Discovery of 2-((2-chloro-6-fluorophenyl)amino)-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)-1-methyl-7,8-dihydro-1*H*-[1,4]dioxino[2',3':3,4]benzo[1,2-d]imidazole-5-carboxamide as potent, selective and efficacious microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitor

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Abstract:

The discovery and SAR of potent, selective dioxane-fused tricyclic benz[*d*]imidazole derivatives as mPGES-1 inhibitor are herein described. Various amide modifications in this series afforded many potent mPGES-1 inhibitors, of which **17d** proved to be suitable for further profiling *in vivo*. Compound **17d** {2-((2-chloro-6-fluorophenyl)amino)-*N*-(3-fluoro-5-(trifluoromethyl)phenyl)-1-methyl-7,8-dihydro-1*H*-[1,4]dioxino[2',3':3,4]benzo[1,2-d]imidazole-5-carboxamide} exhibited excellent mPGES-1 enzyme (IC_{50}: 8 nM), cell (A549 IC_{50}: 16.24 nM) and human whole blood potency (IC_{50}: 249.9 nM). In rodent species, **17d** strongly inhibited guinea pig mPGES-1 (IC_{50}: 10.79 nM), but not the rat and mouse enzyme. Furthermore **17d** displayed excellent *in vitro* selectivity over mPGES-2, cPGES, COX-enzymes (COX-1, 2), selectivity against other prostanoid synthases, favorable hERG and CEREP panel profile. Likewise, our lead **17d** demonstrated good oral pharmacokinetic profiles and good CNS B/P ratio in rat and guinea pig. Lead **17d** also unveiled good efficacy in LPS-induced thermal hyperalgesia pain model with ED_{50} of 36.7 mg/kg, respectively.

Key Words: Rheumatoid arthritis; Benzimidazole; mPGES-1 inhibitor; PGE_2; CSF concentration; Scaffold hopping.

Prostaglandin E_2 (PGE_2) is the important major prostanoid, being produced by a variety of cells and tissues, and has a broad range of biological activity. Recent advances in this field have led to the identification and characterization of various enzymes involved in the biosynthesis of PGE_2, including phospholipase A_2 (PLA_2), cyclooxygenase (COX) and terminal PGE synthase (PGES).[1,2] The terminal prostaglandin-E synthases (PGES), which catalyzes the conversion of PGH_2 to PGE_2, have been characterized as mPGES-1, mPGES-2 and cytosolic PGES (cPGES). Among these, mPGES-1 is an inducible isoform, which is induced during the inflammation. It is a glutathione (GSH) dependent, perinuclear, membrane-bound trimer which is predominantly up-regulated and functionally coupled to COX-2.[3-5] The other two constitutively expressed isoforms mPGES-2, cPGES are also GSH-dependent and structurally, biologically distinct from mPGES-1, which are likely to be involved in the production of PGE_2 responsible for normal physiological reactions.[1, 6-11] Mice deficient in mPGES-1 but not mPGES-2 or cytosolic PGES suppress PGE_2 production, suggesting that mPGES-1 may represent the only enzymatic pathway capable of generating PGE_2 *in vivo*.[2,12-14] Several recent studies using mPGES-1 knockout (KO) mice demonstrated a major role for mPGES-1 in pain and inflammatory responses.[12] Similarly, another set of knockout studies demonstrated that mPGES-1 deletion in mice does not affect blood pressure and hypertension.[3,15]

Though most commonly consumed traditional nonsteroidal anti-inflammatory drugs (NSAIDs) relieves pain and edema associated with arthritis and inflammation, most of the NSAIDs are associated with mild to severe gastro-intestinal (GI) toxicity. Furthermore, currently marketed COX-2 inhibitors are linked to cardiovascular risk, evident from the withdrawal of rofecoxib and valdecoxib from the US market and failure to get approval for etoricoxib. On the other hand, celecoxib now carries explicit warning of its cardiovascular risks.[16-18] It was generally believed that inhibition of COX enzymes led to suppression of various important prostaglandins (PGs) such as prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) along with the pathogenic PGE_2 resulting in GI, renal and hypertension risk.[16] The cardiovascular consequences associated with COX-2 inhibitors[16-18] have stimulated the interest in mPGES-1 as a potential target for the development of next generation anti-inflammatory drugs.[19] A number of excellent reviews have summarized the biology and *in vivo* pharmacology (pre-clinical animal models) of

mPGES-1 and have provided a firm rationale for targeting the enzyme in drug development devoid of GI and cardiovascular risk.[5,12,16-20]

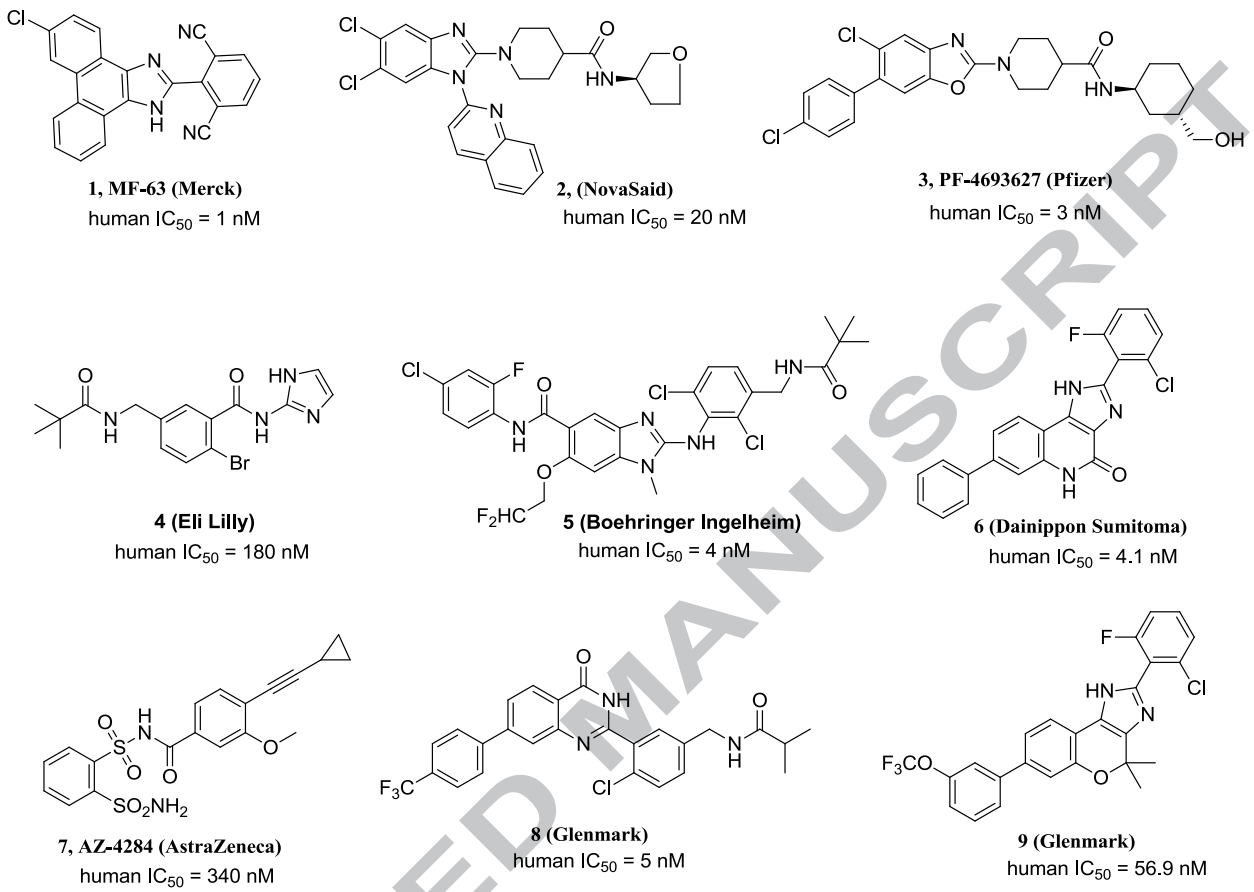


Figure 1. Representative mPGES-1 inhibitors.

