Characterization of 3,3-Dimethyl Substituted N-aryl Piperidines as Potent Microsomal
Prostaglandin E Synthase-1 Inhibitors

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Abstract:

Here we report on novel, potent 3,3-dimethyl substituted N-aryl piperidine inhibitors of
microsomal prostaglandin E synthases-1(mPGES-1). Example **14** potently inhibited PGE₂
synthesis in an *ex vivo* human whole blood (HWB) assay with an IC₅₀ of 7 nM. In addition, **14**
had no activity in human COX-1 or COX-2 assays at 30 μM, and failed to inhibit human
mPGES-2 at 62.5 μM in a microsomal prep assay. These data are consistent with selective
mPGES-1-mediated reduction of PGE₂. In dog, **14** had oral bioavailability (74%), clearance

(3.62 mL/(min*kg)) and volume of distribution ($V_{d,ss} = 1.6$ L/kg) values within our target ranges.

For these reasons, **14** was selected for further study.

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Prostanoids are a class of 20-carbon fatty acid derivatives of arachidonic acid which participate in a wide array of cellular signaling pathways. Transformation of arachidonic acid by cyclooxygenase-1 or -2 (COX-1 or COX-2) yields prostaglandin H₂ (PGH₂), which is further modified by specific prostaglandin synthases to form biologically active products: prostaglandin E₂ (PGE₂), prostaglandin F_{2α} (PGF_{2α}), prostaglandin D₂ (PGD₂), prostacyclin (PGI₂) and thromboxane A₂ (TxA₂).^{1, 2} Each of these products contributes to important cellular activities for homeostasis and cell signaling.^{3, 4} Specifically, the role of PGE₂ in nociception has drawn significant attention, and PGE₂ signaling has been hypothesized as the mechanism by which COX inhibitors exert analgesic activity.⁵ In fact, recent analysis of COX inhibitors has revealed a correlation between the reduction of PGE₂ production in bacterial lipopolysaccharide (LPS) stimulated human whole blood and the corresponding drug plasma concentrations need for analgesic efficacy in humans.⁶

Therapies that target the inhibition of both the COX-1 and COX-2 enzymes; *e.g.*, aspirin and ibuprofen, are effective at treating joint mobility issues and pain associated with chronic inflammation.⁷ Unfortunately, with prolonged use, this class of drugs carries the risk of causing gastric bleeding and ulcerations by inhibiting the synthesis of the normal gastroprotective PGE₂ produced through the COX-1 pathway.⁸ In an attempt to pave the way to safer therapeutics, selective COX-2 inhibitors have been developed. While this approach proved efficacious, increased adverse cardiovascular events led to the withdrawal of selective COX-2 drugs rofecoxib and valdecoxib from the market.^{4,9} (One selective COX-2 inhibitor, celecoxib, remains available to patients.) The reduction of antithrombotic PGI₂, a consequence of disrupting the pathway at COX-2, is speculated to contribute to the adverse cardiovascular outcomes.¹⁰ Because of the efficacy seen with the above therapies, selective reduction of PGE₂ levels continues to be an attractive option for development of therapeutic analgesics.¹¹

Three PGE₂ synthases have been identified to date: microsomal prostaglandin E synthase-1 (mPGES-1), mPGES-2, and cytosolic PGES (cPGES).⁵ While mPGES-2 and cPGES are constitutively expressed, only mPGES-1 is induced during the inflammatory response.¹¹ Moreover, recent evidence has shown that mPGES-2 does not contribute to the production of PGE₂ *in vivo*.¹² Studies with mPGES-1 knockout mice have demonstrated attenuated nocifensive responding, reduced febrile response, and decreased severity to collagen antibody-induced arthritis, each of which was accompanied by decreased PGE₂ levels.¹³ Systemic reduction of PGE₂ by mPGES-1 inhibition has been recognized as an attractive alternative to COX-2 inhibitors, because through this mechanism, PGI₂ should not be suppressed.¹⁴

