ChemMedChem

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

Title: Polysubstituted Pyrimidines as mPGES-1 Inhibitors: Discovery of Potent Inhibitors of PGE2 Production with a Strong Anti-inflammatory Effects in Carrageenan-Induced Rat Paw Edema

Authors: Filip Kalčic, Viktor Kolman, Haresh Ajani, Zdeněk Zídek, and Zlatko Janeba

To be cited as: *ChemMedChem* 10.1002/cmdc.202000258

Link to VoR: https://doi.org/10.1002/cmdc.202000258

WILEY-VCH

FULL PAPER WILEY-VCH

Polysubstituted Pyrimidines as mPGES-1 Inhibitors: Discovery of Potent Inhibitors of PGE_2 Production with a Strong Anti-inflammatory Effects in Carrageenan-Induced Rat Paw Edema

Filip Kalčic,[a,b] Dr. Viktor Kolman,[a] Dr. Haresh Ajani,[a] Dr. Zdeněk Zídek,*[c] and Dr. Zlatko Janeba*[a]

[a] F. Kalčic, Dr. V. Kolman, Dr. H. Ajani, and Dr. Z. Janeba
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences
Flemingovo nám. 2, 166 10 Prague 6, Czech Republic
E-mail: janeba@uochb.cas.cz
[b] F. Kalčic
Department of Organic Chemistry, Faculty of Science
Charles University
Hlavova 8, 128 43 Prague 2, Czech Republic
[c] Dr. Zdeněk Zídek
Institute of Experimental Medicine of the Czech Academy of Sciences
Vídeňská 1083, 142 20 Prague 4, Czech Republic
E-mail: zdenek.zidek@iem.cas.cz

Supporting information for this article is given via a link at the end of the document.

Abstract: We report an extensive structure-activity relationship optimization of polysubstituted pyrimidines that led to a discovery of 5-butyl-4-(4-benzyloxyphenyl)-6-phenylpyrimidin-2-amine, and its difluorinated analogue. These compounds are submicromolar inhibitors of PGE_2 production (IC_{50} as low as 12 nM). In order to identify the molecular target of anti-inflammatory pyrimidines, we performed extensive studies including enzymatic assays, homology modeling and docking. The difluorinated analogue simultaneously inhibits two key enzymes of the arachidonic acid cascade, namely mPGES-1 and COX-2, where the mPGES-1 inhibition represents the principal mechanism of action. Other pyrimidines studied are potent mPGES-1 inhibitors with no observed inhibition of COX-1/2 enzymes. Moreover, two most potent compounds proved to be significantly effective *in vivo* in a model of acute inflammation, suppressing the carrageenan-induced rat paw edema by 36% and 46%. Promising results of this study warrant further preclinical evaluation of selected anti-inflammatory candidates.

Introduction

Prostaglandin E_2 (PGE_2) is a naturally occurring lipid mediator, which plays an important role in various inflammatory processes, fever, pain, and cancer. PGE_2 belongs to the most predominant pro-inflammatory prostanoids. PGE_2 biosynthesis consists of the release of arachidonic acid (AA) from membrane phospholipids by phospholipases (PLAs), oxygenation of AA to prostaglandin G_2 (PGG_2) and subsequent reduction of PGG_2 to prostaglandin H_2 (PGH_2) by cyclooxygenase (COX) enzymes, and conversion of PGH_2 to PGE_2 by inducible microsomal PGE_2 synthase-1 (mPGES-1).

Non-steroidal anti-inflammatory drugs (NSAIDs),[1] which today belong to the most widely used therapeutic agents to treat inflammation, fever, and pain, exert their biological activity *via* non-selective COX enzymes inhibition, i.e. inhibition of both constitutive COX-1 and inducible COX-2.[2-4] The major drawback of traditional NSAIDs (t-NSAIDs) is the occurrence of serious gastrointestinal (GI) complications,[5] namely gastric bleeding, ulceration, perforation and dyspepsia. Thus, selective COX-2 inhibitors (coxibs) were developed and commercialized as promising anti-inflammatory agents in order to avoid the GI adverse events.[6,7] However, coxibs were later related to the increased risk of cardiovascular events like myocardial infarction, stroke, and systemic and pulmonary hypertension.[8]

Due to a wide spectrum of adverse events of t-NSAIDs as well as cardiovascular toxicity of coxibs, researchers intensively searched for new approaches to treat inflammation, pain, and fever. As most t-NSAIDs contained a free carboxylic acid group in their molecule, the GI toxicity seemed to be closely related to their acidic nature. Thus, structural modification of some NSAIDs like indomethacin and meclofenamic acid (mostly by conversion of their free carboxylic acid moiety into ester or amide derivatives) led to a generation of highly selective COX-2 inhibitors with eliminated GI side effects compared to the parent compounds.[9-12] Also synthesis of analogues with lower membrane permeabilization activity produced fewer gastric lesions after their oral administration.[13,14] Other attempts how to enhance gastric safety profile of anti-inflammatory drugs were based on their association with cytoprotective mediators (for comprehensive reviews see[1,15,16]), such as phosphatidylcholine,[17,18] dialkylphosphate,[19] nitric oxide[20-34] or nitroxyl,[35] and hydrogen sulfide.[36-42] Nevertheless, it has been speculated,[43] that the NO release is not required to exert the cytoprotective effects of modified NSAIDs, while the simple formation of NSAIDs prodrugs is a sufficient condition to develop a safer alternative to unprotected NSAIDs.