The discovery of MF-63 (**1**) and its effectiveness in guinea pig hyperalgesia pain model[21,22] further fuelled strong interest in mPGES-1 inhibitor. Numerous examples of potent mPGES-1 inhibitors can be found in the literature including patent application.[21-37,39-42] Some more noteworthy inhibitors exemplified in Figure 1 are: piperidinyl benzoimidazole **2**,[23] PF-4693627 (**3**),[24] imidazole-2-benzamide **4**,[25,26] benzimidazole-5-carboxamide **5**,[27,28] imidazoquinolinone **6**,[29] AZ-4284 (**7**),[30,31] quinazolinone **8**,[32] dihydrochromenoimidazole **9**.[33] Further, compound **8** effectively regulated PGE_2 biosynthesis in the synovial fibroblasts and chondrocytes derived from the patients with rheumatoid arthritis and osteoarthritis.[32] Moreover, our tool compound **9** (IC_{50}: 56.9 nM) exhibited good mPGES-1 potency and moderate in vivo efficacy.[33] However, a poor whole cell potency of **9** (A549 IC_{50}: 839 nM) encouraged us to design a new chemical class of mPGES-1 inhibitor.

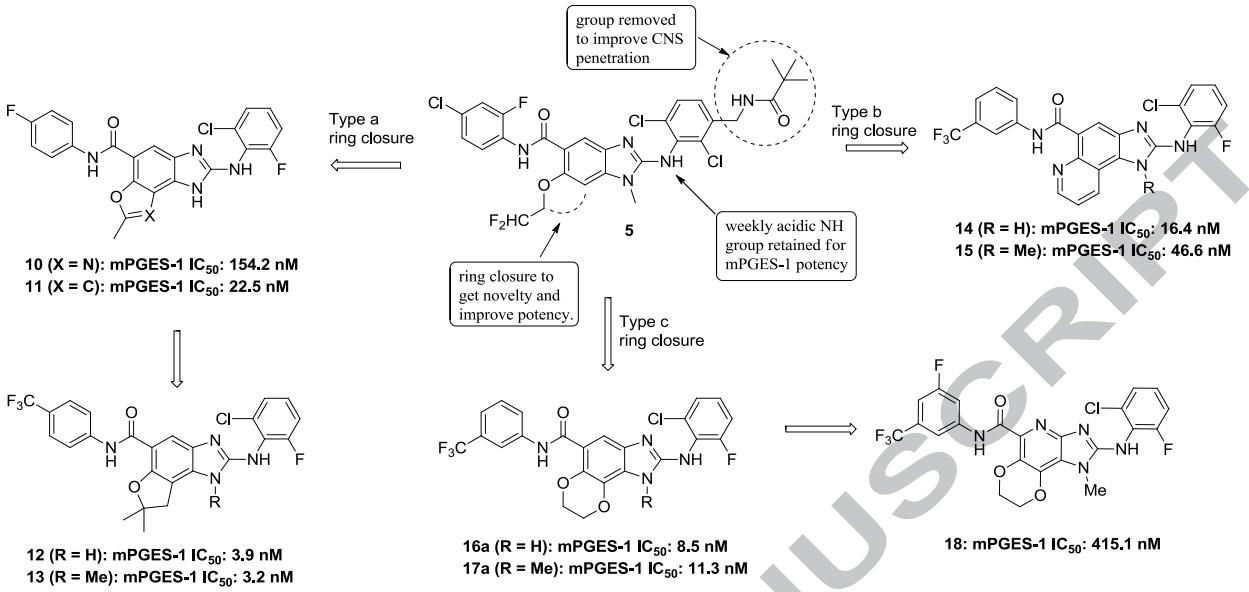
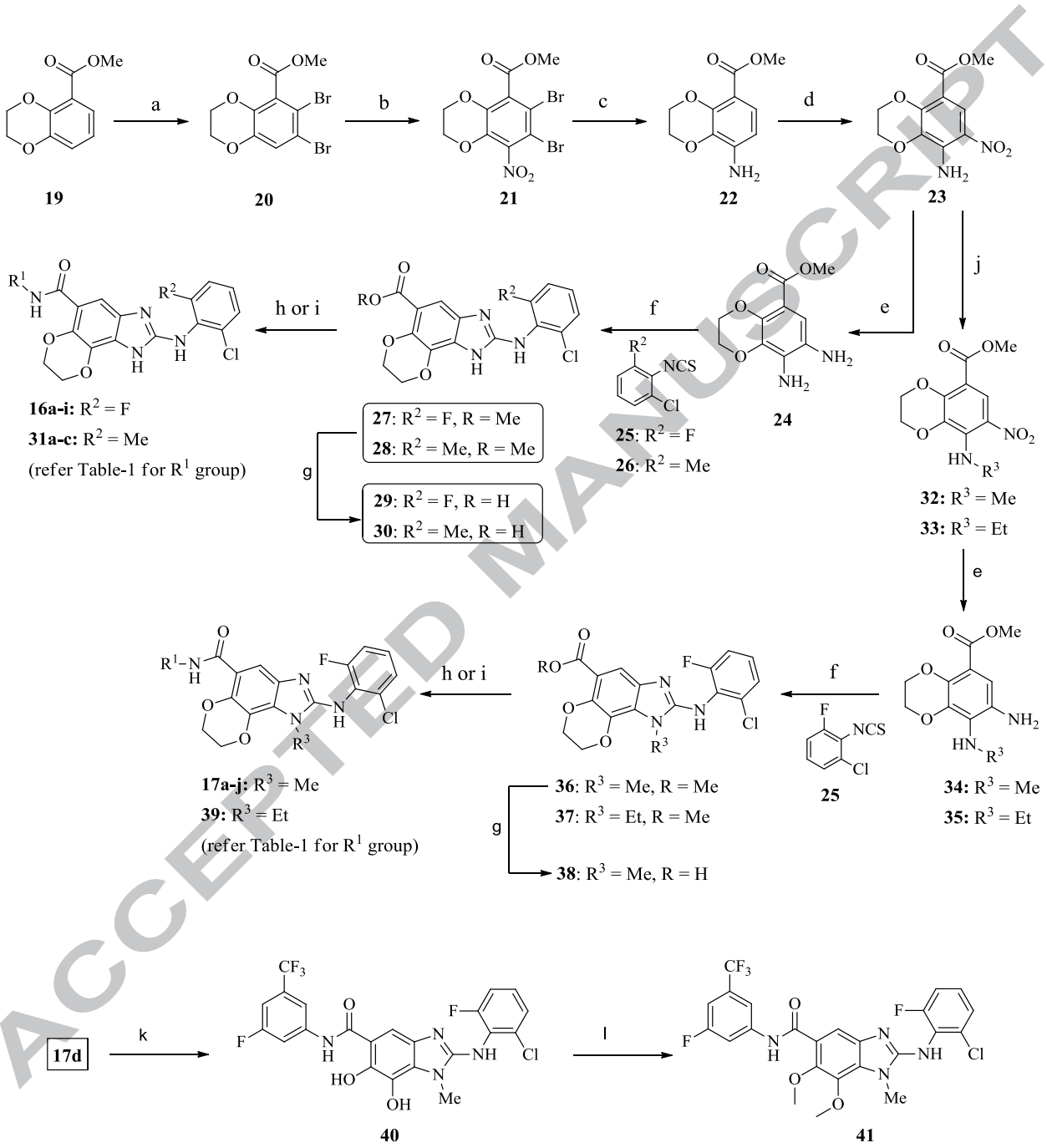


