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Discovery and SAR of PF-4693627, a potent, selective and orally bioavailable *m*PGES-1 inhibitor for the potential treatment of inflammation

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ABSTRACT

Inhibition of *m*PGES-1, the terminal enzyme in the arachidonic acid/COX pathway to regulate the production of pro-inflammatory prostaglandin PGE2, is considered an attractive new therapeutic target for safe and effective anti-inflammatory drugs. The discovery of a novel series of orally active, selective benzoxazole piperidinecarboxamides as *m*PGES-1 inhibitors is described. Structure–activity optimization of lead **5** with cyclohexyl carbinols resulted in compound **12**, which showed excellent in vitro potency and selectivity against COX-2, and reasonable pharmacokinetic properties. Further SAR studies of the benzoxazole ring substituents lead to a novel series of highly potent compounds with improved PK profile, including **23**, **26**, and **29**, which were effective in a carrageenan-stimulated guinea pig air pouch model of inflammation. Based on its excellent in vitro and in vivo pharmacological, pharmacokinetic and safety profile and ease of synthesis, compound **26** (PF-4693627) was advanced to clinical studies.

For patients with inflammatory pain caused by osteoarthritis (OA) and rheumatoid arthritis (RA), the first line of treatment is nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors (COXIBs). The COXIBs are superior to NSAIDs due to their lower incidence of GI side effects, but they are no more effective in symptom remission than NSAIDs and safety issues have surfaced, including renal toxicity and increased cardiovascular risk. Therefore, a drug that displays equal or better efficacy for treating OA and RA relative to NSAIDs and COXIBs, through an alternative biochemical pathway would be an exciting product concept for drug discovery.

Prostaglandin E2 (PGE2) is an important mediator in acute and chronic inflammation, pain, and fever and is found in the synovial fluid of patients with OA and RA.[1] The biosynthesis of PGE_2 begins with the cleavage of arachidonic acid (AA) from membrane phospholipids by phospholipase A_2, followed by the conversion of AA to PGH_2 by cyclooxygenase (COX), and finally to the production of PGE_2 by PGE synthase (PGES).[2] Three forms of PGES have been reported: cytosolic PGES (cPGES), microsomal PGES-1 (*m*PGES-1), and microsomal PGES-2 (*m*PGES-2).[3] Both *c*PGES and *m*PGES-2 are constitutively expressed in a variety of tissues, while mPGES-1 is up-regulated under inflammatory conditions.

A selective inhibitor of *m*PGES-1 would be expected to inhibit PGE2 production induced by inflammation while sparing constitutive PGE2, prostacyclin (PGI2), and thromboxane production. This selectivity should differentiate *m*PGES-1 inhibitors from NSAIDS and COX-2 inhibitors in the treatment of inflammation and arthritis. A number of excellent reviews summarizing the biology, pharmacology of *m*PGES-1, as well as the progress in the development of *m*PGES-1 inhibitors, have recently become available.[4]

Figure 1 shows examples of mPGES-1 inhibitors recently described in the literature. A series of potent and selective mPGES-1 inhibitors, represented by **1** was obtained by optimization of a 5-lipoxygenase-activating protein (FLAP) inhibitor lead with moderate rat and human mPGES-1 inhibition.[5] These compounds are highly protein shifted and thus they have not shown mPGES-1 inhibition in vivo.

The benzoxazole scaffold has been described in the patent literature to yield inhibitors of the MAPEG family enzymes including mPGES-1.[6] The most potent compounds against mPGES-1 disclosed are **2** (IC_{50} = 1.3 μM) and **3** (IC_{50} = 1.5 μM) although no selectivity and efficacy data was reported.