Relatively recent strategy in development of new anti-inflammatory agents is a design of compounds targeting downstream and/or multiple enzymes of the AA cascade. In that matter, selective inhibitors of mPGES-1 have been recently identified,[44-61] as well as selective inhibitors of 5-lipoxygenase (5-LOX),[62] dual COX/5-LOX inhibitors,[34,63-67] dual mPGES-1/5-LOX inhibitors,[53,68-73] and dual thromboxane antagonists–COX-

2 inhibitors.[74] Similarly, dual inhibition of fatty acid amide hydrolase (FAAH) and the COX enzymes led to enhanced analgesic effects of NSAIDs with decreased GI side effects.[75,76] All these findings have raised high expectations for development of novel and safer anti-inflammatory drugs.

We previously reported polysubstituted pyrimidines as potent inhibitors of prostaglandin E_2 (PGE_2) production with potential anti-inflammatory properties.[77–81] Out of them, lead compound 5-butyl-4-(4-methoxyphenyl)-6-phenylpyrimidin-2-amine (**1,** Figure 1) was selected for further evaluation as a preclinical candidate for treatment of inflammation. Moreover, compound **1** served as a starting point for further structure-activity relationship (SAR) studies with the aim to identify even more potent inhibitors of PGE_2 production and, eventually, superior anti-inflammatory agents.

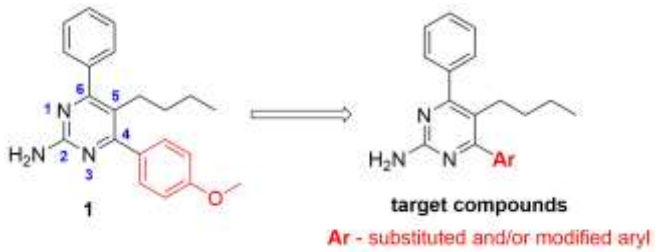


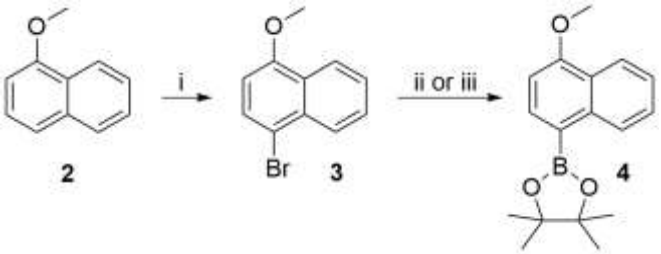
Figure 1. Structure of preclinical anti-inflammatory candidate **1** and a general structure of target compounds prepared within this study.

Herein, we describe a systematic structure-activity relationship optimization of compound **1** *via* preferential introduction of electron-donating groups (e.g. alkyls, amines or ethers) at the phenyl moiety in C4 position of the pyrimidine ring. Other substituents (at positions C2, C5, and C6 of pyrimidine) were kept intact in order not to introduce too many variables. Based on our previous research,[80-81] such compounds were expected to exhibit an increased potential to inhibit PGE_2 production. The potency of prepared compounds to inhibit PGE_2 production was evaluated *in vitro* on mouse peritoneal cells with induced immune response provoked by lipopolysaccharide (LPS) from *Escherichia coli*. The extensive SAR study led to the discovery of very potent inhibitors of PGE_2 production and their efficacy was verified *in vivo* using carrageenan-induced rat paw edema experiments.

Results and Discussion

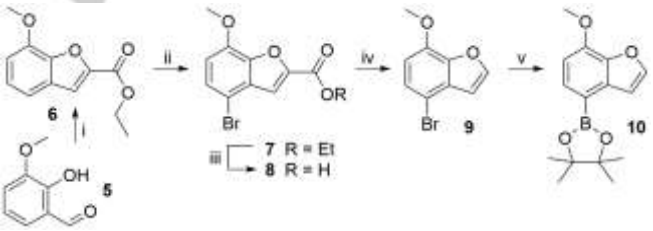
Chemistry. In our laboratory, Suzuki-Miyaura cross-coupling represents a major tool to obtain target polysubstituted pyrimidines bearing two aromatic moieties in the C4 and C6 positions of the pyrimidine ring.[80-81] Various arylboronic acids (or their pinacol esters) can be nowadays purchased from commercial suppliers as a starting material for the Suzuki-Miyaura cross-coupling reactions. Nevertheless, two arylboronic acid pinacol esters had to be prepared in order to be able to synthesize some of the desired final compounds.

