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Tricyclic 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole derivatives as microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitors: SAR and *in vivo* efficacy in hyperalgesia pain model

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ABSTRACT

A series of substituted tricyclic 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole derivatives have been synthesized and their mPGES-1 biological activity has been disclosed in detail. Structure-activity relationship (SAR) optimization provided inhibitors with excellent mPGES-1 potency and low to moderate PGE_2 release A549 cell potency. Among the mPGES-1 inhibitors studied, **7, 9** and **11l** provided excellent selectivity over COX-2 (>200-fold) and >70-fold selectivity for COX-1 except **11l**, which exhibited dual mPGES-1/COX-1 activity. Furthermore, the above tested mPGES-1 inhibitors demonstrated good metabolic stability in liver microsomes, high plasma protein binding (PPB) and no significant inhibition observed in clinically relevant CYP isoforms. Besides, selected mPGES-1 tool compounds **9** and **11l** provided good *in vivo* pharmacokinetic profile and oral bioavailability (%F = 33 and 85). Additionally, the representative mPGES-1 tool compounds **9** and **11l** revealed moderate *in vivo* efficacy in the LPS-induced thermal hyperalgesia guinea pig pain model.

Microsomal prostaglandin E synthase-1 (mPGES-1) is the terminal enzyme in the biosynthesis of PGE_2. Membrane-bound and secretory phospholipase A_2 (PLA_2) isoforms convert phospholipids (PL) to arachidonic acid (AA) in the first step. Next, the COXs convert AA into the unstable intermediate, PGH_2. Finally, terminal PGESs isomerize PGH_2 into Prostaglandin E_2 (PGE_2)[1,2] PGH_2 is the precursor for several structurally related PGs, which are formed by the action of specific prostaglandin synthases.[3] The PGs synthesized by this pathway include the afore-mentioned PGE_2, as well as prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostaglandin I_2 (PGI_2, also known as prostacyclin) and thromboxane A_2 (TXA_2). It is recognized that inhibition of COX-2 activity affects the synthesis of all prostanoids down-stream of PGH_2, whereas selective targeting of mPGES-1 would only reduce PGE_2 production and it should be noted that shunting towards other PG has been observed.[4,5] Furthermore, mPGES-1 expression is low in most normal tissues, although abundant and constitutive expression is detected in a limited number of organs, such as the lung, kidney and reproductive organs.[3b]

PGE_2 plays a significant role in the inflammatory response and is involved in several chronic inflammatory disease conditions, such as inflammation, fever, rheumatoid arthritis, cardiovascular diseases, cancer, bone disorders, periodontitis and pain.[6] Prostaglandin E-synthase (PGES) exist in different isoforms and co-expression studies have demonstrated preferential functional coupling between COX and PGES isoenzymes. Moreover, mPGES-1 is a member of Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) superfamily and is functionally coupled with COX-2. There are three terminal synthases responsible for PGE_2 biosynthesis: one cytosolic isoform cPGES, and two membrane associated enzymes, mPGES-1 and mPGES-2. cPGES and mPGES-2 are constitutively expressed and are likely to be involved in the production of PGE_2 responsible for normal

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physiological reactions.[3b,7] On the other hand mPGES-1 is an inducible isoform, and its expression is dramatically increased in response to a pro-inflammatory stimulus. mPGES-1 knockout (KO) experiments in mice demonstrate the link between mPGES-1 and reduced inflammation, pain and fever response in animal models.[8c] Also, additional knockout studies in mice has shown no abnormalities in thrombogenesis, blood pressure and renal function when the mice were fed a normal salt diet. In the same study, selective inhibition, knockout or mutation of COX-2 was shown to accelerate thrombogenesis and elevated blood pressure in mice.[9]

The therapeutic interventions developed to inflammation so far include either the inhibition of COX-1 and COX-2 enzymes by non-steroidal anti-inflammatory drugs (NSAIDs). Further, COX-2 inhibitors can induce serious cardiovascular risks, plausibly due to upstream blockade of the pathway. However, selective inhibition of terminal mPGES-1 will block the production of PGE_2 in the disease state while sparing other arachidonic acid metabolites, probably without affecting the balance between prostaglandins PGI_2 and TXA_2 in order to have a safer cardiovascular profile.[1–3,9] Both COX-1/2 have severe gastrointestinal side-effects and pronounced effects on the renal function that limit their use in long-term treatments as required in chronic inflammation processes,[10] thus prompting to explore new enzymatic targets in this pathway. In this perspective, selective inhibition of mPGES-1 has emerged as an alternative promising strategy to develop effective and safer agents to treat inflammation devoid of side effects of COX inhibitors.[3b,9,11] Recent study highlighted that, prolonged inhibition of mPGES-1 in dogs did not affect renal function which further support the development of mPGES-1 inhibitors as therapeutic agent.[12]

Recently, our group has published potent mPGES-1 inhibitors (**VII** and **VIII**) with an excellent efficacy (both ex vivo and *in vivo* efficacy) and good oral bioavailability[13,14] in addition to several reported mPGES-1 inhibitors,[7,15] such as MF-63 (**I**),[8a,16] AZ-4284 (**II**),[17,18] AF-3442 and AF-3485 (**III**, **IV**),[19] compound **V**,[20] PF-04693627 (**VI**)[21] as exemplified in Fig. 1. Although many potent mPGES-1 inhibitors were developed in the past decade, only a few inhibitors were published with oral *in vivo* efficacy data.[8,13–16] Furthermore, mPGES-1 inhibitors from Eli Lilly (LY3023703)[22] and our group (GRC27864)[23] has entered human clinical trials for inflammatory pain. As part of an ongoing drug discovery program, we were required to design structurally distinct mPGES-1 inhibitors for SAR optimization and to identify compounds for the pre-clinical development. We observed that most of the recently published mPGES-1 inhibitors were having non-acidic core for the potential activity as exemplified in Fig. 1.[24] Therefore, in our design, inspired from the moderate potency and non-developmental activity of known compound **IX**[25] (mPGES-1 IC_{50}: 56 nM) prodded us to design novel scaffold from it. We applied the ring closure and scaffold-hopping[26] approach (also called lead hopping) on compound **IX** to design novel and conformationally rigid tricyclic 3,4-dihydrochromeno[3,4-*d*]imidazole **X**, which envisioned to provide enhanced mPGES-1 potency over structurally similar compounds like **V**, MF-63 (**I**) and **IX** as displayed in Figs. 1 and 2.

