Bioorganic & Medicinal Chemistry Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Alleviating CYP and hERG liabilities by structure optimization of dihydrofuran-fused tricyclic benzo[*d*]imidazole series – Potent, selective and orally efficacious microsomal prostaglandin E synthase-1 (mPGES-1) inhibitors: Part-2

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ARTICLE INFO

Article history:
Received 17 January 2018
Revised 26 February 2018
Accepted 26 February 2018
Available online xxxx

Keywords:
Rheumatoid arthritis
Bioavailability
Lipophilicity
PGE_2
Thromboxane A
Hyperalgesia
Osteoarthritic pain

ABSTRACT

In an effort to identify CYP and hERG clean mPGES-1 inhibitors from the dihydrofuran-fused tricyclic benzo[*d*]imidazole series lead **7**, an extensive structure-activity relationship (SAR) studies were performed. Optimization of A, D and E-rings in **7** afforded many potent compounds with human whole blood potency in the range of 160–950 nM. Selected inhibitors **21d**, **21j**, **21m**, **21n**, **21p** and **22b** provided selectivity against COX-enzymes and mPGES-1 isoforms (mPGES-2 and cPGES) along with sufficient selectivity against prostanoid synthases. Most of the tested analogs demonstrated required metabolic stability in liver microsomes, low hERG and CYP liability. Oral pharmacokinetics and bioavailability of lead compounds **21j**, **21m** and **21p** are discussed in multiple species like rat, guinea pig, dog, and cynomolgus monkey. Besides, these compounds revealed low to moderate activity against human pregnane X receptor (hPXR). The selected lead **21j** further demonstrated *in vivo* efficacy in acute hyperalgesia (ED_{50}: 39.6 mg/kg) and MIA-induced osteoarthritic pain models (ED_{50}: 106 mg/kg).

Microsomal PGE synthase-1 (mPGES-1) is a dominant source of biologically active PGE_2, during the biosynthesis in the downstream of the COXs in arachidonic acid (AA) pathway. PGES is a terminal enzyme, which is classified into three isoforms, namely microsomal PGES-1 (mPGES-1), microsomal PGES-2 (mPGES-2) and cytosolic PGES (cPGES).[1] The role of prostaglandins (PGs) in inflammatory pain is well established. Binding of PGs to prostanoid receptors (EP1, EP2, EP3 and EP4) sensitizes pain specific neurons to stimulate pain in central nociceptive systems and mPGES-1 expression was strongly up-regulated in the brain and spinal cord during inflammation.[2] An inducible enzyme mPGES-1, which is functionally coupled to COX-2, is responsible for the release of PGE_2 in response to inflammatory stimuli, such as IL-1β, TNF-α, and LPS. A previous study by Akira group has shown that PGE_2 production by LPS is completely suppressed in peritoneal macrophages derived from mPGES-1 knockout mice.[3] This enabled the use of mPGES-1 knockout mice as models for various diseases, such as collagen induced arthritis, pain hypersensitivity and neuropathic pain.[4] An mPGES-1 knockout study in mice exhibits rediversion of the PGH_2 substrate by a PG synthases into several prostanoids ($PGF_{2\alpha}$, PGD_2 and PGI_2), including PGE_2 and thromboxane A_2 (TXA_2).[4] Therefore, it is expected that mPGES-1 inhibitors may not increase the risk of cardiovascular side effects associated with COX-2 inhibitor, as they do not inhibit PGI_2 production.[5] Similarly, additional knockout studies demonstrated devoid of gastrointestinal and renal toxicity, which are associated with COX-1 inhibitors.[6] Further, these knockout animals revealed viable, fertile, and normal phenotype, which signifies that mPGES-1 inhibitors could possess anti-inflammatory potential with minimum or negligible side effect profile.[4] Therefore, selective inhibition of mPGES-1 might be a promising approach for the design of an effective anti-inflammatory drugs lacking NSAID related side effects.[7]

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https://doi.org/10.1016/j.bmcl.2018.02.048
0960-894X/

Although development of novel mPGES-1 inhibitors has received great attention recently, numerous inhibitors with variety of chemo types are reported with only general SAR[8] and the available *in vivo* efficacy data is still limited to very few compounds in the literature. Some notable examples are, MF-63 (**1**) from Merck,[9] pyridine-3-carboxamide (**2**) from Eli Lilly[10] and PF-4693627 (**3**) from Pfizer[11] demonstrated *in vivo* efficacy in guinea pig hyperalgesia model (Fig. 1). As well, we have recently reported *in vivo* efficacy to few potent mPGES-1 inhibitors, such as quinazolinone (**4**), aminobenzimidazole-5-carboxamide (**5**) and 1,4-dihydrochromeno[3,4-*d*]imidazole (**6**) in hyperalgesia pain model.[12,13] Further, mPGES-1 inhibitors from Eli Lilly (LY-3023703)[14] and our group (GRC27864)[15] have completed Ph-I clinical trials for the treatment of pain and an inflammatory diseases.

In the preceding communication,[16] the design, synthesis and initial SAR (structure-activity relationship) optimization of potent dihydrofuran-fused benz[*d*]imidazole series, as exemplified by lead **7** was described (Fig. 2). The mPGES-1 lead **7** and its analogs were highly potent both in human and guinea pig enzymes, cell permeable, selective against COX-enzymes with adequate PGE_2 release human whole blood potency. In addition, it had adequate brain penetration, orally bioavailable and was efficacious in animal models of pain. However, compound **7** and other analogs from this series suffered cytochrome P450 (CYP) enzyme (CYP3A4, CYP2C9 and CYP2C19) and hERG liability, which was expected to cause adverse drug-drug interaction (DDI)[17] and potential QTc prolongation safety issues[18] if taken to clinical development. Overall, analogs from this series with nitrogen incorporated in the E-ring exhibited higher CYP3A4 liability, whereas compounds with *meta*- and *para*-substituted phenyl as A-ring revealed CYP2C9 and CYP2C19 liabilities.[16a] Further, compounds with substituted pyridine as A-ring tested so far had shown decreased enzyme, cell and human whole blood potency along with moderate to low metabolic stability.[16a] Therefore, additional SAR optimization of lead **7** is warranted in order to completely eliminate CYP and hERG liabilities for further development.