Figure 2. Scaffold hopping of benzimidazole for novel mPGES-1 inhibitors

In order to afford better cell permeable compounds and by recognizing excellent mPGES-1 potency of known compound **5**,[27,28] we hypothesized that constraining the difluoroethoxy group in **5** using different ring closure scaffold hopping strategy would provide novel tricyclic compounds such as **10**-**18** with good mPGES-1 potency (Fig. 2).[36,37] As illustrated in **Figure 2**, tricyclic scaffolds **10-18** were likely to provide structural rigidity, novelty and expected to retain mPGES-1 potency. We also envisioned that removal of pivalamide group on compound **5** expected to provide good CNS penetrable mPGES-1 inhibitors. Therefore, we first synthesized oxazole-fused benzo[*d*]imidazole **10** (IC_{50}: 154.2 nM), which was moderately potent. A switch over to furan-fused benzo[*d*]imidazole **11**(IC_{50}: 22.5 nM) afforded higher intrinsic mPGES-1 potency.[36] Further modification on **11** led to dihydrofuran-fused benzimidazole **12** (IC_{50}: 3.89 nM) and **13** (IC_{50}: 3.32 nM) with significantly improved (6-8 fold) mPGES-1 inhibitory activities.[36] In another modification, pyridine fused tricyclic core **14** and **15** showed in the range of 16-46 nM mPGES-1 potency.[37] Fused pyridine compounds **14** and **15** were not explored further due to poor A549 cell potency.[38] Consequently, to circumvent the above issues, we designed dioxane-fused benzo[*d*]imidazole **16a** (IC_{50}: 8.56 nM) and **17a** (IC_{50}: 11.34 nM) which afforded very good intrinsic mPGES-1 potency.[37] Replacing the central phenyl ring of **17a** to a pyridine ring, afforded compound **18** (IC_{50}: 415.1 nM) which unveil a 37-fold drop in mPGES-1 potency.[37] Henceforth, SAR studies and optimization were solely conducted on leads **16a** and

17a with an initial objective of obtaining compounds with good intrinsic mPGES-1 potency (preferable < 10 nM), A549 cell potency (preferable < 20 nM)[47a] and human whole blood potency (preferable < 500 nM) to select compound for the pre-clinical evaluation.

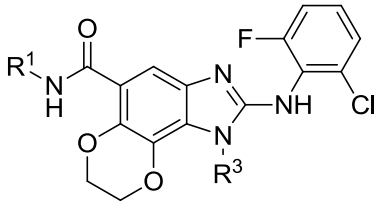


Scheme 1. Reagents and conditions: (a) Br_2, acetic acid, 60-70 ^oC, 18 h, 33%; (b) fuming HNO_3, -50 ^oC, 30 min, 44%; (c) 10% Pd-C, Et_3N, MeOH, H_2, 60 psi, 5 h, 91%; (d) KNO_3, TFA, 0-5 ^oC for 1 h, then rt for 3 h, 69%; (e) Fe, aq. HCl-MeOH, rt, 2 h, 83%; (f) CH_3CN, $FeCl_3$, 70-80 ^oC, 18 h, 30-50%; (g) aq.NaOH, methanol, reflux, 4 h; (h)

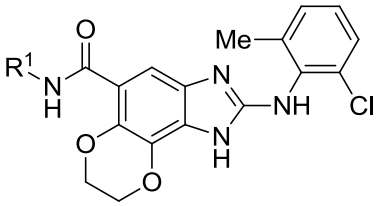
For compounds **16a-f**, **17a-f**, **31a-b** and **40**: carboxylic esters **27**, **28**, **36** and **37** used as starting material (SM): Me_3Al, toluene, rt or 100 ^oC, 12-16 h, 15-40%; (i) For compounds **16g-i**, **17g-j** and **31c**: carboxylic acids **29**, **30** and **38** used as SM: BOP, DIPEA, THF-DCM (1:0.25 ratio), and or DMF-DCM (1:0.25 ratio), rt, 12-18 h, 30-50%; (j) MeI for **32** and EtI for **33**: NaH, THF, rt, 18 h, 40-60%; (k) BBr_3, DCM, rt, 4 h, 32%; (l) MeI, K_2CO_3, DMF, 40 ^oC, 14 h, 20%.

We demonstrate here the design, synthesis, detailed SAR, *in vitro*, *in vivo* PK profiles and an efficacy of selected lead compound in the tricyclic benz[*d*]imidazole series. Compounds tested in this study (**16a-i**, **17a-j**, **31a-c** and **39-41**) were prepared according to Scheme 1. Commercially available dioxane-fused carboxylic ester **19** was brominated with bromine in acetic acid at 60-70 ^oC for 18 h to afford dibromo ester **20** along with ~ 30% carboxylic acid of compound **19** as byproduct[43]. Fuming nitric acid mediated nitration on compound **20** at -50 ^oC provided compound **21**.[43] Compound **21** was further hydrogenated over 10% Pd/C at 60 psi to give de-brominated amine **22**.[44] Subsequently, nitration on **22** yielded **23**, followed by Iron-HCl mediated nitro reduction to give di-amino ester **24**. Condensation of di-amine **24** with 1-chloro-3-fluoro-2-isothiocyanatobenzene **25** and 1-chloro-2-isothiocyanato-3-methylbenzene **26**, individually in the presence of ferric chloride in acetonitrile afforded tricyclic aryl aminoimidazoles **27** and **28**.[45] Next, aryl amides were prepared directly by coupling of esters **27** and **28** with various substituted anilines using trimethylaluminum in toluene,[46] whereas alicyclic amides were synthesized by coupling of the corresponding acids **29** and **30** with alicyclic amines using the standard coupling condition to give **16a-i** and **31a-c** as shown in Scheme 1 and Table 1.[46] On the other hand, alkylation on nitro amine **23** using NaH with MeI and EtI gave N-alkylated derivatives **32** and **33** along with ~ 10-15% of the corresponding N-dialkylated product, which were separated in flash chromatography. Nitro reduction of compounds **32** and **33** afforded diamines **34** and **35**, followed by condensation with 1-chloro-3-fluoro-2-isothiocyanatobenzene **25**[45] provided tricyclic esters **36** and **37**. As described earlier, aryl amines (Table 1) were directly coupled with esters **36** and **37** using trimethylaluminum in toluene, and alicyclic amines were coupled with the corresponding acid **38** using the standard coupling condition to afford tricyclic amide derivatives **17a-j** and **39** (Scheme 1).[46] Furthermore, the dihydroxyimidazole derivative **40** was prepared from **17d** using BBr_3 in DCM, followed by alkylation using K_2CO_3/MeI at 40 ^oC to give methylated product **41** along with some unidentified products. All the synthesized compounds in Scheme 1 were characterized spectroscopically.

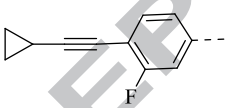
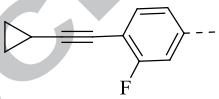
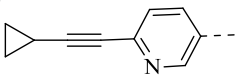
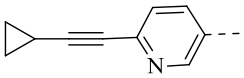
Table 1
SAR of substituted amines at amide position of tricyclic core

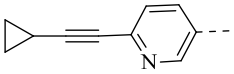


16a-i: R^3 = H
17a-j: R^3 = Me



31a-c

Example	R^1	R^3	mPGES-1 IC_{50} [a, b] (nM)	A549, 2% FBS PGE_2 IC_{50} [a, b] (nM)
16a	3CF_3-Phenyl	H	8.56	41.38
17a	3CF_3-Phenyl	Me	11.34	42.65
16b	4CF_3-Phenyl	H	13.4	38.39
17b	4CF_3-Phenyl	Me	6.59	18.53
16c	3CF_3, 4F-Phenyl	H	9.09	35.07
17c	3CF_3, 4F-Phenyl	Me	7.99	26.69
16d	3CF_3, 5F-Phenyl	H	11.04	14.34
17d	3CF_3, 5F-Phenyl	Me	8.00	16.24
39	3CF_3, 5F-Phenyl	Et	12.53	54.69
16e		H	3.56	16.21
17e		Me	4.19	51.13
16f		H	71.82	-
17f		Me	77.55	-
16g	3CF_3, 5F-Benzyl	H	70.85	-
17g	3CF_3, 5F-Phenyl	Me	49.47	-
16h	4,4-Di-CH_3-Cyclohexyl	H	20.69	64.3
17h	4,4-Di-CH_3-Cyclohexyl	Me	7.20	30.62

16i	Trans-4CF_3-Cyclohexylmethyl	H	103.3	-
17i	Trans-4CF_3-Cyclohexylmethyl	Me	70.96	-
17j	Trans-4CF_3-Cyclohexyl	Me	15.25	65.0
31a	3CF_3, 5F-Phenyl	H	10.41	27.82
31b		H	141.2	-
31c	Trans-4CF_3-Cyclohexyl	H	18.66	128.6
40	--	Me	10.79	> 8000
41	--	Me	14.65	47.38

– : not determined.