Here we report the synthesis and pharmacological characterization of series of 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole derivatives bearing aryl group at C(2)-position and halogens, aryl-, aryl-alkynes at C(7)-position (compounds **4a–d**, **5–9**, **11a–v**, **16a–f**, **20a–c**, **21a–b** and **32a–c**) as depicted in Scheme 1–3.[27] The synthesis of compounds **4a–d** and **5–9** were initiated from the commercially available halogen substituted 2-hydroxyacetophenones **1a–c**, which reacted with acetone in the presence of pyrrolidine at reflux to provide 2,2-dimethylchroman-4-one derivatives **2a–c**. Compounds **2a–c** was further reacted with isopentylnitrite[28] in the presence of conc. HCl to afford compounds **3a–c**, respectively. Compounds **4a–d** were obtained by condensing 2,2-dimethylchroman-3,4-dione derivatives **3a–c** with 2,6-dibromobenzaldehyde and or 2-chloro-6-fluorobenzaldehyde in the presence of NH_4OAc in acetic acid under heating conditions in 53–66% yield (Scheme 1). Further, Suzuki coupling of compound **4c–d** with various *para*- and *meta*-substituted arylboronic acid in the presence of $Pd(Ph_3P)_4$ afforded 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole derivatives **5–9** in 45–70% yield (Scheme 1).

The above synthesized compounds were tested for biological activity before generating more SAR in order to understand scaffold feasibility as mPGES-1 inhibitors. The inhibitory activity[29] of halogenated 3,4-dihydrochromeno[3,4-*d*]-imidazoles **4a** and **4b** with 2,6-dibromophenyl at C(2)-position (Fig. 2) gave moderate to poor enzyme potency with IC_{50s} of 325.2 and 709.5 nM, respec-

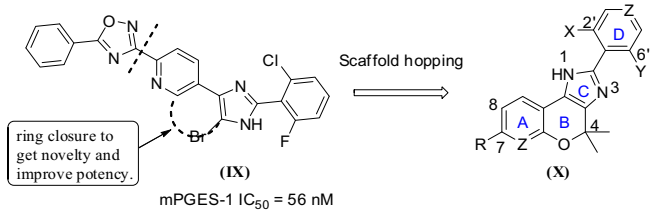


Fig. 2. General structure **X**: R = Cl, Br and I, substituted aryl and substituted aryl-alkynes; X, Y = combination of Cl, F and CN; Z = C and or N.