Phenanthrene imidazole **4** (MF63) was described as a potent inhibitor of human mPGES-1 and showed potency in a LPS-stimulated human whole blood assay where it inhibited PGE2 production, IC_{50} = 1.3 μM. Although **4** is inactive against rat mPGES-1 for unknown reasons, it is active on guinea pig enzyme and showed efficacy in a guinea pig model of LPS-induced hyperalgesia.[7]

In a prior study, we disclosed a novel class of benzoxazoles as inhibitors of *m*PGES-1.[8] The initial HTS hit (**5**, Fig. 2), showed modest activity in our enzyme assay (850 nM), but it lacked potency in

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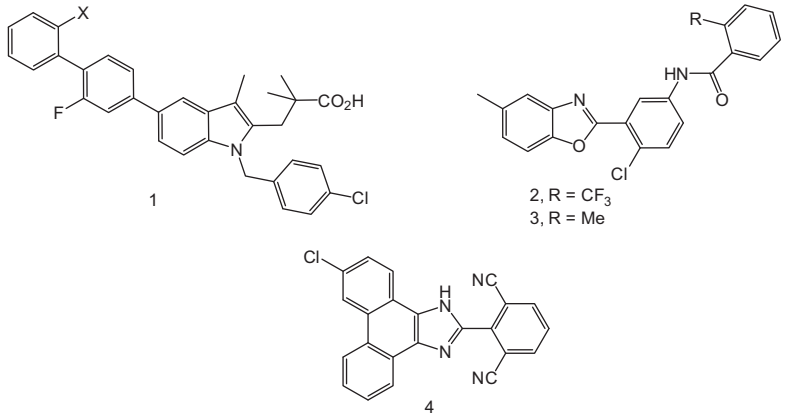


Figure 1. Scaffolds of reported mPGES-1 inhibitors.

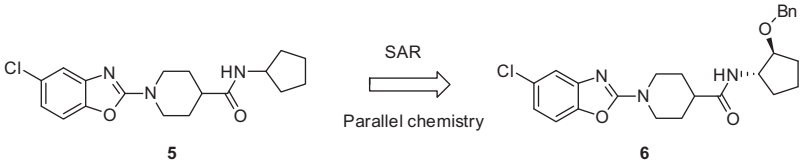
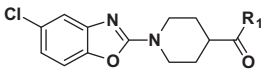


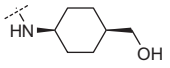
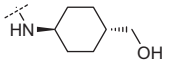
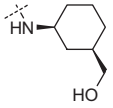
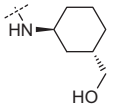
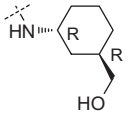
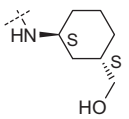
Figure 2. Optimization of benzoxazole HTS hit.

our cell-based assay. A parallel chemistry program ensued to study the SAR of the piperidine and cyclopentane rings of **5**. Based on this work, benzoxazole **6** was identified as a very potent inhibitor (*m*PGES-1 IC_{50} = 18 nM) with equal potency in a fetal fibroblast cell assay.[9] Unfortunately, the compound showed only modest activity in our human whole blood (HWB) cell-based assay (IC_{50} = 7.5 μM). Also, while compound **6** had an acceptable in vivo dog pharmacokinetic (PK) profile; in general, the in vivo PK for compounds possessing a cyclopentane ring on the right-hand side was quite variable. Herein, we describe the design and synthesis of new benzoxazoles, focusing on the SAR of the left-hand benzoxazole and right-cyclopentyl regions of the molecule. Also, we will detail in vitro and in vivo efficacy and safety data that led to the discovery of PF-04693627, a candidate suitable for human studies.

Our structure–activity relationship (SAR) was initially focused on improving the activity of lead **5** in our HWB cell assay. It was hypothesized that the large right-shift of compound **6** on going from the enzyme to HWB cell-based assay was likely due the compound's high *c*log*P* (4.2). We hypothesized that replacing the benzyloxycyclopentane in compound **6** with a smaller, more polar group would lead to an increase in potency in the HWB cell assay. To that end, we focused on compound **7** (Table 1), which was discovered during the hit-to-lead phase of the program.[8] While compound **7** was not as potent as compound **6**, it possessed a more favorable HWB cell to enzyme ratio (36 vs 220), presumably due to the compound's lower *c*log*P* of 1.7. Additional SAR work on the cyclohexyl ring revealed that moving the hydroxymethyl group from the 4-position of the cyclohexane ring to the 3-position in compound **10** resulted in potent activity in both the enzyme and HWB cell assays. It was further determined that the (1*S*,3*S*)-enantiomer (**12**) is 75-fold more potent than the corresponding (1*R*,3*R*)-enantiomer (**11**).[10] Interestingly, while the *cis*-geometry is preferred in the 1,4-disubstituted cyclohexane series (compare compound **7** with compound **8**), the *trans*-geometry (**10**) is preferred in the 1,3-disubstituted cyclohexane series over the