In this manuscript, we describe the synthesis and structure-activity relationship (SAR) optimization based on lead **7**[16a] to afford mPGES-1 inhibitors with low CYP and hERG liability without altering the core scaffold. Therefore, we focused our attention on the A, D and E-rings of lead **7** for optimization (Fig. 2). It is well-known in the literature that reducing lipophilicity (or increasing polarity) and disturbing the geometry

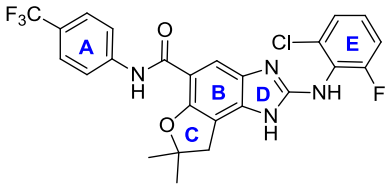


Fig. 2. mPGES-1 lead **7**. IC_{50}: 3.9 nM; A549 cell IC_{50}: 10.4 nM. Human whole blood IC_{50}: 275 nM. Guinea Pig whole blood IC_{50}: 222 nM. CYP liability: 2C9, 2C19 (>50% inhibition @10 μM). hERG liability: 56% inhibition @10 μM.

of molecule while performing lead optimization would provide compounds with reduced hERG and CYP liability.[19] With this hypothesis, we initially synthesized two compounds by introducing 2, 6-difluorophenyl (**21a**) and 2-trifluoromethylphenyl (**21b**) as A-ring along with 2, 6-dichlorophenyl group as E-ring without any modification done on the other rings (Table 1). The biological activity of **21a** and **21b** revealed comparable mPGES-1 enzyme and cell potency (IC_{50}s: <15 nM) similar to earlier lead **7** along with human whole blood potency (HWB IC_{50}s: 660 nM for **21a** and 506 nM for **21b**) similar to COX-2 inhibitor, Celecoxib (HWB IC_{50}: 540 nM).[8c,20] After having comparable human whole blood potency to Celecoxib, compounds **21a** and **21b** were further evaluated for CYP inhibition and unveiled low liability (<50%@ 10 μM) against five major CYP isoforms tested, namely CYP1A2, CYP2D6, CYP3A4, CYP2C9 and CYP2C19, respectively (Table 2).[21] Next analog **21c** with 2-trifluoromethylphenyl as A-ring and 2-chloro-6-fluorophenyl as d-ring afforded single digit enzyme and, cellular potency with a human whole blood IC_{50} of 536 nM, similar to compound **21b**. CYP inhibition study of **21c** also revealed low CYP liability against all five major isoforms tested. Encouraged with these results, additional SAR optimization was initiated to improve human whole blood potency in this series. Therefore, combination of 2-fluoro-5-trifluoromethylphenyl (**21d**), 2-fluoro-5-difluoromethylphenyl (**21e**), 2-fluoro-5-cyclopropyl phenyl (**21f** and **21g**), 2-fluoro-4-trifluoromethylphenyl (**21h**) and 2-methyl-4-trifluoromethyl phenyl (**21i**) analogs were synthesized and tested for mPGES-1 potency. All these compounds demonstrated single digit enzyme and A549 cell potency except **21g**, which displayed slightly lower enzyme potency (IC_{50}: 16 nM) and single digit cell potency (cell IC_{50}: 3.2 nM). Among the analogs (**21d–i**) tested for human whole blood potency, analogs **21d**, **21e**, **21g** and **21i** afforded enhanced whole blood potency (HWB IC_{50}s: <340 nM)

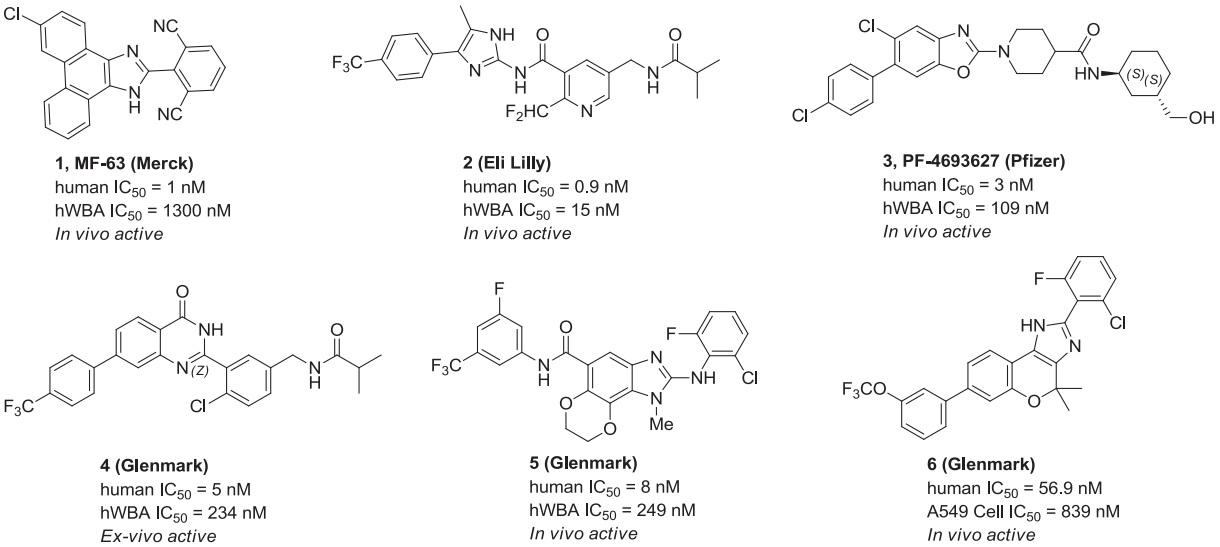
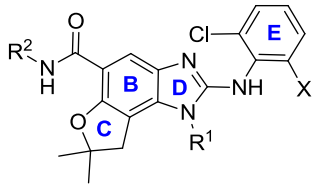


Fig. 1. Representative mPGES-1 inhibitors.

Table 1
Dihydrofuran-fused benzo[*d*]imidazole SAR.



