the *N*-benzenesulfonyl moiety of **6** with the *N*-heteroarylsulfonyl moiety afforded **9**–**12**. *N*-Pyridinesulfonyl analogs **9** and **10** exhibited nearly equipotent binding affinity and antagonist activity relative to **6**. *N*-(5-Methylfuran)-2-sulfonyl analog **11** and *N*-(4-methylthiazole)-2-sulfonyl analog **12** also showed retained binding affinity and antagonist activity. Effect of the substituent on the benzenesulfonamide moiety of **6** was investigated. As summarized in Table 2, *N*-(3,4-disubstituted benzene)sulfonyl analogs **13**–**16** had remarkable improvement in their antagonist activity relative to **6**. Introduction of one or two substituents into the benzene nucleus of the *N*-benzenesulfonyl moiety of **6** seemed to be effective for increasing the antagonist activity. Among them, *N*-(3,4-difluorobenzene)sulfonyl analog **16** was found to exhibit the most potent antagonist activity with an IC_{50} value of 1.2 nM. Compound **16** showed 110 and 480-fold more potent antagonist activity relative to non-substituted analog **6** and our lead compound **1**, respectively. *N*-(3-Cyanobenzene)sulfonyl analog **17** was also more potent relative to **6**. Although introduction of heteroaromatic rings is tolerated, the EP3 receptor prefers hydrophobic parts such as the *N*-benzenesulfonyl moiety.

Optimization of the pyrazol-1-ylmethyl side chain or the ether side chain of **16** and the corresponding in vivo evaluations were shown in Table 3. The in vivo activity was evaluated for their inhibitory effect on PGE_2-induced uterine contraction in pregnant rats, according to the protocol described in our previous paper.[12,14] All the test compounds **18**–**26** listed in Table 3 were found to exhibit excellent subtype selectivity for the EP3 receptor similar to the results for **16**. Compound **16** showed potent in vivo efficacy in a dose dependent manner, with 43% inhibition at 0.3 mg/kg id and 76% inhibition at 1 mg/kg id. Replacement of the 2-(2-naphthyl)ethyloxy moiety of **16** with a *N*-(1-naphthyl)methylcarboxyamide moiety afforded **18** with equipotent in vitro activity relative to **16**, while it showed relatively weak in vivo efficacy. Replacement of the naphthalene ring of **16** with a benzene ring afforded **19** with equipotent binding affinity and 49-fold less potent antagonist activity. Compound **19** showed dose-dependent in vivo efficacy, but its potency was approximately threefold less than **16**. In an effort to improve the potency of **19**, compounds **20**–**22** were synthe-

Table 1
Activity profiles of **1** and its *N*-acylsulfonamide analogs



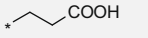
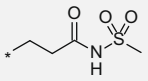
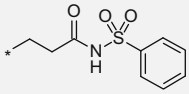
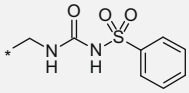
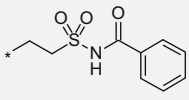
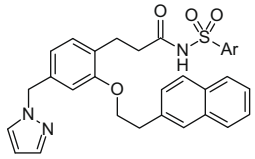
Compd	X	Binding K_i (nM)	Function IC_{50} (nM)
1		21	580
5		22	>10,000
6		0.50	140
7		15	820
8		140	>10,000

Table 2
Effects of the *N*-acylsulfonamide moiety on the binding affinity and the antagonist activity for the EP3 receptor