[a] MF-63 (**1**) was used as a positive control in this experiments and the in-house enzyme IC_{50} = 1.9 nM and A549 cell IC_{50} = 56 nM. For the literature reported potency of MF-63, see Ref. 22a.

[b] IC_{50} values are derived from graphs plotted with data from a minimum of two experiments in duplicates.

The profound understanding of structure activity relationship (SAR) on earlier scaffolds **10-13**[36] provided enough guidance in the optimization of current scaffolds **16a** and **17a** to afford metabolically stable compounds. Therefore, initial SAR optimization was primarily focused on left-hand side amide modification and central benzimidazole substitution along with 2-chloro-6-fluorophenyl at right-hand side intact to get potent mPGES-1 inhibitors. Accordingly, initial set of compounds 3-trifluoromethylphenyl derived amide **16a** and its N-methylbenzimidazole derivative **17a** exhibited excellent mPGES-1 potency (IC_{50s}: 8.56 nM and 11.34 nM) and good cell potency (A549 IC_{50s}: 41.38 nM and 42.65 nM), respectively. Next, 4-trifluoromethylphenyl derived imidazole amide **16b** exhibited comparable enzyme and cell potency as that of **16a** and **17a**. On the other hand, the corresponding N-methylbenzimidazole analog **17b** gave enhanced enzyme (mPGES-1 IC_{50}: 6.59 nM) and cell potency (A549 IC_{50}: 18.53 nM) relative to **16a** and **17a**. Subsequently, a set of di-substituted benzimidazole amides **16c-d** and its corresponding N-methylbenzimidazoles **17c-d** were tested for enzyme and cell potency. Among this, 3-fluoro-5-trifluoromethylphenyl derived amides **16d** and **17d** exemplified ~ 2-fold improvement in A549 cell potency and similar enzyme potency relative to compounds **16a** and **17a**. Furthermore, the corresponding N-ethylbenzimidazole **39** showed two-fold drop in A549 cell potency albeit having good enzyme potency in comparison with N-methylbenzimidazole **17d**. The poor cell potency of compound **39** as well as N-alkylated benzimidazole SAR of our earlier tricyclic scaffolds **10-13**[36] revealed that only N-methyl substitution on benzimidazole was well tolerated

to afford optimum cell potent compounds.[47a] Since, the bulky groups are well tolerated in the left-hand side of the molecule, bulky 4-cyclopropylethynyl-3-fluorophenyl derived amide **16e** demonstrated comparable enzyme (IC_{50}: 3.56 nM) and significantly improved A549 cell potency (IC_{50}: 16.21 nM) in relation to **16a**. Likewise, the corresponding N-methyl benzyimidazole **17e** revealed 3-fold drop in cell potency in comparison with **16e** albeit having similar enzyme potency. Next, the substituted pyridine and benzyl derived amide compounds **16f-g** and **17f-g** afforded ~ 6 to 9-fold lower mPGES-1 potency, which was quite consistent with our earlier SAR on scaffolds **10-13**.[36] Direct comparison between **16e**, **17e** and **16f**, **17f** revealed that pyridine derivatives **16f** and **17f** exhibited inferior mPGES-1 potency than the corresponding phenyl derivatives (Table 1). Next, we evaluated alicyclic amine based benzimidazole compounds **16h-i** and **17h-j** for mPGES-1 potency. Among this, **16h, 17h** and **17j** demonstrated good mPGES-1 enzyme potency and acceptable A549 cell potency[47a] (Table 1). Further, the replacement of 2-chloro-6-fluorophenyl with 2-chloro-6-methylphenyl at right-hand side of **16d** provided **31a**, which revealed very good enzyme (IC_{50}: 10.41 nM) and cell potency (A549 IC_{50}: 27.82 nM). Conversely, other two left-hand side modified phenyl derivatives **31b-c** did not offer good mPGES-1 potency as illustrated in Table 1. In order to emphasize the importance of rigid tricyclic compounds (example: **17d**) on overall mPGES-1 potency, the corresponding bicyclic compounds, such as **40** (A549 IC_{50}: > 8.0 μM) exhibited inferior cell potency, whereas **41** (A549 IC_{50}: 47.38 nM) afforded moderate cell potency in comparison to **17d**. Overall, small subtle changes in the tricyclic benzimidazole revealed discrepancy in A549 cell potency even though majority of compounds in Table 1 demonstrated consistently good enzyme potency (IC_{50}: < 20 nM) excluding few.[47a]

Table 2
HWB data and *in vitro* ADME profiles of key mPGES-1 compounds

Example	HWB IC_{50} (nM)[a]	Metabolic stability (% remaining)[b]			CYP inhibition at 10 µM concentration[c]					PPB (% bound)[d]	
		HLM	RLM	GPLM	1A2	2D6	3A4	2C9	2C19	H	G. Pig
16c	1127	97.64	99.1	99.9	2.08	41.27	19.63	49.12	34.42	-	-
17c	1388	87.74	61.79	93.94	25.86	77.27	15.38	29.76	33.07	-	-
16d	632.5	98.23	93.82	95.79	n.i	7.00	n.i	12.50	n.i	>99.5	>99.5
[e,f]**17d**	249.9	99.03	92.61	92.90	29.97	55.87	13.41	38.54	34.41	98.71	99.42
17h	835	74.1	28.1	16.1	n.i	9.07	n.i	27.78	12.58	-	-
17j	541.9	51.77	23.96	22.82	n.i	n.i	n.i	39.40	16.94	-	-
16e	277.3	62.01	69.0	69.19	n.i	9.04	12.64	27.07	11.46	>99.5	99.8
31a	959.6	74.17	80.87	77.03	-	-	-	-	-	>99.4	>99.5

[a] MF-63 (**1**) was used as a positive control in this experiments and the in-house HWB IC_{50} = 1.36 µM. For the literature reported HWB potency of MF-63, see Ref. 22a.
[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 and minimum of two experiments in duplicates.
[c] Percentage of test compound remaining after 60 min incubation with liver microsomes (human, rat and guinea Pig) at 37 ^{o}C. MS experiment was conducted in triplicates (see supporting info (SI) for details).
[d] Cytochrome P450 (CYP) % inhibition as compared to control (no inhibitor) and conducted in triplicates (see SI for details).
[e] Plasma protein binding (PPB) was determined using equilibrium dialysis method. 10 µM concentrations were used (see SI for details).
[f] Metabolic stability in other species (% remaining): mouse (88.84); dog (94.03) and cynomolgus monkey (78.62).
[g] CYP 2D6 IC_{50}: 2.5 µM.