Compd	R^2 (A-ring)	R^1	X	mPGES-1 IC_{50} (nM)[a,b]	A549, 2% FBS PGE_2 IC_{50} (nM)[a,c]	hWBA IC_{50} (nM)[a,d]
21a	2,6-diF-Phenyl	H	Cl	6.4	15	661
21b	2CF_3-Phenyl	H	Cl	4.9	5.2	506
21c	2CF_3-Phenyl	H	F	5.1	9.7	536
21d	2F, 5CF_3-phenyl	H	F	3.0	9.6	256
21e	2F, 5CHF_2-phenyl	H	F	9.2	6.3	336
21f	2F, 5Cy-phenyl	H	F	3.6	6.0	1377
21g	2F, 5Cy-phenyl	H	Cl	16	3.2	197
21h	2F, 4CF_3-phenyl	H	F	3.1	9.6	570
21i	2CH_3, 4CF_3-Phenyl	H	F	6.1	6	270
21j	4F, 3CF_3-phenyl	H	F	5.0	14	208
21k	4CF_3-phenyl	H	Me	14	11	337
21l	4F, 3CF_3-phenyl	H	Me	5.4	10	876
21m	2F, 5CF_3-phenyl	H	Me	3.3	30	222
21n	2F, 5CHF_2-phenyl	H	Me	10	–	162
21o	2F, 5Cy-phenyl	H	Me	10	–	447
21p	5CF_3-Pyridin-2-yl	H	Cl	8.4	4.1	261
21q	5CF_3-Pyridin-2-yl	H	F	25	14	955
21r	5CF_3-Pyridin-2-yl	H	Me	32	27	–
22a	4CF_3-Phenyl	Me	F	3.3	2.6	897
22b	3CF_3-Phenyl	Me	F	5.7	3.0	405
22c	4Cy-Phenyl	Me	F	4.9	4.3	350
22d	2F, 4CH_3-Phenyl	Me	F	16	3.1	177
22e	5CF_3-Pyridin-2-yl	Me	F	12	4	673

–: not determined.
[a] MF-63 (**1**) was used as a positive control here and the in-house data of MF-63 (**1**) for enzyme IC_{50}: 1.9 nM; A549 cell IC_{50}: 56 nM; hWB IC_{50}: 1.36 μM. For the literature reported potency of MF-63, see Ref. 9.
[b] IC_{50} values are derived from graphs plotted with data from a minimum of two experiments in duplicates.
[c] IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control and derived from the graphs plotted with data from a minimum of two experiments in duplicates.
[d] Lipopolysaccharide (LPS) stimulated human whole blood (hWB) assay. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control and minimum of two experiments in duplicates.

over **21a–c**. In contrast, **21h** (HWB IC_{50}: 570 nM) and **21f** (HWB IC_{50}: 1377 nM) provided lower human whole blood potency.[21] Analogs **21d–i** were further assessed for potential CYP inhibition and exhibited least liability against all five major CYP isoforms studied (Table 2). Then, 4-fluoro-3-trifluoromethylphenyl as A-ring analog **21j** (no 2-substituted phenyl as A-ring) was prepared and tested for CYP activity, which revealed moderate liability against CYP3A4, CYP2C9 and CYP2C19 isoforms (45–55%@10 μM) despite having suitable mPGES-1 enzyme, cell and human whole blood potency (Tables 1 and 2). The CYP data of **21j** further reconfirm our hypothesis that 2-substituted phenyl as A-ring is essential in this series to afford compounds with low or negligible CYP liability.

Next, we focused our attention on the E-ring modification in order to afford additional analogs with low CYP liability (<50%@ 10 μM). In the earlier study, compounds with 2, 6-dichlorophenyl, 2-chloro-6-fluorophenyl and 3, 5-dichloropyridin-4-yl as E-ring of lead **7** delivered mPGES-1 inhibitors with CYP liability (>50%@ 10 μM).[16a] In order to overcome CYP liability, 4-trifluoromethylphenyl (**21k**) and 4-fluoro-3-trifluoromethylphenyl (**21l**) as A-ring analogs having 2-chloro-6-methylphenyl as E-ring were synthesized and tested for mPGES-1 potency and CYP liability. Analog **21k** provided higher human whole blood potency (IC_{50}: 337 nM) than Celecoxib along with robust enzyme and cell potency, whereas **21l** provided > 2-fold reduced whole blood potency (hWB IC_{50}: 876 nM) even though it had <10 nM enzyme and cellular potency. Gratifyingly, low CYP liability (<50%@ 10 μM) were observed to **21k** and **21l** as foreseen. Hence, additional analogs such as 2-fluoro-5-trifluoromethylphenyl (**21m**), 2-fluoro-5-difluoromethylphenyl (**21n**) and 2-fluoro-5-cyclopropylphenyl (**21o**) as A-ring along with 2-chloro-6-methylphenyl as common E-ring analogs were synthesized and tested for CYP liability and mPGES-1 potency. As projected, analogs **21m–o** also displayed diminished CYP inhibition for all major CYP isoforms tested along with noteworthy enzyme, cellular and human whole blood potency (Tables 1 and 2).[21]

Having several potent mPGES-1 inhibitors in hand with low CYP liability (<50%@ 10 μM), additional SAR was generated by introducing *N*-methyl group in the d-ring along with 2-chloro-6-fluorophenyl as E-ring intact for further optimization. Therefore, new analogs having 4-trifluoromethylphenyl (**22a**), 3-trifluoromethylphenyl (**22b**), 4-cyclopropylphenyl (**22c**) and 2-fluoro-4-methylphenyl (**22d**) were synthesized and tested for mPGES-1 potency and CYP inhibition studies. Among these, analogs **22b–d** unveiled human whole blood potency ≤405 nM IC_{50}s along with single digit enzyme and A549 cellular potency, whereas, **22a** (HWB IC_{50}: 897 nM) exhibited fivefold lower human whole blood potency in comparison to **22d** (HWB IC_{50}: 177 nM), despite having ≤15 nM enzyme and cell potency (Table 1). Further CYP inhibition study on *N*-methyl d-ring analogs **22a–c** provided low CYP liability for all five major isoforms tested, while **22d** revealed modest CYP2C9 liability (52% inhibition @10 μM) and other CYP isoforms gave low liability (<50%@ 10 μM). In order to improve the physicochemical properties in our scaffold,[22] substituted pyridine A-ring was introduced in the place of phenyl ring. Therefore, analogs

Table 2
In vitro DMPK data of selected mPGES-1 inhibitors.