Table 1
Amino cyclohexyl carbinol optimization



Compound	R1	*m*PGES-1 IC_{50}[a] (nM)
7		383
8		3620
9		6750
10		206
11		4850
12		65

[a] *m*PGES-1 enzyme assay as described in Ref. 9. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control. Numbers indicate IC_{50} values generated from individual 10-point concentration response relationships in duplicate.

cis-geometry (**9**).[11] While compound **12** displayed a significantly improved HWB cell to enzyme ratio of 5, it lacked in vivo metabolic stability in a rat PK model (vide infra).

At this point, we turned our attention toward optimizing the left-hand benzoxazole region of the molecule with the hope of identifying compounds with equal or better *m*PGES-1 potency and improved rat PK compared to compound **12**.

Over 100 analogs possessing substituent modifications to the benzoxazole core were prepared. A subset of those analogs is detailed in Table 2. The synthesis of benzoxazoles **13**–**18**, and **22** is shown in Scheme 1. Toward this end, treatment of commercially available aminophenols **32** with carbon disulfide afforded 2-mercaptobenzoxazoles **33**. Replacement of the thiol in **33** with ethyl isonipecotate gave rise (80–90%) to piperidinebenzoxazoles **34**. Saponification of the ester in **34** led to carboxylic acids **35**. HBTU-Mediated coupling of acids **35** with (1*S*,3*S*)-3-aminocyclohexylmethanol[10] afforded amides **13**–**18** and **22**.

The synthesis of analogs **19**–**21** is shown in Scheme 2. Thus, bromination of benzoxazole **37** afforded bromide **38**. Suzuki-cross coupling reaction of in situ generated organo-boranes with bromide **38** gave rise (75–80%) to alkyl-substituted benzoxazoles **39**.[14] Saponification of the ester in **39** and coupling with amine **36** led to compounds **19**–**21**.

6-Aryl and heteroaryl substituted benzoxazoles **25**–**31** were prepared as described in Scheme 3. Accordingly, Suzuki cross coupling of bromide **38** with the appropriate aryl or heteroaryl boronic acid afforded coupled products **40**. Treatment of the ester in **40** with sodium hydroxide, followed by subsequent amide coupling with amine **36** led to analogs **25**–**31**.

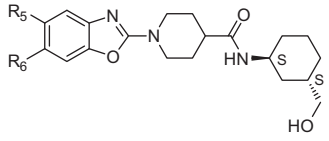
Analog **23** was prepared as shown in Scheme 4. Toward this end, iodination of compound **37** gave iodide **41**; subsequent trifluoromethylation gave rise to **42**.[15] Separation of **42** from unreacted aryl iodide **41** was not possible. However, hydrogenation of the crude product mixture converted unreacted iodide into **37**, which allowed clean isolation of **42** via chromatography. Saponification of the ester in **42** and coupling with amine **36** led to analog **23**.

Difluoromethyl analog **24** was prepared as shown in Scheme 5. Thus, exposure of **37** to hexamethylenetetramine (HMTA) led to a 69% yield of aldehyde **43**.[16] Fluorination of the aldehyde with DAST afforded difluoride **44**. Saponification of the ester and coupling with amine **36** gave rise to analog **24**.

Our original screening hit (**5**) had a chlorine atom at the 5-position of the benzoxazole. Initial attempts to replace this substituent with hydrogen or methyl produced inactive analogs (data not shown). In general the replacement of chlorine at this position was met with limited success. For instance, while other small, lipophilic, electron-withdrawing groups, such as bromo (**13**) or trifluoromethyl (**17**) were tolerated at this position, they did not offer any significant benefit in terms of potency or lower clearance compared to the chloro analog **12**. Replacing the chloro substituent with small, polar substituents, such as cyano (**14**), ethanesulfonyl (**15**) or carboxamido (**16**) caused a significant right-shift in *m*PGES-1 activity.