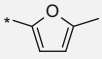
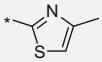
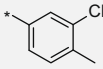
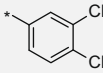
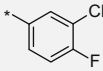
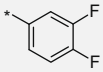
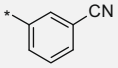
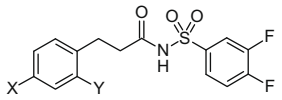
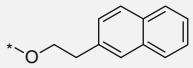
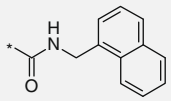
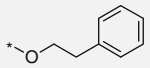
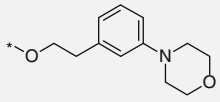
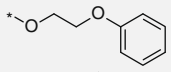
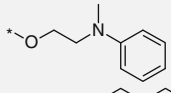
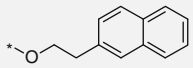
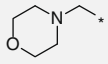
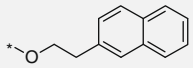
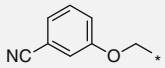
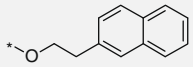
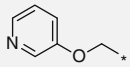
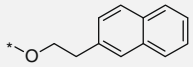
Compd	Ar	Binding K_i (nM)	Function IC_{50} (nM)
6		0.50	140
9		1.6	190
10		1.0	150
11		0.58	88
12		0.35	110
13		0.43	10
14		0.45	7.8
15		0.22	5.5
16		0.086	1.2
17		0.065	18

Table 3
Activity profiles of a series of *N*-acyl 3,4-difluorobenzenesulfonamide analogs

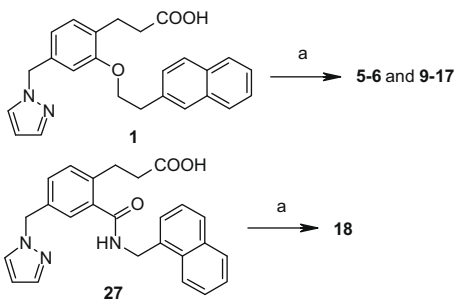


Compd	X	Y	Binding *Ki* (nM)				Function IC_{50} (nM)	In vivo (%inh.)[a]	
			EP1	EP2	EP3	EP4		0.3 mg/kg	1 mg/kg
16			2100	>10,000	0.086	2400	1.2	43	76
18			5300	>10,000	0.13	1800	1.4	NT[b]	30
19			1300	>10,000	0.20	2500	59	29	51
20			560	>10,000	0.54	3700	6.4	NT[b]	33
21			2800	>10,000	0.27	>10,000	51	NT[b]	28
22			260	>10,000	0.12	3500	3.8	NT[b]	50
23			>10,000	>10,000	8.2	>10,000	24	NT[b]	32
24			5000	>10,000	1.2	>10,000	47	NT[b]	35
25			>10,000	>10,000	0.51	2300	24	50	92
26			1700	>10,000	0.16	710	0.40	44	71

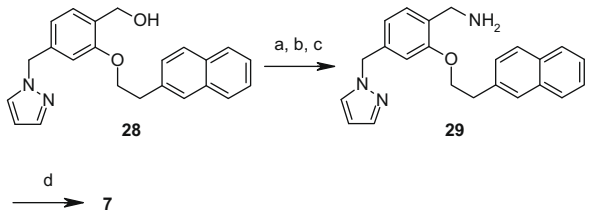
[a] All the values (%inh.) were determined as the mean values of twice of the experiments.
[B] NT: not tested.

sized and evaluated. Replacement of the phenylethyloxy moiety of **19** with the (3-morpholinophenyl)ethyloxy, phenoxyethyloxy or (*N*-methylanilino)ethyloxy moieties afforded **20**–**22**, respectively. Compound **20** showed slightly less potent binding affinity while having 9.2-fold more potent antagonist activity relative to **19**. Compound **21** was nearly equipotency in both of the in vitro evaluations. Compound **22** displayed equipotent binding affinity with an increase in antagonist activity. Regarding in vivo efficacy, **22** exhibited the same potency as **19** at a dose of 1 mg/kg id, while **20** and **21** showed less potency relative to **19**.