Compd	Metabolic stability (% remaining)[a]			% CYP inhibition @10 μM concentration[b]					PPB (% bound)[c]		hERG inh. @ 10 μM[d]	PAMPA Papp (*10^{-6} cm/s)[e]
	HLM	RLM	GPLM	1A2	2D6	3A4	2C9	2C19	H	GPig		
21a	72	–	80	14	n.i	24	35	41	–	–	–	–
21b	97	–	86	18	n.i	17	16	30	–	–	–	–
21c	93	–	89	17	4	11	29	46	> 99.5	>99.5	–	0.12
21d	89	96	79	22	12	2	35	33	99.96	99.65	5.8	0.62
21e	88	86	68	31	−1	4	42	22	99.82	99.5	–	–
21f	100	93	93	36	7	−12	42	22	–	–	–	–
21g	95	92	74	14	5	21	26	10	–	–	–	–
21h	100	–	80	–	–	37	39	16	>99.5	>99.5	–	–
21i	82	91	72	26	16	20	1	38	–	–	16.2	0.31
[c]**21j**	100	91	100	−6	19	46	48	55	99.46	98.64	3.8	0.33
21k	100	–	62	25	6	34	46	19	>99.5	>99.5	–	–
21l	95	97	80	–	–	30	47	31	–	–	–	–
21m	100	97	46	24	−2	−4	15	16	99.97	99.96	12.3	1.34
21n	83	87	60	28	14	16	28	14	99.92	98.42	11.8	–
21o	93	89	55	14	31	−13	12	24	–	–	–	–
21p	91	88	71	4	9	40	56	15	99.96	99.84	5.4	0.63
22a	100	68	68	34	−5	11	44	−7	–	–	–	–
22b	98	89	51	5	29	8	19	36	99.71	99.72	26.5	–
22c	97	96	51	41	25	19	47	24	–	–	–	–
22d	68	71	58	26	14	23	52	24	>99.5	>99.5	12.8	–
22e	58	66	69	14	3	17	52	10	99.85	99.65	–	–

–: not determined.
[a] Percentage of test compound remaining after 60 min incubation with liver microsomes (human, rat and guinea Pig) at 37 °C. MS experiment was conducted in triplicates (see supporting info (SI) for details. HLM: human liver microsomes; RLM: rat liver microsomes; GPLM: guinea pig liver microsomes.
[b] Cytochrome P450 (CYP)% inhibition as compared to control (no inhibitor) and conducted in triplicates (see SI for details).
[c] Plasma protein binding (PPB) was determined using equilibrium dialysis method. 10 μM concentrations were used (see SI for details) and the data provided are% bound. PPB data of **21j**: 99.1 (dog); 99.48 (cynomolgus monkey); PPB data of **21m**: 99.7 (dog); PPB data of **21p**: 99.2 (dog); 99.5 (cynomolgus monkey).
[d] For hERG assay details (patch clamp), see Ref. 23.
[e] PAMPA permeability assay was carried out using 2% lecithin dodecane mixture to determine the passive permeability and the study was conducted in triplicates.

having 5-trifluoromethyl-pyridine-2-yl as common A-ring along with 2,6-dichlorophenyl (**21p**), 2-chloro-6-fluorophenyl (**21q**), 2-chloro-6-methylphenyl (**21r**) and 2-chloro-6-fluoro- phenyl as common E-ring, in combination with *N*-methyl as d-ring (**22e**) were tested for mPGES-1 potency. Among these, compounds **21p** and **22e** retained potency similar to other analogs in the Table 1, whereas **21q** and **21r** revealed substantial drop in hWB potency, slightly lower enzyme and cell potency in comparison to other analogs.[21] Further CYP inhibition study of pyridine analogs **21p** and **22e** revealed moderate CYP2C9 liability (52–56% inhibition @10 μM, Table 2).

Next, most of the analogs from Table 1 were further assessed for metabolic stability in liver microsomes (human, rat and guinea pig), plasma protein binding (PPB), hERG channel activity and PAMPA permeability. As illustrated in Table 2, most of the tested mPGES-1 inhibitors (**21a**–**p** and **22a**–**e**) revealed >50% metabolic stability in across species, > 98% plasma protein binding (PPB,% bound) in both human and guinea pig, and low PAMPA permeability (Table 2).[21] Further, few selected mPGES-1 inhibitors (**21d**, **21i**, **21j**, **21m**, **21n**, **21p**, **22b** and **22d**) demonstrated low activity in the hERG channel assay (≤26% inhibition @10 μM in patch clamp).[23] The notable differences in hERG potency between current series and earlier compound **7** series analogs[16a] were not explainable. However, we believe that the low hERG potency in the current series is due to the introduction of various ortho-substituents in A and E-rings, respectively.[19]

Additionally, the chosen analogs **21d**, **21j**, **21m**, **21n**, **21p** and **22b** were profiled against a battery of *in vitro* and cellular assays to evaluate their selectivity over other prostanoid synthases.[21] As represented in Table 3, selected mPGES-1 inhibitors demonstrated selectivity over COX-1, COX-2, mPGES-2, cPGES, PGDS, PGIS, TXAS synthases and further these compounds did not inhibit cytosolic phospholipase A_2 ($cPLA_2$), an enzyme upstream of arachidonic acid (AA) pathway. However, few compounds (**21d**, **21j** and **22b**) among the selected six showed potent $PGF_{2\alpha}$ inhibition at 10 μM test concentration and the $PGF_{2\alpha}$ potency of **21d** (IC_{50}: 2.47 μM), **21j** (IC_{50}: 2.32 μM) and **22b** (IC_{50}: 3.18 μM) were >450-fold margin over their corresponding mPGES-1 cellular potency (Tables 1 and 3).

Since, most of the reported mPGES-1 inhibitors were not active against rodent enzyme (rat and mouse) except NovaSaid inhibitor,[24] we further assessed chosen compounds (**21d**, **21j**, **21m**, **21n**, **21p** and **22b**) for rat, mouse and guinea pig enzyme potency. As disclosed in Table 4, though the lead compounds were not active against rat and mouse mPGES-1 enzymes, these are highly active in guinea pig enzymes (Guinea pig IC_{50}s: <45 nM and WB IC_{50}s: <465 nM), which is a pre-clinical efficacy species.[21] The observed species selectivity difference in rodents are consistent with previously reported mPGES-1 inhibitors.[9–11,13a,16a] Next, analogs **21j**, **21m** and **21p** among the six leads were further evaluated for dog and cynomolgus monkey whole blood potency and to our surprise, dog exemplified modest whole blood potency (IC_{50}s: 3 to 6 μM range), whereas a lower% inhibition in monkey whole blood (≤50% inhibition @10 μM) was observed as shown in Table 4. The poor potency in dog and monkey perhaps attributed to high plasma protein binding and lipophilicity of lead compounds which may cause serum shift and therefore might have led to lower whole blood potency (see Table 2 Footnote for dog and monkey PPB data).[8a,8g]