Having identified chlorine as an ideal group at C(5), we next explored substitution at the C(6) position of the benzoxazole (Table 2). Analogs **18**–**21** show the effects of placing an alkyl substituent at C(6). We were pleased to find that all of these analogs showed enhanced activity in the *m*PGES-1 enzyme assay. While the methyl (**18**), ethyl (**19**) and isopropyl (**20**) analogs also showed enhanced activity in the HWB cell assay, the isobutyl analog had similar cell activity to compound **12**. In general, as the *c*log*P*

Table 2
Benzoxazole ring optimization

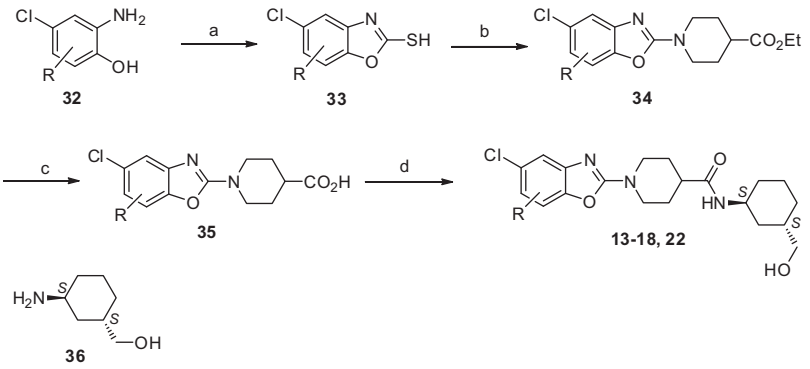


Compound	R5	R6	*m*PGES-1 IC_{50}[a] (nM)	HWB IC_{50}[b] (nM)	HWB/mPGES-1 IC_{50} ratio	*c*log*P*	HLM CLint[c] (mL/min/kg)
12	Cl	H	65	340	5.2	1.74	19.7
13	Br	H	47	761	18.5	1.89	25.6
14	CN	H	5700	9800		0.55	< 8
15	Ethylsulfonyl	H	>33,000	>50,000		0.32	
16	Carboxamide	H	>33,000	>50,000		−0.05	
17	CF3	H	88	510	5.8	1.96	22.2
18	Cl	Me	25	338	13.5	2.24	28.3
19	Cl	Et	20	162	8.1	2.77	64.6
20	Cl	*i*-Pr	6	261	43.5	3.17	46.4
21	Cl	*i*-Bu	15	761	50.7	3.70	44.0
22	Cl	Cl	16	684	42.8	2.36	27.1
23	Cl	CF3	33	174	5.3	2.49	26.5
24	Cl	CHF2	49	336	6.9	1.94	40.2
25	Cl	4-Fluorophenyl	3	119	39.7	3.52	81.9
26	Cl	4-Chlorophenyl	3	109	36.3	4.09	37.1
27	Cl	3-Chlorophenyl	3	240	80	4.09	55.8
28	Cl	4-Ethoxyphenyl	3	133	44.3	3.83	<8
29	Cl	4-(Trifluoromethoxy)phenyl	2	53	26.5	4.41	50.8
30	Cl	4-Methoxypyridyl	18	632	35.1	2.70	52.0
31	Cl	4-(Trifluoromethyl)pyridyl	8	317	39.6	2.91	68.8

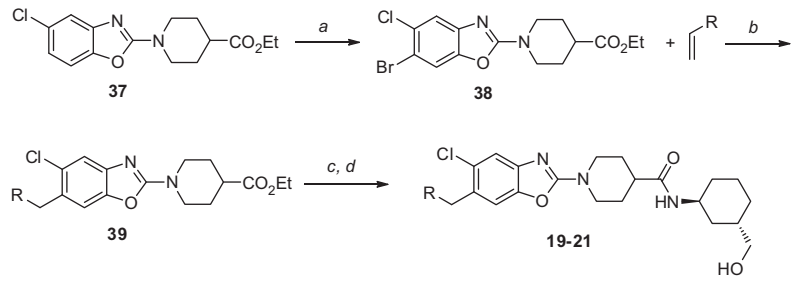
[a] *m*PGES-1 enzyme assay as described in Ref. 9. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control. Numbers indicate IC_{50} values generated from individual 10-point concentration response relationships in duplicate.