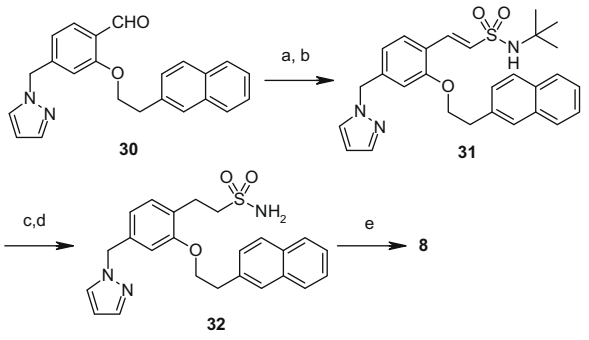
Further optimization was focused on the pyrazol-1-ylmethyl side chain of **16**. Replacement of the pyrazol-1-ylmethyl moiety of **16** with the more hydrophilic dimethylaminomethyl moiety and *N*-morpholinomethyl moiety provided **23** and **24**, respectively, both of which resulted in reduced in vitro and in vivo potencies relative to **16**. Introduction of more hydrophobic groups instead of the hydrophilic groups afforded **25** and **26**. Compound **25** possessing a 3-cyanophenoxymethyl moiety was found to show improved in vivo efficacy relative to **16** compared with its reduced in vitro activity. Compound **26** possessing a 3-pyridyloxymethyl moiety was a subnanomolar EP3 antagonist, with an IC_{50} value of 0.40 nM in the presence of 1% additive BSA and equipotent to **16** in term of in vivo efficacy. For comparison, evaluation of the well-known standard compound **4** (DG-041) was carried out using the same in vivo evaluation system as ours. The inhibitory effect of **4** and **25** for 4 h after their oral administration at 3 mg/kg exhibited



Scheme 1. Synthesis of **5**–**6** and **9**–**18**. Reagents: (a) RSO_2NH_2, EDC·HCl, DMAP, DMF.



Scheme 2. Synthesis of **7**. Reagents: (a) PBr_3, CH_2Cl_2; (b) NaN_3, DMF; (c) H_2, Pd/C, MeOH; (d) $PhSO_2NCO$, toluene.