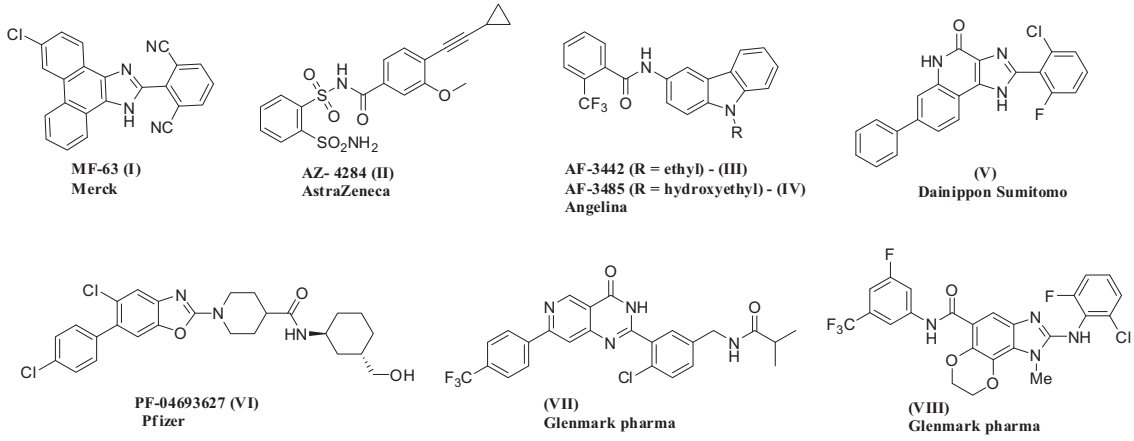
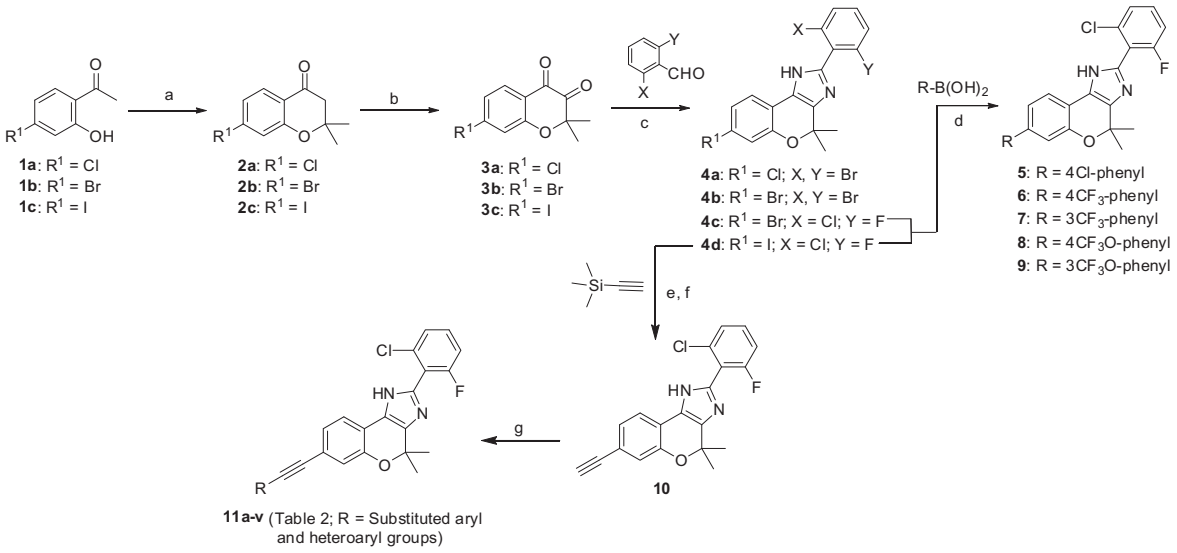
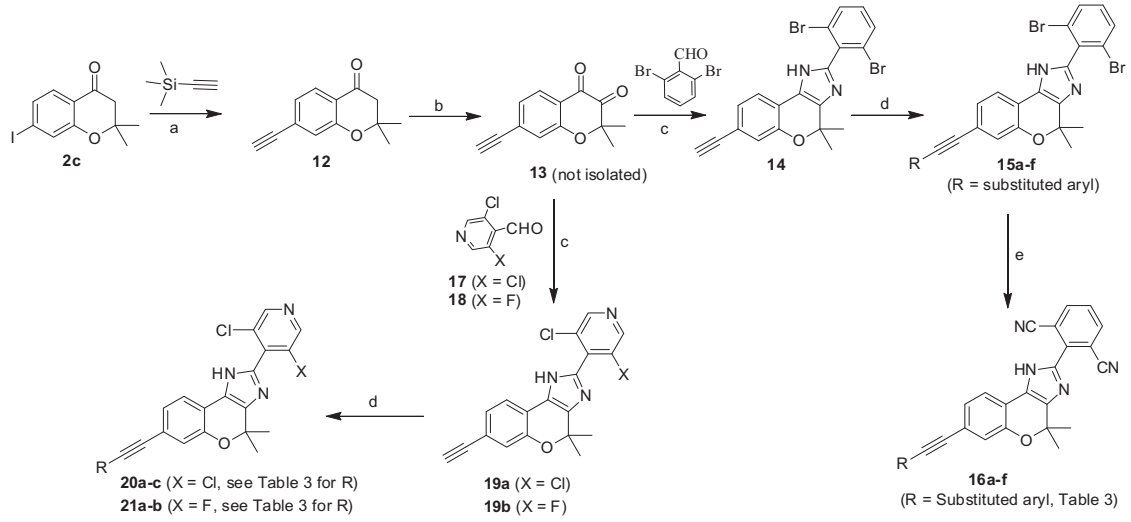


Fig. 1. Structure of reported mPGES-1 inhibitors.



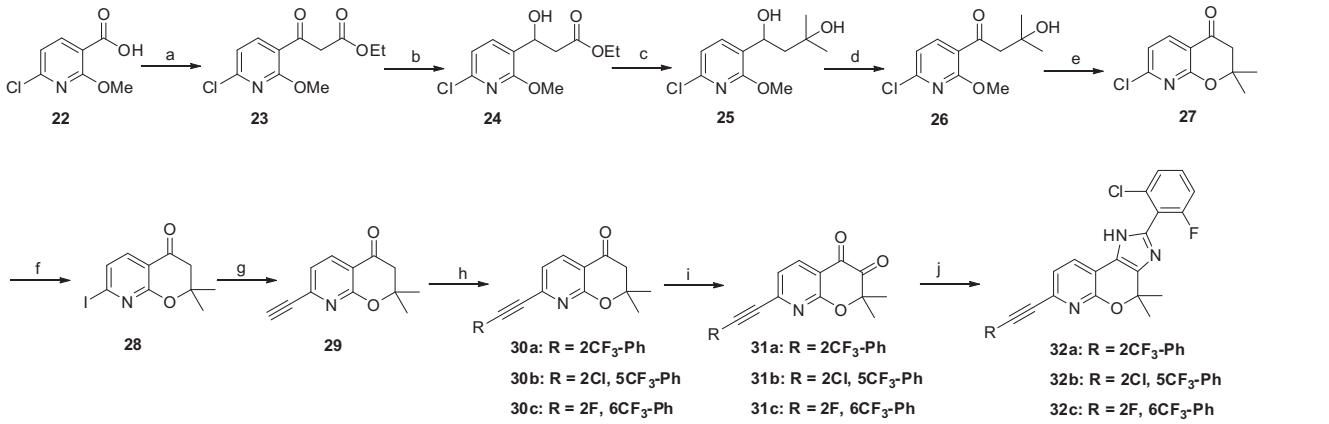
Scheme 1. Reagents and conditions: (a) acetone, pyrrolidine, MeOH, reflux, 4–6 h, 50–65%; (b) isopentyl nitrite, ethanol, conc. HCl, rt, 12–16 h, 57–70%; (c) 2,6-Di-Br-benzaldehyde and or 2Cl-6F-benzaldehyde, acetic acid, NH_4OAc, 100 °C, 6–8 h, 53–66%; (d) R-$B(OH)_2$, K_2CO_3, $Pd(Ph_3P)_4$, DMSO, 90 °C, 8–10 h, 45–70%; (e) CuI, DMSO, $PdCl_2(PPh_3)_2$, TEA, 70 °C, 8 h, 60%; (f) TBAF, THF, rt, 65%; (g) $PdCl_2(PPh_3)_2$, TBAF, R-Br and or R-I (R = substituted phenyl, heteroaryl), 90 °C, 4–8 h, 12–65%.