Table 3
Oral PK data of **17d** in rat and guinea pig

PK parameters[a]	Rat iv dose[b] 1 mg/kg	Rat oral dose (mg/kg) 10[c]	10[d]	30[d]	100[d]	G.Pig oral dose 100 mg/kg[e]
C_{max} (ng/mL)	-	29.0±5	163±41	598±108	2035±840	676±175
AUC (ng.h/mL	-	149±16	2259±450	8403±1560	23114±6608	10739±2385
T_{max} (h)	-	4	4	8	2	6
Bioavailability (%F)	-	1.5	23	29	24	-
T1/2 (h)	6.2	-	-	-	-	-
CL (mL/min/kg)	18.0±4.5	-	-	-	-	-
Vz (L/kg)	10.24±5.9	-	-	-	-	-
CNS b/p ratio	0.96	-	-	-	-	1.75[f]

- : not applicable.
[a] C_{max}, AUC_{0-24}, T_{max} and bioavailability (%F), Clearance (CL), volume of distribution (Vz) were determined in male Sprague-Dawley rats.
[b] *Vehicle for i.v (rat) - 20% NMP+20% Ethanol+60% PEG 200).*The data represented is mean ± SD (n = 3).
[c] *Vehicle for Oral dosing - 0.5% methylcellulose MC suspension.* The data represented is mean ± SD (n = 3).
[d] *Vehicle for solubilized formulation (rat) - 0.5% methylcellulose suspension. Vehicle for i.v - 20% NMP+20% Ethanol+60% PEG 200).*The data represented is mean ± SD (n = 3).
[e] *Vehicle for solubilized formulation (guinea pig) – 20% capmul+32% PG+32% labrasol+2% vitamin E+water.* The data represented is mean ± SD (n = 3).
[f] Guinea pig *i.v* dose - 1 mg/kg.

Additionally, compounds having good enzyme and A549 cellular potencies (≤ 40 nM)[47a] from Table 1 were further assessed for human whole blood (HWB) potency and *in vitro* pharmacokinetic profile (metabolic stability, cytochrome P450 inhibition and plasma protein binding).[47b] Among these, **17d, 17j** and **16e** exemplified good human whole blood IC_{50} in the range of 250 to 550 nM and all other cell potent compounds (**16c**, **17c**, **16d**, **17h** and **31a**) revealed HWB potency with IC_{50} range of 600-1400 nM as shown in Table 2. All these analogs were further evaluated for their metabolic stability (human, rat and guinea pig liver microsome assay), cytochrome P450 (CYP) inhibition and plasma protein binding (PPB) studies, respectively.[47b] As presented in Table 2, most of the selected compounds showed good metabolic stability in liver microsomes excluding alicyclic amide derivatives **17h** and **17j**. Alicyclic amides **17h** and **17j** displayed very poor metabolic stability in rodents (< 30% remaining after 60 mins incubation in rat and guinea pig liver microsomes), even though they possessed adequate human

metabolic stability and HWB potency. All the selected mPGES-1 inhibitors in Table 2 exhibited no significant inhibition against five major isoforms of the cytochrome P450 enzymes (1A2, 2D6, 3A4, 2C9 and 2C19) except **17d**, which revealed negligible CYP2D6 activity (IC_{50}: 2.58 µM). Furthermore, **17d** displayed good metabolic stability in mouse, dog and cynomolgus monkey liver microsomes (> 75% remaining after 60 mins incubation). The plasma protein binding (PPB) of key analogs **16d-e**, **17d** and **31a** were assessed in human and guinea pig plasma and found to have high protein binding (> 98%) in both species. After closely examining the HWB data, *in vitro* PK data, chemical structure of leads **17d** and **16e**, we chose compound **17d** for further profiling.[48] Therefore, the chosen lead compound **17d** was further evaluated for oral pharmacokinetic study in rat and in guinea pig and the results were summarized in Table 3. The initial rat PK data of **17d** in methylcellulose suspension (10 mg/kg) exhibited very poor systemic exposure upon oral administration and poor bioavailability (%F). The high intrinsic lipophilicity of **17d**[49] may have contributed to poor aqueous solubility, which led to poor oral PK. In order to overcome poor pharmacokinetics, oral PK of **17d** was further conducted in rat using a solubilized formulation (Table 3), which exhibited gradual enhancement of C_{max}, mean AUC and oral bioavailability (%F) in dose range (10, 30 and 100 mg/kg) studies. Additionally, **17d** upon intravenous (i.v) administration revealed moderate plasma clearance (Cl = 18 mL/min/kg), large volume of distribution (V_z = 10.24 L/kg), good $T_{1/2}$ (6.2 h) and adequate CNS brain penetration (rat B/P ratio: 0.96), respectively. On the other hand, the oral PK of **17d** in guinea pig (100 mg/kg) displayed 3-fold lower C_{max} and 1-fold lower mean AUC in comparison with 100 mg/kg rat oral dose. Likewise, 1 mg i.v administration of **17d** exemplified good CNS brain penetration in guinea pig and the brain to plasma (B/P) ratio was found to be 1.75, respectively (Table 3).

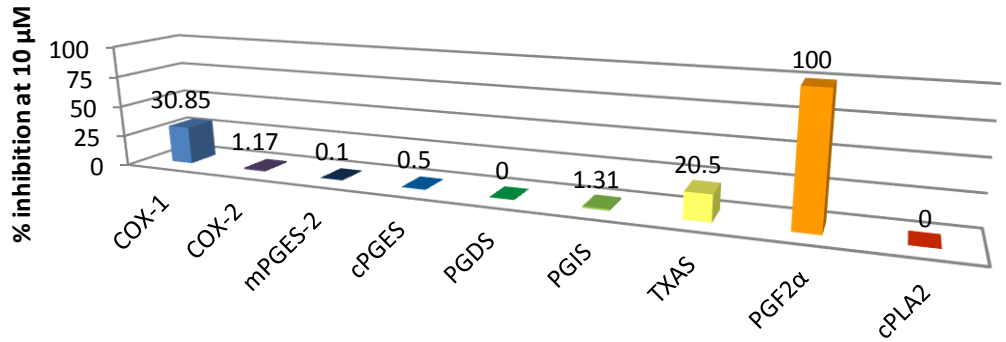


Figure 3. COX-1/2 and Prostanoid synthases selectivity of **17d** (% inhibition values are means of at least two experiments in duplicates, see SI for experimental details).

Table 4
Human, rodents (guinea pig, rat, mouse) and dog mPGES-1 potency of **17d**

Species	mPGES-1 inhibition IC_{50}[a] (nM)	A549, 2% FBS PGE_2 IC_{50}[a] (nM)	WB IC_{50} (nM)[b] or % inh.@ 10μM
Human	8.0	16.24	249.9
G. Pig	10.79	ND	380.9
Rat	>10,000	-	-
Mouse	>10,000	-	-
Dog	-	-	48.5%

– : not determined.
[a] IC_{50} values are derived from graphs plotted with data from a minimum of two experiments in duplicates.
[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 and minimum of two experiments in duplicates. Same positive control was used here as in the Table 1.

Furthermore, the lead compound **17d** was profiled in a battery of *in vitro* and cellular assays[47a,b] to evaluate its selectivity over other prostanoid synthases. As illustrated in Figure 3, **17d** demonstrated good selectivity (approximately > 1000 fold) over COX-1, COX-2, mPGES-2, cPGES, PGD_2, PGI_2, and TXA_2 synthases. An elevated level of $PGF_{2\alpha}$ (100% inhibition @ 10 μM test concentration, which translated to IC_{50} of 1.90 μM) observed was attributable to probable shunting of PGH_2, as professed with MF-63[21] and PF-9184.[24] Also, **17d** did not inhibit cytosolic phospholipase A_2 ($cPLA_2$), an upstream of arachidonic acid (AA) pathway (Fig. 3). Since many of known mPGES-1 inhibitors were active against human enzyme and not against rodents, current lead **17d** was further evaluated for rodent (rat, mouse and guinea pig) enzyme potency and dog whole blood potency (Table 4).[47b] As foreseen, our lead **17d** did not show any mPGES-1 inhibitory activity in rodents (rat and mouse) as illustrated in the literature.[21,24,50] In contrast to rat and mouse, **17d** displayed a significant mPGES-1 enzyme (IC_{50}: 10.79 nM) and LPS induced whole blood potency (IC_{50}: 380.9 nM) in guinea pig. Surprisingly, dog exemplified only modest inhibition of PGE_2 release in the LPS induced dog whole blood assay (Table 4).