[b] Lipopolysaccharide (LPS) stimulated human whole blood (HWB) cell assay. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control, see Ref. 12 for further details. Numbers indicate IC_{50} values generated from individual 10-point concentration response relationships in duplicate.

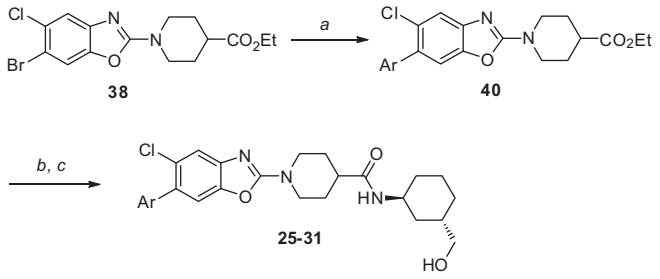
[c] Human liver microsomal stability assay. CLint refers to total intrinsic clearance obtained from scaling in vitro half-lives in human liver microsomes.[13]



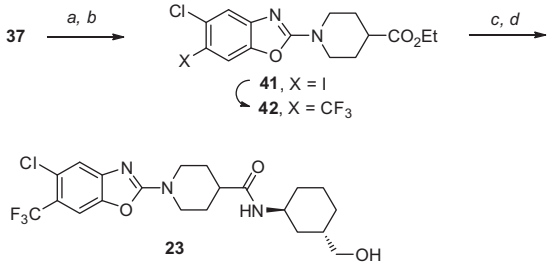
Scheme 1. Preparation of compounds **13**–**18** and **22**. Reagents and conditions: (a) CS_2 (2.0 equiv), KOH (2.1 equiv), EtOH, reflux, 16 h, 80–95%; (b) ethyl isonipecotate (1.1 equiv), toluene, reflux, 16 h, 80–90%; (c) 2.5 N NaOH (2.0 equiv), MeOH/H_2O 9:1, rt, 8 h, 90–98%; (d) (1*S*,3*S*)-3-aminocyclohexylmethanol (**36**), HBTU (1.2 equiv), Et_3N (1.5 equiv), DMF, rt, 16 h, 70–90%.



Scheme 2. Preparation of compounds **19**–**21**. Reagents and conditions: (a) Br_2 (1 equiv), AcOH, rt, 1 h, 99%; (b) olefin, 2.5 M 9-BBN/THF, rt, 15 min; **38**, $Pd(dppf)_2Cl_2$ (cat.), K_3PO_4, 60 °C, 4 h, 75–80%; (c) 2.5 N NaOH (2.0 equiv), MeOH/H_2O 9:1, rt, 8 h, 90–98%; (d) **36**, HBTU (1.2 equiv), Et_3N (1.5 equiv), DMF, rt, 16 h, 70–90%.



Scheme 3. Preparation of compounds **25**–**31**. Reagents and conditions: (a) aryl boronic acid (1.1–1.5 equiv), $Pd(P(Ph_3)_4$ (cat.); Na_2CO_3 (2 equiv), DMF, reflux, 8 h, 60–85%; (b) 2.5 N NaOH (2.0 equiv), MeOH/H_2O 9:1, rt, 8 h, 90–98%; (c) **36**, HBTU (1.2 equiv), Et_3N (1.5 equiv), DMF, rt, 16 h, 70–90%.



