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Trisubstituted ureas as potent and selective mPGES-1 inhibitors

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ABSTRACT

A novel series of trisubstituted ureas has been identified as potent and selective mPGES-1 inhibitors. These compounds are selective over other prostanoid enzymes such as PGF synthase and TX synthase. This series of inhibitors was developed by lead optimization of a hit from an internal HTS campaign. Lead compound **42** is potent in A549 cell assay (IC_{50} of 0.34 μM) and in human whole blood assay (IC_{50} of 2.1 μM). An efficient and versatile one-pot strategy for the formation of ureas, involving a reductive amination, was developed to generate these inhibitors.

Prostaglandin E_2 (PGE_2) is a key mediator in fever, pain and the inflammatory response.[1] Like all prostaglandins, PGE_2 is produced from arachidonic acid (AA), a fatty acid present in the phospholipids of cell membranes. First, AA is converted to PGH_2 by cyclooxygenases (COX-1/COX-2), followed by the subsequent isomerization of PGH_2 into PGE_2 by microsomal prostaglandin E_2 synthase (mPGES-1).[2] Selective inhibition of mPGES-1 would be expected to preclude PGE_2 production[3] without any potential side effects, resulting from the inhibition of PGD_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 biofunctions by the blockade of their respective prostanoid enzymes, PGD synthase, PGF synthase, PGI synthase and TX synthase.[4]

We recently reported that the phenanthrene imidazoles **1** and **2** are potent and selective mPGES-1 inhibitors (Fig. 1).[5] Furthermore, compound **2** also have a good pharmacokinetic profile in rats ($t_{1/2}$ = 2.3 h; F = 68%; iv: 5 mg/kg; po: 20 mg/kg). As part of an ongoing process aimed at the discovery of another clinical candidate for the program, a high throughput screening campaign was conducted on our in-house sample collection in order to identify a new structural class of mPGES-1 inhibitors. From that initiative, trisubstituted urea **3** was identified as a moderate inhibitor of recombinant human mPGES-1, achieving 88% enzyme inhibition at 10 μM.[6] Herein, we wish to report on the structure–activity relationship (SAR) of trisubstituted urea analogs that lead to selective and potent mPGES-1 inhibitors.

Starting from lead compound **3**, early exploratory efforts demonstrated that replacing both the ethylpyridyl and *N*-cyclopentyl moieties with benzyl and isopropyl groups in **4**, increased the potency (IC_{50}) in the enzyme assay from 10 μM to 1.7 μM (data not shown). For subsequent potency optimization, we chose to fix the identity of these two groups, while studying the impact of introducing a *meta*-substituent on the lower *N*-benzyl group in **4** (Table 1). It is interesting to note that *ortho*- and *para*-substituted regioisomeric analogs of compound **4** are not active against mPGES-1 (IC_{50} >10 μM). Replacement of the *meta* phenyl group in **4** by a variety of polar groups such as an amide (**6**), or an ether (**8** and **9**) provided inhibitors of comparable enzyme and cellular potency. Installation of a sulfonamide in **7** yielded a slight increase in intrinsic potency, however, this modification resulted in a loss of activity in our cell-based assay, which measured the production of PGE_2 in an A549 cell line under high serum conditions.[7] Incorporation of a rigid alkyne linker in lead compound **4** to give tolane analog **10** increased potency in the enzyme assay by 300-fold (IC_{50} = 0.008 μM) and in the cellular assay by 10-fold (IC_{50} = 0.91 μM). Walking substituents around the distal phenyl ring of **10** indicated that substitution was best tolerated in the *meta*- and *para*-positions in the lower *N*-benzyl group (compounds **11**–**17**). Introduction of an electron-donating methoxy substituent in **12** preserved enzyme potency and low micromolar activity in the cell assay. Similar observations were made when the methoxy