Scheme 2. Reagents and conditions: (a) CuI, DMSO, $PdCl_2(PPh_3)_2$, TEA, 70 °C, 6 h, then TBAF, THF, rt, 62%; (b) ethanol, isopentyl nitrite, conc. HCl, 0 °C to rt, 60% (crude); (c) NH_4OAc, acetic acid, 100 °C, 29–38%; (d) $PdCl_2(PPh_3)_2$, TBAF, R-Br and or R-I (R = substituted phenyl), 90 °C, 4–6 h, 19–42%; (e) CuCN, *N*-methyl-2-pyrrolidinone or DMF, 100–120 °C, 9–28%.

tively. Further, halogenated (Br- and I-) compounds **4c** and **4d** with 2-chloro-6-fluorophenyl linked at C(2)-position provided lower % inhibition at 10 μM test concentration. Concurrently, we replaced the bromine and/or iodine in compounds **4c–d** with substituted phenyl afforded compounds **5–9**, which furnished moderate to potent mPGES-1 inhibitors. Among them, *meta*-substituted phenyl compounds **7** (IC_{50} = 92.94 nM) and **9** (IC_{50} = 56.89 nM) exhibited improved mPGES-1 enzyme potency in comparison to *para*-substituted phenyl derivatives **5**, **6** and **8**, as well as halogen analogs **4a** and **4b** as disclosed in Table 1 (Similar structure-activity relationship was disclosed for compounds **V** and **VII** which unveiled enhanced enzyme potency over their halogen-substituted analogs).[20,13] In order to understand our scaffold further as mPGES-1 inhibitors, enzyme potent compounds **7** and **9** were further assessed for cell-based activity.[29,30] Therefore, microsomes of IL-1β stimulated human A549 epithelial lung cells expressing mPGES-1 were pre-incubated, and the amount of PGE_2 release was measured using HTRF® (Homogeneous Time Resolved Fluorescence) assay kit. Compounds **7** and **9** revealed moderate PGE_2 release with an IC_{50}s of 616.1 and 839.7 nM, respectively (Foot note in Table 1). Next, introduction of heterocyclic groups and aryl amides at C(7)-position of tricyclic core (Fig. 2) revealed diminished mPGES-1 potency (see supporting info for the trivial SAR and enzyme potency details as in Table 7).[27]

Subsequently, we next explored the possibility of improving the mPGES-1 potency by attaching the substituted aryl-alkyne group at C(7)-position of 2-(2-chloro-6-fluorophenyl)-4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole as shown in Scheme-1. Iodo compound **4d** was subjected to Sonogashira coupling with trimethylsilylacetylene, followed by trimethylsilyl (TMS) cleavage to provide key alkyne intermediate **10.** Palladium-catalyzed cross-coupling of alkyne compound **10** with commercially available substituted aryl, heteroaryl bromides and or iodides provided various aryl-alkyne appended 3,4-dihydrochromeno[3,4-*d*]



Scheme 3. Reagents and conditions: (a) CDI, potassium 3-ethoxy-3-oxopropanoate, $MgCl_2$, TEA, THF, acetonitrile, 0 °C-rt, 72%; (b) $NaBH_4$, THF, 0 °C, 77%; (c) CH_3MgCl, THF, 0 °C, 60%; (d) PCC, DCM, rt, 5 h, 63%; (e) 48% HBr in acetic acid, 80 °C, 2 h, then $POCl_3$, 100 °C, 5 h, 85% (single flask); (f) 4 M HCl, dioxane, rt, 5 h, then NaI, THF, 100 °C, 2 h, 58%; (g) CuI, DMSO, TEA, trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, 90–100 °C, 12 h; then TBAF, rt, 4 h, 40% for two steps; (h) R-I, $PdCl_2(PPh_3)_2$, CuI, TEA, DMSO, 100 °C, 4 h, 28–46%; (i) ethanol, isopentyl nitrite, conc. HCl, rt, 6 h, 80–86%; (j) NH_4OAc, 2-chloro-6-fluorobenzaldehyde, acetic acid, 90 °C, 12 h, 27–52%.

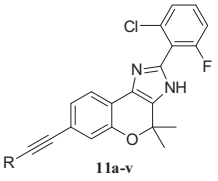
Table 1
SAR of halogens and substituted aryl on A-ring.

Entry	Compd	mPGES-1% inhibition[a]		mPGES-1 IC_{50}[b,c] (nM)
		@ 1 μM	@ 10 μM	
1	**4a**	73.98	88.98	325.2
2	**4b**	68.56	91.97	709.5
3	**4c**	40.80	94.09	–
4	**4d**	44.30	84.21	–
5	**5**	76.04	94.80	–
6	**6**	81.37	83.31	191.4
[d] 7	**7**	91.41	94.62	92.94
8	**8**	70.08	86.89	–
[d] 9	**9**	97.93	91.77	56.89

–: Not determined.
[a] % Inhibition values are means of two experiments in duplicates.
[b] MF-63 (**1**) was used as a positive control in this experiment and for MF-63 in-house enzyme and A549 cell IC_{50s} are 1.9 nM and 56 nM. For the reported potency of MF-63, see Ref. 16a.
[c] IC_{50} values are derived from graphs plotted with data from a minimum of two experiment in duplicates.
[d] A549 whole cell IC_{50s} of **7** and **9** are 616.1 and 839.7 nM respectively.