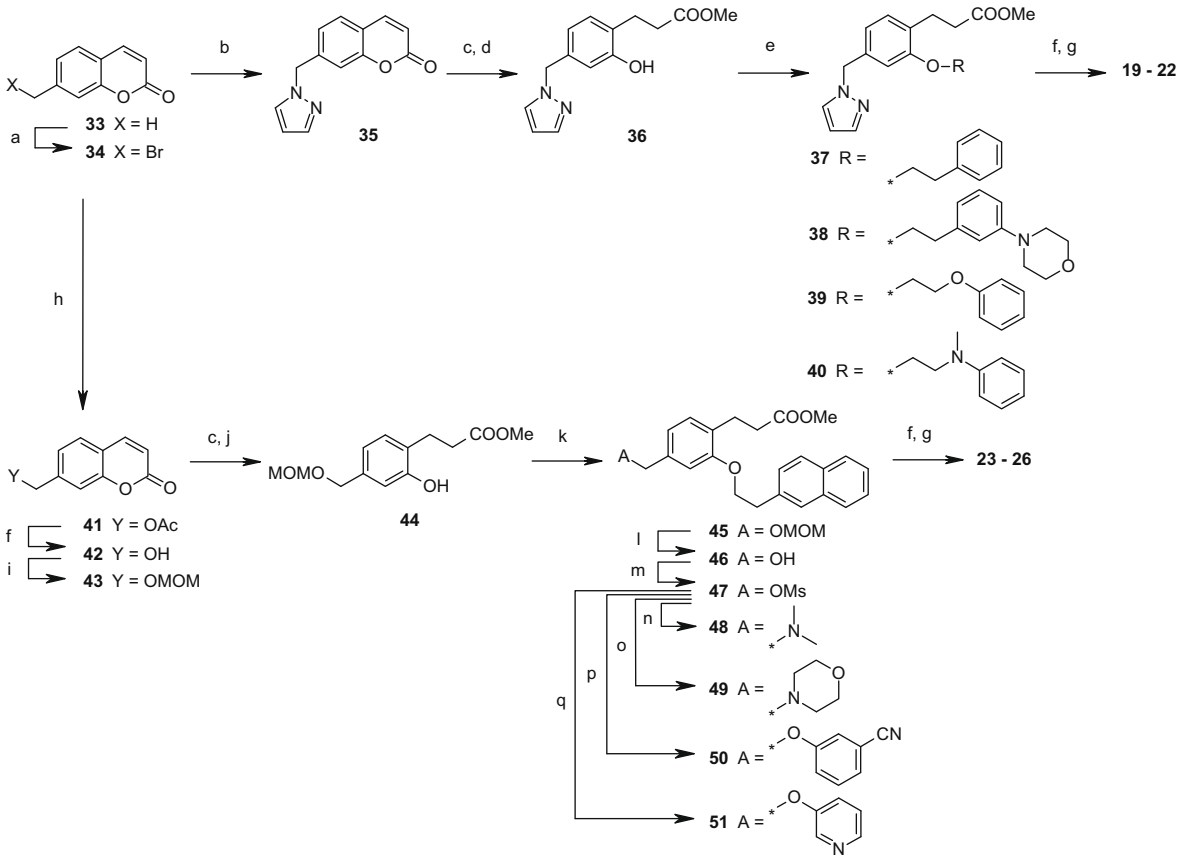


Scheme 3. Synthesis of **8**. Reagents: (a) $MeSO_2NH(t\text{-}Bu)$, *n*-BuLi, THF; (b) MsCl, TEA, $ClCH_2CH_2Cl$; (c) H_2, PtO_2, EtOH; (d) TFA, anisole; (e) PhCOOH, EDC·HCl, DMAP, DMF.

70 ± 6.5% and 66 ± 12%, respectively. Both of them showed equipotent efficacy in the in vivo assessment.

Synthesis of *N*-acylsulfonamide analogs listed in Tables 1–3 is shown in Schemes 1–4. As shown in Scheme 1, preparation of *N*-acylsulfonamides **5**–**6** and **9**–**18** was achieved using a dehydrative condensation reaction of the corresponding carboxylic acid **1** or **27**[12] with an optional sulfonamide using EDC·HCl in the presence of DMAP. As shown in Scheme 2, compound **7** was synthesized from previously reported alcohol **28**,[7] which was converted into amine **29** in three steps. Reaction of **29** with benzenesulfonyl isocyanate in toluene afforded **7**. Synthesis of reverse *N*-acylsulfonamide **8** is shown in Scheme 3. Reaction of aldehyde **30**[7] with an anion prepared from *N*-(*t*-butyl)methanesulfonamide in the presence of *n*-BuLi, followed by dehydration with methanesulfonyl chloride in the presence of triethylamine afforded unsaturated sulfonamide **31**. Catalytic hydrogenation of **31** in the presence of platinum oxide followed by acidic deprotection of *t*-butyl group resulted in saturated sulfonamide **32**, *N*-benzoylation of which was carried out to afford **8** according to the same procedure as described above.

Compounds **19**–**26** were synthesized as outlined in Scheme 4. Bromination of 7-methylcoumarin (**33**) followed by the substitution reaction with pyrazole afforded **35**. Methanolysis of **35** with sodium methoxide followed by catalytic hydrogenation afforded a common intermediate **36** for etherification. O-Alkylation of **36** with an optional alcohol under Mitsunobu reaction conditions afforded **37–40**, respectively, alkaline hydrolysis of which followed by the dehydrative condensation with 3,4-difluorobenzenesulfonamide provided **19**–**22**, respectively. 7-Bromomethylcoumarin (**34**) described above was converted into **41** by a substitution reaction with potassium acetate, alkaline hydrolysis of which provided **42**. O-Alkylation of **42** with methoxymethyl chloride afforded **43**. Methanolysis of **43** followed by sodium borohydride reduction in the presence of nickel chloride resulted in **44**. O-Alkylation of **44** with 2-(naphthalene-2-yl)ethanol under Mitsunobu reaction conditions afforded **45**, acidic deprotection of which produced **46**. O-Methanesulfonylation of **46** afforded a common intermediate **47** for the preparation of the title compounds. Substitution reaction of the methanesulfonate **47** with dimethylamine, morpholine, 3-cyanophenol or 3-hydroxypyridine resulted in **48**–**51**, respectively. Alkaline hydrolysis of **48**–**51** followed by the condensation reac-