After assessing *in vitro* pharmacology, pharmacokinetics and prostanoid synthases selectivity (Tables 2–4), chosen analogs **21j**, **21m** and **21p** were subjected to pharmacokinetic (PK) study in rat, guinea pig, dog, and cynomolgus monkey in 10 mg/kg oral dose (Table 5).[21,25] The plasma PK of compound **21j** was determined in rat, dog and cynomolgus monkey following intravenous (i.v) and oral administration.[25] The intravenous clearance was 11.2 mL/min/kg in rats, 1.61 mL/min/kg in dogs and 3.35 mL/min/kg in monkeys. The volume of distribution (Vz) was 3.85, 1.94 and 1.83 L/kg in rat, dog and monkey, respectively. The oral half-life

Table 3
COX-1/2 and prostanoid synthases selectivity of selected mPGES-1 inhibitors.

Compd	% inhibition @ 10 μM test concentration[a]								
	COX-1	COX-2	mPGES-2	cPGES	PGDS	PGIS	TXAS	$PGF_{2\alpha}$	$cPLA_2$
21d	14.7	28.7	13.5	4.2	5.8	5.6	4.6	99.2[b]	22.9
21j	8.27	23.1	11.6	0.0	0.0	2.5	25.5	100[c]	−14.9
21m	14.6	31.0	23.5	21.1	19.5	7.5	0.87	17.9	5.1
21n	38.2	43.2	3.8	11.0	27.6	20.1	17.2	−20.1	−30.7
21p	7.8	35.9	7.8	0	15.1	4.3	1.3	15.4	4.3
22b	19.7	6.5	7.9	24	16.5	3.9	22.1	61.4[d]	10.6

[a] % inhibition values are means of at least two experiments in duplicates, see SI for experimental details.
[b] $PGF_{2\alpha}$ IC_{50}: 2.47 μM (740-fold over A549 cell potency).
[c] $PGF_{2\alpha}$ IC_{50}: 2.32 μM (461-fold over A549 cell potency).
[d] $PGF_{2\alpha}$ IC_{50}: 3.18 μM (556-fold over A549 cell potency).

Table 4
Cross-species activity of selected mPGES-1 inhibitors.

Compd	hIC_{50} (nM)[a]	A549 cell IC_{50} (nM)[b]	hWBA IC_{50} (nM)[c]	GPig IC_{50} (nM)[d,e]	GPigWBA IC_{50} (nM)[d,f]	Dog WBA IC_{50} (nM)[g]	% inhibition @10 μM concentration[h]		
							Rat [h]	Mouse [h]	Monkey [i]
21d	3.0	9.6	256	14	204	–	11.7	14.4	–
21j	5.0	14	208	24	465	6221	10.5	22.1	50.4
21m	3.3	30	222	19	340	3029	0.0	8.2	48.2
21n	10	–	162	46	222	–	0.7	2.5	–
21P	8.4	4.1	261	12	259	3538	7.1	10.9	37
22b	5.7	3	405	4.3	188	–	3.4	5.2	–

–: not determined.
[a,b,c] See Table 1 foot note for details.
[d] Same positive control was used for guinea pig enzyme and WBA potency as shown in the foot note of Table 1.
[e] IC_{50} values are derived from graphs plotted with data from a minimum of two experiments in duplicates.
[f] Lipopolysaccharide (LPS) stimulated guinea pig (GPig) whole blood assay (WBA). IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control and minimum of two experiments in duplicates.
[g] Lipopolysaccharide (LPS) stimulated dog whole blood assay. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control (Celecoxib) and minimum of two experiments in duplicates. Study was conducted at Vimta Labs, Hyderabad, India.
[h] Study protocol was done similar to human and guinea pig enzyme assay (see Table 1 foot note for details).
[i] Lipopolysaccharide (LPS) stimulated Rhesus monkey whole blood assay. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control (Celecoxib) and minimum of two experiments in duplicates. Study was conducted at Lonza India Ltd, Hyderabad, India.

Table 5
Oral Pharmacokinetics (PK)[a] of selected mPGES-1 inhibitors- **21j**, **21m** and **21p**.

Example	Species[b]	C_{max} (ng/mL)	$AUC_{0\text{-}24}$ (ng.h/mL)	T_{max} (h)	$T_{1/2}$ (h)	CL (mL/min/kg)	Vz (L/kg)	% F	CNS b/p[g]
21j	Rat[c]	995 ± 82	11020 ± 2289	2	4 ± 0.5	11.2 ± 0.4	3.85 ± 0.6	91	0.18
	G.Pig[*,d]	361 ± 91	4125 ± 2337	2	–	–	–	–	0.44
	Dog[e]	492 ± 183	8236 ± 3235	12	14.4 ± 3.6	1.61 ± 0.3	1.94 ± 0.13	15	0.72
	Monkey[f]	680 ± 326	9669 ± 3623	24	6.45 ± 1.13	3.35 ± 0.87	1.83 ± 0.41	44	0.32
21m	Rat[c]	210 ± 24	2579 ± 296	4	7.0 ± 0.9	8.2 ± 1.3	4.84 ± 0.15	14	0.04
	G.Pig[d]	80 ± 21	408 ± 164	1	–	–	–	–	BQL
	Dog[e]	843 ± 111	12933 ± 2507	8	20 ± 3	3.5 ± 0.5	9.17 ± 1.8	40	ND
	Monkey[f]	499 ± 11	7507 ± 1937	12	12 ± 2.6	1.6 ± 0.3	2.1 ± 0.3	11	0.16
21p	Rat [c]	344 ± 52	4025 ± 365	4	2.7 ± 0.8	16.2 ± 2.4	3.77 ± 0.8	43	0.16
	G.Pig[d]	91 ± 78	639 ± 410	12	–	–	–	–	0.58
	Dog[e]	336 ± 106	4432 ± 1543	12	17 ± 5	3.9 ± 1.5	5.4 ± 0.79	25	0.32
	Monkey[f]	323 ± 195	3669 ± 2180	6	5.3 ± 3.6	8.5 ± 1.1	4.1 ± 3.3	21	0.37