imidazole derivatives **11a–v**, as shown in Table 2.[31] Also, we focused our attention on the d-ring modification (Fig. 2) to improve the mPGES-1 enzyme potency (Schemes 2 and 3). Accordingly, 7-ethynyl-2,2-dimethylchroman-3,4-dione **13** was prepared by coupling of 7-iodochromanone **2c** with trimethylsilylacetylene using Sonogashira conditions and further TMS-cleavage using tetrabutylammonium fluoride (TBAF), followed by oxidation using isopentyl nitrite[28] as described earlier. Compound **13** was further condensed with 2,6-dibromobenzaldehyde in the presence of ammonium acetate (NH_4OAc) to afford alkyne derivative **14**. A direct conversion of dibromo group to dicyano functionality in compound **14** did not work satisfactorily.[32] Therefore, compound **14** was first coupled with substituted aryl iodides and or bromides using palladium-catalyzed coupling conditions yielded compounds **15a–f**, respectively. Then, the dibromo group in compound **15a–f** was individually converted to dicyanophenyl derivatives **16a–f** using Cu(I)CN in DMF[16a] as displayed in Scheme 2. Furthermore, intermediate **13** was condensed independently with 3,5-dichloroisonicotinaldehyde (**17**) and 3-chloro-5-fluoroisonicotinaldehyde (**18**)[33] in the presence of NH_4OAc to afford compounds **19a** and **19b** in 29–38% yield. Then, compounds **19a** and **19b** were subjected to Sonogashira coupling with substituted aryl iodides or bromides to provide compounds **20a–c** and **21a–b**, correspondingly (Scheme 2 and Table 3).

Table 2
SAR of substituted aryl alkyne on A-ring.

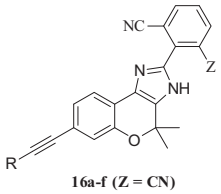


Compd	R	mPGES-1 IC_{50}[a,b] (nM)	A549, 2% FBS PGE_2 IC_{50}[a,b] (nM)
11a	Phenyl	83.02	–
11b	2Cl-Phenyl	64.68	–
11c	3Cl-Phenyl	39.16	–
11d	4Cl-Phenyl	38.24	–
11e	$2CF_3O$-Phenyl	19.85	578.0
11f	$2CF_3$-Phenyl	15.61	692.6
11g	$3CF_3$-Phenyl	29.31	–
11h	$4CF_3$-Phenyl	61.81	–
11i	2, 3-Di-Cl-Phenyl	35.55	–
11j	2, 5-Di-Cl-Phenyl	9.30	1416.0
11k	2, 6-Di-Cl-Phenyl	11.22	–
11l	2F, 5Cl-Phenyl	36.28	838.7
11m	$2CF_3$, 4Cl-Phenyl	7.76	2828.0
11n	$2CF_3$, 5F-Phenyl	21.02	705.7
11o	$2CF_3$, 6F-Phenyl	12.28	691.5
11p	$3CF_3$, 4F-Phenyl	29.65	1252.0
11q	2F, $5CF_3$-Phenyl	25.04	461.0
11r	2Cl, $4CF_3$-Phenyl	28.20	–
[c]**11s**	$5CF_3$-Pyridin-2-yl	320.0	–
11t	3F-Pyridin-4-yl	640.8	–
11u	Pyrimidin-5-yl	796.7	–
11v	$5CH_3$-Thiazole	464.8	–

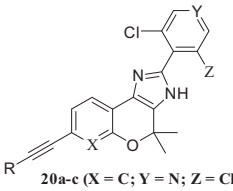
–: not determined.
[a] MF-63 (**1**) was used as a positive control in this experiment and the in-house enzyme and A549 cell IC_{50}s are 1.9 nM and 56 nM. For the reported potency of MF-63, see Ref. 16a.
[b] IC_{50} values are derived from graphs plotted with data from a minimum of two experiment in duplicates.
[c] Check Ref. 29 for details.

Scheme 3 describes the preparation of tricyclic chromenoimidazoles **32a–c** with nitrogen incorporated in the A-ring (Fig. 2). Commercially available 6-chloro-2-methoxynicotinic acid **22** was converted into nicotinoyl acetate **23** using ethyl potassium malonate and magnesium chloride ($MgCl_2$) in the presence of 1,1′-carbonyldiimidazole (CDI) in 72% yield.[34] The carbonyl group in

Table 3
SAR of D- and A-ring modification.



16a-f (Z = CN)



20a-c (X = C; Y = N; Z = Cl)
21a-b (X = C; Y = N; Z = F)
32a-c (X = N; Y = C; Z = F)

Compd	R	Z	mPGES-1 IC_{50} (nM)[b]or% inh.@1/10 μM[a]	A549, 2% FBS PGE_2 IC_{50} (nM)[b]
16a	Phenyl	CN	24.76	984.0
16b	2CF_3-Phenyl	CN	15.90	297.6
16c	2CF_3O-Phenyl	CN	11.77	266.7
16d	2,5-Di-Cl-Phenyl	CN	12.72	2320.0
16e	2F, 6CF_3-Phenyl	CN	12.65	835.9
16f	2Cl, 5CF_3-Phenyl	CN	3.89	1196.0
20a	2CF_3-Phenyl	Cl	81.28	–
20b	2Cl, 5CF_3-Phenyl	Cl	33.06	–
20c	5F, 2CF_3-Phenyl	Cl	31.50	–
21a	2Cl, 4CF_3-Phenyl	F	73.89	–
21b	2CF_3-Phenyl	F	169.7	–
32a	2CF_3-Phenyl	F	407.0	–
32b	2Cl, 5CF_3-Phenyl	F	65%/63%	–
32c	2F, 6CF_3-Phenyl	F	75%/76%	–