Moreover, **17d** displayed low to moderate activity in the hERG channel (patch clamp assay, 17% and 46% inhibition at 1 and 10 μM test concentration),[51] suggesting a lower probability of QT prolongation effect in human. Likewise, **17d** did not reveal any significant off-target liabilities based on the testing against broad panel of receptors, ion channel binding and enzyme activity assay.

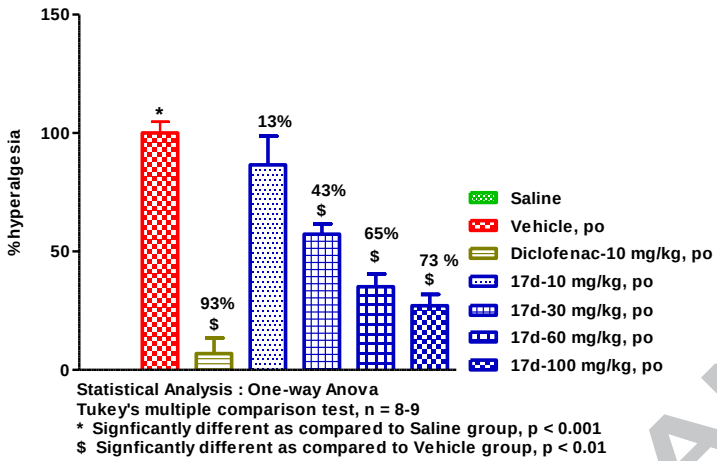


Figure 4. Analgesic effects of **17d** in the guinea pig hyperalgesia pain model. Data are expressed as percentage of hyperalgesia, with the naive group (injected intraplantarly with saline) as 0% and the vehicle-treated LPS-injected group as 100% (Results are shown as mean ± SEM, n = 8-9 animals per dose group).

Table 5

Plasma and CSF concentration of **17d** at PD time point

Dose (mg/kg p.o)	Plasma concentration @PD time point (nM)[a]	CSF concentration @ PD time point (nM)[a]
10	425	0.62
30	1054	2.00
60	2162	2.32
100	2867	13.12

[a] Concentrations are means of n = 8-9 animals per dose group. Study protocol is provided in the SI.

Having achieved good mPGES-1 potency in human and guinea pig (enzyme, cell and human whole blood potency), metabolic stability, cytochrome P450 selectivity against major five isoforms, selectivity against many prostanoid synthases, favorable off-target selectivity and

reasonable pharmacokinetic profile in rat and guinea pig, the advanced lead **17d** was further evaluated for *in vivo* efficacy in the LPS-induced thermal hyperalgesia pain model (acute study). In order to understand the efficacy of lead **17d**, it was studied over wide range of doses (10, 30, 60 and 100 mg/kg) in the guinea pig hyperalgesia pain model and the results were summarized in Figure 4 and Table 5. Injection of LPS into the plantar caused a significant increase in thermal hyperalgesic response compared to saline injected animals. When administered orally at 1 h before LPS injection, the standard NSAID pain drug, diclofenac demonstrated 93% inhibition of hyperalgesic response at 10 mg/kg dose (Fig. 4). On the other hand, our lead **17d** significantly inhibited hyperalgesic response in a dose-dependent manner with maximum inhibition of 73% at 100 mg/kg. However, lower doses 10 and 30 mg/kg inhibited < 50% of hyperalgesic response in comparison with diclofenac (Fig. 4). The calculated ED_{50} in the dose range efficacy study was found to be 36.7 mg/kg, respectively. As depicted in Table 5, the plasma concentration at pharmacodynamics (PD) time point of **17d** at 10 mg/kg was at par with guinea pig whole blood potency (WB IC_{50}: 380.9 nM), while CSF concentration (0.62 nM) was well below their guinea pig mPGES-1 potency (IC_{50}: 10.79 nM). Nevertheless, gradual increase in doses (30 and 60 mg/kg) ensued roughly 3 to 5-fold higher plasma concentration and 4-fold higher CSF concentration as compared to initial 10 mg/kg dose. The plasma and CSF concentration was significantly enhanced at 100 mg/kg and the obtained plasma concentration was 7-fold above over guinea pig whole blood potency and the corresponding brain CSF level was at par with guinea pig mPGES-1 potency. The thermal hyperalgesic response in our experiment was believed to be mediated by both peripheral and central PGE_2 inhibition.[21,53,54]

In summary, we have described the synthesis and SAR evaluation of a novel series of dioxane-fused benzo[*d*]imidazole amides as mPGES-1 inhibitors, based on scaffold-hopping approach from the known compound **5**. SAR optimization afforded many potent mPGES-1 inhibitors and compounds **16d**, **17d**, **17h**, **17j**, **16e** and **31a** were further selected for evaluating human whole blood potency based on the good enzyme and cell potency along with minimum inhibition of five major cytochrome P450 isoforms. Among these, **17d** was identified as pre-clinical lead based on favorable human whole blood potency (HWB) and good metabolic stability in across species. The identified lead **17d** demonstrated excellent *in vitro* selectivity (mPGES-2, COX-1 and COX-2), prostanoid selectivity and minimal off-target activity in CEREP panel and acceptable hERG activity, apart from good pharmacokinetic profile.

Furthermore, the *in vivo* efficacy of **17d** displayed a significant inhibition of LPS-induced hyperalgesic response in a dose range study. Consequently, **17d** was selected for pre-clinical toxicology study and will be further evaluated for OA and RA pain model.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at

References and notes

1. Murakami, M.; kudo, I. *Prog. Lipid Res*. **2004**, 43, 3.
2. Kamei D.; Yamakawa, K.; Takegoshi, Y.; Mikami-Nakanishi, M.; Nakatani, Y.; Oh-ishi, S.; Yasui, H.; Azuma, Y.; Hirasawa, N.; Ohuchi, K.; Kawaguchi, H.; Ishikawa, Y.; Ishii, T.; Uematsu, S.; Akira, S.; Murakami, M.; Kudo, I. *J. Biol. Chem*. **2004**, 279, 33684.
3. Cheng, Y.; Wang, M.; Yu, Y.; Lawson, J.; Funk, C. D.; Fitzgerald, G. A. *J. Clin. Invest.* **2006**, 116, 1391.
4. Korotkova, M.; Jakobsson, P.-J. *Basic Clin. Pharmacol. Toxicol.* **2014**, 114, 64.
5. Samuelsson, B.; Morgenstern, R.; Jakobsson, P. *J. Pharmacol. Rev*. **2007**, 59, 207.
6. Jachak, S. M. *Curr Opin. Invest. Drugs.* **2007**, 8, 411.
7. Chang, H. H.; Meuillet, E. J. *Future Med. Chem*. **2011**, 3, 1909.
8. Murakami, M.; Nakatani, Y.; Tanioka, T.; Kudo, I. *Prostaglandins Other Lipid Mediat.* **2002**, 68-69, 383.
9. Jakobsson, P. J.; Thoren, S.; Morgenstern, R.; Samuelsson, B. *Proc.Natl. Acad. Sci. U.S.A.* **1999**, 96, 7220.
10. Filion, F.; Bouchard, N.; Goff, A. K.; Lussier, J. G.; Sirois, J. *J. Biol.Chem.* **2001**, 276, 34323.
11. Kamei, D.; Murakami, M.; Nakatani, Y.; Ishikawa, Y.; Ishii, T.; Kudo, I. *J. Biol. Chem.* **2003**, 278, 19396.
12. 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.
13. Jania, L. A.; Chandrasekharan, S.; Backlund, M. G.; Foley, N. A.; Snouwaert, J.; Wang, I. M.; Clark, P.; Audoly, L. P.; Koller, B. H. *Prostaglandins Other Lipid Mediat*. **2008**, 88, 73.
14. Nakatani, Y.; Hokonohara, Y.; Kakuta, S.; Sudo, K.; Iwakura, Y.; Kudo, I. *Biochem Biophys Res Commun*. **2007**, 362, 387.
15. Wang, M.; Lee, E.; Song, W.; Ricciotti E.; Rader, D. J.; Lawson, J. A. Pure, E.; FitzGerald, G. A. *Circulation* **2008**, 117, 1302.
16. (a) Grosser, T.; Fries, S.; FitzGerald, G. A. *J Clin Invest*. **2006**, 116, 4; (b) Wang, M.; Song, W. L.; Cheng, Y.; Fitzgerald, G. A. *J. Intern. Med*. **2008**, 263, 500; (c) Marnett, L. *J. Annu. Rev. Pharmacol. Toxicol.* **2009**, 49, 265.
17. Harris, R. C.; Breyer, M. D. *Clin J Am Soc. Nephrol*. **2006**, 1, 236.
18. Marcus, A. J.; Broekman, M. J.; Pinsky, D. J. *New. Engl. J Med.* **2002**, 347, 1025.
19. Serhan, C. N.; Levy, B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, 100, 8609.