Multiple mPGES-1 inhibitor chemotypes have been identified in the course of research into this high value target (Figure 1). A group at NovaSAID published a series of substituted

benzimidazoles, exemplified by compound **1** with a reported IC_{50} of 25 nM in a microsomal enzyme assay,¹⁵ and Daiichi Sankyo's recent patent explored 5-phenylfuro[2,3-b]pyridines exemplified by **2**.¹⁶ Example **3** from Pfizer has a reported IC_{50} of 3 nM.¹⁷ In addition, we have previously reported on a series of 2-(acylamino)imidazoles such as **4** (IC_{50} = 0.9 nM).¹⁸

Figure 1. Structures of known mPGES-1 inhibitors.

In this manuscript, we describe our continued efforts toward identifying novel inhibitors of human mPGES-1. To characterize the potency and selectivity of our ligands, we utilized a series of three assays. First, enzyme inhibition was measured *in vitro* using a cell-free microsomal mPGES-1 enzyme preparation where PGE_2 concentration was evaluated by LC/MS. Secondly, we tested compound efficacy and selectivity in hIL-1 β stimulated A549 (epithelial carcinoma) cells, which have previously shown to produce a variety of prostanoids, including PGE_2 and PGI_2 . The prostanoids secreted into the medium were assayed with ELISA kits. In this assay, coxibs inhibited both PGE_2 and PGI_2 while mPGES-1 inhibitors blocked only the

synthesis of PGE₂. Therefore, this assay served to determine selectivity of action and was used as a basis for excluding compounds. Finally, compounds were incubated in LPS-stimulated single-donor human whole blood (HWB), which was then monitored for levels of PGE₂, TxB₂, and PGF_{2α} by LC/MS/MS. This assay provided a direct measurement of target engagement in a relevant human tissue, obviating the need to factor in additional drug concentration corrections such as fraction unbound in the determination of potency. The relationship between PGE₂ reduction (as measured in patient plasma samples) and clinical benefit, which was known from previous work with COX-2 inhibitors, was used in combination with PK data to project an efficacious oral dose.⁶ We chose an efficacious dose of celecoxib as the benchmark for both PGE₂ reduction and duration of coverage by which we would compare our inhibitors.

Celecoxib, a widely prescribed and efficacious COX-2 inhibitor, is reported to have a HWB IC₅₀ of 540 ± 70 nM (monitored by PGE₂ levels) and an oral exposure in humans after a therapeutic dose (200 mg) that affords plasma drug concentrations exceeding the HWB IC₅₀ for 8–12 hours. We therefore set out to develop inhibitors capable of delivering a celecoxib-like profile, but whose PK properties would potentially maintain plasma concentrations which exceeded their HWB IC₅₀ values for 24 hours after oral dosing.

As part of ongoing efforts, we sought ligands structurally distinct from those found in our previous work. Here we report on novel, potent 3,3-dimethyl substituted N-aryl piperidine inhibitors of mPGES-1 (Table 1).¹⁹ The piperidine stereocenter was assigned based on X-ray co-crystal data (*vide infra*) and the less potent isomer was assigned the opposite configuration by deduction.²⁰ Initial evaluation of stereoisomeric pairs **5–6** and **7–8** revealed a significant preference for the (*S*) configuration at the piperidine stereocenter. Inhibition of PGE₂ production generally was consistent across the three screening assays, and for the reasons given above, our

focus was ultimately on potency in the HWB assay. Replacement of the 8-chloroquinoline of **5** with an 8-methylquinoline (**9**) improved the HWB activity to 2 nM. Changing the 4-hydroxymethylcyclohexane of **9** to a tetrahydropyran (**10**) or tetrahydrofuran (**11**) decreased potency by more than an order of magnitude in the HWB assay. Acyclic analog **12** was approximately as potent as its cyclic congeners (**11–12**) in HWB (IC₅₀ 77 nM). The combination of the 3-hydroxymethylcyclohexane from **7** and the 8-methylquinoline from **9** produced the most potent compound (**13**) in the HWB assay, with an IC₅₀ of 1 nM. Replacing a methylene in the cyclohexyl ring of **13** with an oxygen atom resulted in a potency loss in HWB (**14**).