–: not applicable; ND: not determined; BQL: Below quantification level.
[*] – Female guinea pig was used.
[a] C_{max}, $AUC_{0\text{-}24}$, T_{max}, and $T_{1/2}$ were determined based on oral dosing and clearance (CL), volume of distribution (Vz) were determined based on i.v dosing. Bioavailability (% F) was determined based on both oral and i.v dosing. This is applicable to all species.
[b] Rat, guinea pig, dog and cynomolgus monkey i.v dose is 1 mg/kg and p.o dose is 10 mg/kg.
[c] Vehicle for rat Oral dosing- 0.5% methylcellulose (MC) suspension; Vehicle for rat i.v- 20% NMP + 20% Ethanol + 60% PEG 200).The data represented is mean ± SD (n = 3). Male Sprague-Dawley rats were used.
[d] Vehicle for guinea pig oral dosing- 20% NMP + 0.5% MC suspension. The data represented is mean ± SD (n = 3).
[e] Vehicle for beagle dog oral dosing- 2.5 μL/mL Tween 80 + 0.5 (w/v) MC suspension. Vehicle for dog i.v- 20% NMP + 20%EtOH + 10%PG + 50% premix solvent (PEG 200: Milli-Q water, 3:2). See Ref. 25.
[f] Vehicle for cynomolgus monkey oral dosing- 2.5 μL/mL Tween 80 + 0.5 (w/v) MC suspension. Vehicle for cynomolgus monkey i.v dosing- 10% NMP + 10% ethanol + 10% PEG + 70% premix solvent (v/v mixture of 3:2 PEG200: Milli-Q water). See Ref. 25.
[g] See supporting info for the experimental details.

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($T_{1/2}$) is ranged from approximately 4–14 h, while absorption was moderate to slow (T_{max} = 2–24 h) in all three species. The oral bioavailability of **21j** was relatively higher in rat and monkey (%F = 91 and 44), whereas dog exemplified poor oral bioavailability (%F = 15).[25b] Plasma C_{max} was higher to moderate in rat, dog and monkey, whereas AUC_{0-24} was comparable in across species. The guinea pig PK of **21j** demonstrated lower C_{max} and AUC_{0-24} in comparison to rat, dog and monkey PK values. Another lead **21m** demonstrated moderate to low clearance (CL = 8.2 –1.6 mL/min/kg), low volume of distribution (Vz = 9.2–2.1 L/kg) in rat, dog and monkey, and longer oral half-life ($T_{1/2}$ = 7–20 h) in across species compared to analog 21**j**. The oral bioavailability of **21m** in rat and monkey was poor (%F = 14–11%), whereas dog demonstrated 40% oral bioavailability. In addition, third lead **21p** divulged comparable CL, Vz and oral $T_{1/2}$ in across species similar to earlier leads **21j** and **21m**, respectively. The oral bioavailability of **21p** was as comparable to **21m** and inferior to **21j**. Plasma C_{max} and AUC_{0-24} of **21m** and **21p** was lower compared to **21j** in across species. The guinea pig oral PK of **21m** and **21p** also revealed inferior to compound **21j** as shown in Table 5. Based on the oral PK data, compound **21j** appeared better than other two analogs. mPGES-1 is functionally coupled to COX-2 in the arachidonic acid (AA) pathway and it is well known that peripheral injury elicits a predominant increase in mPGES-1 expression and PGE_2 levels in the CNS, which plays a role in pain mitigation.[2d] Therefore, mPGES-1 inhibitor should probably have CNS penetration for its *in vivo* efficacy similar to Celecoxib (COX-2 inhibitor).[20,26] Among the compounds (**21j**, **21m** and **21p**) evaluated for CNS penetration in across species (rat, guinea pig, dog and monkey), compounds **21j** and **21p** revealed comparable brain to plasma ratio (B/P) in across species, whereas **21m** unveiled lower brain to plasma ratio (Table 5). Next, the advanced leads (**21j**, **21m** and **21p**) were further evaluated for human PXR (pregnane X receptor) activation. Among this, **21m** and **21p** were found to be moderate PXR activator, whereas **21j** exhibited low PXR activity relative to the effect of rifampicin at 10 μM test concentration (see reference section for data).[27]

Finally, lead **21j** was further selected for *in vivo* efficacy study (acute hyperalgesia and monosodium iodoacetate (MIA)-induced osteoarthritis pain models)[21] over other leads **21m** and **21p** due to the following reasons: (i) a better whole blood potency, (ii) a lower hERG and PXR activity, and (iii) a better oral PK and CNS penetration in across species. In the acute hyperalgesia model, the antihyperalgesic effect of compound **21j** was studied at 10, 30 and 100 mg/kg in guinea pig and the results are summarized in Fig. 3 and Table 6. Injection of LPS into the plantar region of right paw of guinea pig caused a significant thermal hyperalgesic response as compared to saline injected animals. When administered orally at 2 h before LPS injection, the compound **21j** significantly inhibited the hyperalgesic response in a dose-dependent manner with maximum inhibition of 66% at 100 mg/kg (Fig. 1). The calculated ED_{50} was found to be 39.6 mg/kg. As depicted in Table 6, the plasma and brain concentrations of **21j** for 100 mg/kg, at 7 h post compound treatment (pharmacodynamics time

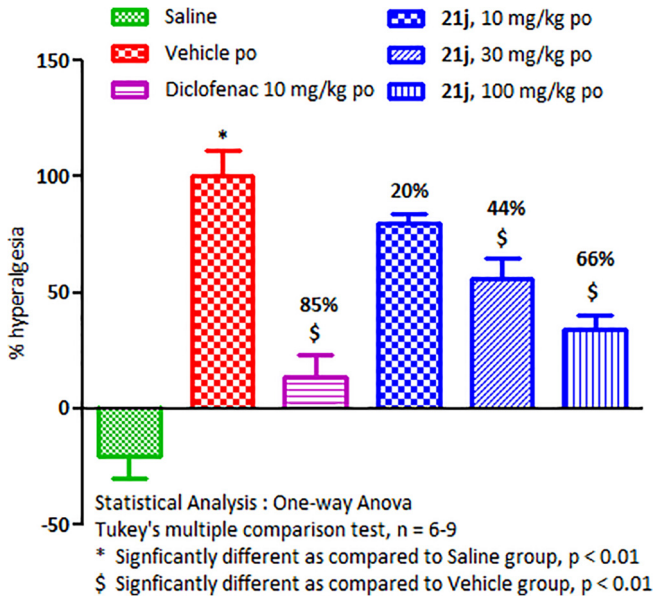


Fig. 3. Analgesic effects of **21j** in the guinea pig hyperalgesia pain model. Data are expressed as percentage of hyperalgesia, with the naive group (injected intraplantarly with saline) as 0% and the **vehicle**-treated LPS-injected group as 100%. Results are shown as Mean ± S.E.M. (n = 6–9/group).