–: Not determined;
[a] % Inhibition values are means of two experiments in duplicates.
[b] IC_{50} values are derived from graphs plotted with data from a minimum of two experiments in duplicates. Positive control data is same as in the Tables 1 and 2.

compound **23** was reduced to alcohol **24** using sodium borohydride reduction, followed by addition of an excess methylmagnesium chloride (Grignard reaction) to afford compound **25**. The secondary alcohol in compound **25** was oxidized using pyridinium chlorochromate (PCC) to ketone **26**, followed by one-pot methyl ether cleavage and cyclization using 48% HBr in acetic acid and further reaction with $POCl_3$ (single flask conversion) afforded 7-chloro-2,2-dimethyl-2H-pyrano[2,3-*b*]pyridin-4(3H)-one **27**. Compound **27** was further converted to iodo derivative **28** using NaI, followed by coupling with trimethylsilylacetylene using Sonogashira condition and further trimethylsilyl(TMS) cleavage to afford 7-ethynyl-2,2-dimethyl-2H-pyrano[2,3-*b*]pyridin-4(3H)-one **29**. Palladium-catalyzed, Sonogashira coupling of alkyne **29** with various substituted aryl bromides gave aryl-alkyne derivatives **30a–c** in moderate yield. Compounds **30a–c** were independently oxidized using isopentylnitrite[28] to yield 3,4-diketo derivatives **31a–c**, followed by condensation with 2-chloro-6-fluorobenzaldehyde, as described previously yielded compounds **32a–c**, respectively.

Having accomplished moderate enzyme potency for aryl linked 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole derivatives **5–9** (Table 1), we further evaluated various aryl-alkyne linked compounds **11a–v** for increased mPGES-1 potency (Table 2). While simple phenyl-alkyne linked compound **11a** (IC_{50}: 83.02 nM) retained enzyme potency similar to aryl linked analog **7**, *ortho*-, *meta*- and *para*-substituted chlorophenyl-alkyne derivatives (**11b–d**) showed onefold higher intrinsic mPGES-1 potency in comparison with **7** having IC_{50} of 92.9 nM, respectively. Next, *ortho*-substituted phenylalkyne analogs such as, 2-(trifluoromethoxyphenyl)ethynyl linked compound **11e** (IC_{50}: 19.8 nM) and 2-trifluoromethylphenylethynyl linked compound **11f** (IC_{50}: 15.6 nM) offered 4-fold higher potency over phenyl alkyne **11a**. Further, among the *m*- and *p*-trifluoromethylphenylethynyl derivatives tested, **11g** (IC_{50}: 29.3 nM) retained strong mPGES-1 inhibitory potency, whereas **11h** potency was dropped to 61.8 nM IC_{50} (Table 2). In another variation, dichloro-substituted phenyl alkyne derivatives (**11i–k**) tested, **11j** and **11k** (IC_{50}s = 9.3 and 11.2 nM) displayed 8-fold improvement in the enzyme potency over **11a**. Another set of di-substituted phenyl-alkyne analogs having bulky trifluoromethyl in combination with chloro- and fluoro-derived compounds **11l–r** exemplified excellent mPGES-1 potency (<36 nM IC_{50}, Table 2). Among this, 4-chloro-2-trifluoromethylphenyl alkyne derivative **11m** (IC_{50}: 7.76 nM) demonstrated greater than 10-fold mPGES-1 potency and 6-fluoro-2-trifluoromethylphenyl alkyne derivative **11o** (IC_{50}: 12.2 nM) provided 7-fold higher potency related to un-substituted phenyl alkyne derivative **11a** (IC_{50} = 83 nM). Furthermore, in order to reduce the high lipophilicity of aryl alkyne analogs (Table 2), six- and five-membered heterocycle alkynes **11s–v** were introduced[31] and tested for its mPGES-1 activity. Among the alkynes **11s–v** tested, substituted pyridine-alkyne derivatives **11s** and **11t** displayed >5-fold drop in mPGES-1 potency over similar derivative **11h**, whereas pyrimidine (**11u**) and thiazole (**11v**) derived alkyne analogs revealed substantial loss of mPGES-1 potency in comparison with high potent analogs **11j** and **11m**, suggesting a trend towards incompatibility of polar groups at C(7)-position of 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole core (**X**, Fig. 2). Subsequently, some of the potent mPGES-1 inhibitors (**11e**, **11f** and **11j–q**) were further evaluated for PGE_2 formation (mPGES-1 biomarker) in human A549 epithelial lung carcinoma cell lines. All the tested analogs as shown in Table 2 unveiled only moderate PGE_2 release and the IC_{50}s in the cellular assays were significantly lower in comparison to their in vitro enzyme potency.[29,30]

Therefore, in order to improve the physicochemical properties and the cellular potency of aryl-alkyne analogs, we introduced 2,6-dicyanophenyl, 3,5-dichloropyridyl, 3-chloro-5-fluoropyridyl groups as D-ring and incorporated nitrogen in the A-ring as shown in Fig. 2, Table 3 and these alkyne analogs were evaluated for mPGES-1 in vitro potency (Schemes 2 and 3).[29] Simple phenyl-alkyne derivative **16a** (IC_{50}: 24.76 nM and A549 IC_{50}: 984 nM)

Table 4
In vitro PGE_2 release activity, COX-selectivity and in vitro ADME profiles of selected compounds.