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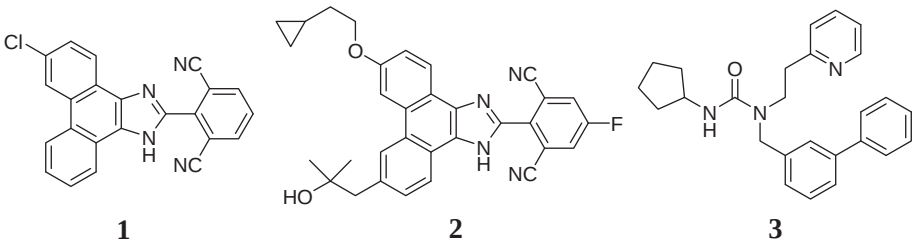
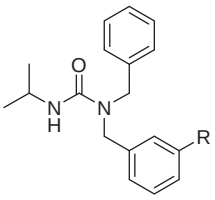
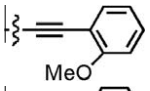
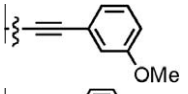
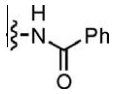
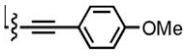
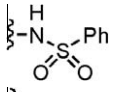
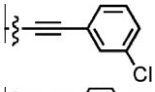
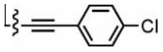
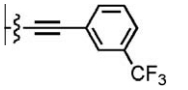
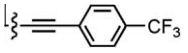


Figure 1. Phenanthrene imidazoles and trisubstituted urea mPGES-1 inhibitors.

Table 1
Lower *N*-benzyl group SAR



Compd	R	mPGES-1 inhibition IC$_{50}$[a] (μM)	A549, 50% FBS PGE$_2$ IC$_{50}$[a] (μM)	Compd	R	mPGES-1 inhibition IC$_{50}$[a] (μM)	A549, 50% FBS PGE$_2$ IC$_{50}$[a] (μM)
4		1.7	11.9	**11**		0.15	–
5		4.5	–	**12**		0.027	1.3
6		3.5	4.3	**13**		0.072	4.2
7		0.66	>50	**14**		0.013	3.7
8		1.1	11.9	**15**		0.022	2.0
9		1.8	8.6	**16**		0.009	1.8
10		0.008	0.91	**17**		0.013	0.99

[a] Values are means of at least two experiments.

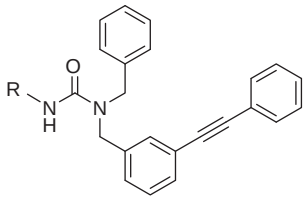
groups in **12** and **13** were replaced by electron-poor chlorines (**14** and **15**) or trifluoromethyl groups (**16** and **17**).

Next, we moved our attention to the substituent located on the monosubstituted nitrogen of the urea starting with compound **10** as our reference point. The results of these explorations are documented in Table 2. We found that replacement of the isopropyl substituent with a larger phenyl in **18** or a benzyl group in **19** led to a complete loss of activity against isolated mPGES-1 enzyme. Generally speaking, a variety of small aliphatic groups were found to be suitable surrogates for the isopropyl in **10**. For example, compounds **20** through **24** were within 1 to 6-fold of the enzyme and whole cell potency observed for **10**.

Replacement of the urea core in **10** with a number of isosteres was also investigated (Table 3). Guanidine analog **25** suffered a 1000-fold loss of potency in the enzyme assay, but only experienced a twofold shift in the cell-based assay compared to **10**. *N*-Boc guanidine **26** was 40-times more active than **25** against mPGES-1, but was more highly shifted in the A549 assay. Switching the *N*-Boc for an *N*-cyano in **27** preserved mPGES-1 activity and reduced the cell shift to sixfold thereby achieving a whole cell potency comparable to that observed with **10** despite a 33-fold lower enzyme potency. The most potent compound against isolated mPGES-1 in this series was thiourea **28**. However, this compound was three times less potent than **10** in the cell assay. As such, we continued our optimization for potency using the initially discovered urea scaffold.