Compd	R ¹	R ²	Piperidine Chirality	mPGES-1 IC ₅₀ (μM) ^a	A549 PGE ₂ IC ₅₀ (μM)	HWB PGE ₂ IC ₅₀ (μM) ^b
5			<i>S</i>	0.004	0.03 (1)	0.023
6			<i>R</i>	3.72	>10.0 (1)	
7			<i>S</i>	0.003	0.011 (1)	0.005
8			<i>R</i>	0.504	4.5 (1)	
9			<i>S</i>	0.001	0.002 (1)	0.002

10			<i>S</i>	0.007	0.072	0.036
11			<i>S</i>	0.006	0.023 (1)	0.051
12			<i>S</i>	0.012	0.089 (1)	0.077
13			<i>S</i>	0.001	0.003	0.001
14			<i>S</i>	0.002	0.005	0.007

Table 1. Values are reported as geometric means with a minimum of three replicates unless noted by the trailing number in parentheses. All geometric sample standard deviations ($\times/\div$) were less than 2.0 for mPGES-1 enzyme and A549 assay data and less than 3.0 for HWB data.

To better understand the SAR and interactions observed with our ligands, we generated an X-ray co-crystal structure of **14** with human mPGES-1. The structure revealed a water-mediated hydrogen bond network between w26, Gln36, Ile32, and the amide carbonyl of **14** (Figure 2A). Additionally, His53 shares a hydrogen bond with the hydroxyl group of **14**. The space above the glutathione cofactor is occupied by the tetrahydropyran with interactions dominated by van der Waals contacts in a similar manner to previously reported cocrystal structures of mPGES-1 inhibitors.^{21, 22} A surface map reveals a significant contact area with Leu39 and Ile32, forming the top of a cleft in which the ligand sits. Notably, the methyl substituent of the quinoline ring fits deeply in the protein surface, displacing a conserved water molecule seen in previously reported cocrystal structures (PDB codes 4YK5, 4YL0, 4YL1 and 4YL3).²¹

Figure 2. (A) Monomer 1 is colored orange. Monomer 2 is colored cyan. Protein side chains and backbone atoms, shown as sticks, are colored by atom (O, red; N, blue; C, same as respective monomer). **14** is represented by sticks and colored by atom (O, red; N, blue; C, yellow). GTT is represented by sticks and colored by atom (O, red; N, blue; C, magenta; S, tan). Waters are represented by spheres (red). Hydrogen bonds are indicated by green dashes. (B) The binding site is represented as a surface with the color scheme as is in (A). GTT is depicted by spheres. Polar areas are represented by blue (N) and red (O), and nonpolar surfaces are represented by cyan in monomer 1 and orange in monomer 2. The 8-methyl of the quinolone points inwards into a water channel at the back of the active site.

For compounds **5–14**, PGI₂ production in A549 cells was not decreased at up to 10 μ M test concentrations. Similarly, PGF_{2 α} and TxB₂ were not decreased at up to 30 μ M compound concentrations in the HWB assay. These observations suggest that the compounds do not inhibit the COX enzymes. In line with these observations, **14** had no activity in human COX-1 or COX-2 assays at 30 μ M, and examples **10**, **13**, and **14** failed to inhibit human mPGES-2 at 62.5 μ M in a microsomal prep assay. These data are consistent with selective mPGES-1-mediated reduction of PGE₂ in stimulated whole blood.

Compounds **9**, **13**, and **14** were selected for additional characterization (Table 2). Microsomal metabolism in rat, dog, and human hepatocytes was moderate for **9** and **13**, while **14** was less metabolized. In addition, **14** exhibited less CYP2C9 and CYP3A4 inhibition than did **9** and **13** (Table 2).