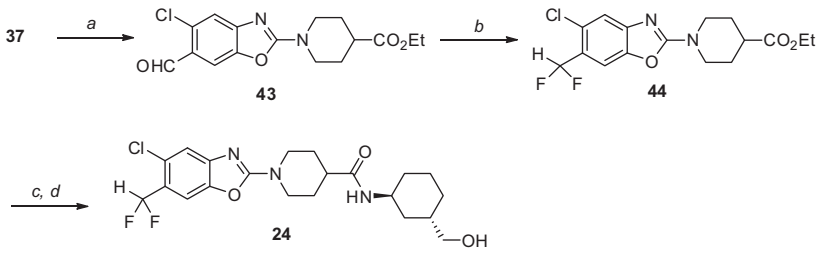
Scheme 4. Preparation of compound **23**. Reagents and conditions: (a) NIS (1.1 equiv), AcOH, rt, 16 h, 91%; (b) methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (3.0 equiv), CuI (1.5 equiv), DMF, 80 °C, 24 h; H_2, 10% Pd/C, MeOH, 24 h, 40%; (c) 2.5 N NaOH (2.0 equiv), MeOH/H_2O 9:1, rt, 8 h, 90–98%; (d) **36**, HBTU (1.2 equiv), Et_3N (1.5 equiv), DMF, rt, 16 h, 70–90%.

increased from 2.2 to 3.7 for methyl compound **18** to isobutyl analog **21**, the cell-to-enzyme ratio also increased from 3 to 51. Replacing the alkyl group at C(6) with a chloro group produced analog **22**. This compound showed potent enzyme activity. With its lower *c*log*P* of 2.4, we had hoped that this compound would have a desirable cell-to-enzyme ratio. Unfortunately, compound **22** showed a poor cell-to-enzyme ratio of 43 however, substituting the C(6) chloro group in **22** for trifluoromethyl gave compound **23**, which showed enhanced activity in both the enzyme and cell assays. In this case, the lower *c*log*P* of compound **23** translated to a lower cell-to-enzyme ratio of 5. Difluoromethyl compound **24** also showed good enzyme and cell activity although the compound showed higher in vitro clearance in HLM. In general, it was found that a chlorine atom at C(5) and a non-polar substituent at C(6) had a synergistic effect on *m*PGES-1 activity.

Continuing our search for potent compounds with reduced clearance, we next considered substituted phenyl rings at the C(6)-position (**25**–**29**, Table 2). We were encouraged to find that these analogs were significantly more potent in the *m*PGES-1 enzyme assay than many of the alkyl-substituted analogs. Due to their high *c*log*P*'s, many of the phenyl-substituted analogs showed a high cell-to-enzyme ratios (27–80). Despite their high *c*log*P* (3.5–4.4) and high cell-to-enzyme ratios, many of the aryl-analogs (**25, 26, 28, 29**) showed very potent activity in the HWB cell assay and in general these analogs showed a range of in vitro metabolic stabilities in HLM.

Pyridyl-substituted analogs **30** and **31** were prepared in an attempt to lower the *c*log*P* while maintaining the excellent potency seen in the phenyl-substituted analogs (Table 2). Unfortunately, both analogs showed a right shift in enzyme and cell potency, and both analogs showed high in vitro clearance.

Rat pharmacokinetic (PK) data for compound **12** and a selected group of potent analogs from Table 2 is shown in Table 3. Compound **12** showed good bioavailability, however, its low volume of distribution and modest half life resulted in an unfavorable



Scheme 5. Preparation of compound **24**. Reagents and conditions: (a) HMTA (2.2 equiv), CF_3CO_2H, 120 °C, 18 h, 69%; (b) DAST (1.1 equiv), CH_2Cl_2, −78 °C, 6 h, 80%; (c) 2.5 N NaOH (2.0 equiv), MeOH/H_2O 9:1, rt, 8 h, 90–98%; (d) **36**, HBTU (1.2 equiv), Et_3N (1.5 equiv), DMF, rt, 16 h, 70–90%.

Table 3

Compound	Rat in vivo pharmacokinetic properties[a]				
	CL (mL/min/kg)	Vd_{ss} (L/kg)	Half-life (h)	MRT (h)	*F* (%)
12	17	0.72	0.51	0.70	66
19	82	2.1	0.40	0.44	
23	34	14	19.3	7.22	42
24	60	1.4	1.4	0.39	
26	12	3.0	3.7	4.42	59
28	28	2.4	1.4	6.26	17
29	14	6.4	6.7	7.78	86

[a] Clearance (CL), volume of distribution (V_{ss}) and half-life pharmacokinetic data were determined following a 1.0 mg/kg dose iv in Sprague–Dawley rats. Mean residence time (MRT) and bioavailability (%F) were determined following a 1.0 mg/kg po dose in Sprague–Dawley rats.