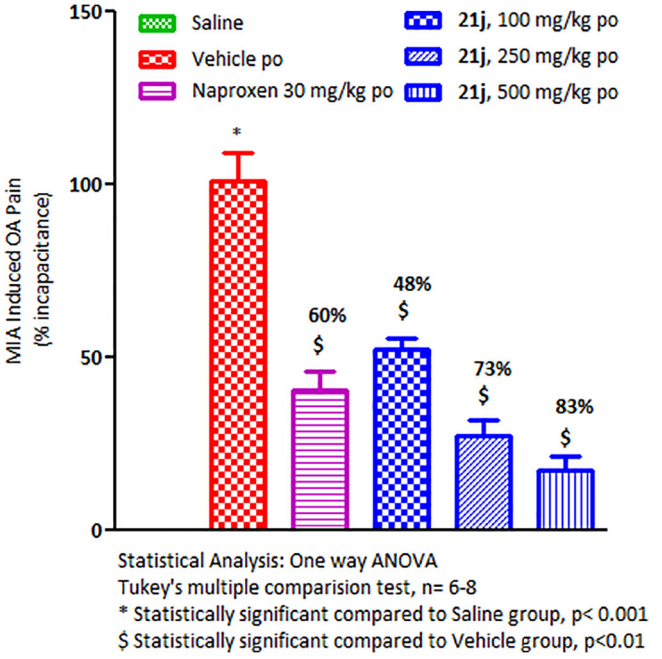


Fig. 4. Analgesic effects of **21j** in the guinea pig MIA induced OA pain model. Data are expressed as percentage of incapacitance, with the naive group (injected with saline in shoulder joint) as 0% and the vehicle-treated MIA-injected group as 100%. Results are shown as Mean ± S.E.M. (n = 6–8/group).

Table 6
Plasma concentration of **21j** at PD time point (HA model).

Dose (mg/kg, po, od)	ED_{50} (mg/kg)	% Hyperalgesia inhibition	Plasma concentration @PD time point (ng/ml)[a]	Brain concentration @PD time point (ng/g)[a]	Brain to Plasma Ratio (B/P)
10		20	94	96	1.02
30	39.6	44	145	87	0.60
100		66	581	616	1.06

[a] Concentrations are means of n = 6–9 animals per dose group. The data represented is from a single experiment. Study protocol is provided in the SI.

point)[21] was found to be 581 ng/mL (equivalent to 1084 nM in plasma) and 616 ng/mL (equivalent to 1149 nM in brain), respectively with brain to plasma (B/P) ratio of 1.06. The plasma concentration was found to be greater than 2-fold over guinea pig whole blood potency and roughly 5-fold higher than its human whole blood potency at steady state and also unveiled more than adequate concentration in the brain.

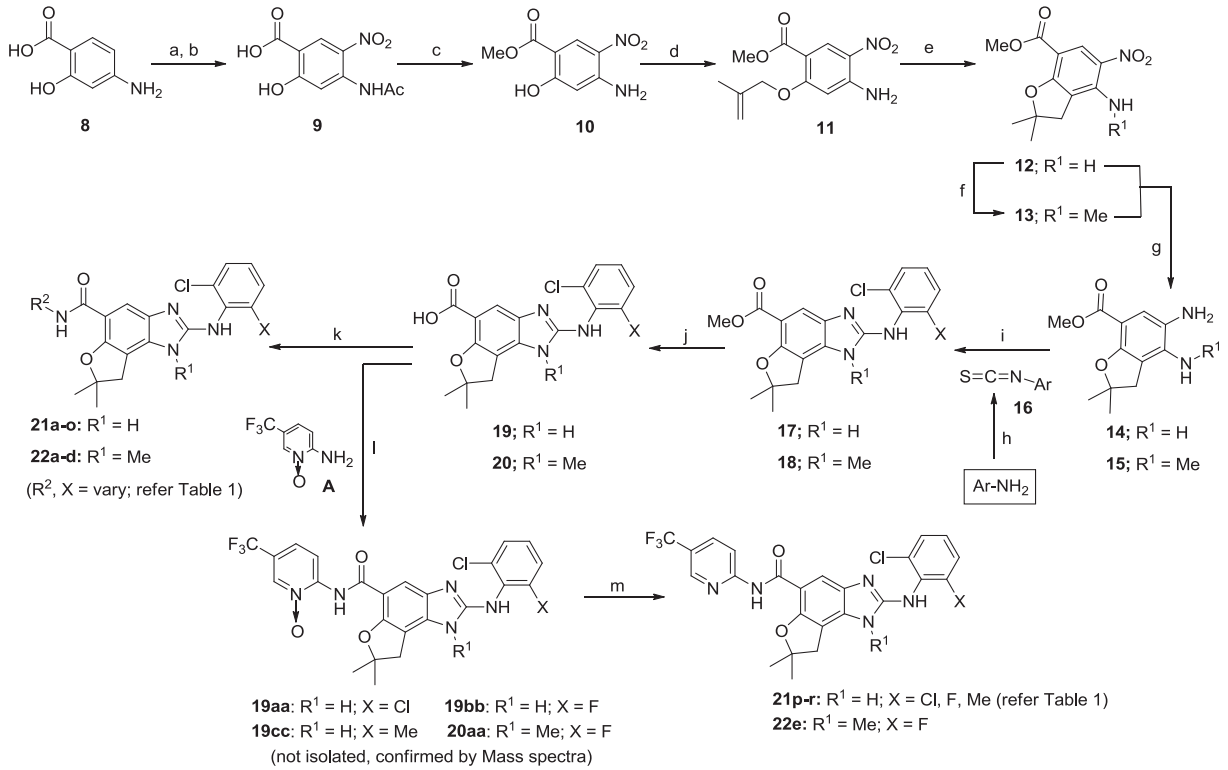
The analgesic property of compound **21j** was further evaluated in a guinea pig model of MIA-induced osteoarthritic pain.[21] Analgesic effect of compound **21j** was studied at 100, 250 and 500 mg/kg and the results are summarized in Fig. 4 and Table 7. Intra-articular injection of monoiodo acetate (MIA) in the right shoulder joint of guinea pig on day 0 caused inflammation and pain, resulting a decreased weight bearing or incapacitance on the injected forelimb by day 3 as compared to saline injected animals. When tested on day 3, compound **21j** dose-dependently reversed incapacitance with maximum reversal of 83% at 6 h after dosing at 500 mg/kg (Fig. 2). The calculated ED_{50} was found to be 106 mg/kg. As depicted in Table 7, the plasma concentration at PD time point (2h post compound treatment) of **21j** for 500 mg/kg was found to be 1896 ng/mL (equivalent to 3537 nM in plasma), which was 7-fold higher than its guinea pig whole blood potency and 17-fold higher than its human whole blood potency. Overall, compound **21j** showed efficient analgesic activity in both LPS-induced hyperalgesia and MIA-induced osteoarthritic pain models when administered orally and thus suppresses both peripheral and centrally mediated PGE_2 synthesis[9,28]