Cmpd	clogP ^a	Rat% ^b	Dog% ^b	Human% ^b	CYP2C9% Inh ^c	CYP2D6% Inh ^c	CYP3A4% Inh ^c
9	4.31	64	60	46	54	4	35
13	4.31	73	57	50	64	0	43
14	2.91	16 ^d	13 ^d	20 ^d	15 ^d	0 ^d	10 ^d

Table 2. a) Calculated using Marvin and calculator plugin freewar (www.chemaxon.com, ChemAxon Kft. Budapest, Hungary); b) Percent metabolized in hepatic microsomes at 4 μ M test concentration; c) Percent inhibition of probe substrate metabolism in human hepatic microsomes at 10 μ M test concentration; d) Data is an average of two separate runs.

Next, we evaluated the pharmacokinetic (PK) properties for these ligands in rats (Table 3).

Compounds were dosed orally at 5 mg/kg or intravenously at 1 mg/kg. Steady state volumes of distribution ranged between 1.3 and 2.3 L/kg. While **9** and **13** both had bioavailability greater than our target of 20%, **14** proved superior. The clearance and $C_{\max}$ values were within ranges we considered desirable.

Cmpd	1 mg/kg IV			5 mg/kg PO	
	CL (mL/(min*kg))	$t_{1/2}$ (h)	$V_{d,ss}$ (L/kg)	$C_{\max}$ (nM)	%F
9	21 $\pm$ 1.56	2.6 $\pm$ 0.19	1.4 $\pm$ 0.05	1450 $\pm$ 125	54
13	34 $\pm$ 2.49	0.6 $\pm$ 0.01	1.3 $\pm$ 0.07	1430 $\pm$ 193	30
14	14 $\pm$ 4.07	2.3 $\pm$ 0.22	2.3 $\pm$ 0.19	2050 $\pm$ 176	100

Table 3. Pharmacokinetics in rats. Data are reported as mean of observations from three animals with standard deviation.

Further profiling of **9**, **13**, and **14** revealed a measurable time dependent inhibition (TDI) of CYP3A4 when tested at a 10 μ M concentration in human hepatic microsomes. Inhibition of 75% and 82%, for **9** and **13** respectively, represented a potential risk factor for drug-drug interactions with prolonged drug exposure. With a corresponding value of 39%, compound **14** appeared to differentiate. At this point, our focus turned to **14** for further study.

To investigate possible species differences, we evaluated **14** in rat (IC_{50} = 7.87 μ M $\times/\div$ 1.16) and dog (IC_{50} = 11 nM $\times/\div$ 1.17) mPGES-1 enzyme assays. The species selectivity

difference is consistent with previously reported mPGES-1 inhibitors.^{17, 18, 23} The dog enzyme and whole blood ($IC_{50} = 28 \text{ nM} \times / \div 1.53$) potencies were similar to those observed in the corresponding human assays. This suggested the possibility of exploring preclinical pharmacology in dogs and prompted us to evaluate the pharmacokinetic profile of **14** in that species.

In dog, the clearance ($CL = 3.62 \pm 0.36 \text{ mL}/(\text{min} \cdot \text{kg})$) and volume of distribution ($V_{d,ss} = 1.6 \pm 0.15 \text{ L/kg}$) of **14** after IV (1 mg/kg) administration and the bioavailability ($\%F = 74\%$) after a 15 mg/kg oral dose were within our target ranges. In a separate dog PK study (Figure 3), after a 3 mg/kg oral dose, drug plasma levels exceeded the DWB IC_{50} for 24 hr. Thereby, compound **14** met our exposure criterion in a preclinical species.

Figure 3. PK of **14** in beagle after 3 mg/kg po dosing (n = 3). Diamonds indicate means and error bars represent standard deviation of measured values. Dog whole blood potency noted for comparison purposes.