20. Jachak, S. M. *Curr. Opin. Invest. Drugs.* **2007**, *8*, 411.
21. Xu, D.; Rowland. S. E.; Clark, P.; Giroux, A.; Cote, B.; Guiral, S.; Salem, M.; Ducharme, Y.; Friesen, R.W.; Méthot, N.; Mancini, J.; Audoly, L.; Riendeau, D. *J. Pharmacol Exp. Ther.* **2008**, *326, 754.*
22. (a) Cote, B.; Boulet, L.; Brideau, C.; Claveau, D.; Ethier, D.; Frenette, R.; Gagnon, M.; Giroux, A.; Guay, J.; Guiral, S.; Mancini, J.; Martins, E.; Massé, F.; Methot, N.; Riendeau, D.; Rubin, J.; Xu, D.; Yu, H.; Ducharme, Y.; Friesen, R.W. *Bioorg. Med. Chem. Let.* **2007**, 17, 6816; (b) MF-63 was designed and derived from Merck's JAK kinase inhibitor program and has some kinase selectivity. Check references 21, 22a and further cited therein for details.
23. Wannberg, J.; Alterman, M.; Malm, J. Piperidinyl benzoimidazole derivatives as MPGES-1 inhibitors. PCT Intl. Appl. WO/2012/117062, 2012.
24. (a) Arhancet, G. B.; Walker, D. P.; Metz, S.; Fobian, Y. M.; Heasley, S. E.; Carter, J. S.; Springer, J. R.; Jones, D. E.; Hayes, M. J.; Shaffer, A. F.; Jerome, G. M.; Baratta, M. T.; Zweifel, B.; Moore, W. M.; Masferrer, J. L.; Vazquez, M. L. *Bioorg. Med. Chem. Lett.* **2013**, 23, 1114; (b) Walker, D. P.; Arhancet, G. B.; Lu, H-F.; Heasley, S. E.; Metz, S.; kablaoui, N. M.; Franco, F. M.; hanau, C. E.; Scholten, J. A.; Springer, J. R.; Fobian, Y. M.; Carter, J. S.; Xing, L.; Yang, S.; Shaffer, A. F.; Jerome, G. M.; Baratta, M. T.; Moore, W. M.; Vazquez, M. L. *Bioorg. Med. Chem. Lett.* **2013**, 23, 1120.
25. (a) Schiffler, M. A.; Antonysamy, S.; Bhattachar, S. N.; Campanale, K. M.; Chandrasekhar, S.; Condon, B.; Desai, P. V.; Fisher, M. J.; Groshong, C.; Harvey, A.; Hickey, M. J.; Hughes, N. E.; Jones, S. A.; Kim, E. J.; Kuklish, S. L.; Luz, J. G.; Norman, B. H.; Rathmell, R. E.; Rizzo, J. R.; Seng, T. w.; Thibodeaux, S. J.; Woods, T. A.; York, J. S.; Yu, X-P. *J. Med. Chem.* **2016**, 59, 194; (b) Kuklish, S. L.; Schiffler, M. A.; York, J. S. Novel imidazole-2-benzamide compounds useful for the treatment of osteoarthritis. PCT Intl. Appl. WO/2012/087771, 2012.
26. Hughes, N. E.; Woods, T. A.; Norma, B. H. Novel imidazole derivatives useful for the treatment of arthiritis. PCT Intl. Appl. WO/2012/161965, 2012.
27. Priepke, H.; Doods, H.; Kuelzer, R.; Pfau, R.; Stenkamp, D.; Pelcman, B.; Roenn, R.New compounds. PCT Intl. Appl. WO/2012/022793, 2012.
28. Pfau, R.; Arndt, K.; Doods, H.; Klinder, K.; Kuelzer, R.; Lubriks, D.; Mack, J.; Pelcman, B.; Priepke, H.; Roenn, R.; Stenkamp, D.; Suna, E. 3H-Imidazo[4,5-c]pyridine-6-carboxamides as anti-inflammatory agents. PCT Intl. Appl. WO/2010/100249, 2010.
29. (a) Shiro, T.; Takahashi, H.; Kakiguchi, K.; Inoue, Y.; Masuda, K.; Nagata, H.; Tobe, M. *Bioorg. Med. Chem. Lett.* **2013**, 22, 285; (b) Shiro, T.; Kakiguchi, K.; Takahashi, H.; Nagata, H.; Tobe, M. *Bioorg. Med. Chem.* **2013**, 21, 2868.
30. Bylund, J.; Ek, M. E.; Holenz, J.; Kers, A.; Ohberg, L. Bis-(Sulfonylamino) derivatives for Treatment of Pain and Inflammation. PCT Intl. Appl. WO/2010/ 132016, 2010.
31. Bylund, J.; Annas, A.; Hellgren, D.; Bjurstrom, S.; Andersson, H.; Syanhagen, A. *Drug Metab. Dispos*. **2013**, 41, 634.
32. Banerjee, A.; Pawar, M.Y.; Patil, S.; Yadav, P. S.; Kadam, P. A.; Kattige, V. G.; Deshpande, D. S.; Pednekar, P. V.; Pisat, M. K.; Gharat, L. A. *Bioorg. Med. Chem. Let.* **2014**, 24, 4838.
33. Muthukaman, N.; Tambe, M.; Shaikh, M.; Pisal, D.; Deshmukh, S.; Tondlekar, S.; Sarode, N.; Narayana, L.; Gajera, J. M.; Kattige, V. G.; Honnegowda, S.; Karande, V.; Kulkarni, A.; Jadhav, S.; Behera, D.; Gudi, G. S.; Joshi, N. K.; Gharat, L. A. *Bioorg. Med. Chem. Let.* **2016**, communicated.
34. (a) Wang, J.; Limburg, D.; Carter, J.; Mbalaviele, G.; Gierse, J.; Vazquez, M. *Bioorg. Med. Chem. Let.* **2010**, 20, 1604; (b) Hamza, A.; Xinyun, Z.; Tong, M.; Tai, H-H.; Zhan, C-G. *Bioorg. Med. Chem*. **2011**, 19, 6077; (c) Giroux, A.; Boulet, L.; Brideau, C.; Chau, A.; Claveau, D.; Cote, B.; Ethier, D.; Frenette, R.; Gagnon, M.; Guay, J.; Gujral, S.; Mancini, J.; martins, E.; Masse, F.; Methot, N.; Riendeau, D.; Rubin, J.; Xu, D.; Yu, H.; Ducharme, Y.; Friesen, R. *Bioorg. Med. Chem. Lett*, **2009**, 19, 5837.