Table 5
Compound **26** selectivity against relevant enzymes as described in Ref. 18

Compound	HWB-1483 IC_{50} (μM)				Fetal fibroblast IC_{50} (μM)	
	mPGES-1	PGDS	TXAS	5-LO	mPGES-1	COX-2
26	0.18	>50	>50	>50	0.006	>10

metabolic profile. Substitution with an ethyl group at the C(6)-position (**19**) resulted in very high clearance while replacement with trifluromethyl produced a more metabolically stable compound (**23**). Aryl substitution at C(6) produced compounds (**26**, **28**, **29**) with good clearance and improved half-lives compared to **12** while maintaining good bioavailability.

To date, no selective *m*PGES-1 inhibitors have been reported in the published literature that are active on the rodent enzyme. Hence, inflammation models historically used to evaluate NSAIDs and COX-2 inhibitors could not be utilized. As a result, the carrageenan-stimulated air pouch model was established in the guinea pig in order to assess in vivo efficacy data.[17] Compounds **23**, **26,** and **29** were tested in the air pouch inflammation model at 10 mg/kg administered orally. The potent NSAID, Naproxen, was used as a reference. We were pleased to find that all three benzoxazole compounds showed good inhibition of PGE_2 production in this assay while no inhibition of 6-keto-$PGF_{1\alpha}$ was observed (Table 4).

Overall compounds **26** and **29** had the best combination of in vitro cell potency, PK, in vivo efficacy and pharmaceutical properties. Furthermore, since the synthesis of compound **26** was more attractive than compound **29**; it was chosen for further profiling. The selectivity of **26** was profiled against several relevant human targets in the following assays: (a) HWB/1483 for selectivity against TXAS, PGDS, 5-LOX, 15-LOX and 12-LOX; (b) Human fetal fibroblasts for selectivity against COX-2. Results are summarized in Table 5. The data show that **26** is selective against relevant human enzymes. Compound **26** was also submitted to a broad panel screen (CEREP) and no significant findings were observed. Based on these data, compound **26** was selected as a clinical candidate. Currently, **26** is undergoing additional in vitro and in vivo safety studies in anticipation of human studies.

Table 4
PGE2 inhibition

Compound	Guinea pig carrageenan stimulated air pouch		
	Dose (mg/kg)	Plasma concd (μM)	PGE2 (%) inh.
Naproxen	10	153.9	60
23	10	1.6	66
26	10	1.2	63
29	10	0.51	52

Carrageenan-simulated air pouch performed as described in Ref. 17.

In summary, we have described a novel class of benzoxazoles as *m*PGES-1 inhibitors with excellent potency and selectivity. Careful modification of the right-hand portion of HTS-hit **5** led to compound **12**. Introduction of fluoroalkyl and aryl substituents at the C(6)-position of the benzoxazole ring led to the discovery of compounds **23**, **26**, and **29** with a remarkably improved potency in the HWB assay and favorable PK profiles. These compounds were further profiled in a Guinea pig carrageenan-stimulated air pouch model of inflammation. As a result of its in vitro potency and selectivity, in vivo efficacy, pharmacokinetic properties, and preclinical safety profile, benzoxazole **26** (PF-4693627) was selected as a clinical candidate for the treatment of inflammation caused by OA and RA.