Compd	Cell A549, 2% FBS PGE_2, IC_{50} (nM)[a]	COX-1 IC_{50} (μM)[b]	COX-2 IC_{50} (μM)[b]	Metabolic stability in liver microsomes (% remaining)[c]	CYP inhibitions at 10 μM concentrations (%)[d]					PPB (% bound)[e]
				H/R/GP	1A2	2D6	3A4	2C9	2C19	H/G.Pig
7	616.1	8.35	>10.0[f]	89/99/93	–	56	37	35	–	–
9	839.7	6.75	>10.0[g]	84/83/80	NI	NI	8.4	45.4	27.2	>99.5/>99.5
11l	838.7	0.06	9.88	76/44/73	33.9	26.6	31	33.3	17/26	>95.5/>99.5
16b	297.6	–	–	81/–/100	–	29	24	32	–	–

–: not determined; NI: no inhibition.
[a] MF-63 (**1**) was used as a positive control in this experiments and the in-house A549 cell IC_{50} = 56 nM. For the literature reported potency of MF-63, see Ref. 16a. IC_{50} values represent the concentration to inhibit 50% of PGE_2 relative to vehicle control and derived from graphs plotted with data from a minimum of two experiments in duplicates.
[b] IC_{50} values are derived from graphs plotted with data from a 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 °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] COX-2% inhibition values are 2.65%@1 μM and 27.45%@ 10 μM.
[g] COX-2% inhibition values are 2.37%@1 μM and 24.21%@ 10 μM.

having 2,6-dicyanophenyl as D-ring revealed approximately four-fold higher mPGES-1 potency and moderate cell potency in comparison to structurally similar analog **11a**. Furthermore, *ortho*-substituted phenyl alkyne derivatives **16b** (IC_{50}: 15.9 nM and A549 IC_{50}: 297.6 nM) and **16c** (IC_{50}: 11.77 nM and A549 IC_{50}: 266.7 nM) exemplified good enzyme and enhanced cell potency in comparison with **11f** and **11e** (A549 cell IC_{50}s are 692.6 and 578 nM), which were structurally similar except for the D-ring modification. Among the di-substituted phenyl alkyne derivatives tested, analogs **16d–f** demonstrated excellent enzyme potency (<13 nM). However, their A549 cellular potency (>800 nM) was significantly less in comparison with other dicyanophenyl as D-ring analogs **16b** and **16c** (<300 nM) and the reason for the cell potency discrepancy was not readily explainable (Table 3). Similarly, 3,5-dichloropyridyl (as D-ring) derived alkyne compounds **20a–c** exhibited slight drop in mPGES-1 potency in relation to similar alkyne derivatives **11f** and **11n** (enzyme IC_{50}s are 15.6 and 21 nM), respectively. In another variation, 3-chloro-5-fluoropyridyl (as D-ring) derived alkyne analogs **21a** (IC_{50}: 73.89 nM) and **21b** (IC_{50}: 169.7 nM) displayed roughly 3 to 11-fold lower mPGES-1 potency compared to **11r** and **11f** having identical substitution at C(7)-position of A-ring. Next, the impact of nitrogen on the A-ring of tricyclic 4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*]imidazole (Table 3) was assessed for the mPGES-1 potency. Thus, 2-trifluoromethylphenyl alkyne derivative **32a** (IC_{50}: 407 nM) provided ~ 37-fold lesser potency than the corresponding non-pyridyl analog **11f**. In contrast to compounds having nitrogen at D-ring (**20a–c** and **21a–b**), nitrogen inserted at A-ring derivatives **32b** and **32c** revealed complete loss of potency (only 65–75% mPGES-1 inhibition at 1 μM test concentration). Albeit many compounds divulged good mPGES-1 enzyme potency in the alkyne series (<30 nM), A549 cellular potency was not significantly improved as anticipated (Tables 2 and 3) with all the projected D-ring modifications.

As part of the lead optimization strategy, selected potent compounds were further screened for COX enzymes selectivity and in vitro ADME profiles (Table 4). Most of the tested compounds (**7**, **9** and **11l**) exhibited >150-fold selectivity for COX-2 inhibition

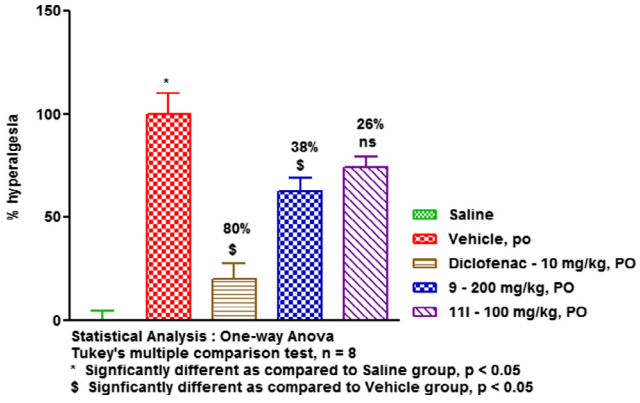


Fig. 3. Analgesic effects of **9** and **11l** in the guinea pig hyperalgesia pain model. Data are expressed as percentage of hyperalgesia, with the naive group (injected intraplantarly with saline) as 0% and the vehicle-treated LPS-injected group as 100% (Results are shown as mean ± SEM, n = 8 animals per dose group).

Table 5
Oral PK of **9** and **11l** in rat and guinea pig.

PK parameters[a]	Compd: **9**[a,b,c]			Compd: **11l**[a,b]	
	Rat i.v dose (5 mg/kg)	Rat oral dose (10 mg/kg)	G.Pig oral dose (100 mg/kg)	Rat IV dose (5 mg/kg)	Rat oral dose (10 mg/kg)
C_{max} (ng/mL)	–	826 ± 125	803 ± 201	–	1176 ± 0.94
AUC (ng h/mL)	–	12,562 ± 1100	13,427 ± 2576	–	12,380 ± 491
T_{max} (h)	–	4	24	–	4
Bioavailability (%F)	–	33	–	–	85
$T_{1/2}$ (h)	9.87 ± 0.41	11.2 ± 1.95	ND	5.50 ± 0.16	6.77 ± 0.87
CL (mL/min/kg)	3.63 ± 0.60	–	–	11.14 ± 0.39	–
Vz (L/kg)	3.11 ± 0.64	–	–	5.30 ± 0.10	–

–: not applicable; ND: Not determined.
[a] C_{max}, AUC_{0-24}, T_{max}, $T_{1/2}$, bioavailability (%F), Clearance (CL) and volume of distribution (Vz) were determined in male Sprague-Dawley rats.
[b] *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)
[c] *Vehicle for guinea pig Oral dosing – 0.5% methylcellulose suspension.* The data represented is mean ± SD (n = 3).