We next focused on optimizing the upper *N*-benzyl portion of the inhibitor as shown in Table 4. Introduction of a methoxy group on the phenyl ring (**29**–**31**) revealed that the *meta* position is the most potent (**31**). Substitution of the methoxy group in **31** by different moieties such as trifluoromethyl (**32**), methyl (**33**) or phenyl (**34**) provided compounds with similar cellular potencies. Incorporation of a second alkyne at this same position in the molecule generated inhibitor **35.** This compound exhibited an

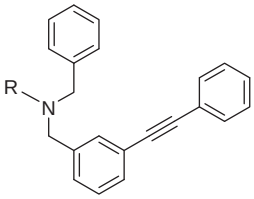
Table 2
Monosubstituted nitrogen SAR

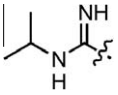
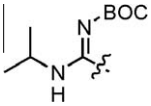
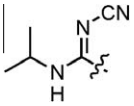
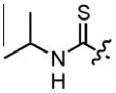


Compd	R	mPGES-1 inhibition	A549, 50% FBS PGE_2
		IC_{50}[a] (μM)	
10		0.008	0.91
18		>10	–
19		>10	–
20		0.008	2.9
21		0.012	2.4
22		0.031	1.6
23		0.032	5.2
24		0.048	3.7

[a] Values are means of at least two experiments.

Table 3
Urea isosteres



Compd	R	mPGES-1 inhibition	A549, 50% FBS PGE_2
		IC_{50}[a] (μM)	
25		7.9	17
26		0.19	27
27		0.26	1.6
28		0.043	2.7

[a] Values are means of at least two experiments.

eightfold potency increase in the enzyme assay (IC_{50} = 0.001 μM) and a fivefold boost activity in the cellular assay (IC_{50} = 0.16 μM) over lead **10**. Replacement of the phenyl group proximal to the urea core in **35** with a pyridyl group in **36** resulted in a loss in enzyme and cellular potency. The replacement of the terminal cyclopropyl with a cyclopentyl in **37** or a phenyl in **38** did not improve the potency over **36**.

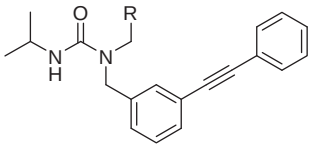
The previously described human whole blood assay (HWB)[8] was utilized to evaluate our trisubstituted urea inhibitors of mPGES-1 in a more physiologically relevant context. This assay was designed to monitor the inhibition of PGE_2 production arising from the addition of lipopolysaccharide (LPS) to freshly isolated blood. The HWB assay also provided a means to concurrently monitor selectivity against TXB_2 inhibition, while $PGF_{2\alpha}$ selectivity was monitored in A549 cell-based assay. It was found that the best balance between potency and selectivity was attained with bis-tolane inhibitors as shown in Table 5. Optimal potencies and selectivities were achieved with a terminal pyridyl group in the upper tolane and a terminal phenyl group substituted with an electron withdrawing group in the lower tolane. All the inhibitors are highly active against mPGES-1 enzyme, moderately active in the cell assay and selective in the $PGF_{2\alpha}$ cellular conterscreen as well as in the human whole blood TXB_2 conterscreen. Compound **42** is a selective mPGES-1 inhibitor and exhibited good human whole blood activity (IC_{50} = 2.1 μM). The potency and selectivity profile of this compound is similar to our first generation mPGES-1 inhibitor, phenanthrene imidazole **1**.[5]

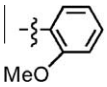
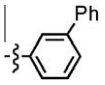
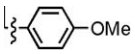
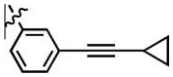
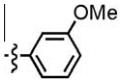
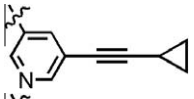
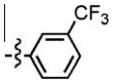
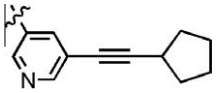
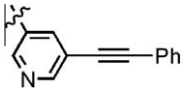
Synthesis of these trisubstituted ureas was accomplished with a stepwise approach as shown in Scheme 1. First, the imine was formed by the addition of benzylamine **43** on aldehyde **44** followed with stirring overnight. The isolated imine was then reduced to **45** with sodium borohydride. Subsequent addition of isopropylisocyanate to **45** yielded the desired trisubstituted urea (**46**). As the yields for that process were found to be highly substrate dependant, especially with electron-poor benzylamines, we sought to develop a one-pot procedure in order to increase the reaction throughput and versatility. A low yield was obtained (20%) when using a standard reductive amination protocol with sodium triacetoxyborohydride[9] followed by in situ urea formation with the appropriate isocyanate. We then chose to preform the iminium intermediate **47**, reduce it in situ, and then add the isocyanate to the mixture to generate the urea in a one-pot fashion (80% Scheme 2). We ultimately found that running the iminium formation overnight gave us better yields with problematic electron-poor benzylamines.