35. (a) Chiasson, J-F.; Boulet, L.; Brideau, C.; Chau, A.; Claveau, D.; Cote, B.; Ethier, D.; Giroux, A.; Guay, J.; Guiral, S.; Mancini, J.; Masse, F.; Methot, N.; Riendeau, D.; Roy, P.; Rubin, J.; Xu, H.; Yu, H.; Ducharme, Y.; Friesen, R. W. *Bioorg. Med. Chem. Lett*, **2011**, 21; 1488; (b) Wang, J.; Limburg, D.; Carter, J.; Mbalaviele, G.; Gierse, J.; Vazquez, M. *Bioorg. Med. Chem. Let.* **2010**, 20; 1604; (c) Hieke, M.; Greiner, C.; Thieme, T. M.; Schubert-Zsilavecz, M.; Werz, O.; Zettl, H. *Bioorg. Med. Chem*. **2011**, 21, 1329; (d) Koeberle, A; Laufer, S. A.; Werz, O. *J. Med. Chem.* **2016**, 59, 5970; (e) Luz, J. G.; Antonysamy, S.; Kuklish, S. L.; Condon, B.; Lee, M. R.; Allison, D.; Yu, X-P.; Chandrasekhar, S.; Backer, R.; Zhang, A.; Russell, M.; Chang, S. S.; Harvey, A.; Sloan, A. V.; Fisher, M. J. *J. Med. Chem.* **2015**, 58, 4727.
36. Gharat, L. A.; Muthukaman, N.; Narayana, L.; Khairatkar-Joshi, N.; Kattige, V. G. Tricyclic compounds as mPGES-1 inhibitors. PCT Intl. Appl. WO/2012/055995, 2012.
37. Gharat, L. A.; Muthukaman, N.; Deshmukh, S.; Khairatkar-Joshi, N.; Kattige, V. G. Tricyclic compounds as mPGES-1 inhibitors. PCT Intl. Appl. WO/2013/153535, 2013.
38. Compounds **14** (A549 IC_{50}: 43.1 nM) and **15** (A549 IC_{50}: 153.2 nM) had moderate to poor PGE_2 release cell potency and most of the synthesized pyridine-fused derivatives exhibited solubility problem during *in vitro* experiments.
39. Choi, E. B.; Lee, H. K.; Lee, E. S.; Pak, C. S.; Shin, D. K.; Bank, M. Y.; Ro, S.; Cho, J. M. Novel Benzimidazole compound, preparation method thereof and pharmaceutical composition comprising the same. PCT Intl. Appl. WO/2011/099832, 2011.
40. Priepke, H.; Doods, h.; Heim-Riether, A.; Kuelzer, R.; Pfau, R.; Rudolf, K.; Stenkamp, D. 6-Amino-2-Phenylamino-1H-Benzimidazole-5-carboxamide derivatives and their use as Microsomal Prostaglandin E2 Synthase-1 inhibitors. PCT Intl. Appl. WO/2012/076673, 2012.
41. Gharat, L. A.; Gajera, J. M.; Narayana, L.; Khairatkar-Joshi, N.; Kattige, V. G. Tricyclic compounds as mPGES-1 inhibitors. PCT Intl. Appl. WO/2012/110860, 2012.
42. Gharat, L. A.; Banerjee, A.; Pawar, M. Y.; Khairatkar-Joshi, N.; Kattige, V. G. Substituted Bicyclic Heteroaryl compounds as mPGES-1 Inhibitors. PCT Intl. Appl. WO/2013/038308, 2013.
43. (a) Kowalczyk, B. A.; Roberts, P. N.; McEwen , G. K.; Robinson III, J. *Org. Process Res. Dev.* **1997**, 1, 355; (b) Kowalczyk, B. A.; Robinson III, J.; Gardner, J. O. *Org. Process Res. Dev.* **2001**, 5, 116.
44. One-pot nitro reduction and de-bromination with Pd/C on **21** carried out in the presence of triethylamine (basic condition). Hydrogenation in the absence of base gave a mixture of compounds **22** and **19** (~1:2 ratios).
45. (a) Yoshiizumi, K.; Seko, N.; Nishimura, N.; Ikeda, S.; Yoshino, K.; Kondo, H.; Tanizawa, K. *Bioorg. Med. Chem. Let.* **1998**, 8, 3397; (b) Khatik, G. L.; Pal, A.; Mobin, S. M.; Nair, V. A. *Tetrahedron Lett*. **2010**, 51, 3654; (c) Pak, C. S.; Youn, I. K.; Lee, Y. S. *Synthesis*. **1982**, 969.
46. Most of the aryl amines were directly coupled with tricyclic carboxylic esters **27**, **28**, **36** and **37** as detailed in Scheme 1. Alicyclic amines were coupled with tricyclic carboxylic acids **29**, **30** and **38** using the standard coupling conditions.
47. (a) The mPGES-1 enzyme assay evaluates the specific interaction of the compound with mPGES-1 target whereas A549 cell assay evaluates the compound's permeability and provides crucial information about its downstream effects. There is abundant literature to show that upon IL-1β stimulation, mPGES-1 overexpression and increased release of pro-inflammatory mediator PGE_2 occurs in A549 cells. Hence this assay is a standard assay to study the anti-inflammatory effects of mPGES-1 inhibitors. For further details, see: (a) Sweeney, F. J.; Wachtmann, T. S.; Eskra, J. D.; Verdries, K. A.; Lambalot, R. H.; Carty, T. J.; Perez, J. R.; Audoly, L. P. *Mol. Cell. Endocrinol*. **2003**, 205, 151; (b) Friesen, R. W.; mancini, J. A. *J. Med. Chem*, **2008**, 51, 4059. (b) See supporting information for in vitro, in vivo pharmacology, in vitro pharmacokinetics and oral PK experimental details.
48. Ethynylcyclopropane-linked compound **16e** likely to exhibit nephrotoxicity during the compound development stage. This was evidenced from ethynylcyclopropane containing drug Efavirenz (NNRT inhibitor), which displayed nephrotoxicity in rat. For the related reference, see: Mutlib,

A. E.; Gerson, R. J.; Meunier, P. C.; Haley, P. J.; Chen, H.; Gan, L. S.; Davies, M. H.; Gemzik, B.; Christ, D. D.; Krahn, D. F.; Markwalder, J. A.; Seitz, S. P.; Robertson, R. T.; Miwa, G. T. *Toxicol. Appl. Pharmacol*, **2000**, 169, 102.

49. The calculated Log D and PSA of compound **17d** was found to be 5.05±1.43 and 77.41, respectively. Log P, Log D and PSA were calculated using ACD product version 12.5 at pH = 7.4.
50. Pawelzik, S-C.; Uda, N. R.; Spahiu, L.; Jegerschold, C.; Stenberg, P.; Hebert, H.; Morgenstern, R.; Jakobsson, P-J. *J. Biol. Chem*. **2010**, 285, 29254.
51. hERG channel assay was carried out at Eurofins labs-France (previously known as Cerep SA, France)- a pharma discovery services CRO (www.eurofins.com).
52. In CEREP safety screen-44 assay (binding assay), compound **17d** inhibited benzodiazepine (BzD)-peripheral (63.2%@ 10 µM), A3 (80.2%@ 10 µM) and Cl^- channel (65%@ 10 µM) activity only. POR study was conducted at Eurofins labs-France (previously known as Cerep SA, France; www.eurofins.com).
53. Yaksh, T. L.; Kokotos, G.; Svensson, C. I.; Stephens, D.; Kokotos, C. G.; Fitzsimmons, B.; Hadjipavlou-Litina, D.; Hua, X. Y.; Dennis, E. A. *J. Pharmacol Exp. Ther.* **2006**, 316, 466.
54. Futaki, D. N.; Harada, M.; Sugimoto, M.; Hashimoto, Y.; Honma, Y.; Arai, I.; Nakaike, S.; Hoshi, K. *J. Pharm and Pharmacol*. **2009**, 61, 607.