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Table 6
Plasma and CSF concentration of **9** and **11l** at PD time point.

Compd	Dose (mg/kg, po, od)	% Hyperalgesia inhibition	Plasma concentration @PD time point (nM)[a]	CSF concentration @ PD time point (nM)[a]
9	200	38	7541	9.22
11l	100	26	13,831	2.63

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

and >70-fold selectivity for COX-1 inhibition except **11l**, which revealed dual mPGES-1 (IC_{50}: 36.2 nM) and COX-1 (IC_{50}: 60 nM) activity. The in vitro metabolic stability of compounds **7**, **9**, **11l** and **16b** were assessed in human, rat and guinea pig liver microsomes using the standard procedure.[29] Briefly, these compounds were incubated at a concentration of 1.0 μM with 1.0 mg/mL protein at 37 °C for 60 min, then samples analyzed using LC/MS/MS and further, the analysis of% remaining as disclosed in Table 4. All the compounds were found to be metabolically stable except **11l** which showed moderate stability in rat liver microsome. Compounds **7**, **9**, **11l** and **16b** were further evaluated for the inhibition of clinically relevant CYP isoforms (1A2, 2D6, 3A4, 2C9, 2C19) in 1 and 10 μM concentrations and no significant inhibition was observed. Further, compounds **9** and **11l** were found to be highly protein binding as illustrated in Table 4, which plausibly explains the poor cellular potency.[35]

Earlier, our group had studied several cell potent mPGES-1 inhibitors (A549 cell IC_{50}s: <20 nM) in the *in vivo* guinea pig animal model which demonstrated excellent efficacy.[13,14,23,36–38] Similarly, in order to understand the correlation between PGE_2 release cell potency and *in vivo* efficacy, compounds **9** and **11l** with poor A549 cellular potency[39,40] were further selected for *in vivo* oral pharmacokinetics (PK) and efficacy study.[38] Selected compounds **9** and **11l** were evaluated in rat and guinea pig for oral pharmacokinetic studies[29] and the results are disclosed in Table 5. The rat oral PK profile of **9** (10 mg/kg) revealed adequate C_{max} (826 ng/mL), $AUC_{0\text{-inf}}$ (12562 ng h/mL) and moderate bioavailability (33%), whereas **11l** exemplified enhanced C_{max}, (1176 ng/mL) and oral bioavailability (85%) with an $AUC_{0\text{-inf}}$ (12,380 ng h/mL) comparable to **9**. Besides, both compounds (**9** and **11l**) exhibited delayed absorption (T_{max} = 4 and 4 h), low *in vivo* clearance (3.6 and 11.1 mL/min/kg, respectively), volume of distribution (3.11 and 5.3 L/kg, respectively) and adequate half-life ($T_{1/2}$) in rat. The guinea pig oral PK of **9** (100 mg/kg) was comparable to its 10 mg/kg rat PK study and revealed slow absorption (T_{max} = 24 h, respectively). Overall, both compounds exhibited favorable pharmacokinetic profile thus suitable for *in vivo* efficacy study.

Having established favorable oral pharmacokinetics in rat and guinea pig, the selected compounds **9** and **11l** were further evaluated in the LPS-induced hyperalgesia guinea pig pain model[8,21] to assess analgesic effects (Fig. 3, Table 6). Injection of LPS into the plantar caused a significant increase in thermal hyperalgesic response compared to saline injected animals. The clinically approved pain drug, diclofenac inhibited 80% of the hyperalgesic response at the dose of 10 mg/kg when administered orally at 1 h before LPS injection, whereas tool compounds **9** (200 mg/kg) and **11l** (100 mg/kg) revealed only 38% and 26% inhibition of hyperalgesic response relative to vehicle treated animal. The plasma concentration at pharmacodynamics (PD) time point for the compounds **9** and **11l** were found to be 7.5 μM and 13.8 μM, respectively. Despite having adequate plasma PD concentrations, the cerebrospinal fluid (CSF) concentrations of **9** and **11l** at PD time point were found to be very low (Table 6). Therefore, moderate efficacy observed with compounds **9** and **11l** might be due to several factors including low CSF concentration, poor cellular potency and unspecific high plasma protein binding,[35] as thermal hyperalgesia response is not only mediated by peripheral PGE_2, but also significantly involves centrally mediated PGE_2.[41,42]

In summary, we have described a novel class of tricyclic 2-(2-chloro-6-fluorophenyl)-4,4-dimethyl-3,4-dihydrochromeno[3,4-*d*] imidazoles with aryl- and aryl alkyne-substituted compounds as mPGES-1 inhibitor with excellent enzyme potency and moderate to poor cellular potency. Careful modification on the D-ring afforded several mPGES-1 inhibitors with improved cell potency. Furthermore, the representative tool compounds **9** and **11l** exhibited good selectivity over COX enzymes and favorable in vitro and *in vivo* pharmacokinetic properties and also demonstrated moderate efficacy in the LPS-induced thermal hyperalgesia pain model (acute study) in comparison to diclofenac (approved COX-1 inhibitor and pain drug). Therefore, further SAR optimization and core scaffold alteration is warranted to afford compounds with improved cellular potency, favorable plasma protein binding and acceptable lipophilicity for further therapeutic utility as mPGES-1 inhibitors.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.03.068.

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