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References and notes

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11. A detailed study looking at the conformation analysis of the 1,4- and 1,3-disubstituted cyclohexane analogs is published in the adjoining paper.
12. IC_{50} values for inhibition of PGE_2 production in the LPS-stimulated human whole blood assay: Human whole blood was collected from healthy donors in heparinized tubes and stimulated with 20 μg/ml LPS in the presence of varying concentrations of inhibitor and 1% DMSO. Following incubation overnight (20–24 h) at 37 °C in 95% air/5% CO_2, assay plates were centrifuged and the plasma removed for quantitation of PGE_2 by ELISA. Data were expressed as% inhibition of PGE_2 with 10 μM indomethacin used for maximal inhibition of PGE_2. Inhibitors were evaluated at 10 concentrations in duplicate with threefold serial dilutions and IC_{50} values calculated using a 4-parameter logistic model. Each IC_{50} value represents the average of $n \geqslant 4$ experiments. Standard error of the IC_{50} was generally less than 30%.
13. Assay conditions adopted from published protocols, see: (a) Riley, R. J.; McGinnity, D. F.; Austin, R. P. *Drug Metab. Dispos.* **2005**, *33*, 1304; (b) Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350; (c) Hosea, N. A.; Collard, W. T.; Cole, S.; Maurer, T. S.; Fang, R. X.; Jones, H.; Kakar, S. M.; Nakai, Y.; Smith, B. J.; Webster, R.; Beaumont, K. *J. Clin. Pharmacol.* **2009**, *49*, 513.
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17. Use of the animals in these studies was reviewed and approved by the Pfizer Institutional Animal Care and Use Committee. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. Male Duncan Hartley guinea pigs (250–300 g), (Charles River Laboratories, Wilmington, MA) were used in these studies. Animals were acclimated in the animal facility for one week prior to experimentation. Air pouches were produced by subcutaneous injection of 5 mL of sterile air into the intrascapular area of the back. The next day pouches were injected with 3–5 mL of sterile air to maintain pouches. Pouches were allowed to develop for 2 days from initial injection. Animals (6 per group) were fasted with free access to water for 16 to 24 h prior to drug administration. Compounds or vehicle (0.5% MetheIcellulose, 0.025% Tween-20) were administered by oral gavage (1 mL) 2 h after injection into the pouch of 2 mL of a 1% suspension of carrageenan (FMC BioPolyer, Philadelphia, PA Cat# GP209) dissolved in saline. At 6 h post-carrageenan injection, 1.5 mL of 100 μM of arachidonate acid (NU-Chek PREP, Inc., Elysian MN) dissolved in saline was injected into the pouch and after 10 min fluid was collected and volume measured. The fluid was centrifuged at 800 g (Beckman Coulter, Allegra X-15R) for 10 min at 4 °C, and the supernatants were collected for analysis of PGE_2 and 6-keto-$PGF_{1\alpha}$. PGE_2 and 6-keto-PGF1α levels were quantitated by ELISA (Cayman Chemical Company, Ann Arbor, MI) by multiplying ng/ml by total pouch volume (ml) to normalize for total pouch ng. Each point represents the mean ± S.E.M. (n = 5–12). ED_{50} and ED_{80} values for each experiment were calculated using a four-parameter sigmoid model. Naproxen was used as a reference compound at 10 mg/kg.
18. *HWB/1483 assay*: Human head and neck squamous cell carcinoma, 1483 cells, were maintained in cultures at 37 °C in 95% air, 5% CO_2 atmosphere in High Glucose D-MEM (Invitrogen, Grand Island, NY) containing extra 10 mM HEPES buffer (Invitrogen), Penicillin–Streptomycin–Glutamine (Invitrogen), and 15% FBS (Sigma, St. Louis, MO). On the day of the experiments, 1483 cells were trypsinized, centrifuged at 400×*g*, and the pellets were resuspended with human whole blood (HWB). HWB was collected from healthy human donors in 10 ml heparinized tubes (Vacutainer tubes, Becton Dickenson, Franklin Lakes, NJ). The cell mixtures were pre-incubated with compounds for 15 min (at a final concentration of 1% DMSO) followed by incubation with 30 mM arachidonic acid. The levels of eicosanoids were determined by LC/MS/MS. Human fetal fibroblasts assay: Cells were stimulated with 1 ng/ml IL-1β for 24 h. The conditioned media were removed, and cells were washed twice with serum-free media. Cells were then treated with the serum-free media containing either the vehicle (at a final concentration 1% DMSO) or the compounds (1% DMSO) for 50 min, and with 10 μM arachidonic acid for additional 10 min, at 37 °C in 95% air, 5% CO_2 atmosphere. To measure $PGF_{2\alpha}$ levels, $SnCl_2$ was added to the cells 40 min after addition of the compounds (or 10 min before addition of arachidonic acid). PG levels in the supernatants were assayed by ELISA.