Table 7
Plasma concentration of **21j** at PD time point (MIA model).

Dose (mg/kg, po, od)	ED_{50} (mg/kg)	% Incapacitance reversal	Plasma concentration @PD time point (ng/ml)[a]
100		48	678
250	106	73	1508
500		83	1896

[a] Concentrations are means of n = 6–8 animals per dose group. The data represented is from a single experiment. Study protocol is provided in the SI.

The synthetic strategies employed in the preparation of compounds exemplified in Tables 1–4 are described in Scheme 1..[16,21] The synthetic route began with acetylation of commercially available 4-aminosalicylic acid **8** using Ac_2O, followed by nitration in TFA using sodium nitrite ($NaNO_2$) to afford nitrated compound **9**. Esterification of compound **9** with conc.H_2SO_4 in methanol at 90 °C gave *N*-deacetylated ester product **10** in good yield.[29] The intermediate **10** was alkylated using methallyl chloride in the presence of K_2CO_3 in DMF at 80 °C to afford compound **11** in 55% yield. Claisen rearrangement of **11** in *N, N*-dimethylaniline and subsequent treatment with formic acid at 100 °C gave dihydrobenzofuran derivative **12**.[30] Compound **12** was methylated in parallel, using MeI in NaH/DMF to afford compound **13**. Nitro reduction of compounds **12** and **13** in Fe/aq.HCl afforded diamine compounds **14** and **15**. Aryl isothiocyanate **16**,[31] prepared from the corresponding aryl aniline using thiophosgene and diisopropylethyl amine, reacted with diamines **14** and **15** independently in the presence of *N,N′*-diisopropylcarbodiimide to afford tricyclic dihydrobenzofuroaminoimidazole derivatives **17** and **18** in 40–70% yield (for two steps).[32] The ester derivatives **17** and **18** were hydrolyzed to carboxylic acid derivatives **19** and **20** in the presence of 10% NaOH solution, suitable for amide formation. The conversion of carboxylic acids (**19** and **20**) to acid chlorides using thionyl chloride, followed by reaction with substituted anilines afforded aryl amides **21a–o** and **22a–d** in 20–37% yield. Standard coupling reagents (TBTU/HOBT, EDCI/HOBT, T_3P/DIPEA and $SOCl_2$ etc.) did not work



Scheme 1. Reagents and conditions: (a) Ac_2O, EtOH, reflux; (b) $NaNO_2$, TFA, −5 to 0 °C, 2 h; (c) MeOH, conc.H_2SO_4, reflux; (d) methallyl chloride, K_2CO_3, DMF, 80 °C, 3 h, 55%; (e) *N,N*-dimethylaniline, reflux, 1.5 h, then formic acid, 100 °C, 3–4 h, 25%; (f) MeI, 60% NaH in mineral oil, DMF, 0 °C to rt, 3 h, 60%; (g) Fe, aq.HCl, 0 °C to rt, 1 h, 45%; (h) thiophosgene, DIPEA, DCM, 0 °C to rt, 4–6 h; 50–70%; (i) *N,N′*-diisopropylcarbodiimide, Ar-NCS, CH_3CN, rt, 24 h, 40–70%; (j) 10% NaOH, MeOH, 60 °C, 8–10 h, 65–80%; (k) $SOCl_2$, DCM, reflux, 2 h, then DIPEA, DCM, aryl amine, 8–12 h, 20–37% - used for **21a–o** and **22a–d**; (l) COMU, DIPEA, 1 h, then 5-(trifluoromethyl)pyridin-2-amine-*N*-oxide (**A**), rt, 12–16 h; (m) Iron power, AcOH, 70 °C, 3 h, 50–60% (for two steps) – used for **21p–r** and **22e**.

in our hand for the direct synthesis of pyridine amide derivatives **21p**–**r** and **22e** (Table 1). Therefore, amide derivatives **19aa**–**cc** and **20aa** were prepared by coupling of 5-(trifluoromethyl)pyridin-2-amine *N*-oxide (**A**)[33a] with corresponding aryl carboxylic acids **19** and **20** using COMU/DIPEA based coupling reagent, followed by *N*-oxide cleavage[33b] with Fe powder in acetic acid to afford aryl amides **21p**–**r** and **22e** in 50–60% yield over two steps.

In summary, SAR optimization of A, D and E-rings of dihydrofuran-fused tricyclic benzo[*d*]imidazole **7** afforded many potent mPGES-1 inhibitors with low CYP and hERG liability. Several identified lead compounds (**21d**, **21j**, **21m**, **21n**, **21p** and **22b**) exhibited selectivity over COX-enzymes, mPGES-2 and prostanoid synthases. The chosen pre-clinical lead **21j** demonstrated enviable oral pharmacokinetics, adequate CNS penetration in multiple species like rat, guinea pig, dog and monkey, and exhibited oral bioavailability in rat and monkey. Besides, **21j** exemplified *in vivo* efficacy in acute hyperalgesia and MIA-induced osteoarthritis pain models, and further revealed low PXR activity. Detailed toxicology and *in vivo* study of all lead compounds will be the subject of future disclosure.

Acknowledgements

We thank Dr. Sanjib das for the review of this manuscript and valuable inputs. We also thank scientists from the analytical support group for their help in compound characterization.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.02.048.

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