The synthesis of the best trisubstituted urea (**42**) is outlined in Scheme 3. The synthetic sequence starts with BOC protection of the amine in starting material **48** to generate **49** in 80% yield. Sonogoshira coupling followed by deprotection of the BOC group with HCl afforded bisphenylacetylene **50** in 70% yield**.** This amine was then transformed to the trisubstituted urea **51** using our one-pot procedure.[10] The amine was stirred together with 3-iodobenzaldehyde, acetic acid and triethylamine in dichloroethane overnight. Sodium triacetoxyborohydride was added followed by isopropylisocyanate to generate **51** in 75% yield. A subsequent sonogashira coupling using 4-ethylpyridine hydrochloride salt gave the final compound **42** with 70% yield.

In summary, trisubstituted ureas have been identified as a new series of potent and selective mPGES-1 inhibitors. Synthesis of these inhibitors was achieved using a convenient and versatile one-pot synthesis approach. Sequential optimization of all three substituents on the urea core of HTS lead **3** ultimately led to the highly potent and selective mPGES-1 inhibitor **42** (mPGES-1 IC_{50} = 0.002 μM; A549 IC_{50} = 0.34 μM; HWB IC_{50} = 2.1 μM). Further study of this series is in progress with the aim to improve whole blood activity and to provide inhibitors with adequate pharmacokinetic properties for further development.

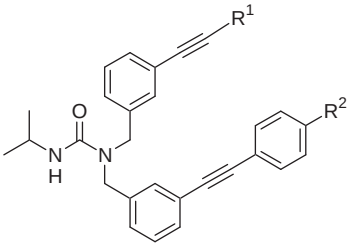
Table 4
Upper *N*-benzyl group SAR

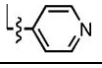


Compd	R	mPGES-1 inhibition	A549, 50% FBS PGE_2	Compd	R	mPGES-1 inhibition	A549, 50% FBS PGE_2
		IC_{50}[a] (μM)				IC_{50}[a] (μM)	
29		0.58	10.6	**34**		0.003	3.7
30		0.15	14.8	**35**		0.001	0.16
31		0.046	3.6	**36**		0.018	0.49
32		0.018	4.1	**37**		0.003	0.49
33		0.015	3.0	**38**		0.002	0.36

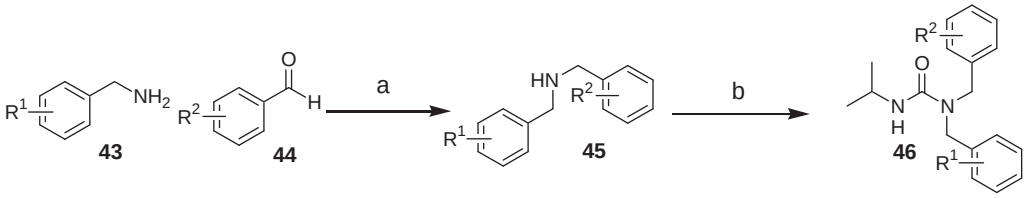
[a] Values are means of at least two experiments.