Preparation of **14** was achieved by a convergent synthesis as shown in Schemes 1–3. The chirally pure acid **21** was prepared as follows: ketone **15** was converted to vinyl triflate **16** followed by palladium mediated carbonylation in the presence of methanol to provide the methyl ester **17**. Attempts to hydrogenate the olefin stereoselectively were unsuccessful; therefore, we continued with racemic **18**. Deprotection of **18** followed by nucleophilic aromatic substitution on 2-chloro-8-methylquinoline gave *rac*-**20**, which was subjected to chiral chromatography to provide methyl ester (*S*)-**20** as a single enantiomer. Acidic hydrolysis of the ester gave rise to the advanced intermediate (*S*)-**21**. The acidic conditions were employed because they did not epimerize the stereocenter alpha to the carbonyl.

Scheme 1. Synthesis of **21**. Reagents and conditions: (a) $\text{ Tf}_2\text{NPh}$, BuLi , THF , 75%; (b) CO , $\text{ CH}_3\text{OH}$, $\text{ Pd}(\text{OAc})_2$, dppf , $\text{ Et}_3\text{N}$, 88%; (c) H_2 , Pd/C , $\text{ CH}_3\text{OH}$, 85%; (d) HCl , dioxane, 92%; (e) 2-chloro-8-methylquinoline, $\text{ K}_2\text{CO}_3$, DMSO , chiral separation, 43%; (f) $\text{ H}_2\text{SO}_4$ (aq), 96%.

Synthesis of the 4-aminotetrahydropyran **22** (Scheme 2) began with a hetero-Diels–Alder cycloaddition between aldehyde **23** and diene **24** to provide dihydropyranone **25**. Palladium catalyzed hydrogenation of the olefin moiety of **25** gave ketone **26**, which was reduced with LAH to afford *rac*-**27**. The racemate was resolved by chiral chromatography to give chirally pure (2*S*,4*R*)-**27**. Mesylation to furnish (2*S*,4*R*)-**28**, followed by S_N2 displacement with sodium azide, yielded (2*S*,4*R*)-**29**, which was hydrogenated to provide (2*S*,4*R*)-**22**. Finally, (S)-**21** and (2*S*,4*R*)-**22** were coupled to give the desired amide **14** (Scheme 3).

Scheme 2. Synthesis of intermediate **22**. Reagents and conditions: (a) ZnCl₂, THF/Toluene, 73%; (b) H₂, Pd/C, EtOAc, Et₃N, 84%; (c) LAH, THF; (d) Chiral Chromatography, 42% two steps; (e) MsCl, DIEA, CH₂Cl₂, 97%; (f) NaN₃, DMF; (g) H₂, PtO₂, EtOAc, 85%.

Scheme 3. a) Synthesis **14**. Reagents and conditions: (a) PyBOP, Et₃N, DMF, 76%

In summary, we have identified a novel series of 3,3-dimethylpiperidine-1-quinolines as inhibitors of mPGES-1. Importantly, these compounds are capable of selectively inhibiting PGE₂ production at low nanomolar concentrations in HWB. The potency of **14**, taken along with its pharmacokinetic profile, suggests that this compound would be amenable to clinical investigation for anti-inflammatory therapy.

Accession Codes

Atomic coordinates and structure factors for compound **14** with mPGES-1 are available from the RSCB Protein Data Bank with accession code 5K0I.

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- 20 Compounds **5–8** were synthesized in an analogous method to **14** but carried through as 1:1 mixtures at the piperidine center, and ultimately separated by chiral chromatography as the final step. We desired a more efficient route and chose to synthesize advanced intermediate **21** as a single enantiomer (Scheme 3). Protein Co-crystal structure of thereby prepared **14** revealed the

configuration of the preferred stereo center to be S (figure 2). The remaining products (**9–13**) were prepared from the preferred isomer.

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