Scheme 4. Synthesis of **19**–**26**. Reagents: (a) NBS, AIBN, CCl_4; (b) pyrazole, NaH, DMF; (c) NaOMe, THF, MeOH; (d) H_2, Pd–C, MeOH; (e) R–OH, DEAD, Ph_3P, THF; (f) NaOHaq, THF, MeOH; (g) 3,4-difluorobenzenesulfonamide, EDC·HCl, DMAP, DMF; (h) AcOK, DMF; (i) MOMCl, *i*-Pr_2NEt, $ClCH_2CH_2Cl$; (j) $NaBH_4$, $NiCl_2{\cdot}6H_2O$, THF, MeOH; (k) 2-(naphthalene-2-yl)ethanol, DEAD, Ph_3P, THF; (l) HCl-dioxane, MeOH; (m) MsCl, Et_3N, THF; (n) dimethylamine, K_2CO_3, DMF; (o) morpholine, K_2CO_3, DMF; (p) 3-cyanophenol, NaH, DMF; (q) 3-hydroxypyridine, NaH, DMF.

tion with 3,4-difluorobenzenesulfonamide produced **23**–**26**, respectively.

In conclusion, starting with further optimization of the carboxylic acid of the previously reported EP3 antagonist **1**, we identified a series of novel *N*-acylsulfonamide analogs as more potent and selective EP3 antagonists. Based on the SAR described above, the optimized *N*-acylsulfonamide moieties were considered to play a role of not only a bioisostere of the carboxylic acid but also another interaction site through the arylsulfonamide moiety for the EP3 receptor. Among them, the *N*-(3,4-difluorobenzene)sulfonyl moiety was found to be the most optimized one for the EP3 receptor antagonist activity. A series of *N*-acyl 3,4-difluorobenzenesulfonamide analogs exhibited potent in vivo efficacy, which was indicated as the inhibitory effect on the PGE_2-induced uterine contraction in pregnant rats. Compounds **16**, **25,** and **26** exhibited the more potent in vivo efficacy among those tested.