Table 5
Optimized trisubstituted ureas

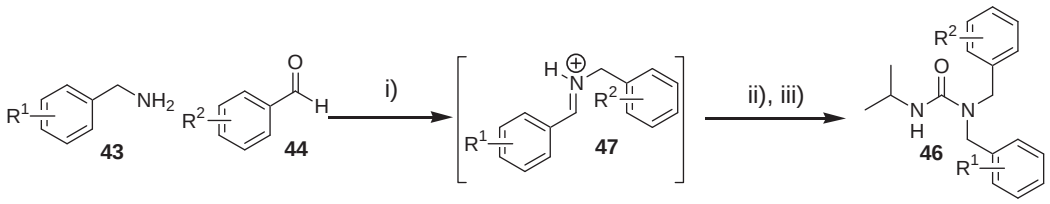


Compd	R^1	R^2	mPGES-1 inhibition	A549, 50% FBS PGE_2	A549, 50% FBS PGF_2	Human whole blood PGE_2	Human whole blood TXB_2
			IC_{50}[a] (μM)				
1	—	—	0.001	0.42	>50	1.3	>40
39			0.001	0.48	17.6	6.7	>40
40			0.001	0.24	8.6	9.7	>40
41			0.003	0.33	10.6	7.7	>40
42			0.002	0.34	10.7	2.1	>40

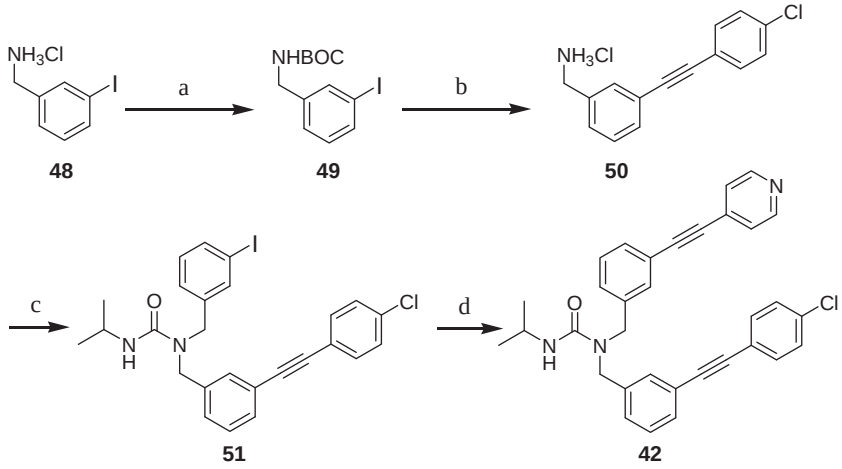
[a] Values are means of at least two experiments.



Scheme 1. Reagents and conditions: (a) (i) toluene, reflux, 18 h; (ii) $NaBH_4$, MeOH, 10 min 85%; (b) isopropylisocyanate, hexanes, 18 h, 80%.



Scheme 2. One-pot procedure. Reagents and conditions: (i) AcOH, DCE, 18 h; (b) (ii) $Na(OAc)_3BH$, 1 h; (iii) isopropylisocyanate, 4 h, 80%.



Scheme 3. Synthesis of urea **42**. Reagents and conditions: (a) (i) $NaHCO_3$, EtOAc; (ii) $(BOC)_2O$, THF, 80%; (b) (i) 1-chloro-4-phenylacetylene, $Pd(Ph_3)_4$, CuI, Et_3N, DMF; (ii) 4 N HCl/dioxane, 70%; (c) (i) 3-iodobenzaldehyde, AcOH, Et_3N, $Na(OAc)_3BH$, DCE; (ii) isopropylisocyanate (one-pot), 75%; (d) 4-ethynylpyridine hydrochloride salt, $Pd(Ph_3)_4$, CuI, Et_3N, DMF, 70%.

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References and notes

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10. *Typical procedure*: To a stirred solution of the amine (1.0 equiv) and the aldehyde (1.0 equiv) in dichloroethane (0.2 M) was added acetic acid (5.0 equiv). The solution was stirred for 18 h, then sodium triacetoxyborohydride (1.2 equiv) was added and the mixture was stirred for 1 h. The appropriate isocyanate (1.5 equiv) was added and the mixture was stirred for an additional 4 h before being quenched with a saturated aqueous solution of sodium bicarbonate. The layers were separated and the aqueous layer was extracted three times with dichloromethane. The organic layers were combined and dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude was purified on silica gel.