References and notes

1. Minami, T.; Nakano, H.; Kobayashi, T.; Sugimoto, Y.; Ushikubi, F.; Ichikawa, A.; Narumiya, S.; Ito, S. *Br. J. Pharmacol.* **2001**, *133*, 438.
2. Ushikubi, F.; Segi, E.; Sugimoto, Y.; Murata, T.; Matsuoka, T.; Kobayashi, T.; Hizaki, H.; Tuboi, K.; Katsuyama, M.; Ichikawa, A.; Tanaka, T.; Yoshida, N.; Narumiya, S. *Nature* **1998**, *395*, 281.
3. (a) Senior, J.; Marshall, K.; Sangha, R.; Baxter, G. S.; Clayton, J. K. *Br. J. Pharmacol.* **1991**, *102*, 747; (b) Goureau, O.; Tanfin, Z.; Marc, S.; Harbon, S. *Am. J. Physiol.* **1992**, *263*, C257.
4. Chen, M. C. Y.; Amirian, D. A.; Toomey, M.; Sanders, M. J.; Soll, A. H. *Gastroenterology* **1988**, *94*, 1121.
5. Ma, H.; Hara, A.; Xiao, C. Y.; Okada, Y.; Takahata, O.; Nakaya, K.; Sugimoto, Y.; Ichikawa, A.; Narumiya, S.; Ushikubi, F. *Circulation* **2001**, *104*, 1176.
6. Gross, S.; Tilly, P.; Hentsch, D.; Vonesch, J. L.; Fabre, J. E. *J. Exp. Med.* **2007**, *204*, 311.
7. Asada, M.; Obitsu, T.; Nagase, T.; Sugimoto, I.; Yamaura, Y.; Sato, K.; Narita, M.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2009**, *17*, 6567.
8. Castaner, J.; Wescott, A. L. *Drugs Fut.* **1978**, *3*, 59.
9. (a) Juteau, H.; Gareau, Y.; Labelle, M.; Sturino, C. F.; Sawyer, N.; Tremblay, N.; Lamontagne, S.; Carriere, M. C.; Denis, D.; Metters, K. M. *Bioorg. Med. Chem.* **2001**, *9*, 1977; (b) Gallant, M.; Carriere, M. C.; Chateauneuf, A.; Denis, D.; Gareau, Y.; Godbout, C.; Greig, G.; Juteau, H.; Lachance, N.; Lacombe, P.; Lamontagne, S.; Metters, K. M.; Rochette, C.; Ruel, D.; Slipetz, D.; Sawyer, N.; Tremblay, N.; Labelle, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2583; (c) Belley, M.; Chan, C. C.; Gareau, Y.; Gallant, M.; Juteau, H.; Houde, K.; Lachance, N.; Labelle, M.; Sawyer, N.; Tremblay, N.; Lamontagne, S.; Carriere, M. C.; Denis, D.; Greig, G. M.; Slipetz, D.; Gordon, R.; Chauret, N.; Li, C.; Zamboni, R. J.; Metters, K. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5639.
10. (a) Zhou, N.; Zeller, W.; Krohn, M.; Anderson, H.; Zhang, J.; Onua, E.; Kiselyov, A. S.; Ramirez, J.; Halldorsdottir, G.; Andresson, T.; Gurney, M. E.; Singh, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 123; (b) O'Connell, M.; Zeller, W.; Burgeson, J.; Mishra, R. K.; Ramirez, J.; Kiselyov, A. S.; Andresson, T.; Gurney, M. E.; Singh, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 778; (c) Zhou, N.; Zeller, W.; Zhang, J.; Onua, E.; Kiselyov, A. S.; Ramirez, J.; Palsdottir, G.; Halldorsdottir, G.; Andresson, T.; Gurney, M. E.; Singh, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1528.
11. (a) Zegar, S.; Tokar, C.; Enache, L. A.; Rajagopol, V.; Zeller, W.; O'Connell, M.; Singh, J.; Muellner, F. W.; Zembower, D. E. *Org. Process Res. Dev.* **2007**, *11*, 747; (b) Singh, J.; Zeller, W.; Zhou, N.; Hategen, G.; Mishra, R.; Polozov, A.; Yu, P.; Onua, E.; Zhang, J.; Zembower, D.; Kiselyov, A.; Ramirez, J. L.; Sigthorsson, G.; Bjornsson, J. M.; Thorsteinsdottir, M.; Andresson, T.; Bjarnadottir, M.; Magnusson, O.; Fabre, J. E.; Stefansson, K.; Gurney, M. E. *ACS Chem. Biol.* **2009**, *4*, 115.
12. Asada, M.; Obitsu, T.; Nagase, T.; Tanaka, M.; Yamaura, Y.; Takizawa, H.; Yoshikawa, K.; Sato, K.; Narita, M.; Ohuchida, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2010**, *18*, 80.
13. Evaluation of the binding affinity (K_i values) was carried out in the absence of BSA, while evaluation of the antagonist activity (IC_{50} values) was carried out in the presence of 1% BSA to estimate more exact in vivo potency.
14. More than 95% of homology between the mouse EP3 receptor and the rat EP3 receptor is reported: Neuschafer-Rube, F.; DeVries, C.; Hanecke, K.; Jungermann, K.; Puschel, G. P. *FEBS Lett.* **1994**, *351*, 119.