Firstly, synthesis of (4-methoxynaphthalen-1-yl)boronic acid pinacol ester (**4**, Scheme 1) started with a selective bromination of 1-methoxynaphthalene using *N*-bromosuccinimide (NBS) to give bromo derivative **3** quantitatively.[82] Subsequent lithiation of intermediate **3**, followed by transmetallation using pinacol diboronate,[83] afforded pinacol ester **4** (Scheme 1) in a 12% yield. However, the desired product **4** was obtained in a 66% yield when we employed conditions of catalytic borylation reported by Harada et al.[84]



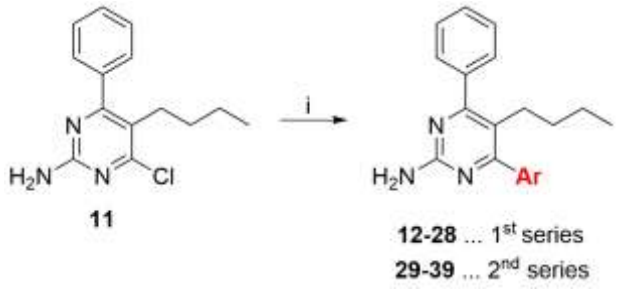
Scheme 1. Synthesis of boronic acid pinacol ester **4**. Reagents and conditions: (i) NBS, MeCN, 98%; (ii) BuLi, B_2pin_2, THF, -78 °C, 12%; (iii) B_2pin_2, K_3PO_4, $Pd(PPh_3)_4$, DMF, 120 °C, 66%.

Secondly, commercially available *o*-vanillin (2-hydroxy-3-methoxybenzaldehyde, **5**) was used as a starting material in order to prepare novel (7-methoxybenzofuran-4-yl)boronic acid pinacol ester (**10**, Scheme 2). The condensation of *o*-vanillin with ethyl 2-chloroacetate afforded ethyl carboxylate **6** in an 84% yield,[85,86] although Yamaguchi et al.[87] have reported the carboxylic acid derivative as the only product of the aforementioned condensation. Intermediate **6** was selectively brominated with NBS to yield quantitatively bromo derivative **7**, which was subsequently hydrolyzed to give carboxylic acid **8** in a 94% yield. Microwave-assisted decarboxylation of acid **8** was performed according to Musser et al.[88] to afford intermediate **9** (Scheme 2). Finally, catalytic borylation[84] of bromo derivative **9** gave the novel pinacol ester **10** in a 75% yield.



Scheme 2. Synthesis of boronic acid pinacol ester **10**. Reagents and conditions: (i) ethyl 2-chloroacetate, Cs_2CO_3, DMF, 80 °C, 84%; (ii) NBS, MeCN, 99%; (iii) NaOH, acetone-H_2O (4:1), AcOH, 94%; (iv) Cu^0, quinoline, MW 180 °C, 89%; (v) B_2pin_2, K_3PO_4, $Pd(PPh_3)_4$, DMF, 120 °C, 75%.

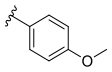
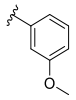
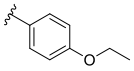
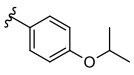
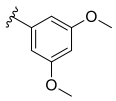
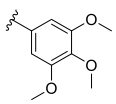
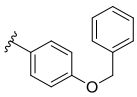
Having all the desired arylboronic acids and pinacol boronates in hand, the previously reported synthetic methodology[80,81] was employed for the synthesis of target 2-amino-5-butyl-6-phenylpyrimidines bearing a substituted/modified phenyl group in the C4 position of the pyrimidine moiety. Firstly, 2-amino-5-butyl-4-chloro-6-phenylpyrimidine [81] (**11**, Scheme 3) was treated with the selected arylboronic acids (and prepared pinacol boronates **4** and **10**), $Pd(PPh_3)_4$ and Cs_2CO_3 in a 1,4-dioxane-H_2O (4:1) mixture at 110 °C to obtain compounds **12**–**28** in 26–85% yields (Table 1).

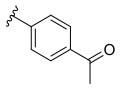
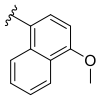
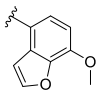
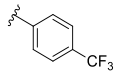
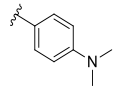
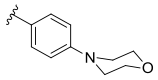
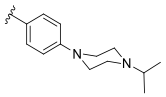
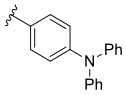
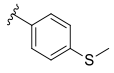


Scheme 3. Synthesis of target compounds **12–39**. Reagents and conditions: (i) arylboronic acid or pinacol boronate, $Pd(PPh_3)_4$, Cs_2CO_3, 1,4-dioxane-H_2O (4:1), 110 °C, yields are summarized in Table 1 and Table 2.

Being encouraged by the promising data generated by biological evaluation of the first series of compounds (**12**–**28**, Table 1, see the discussion below), the second series of compounds was designed based on the most potent 4-(benzyloxy)phenyl derivative **18**. Compounds **29**–**39** (Scheme 3, Table 2) were then prepared in 36–99% yields starting from 4-chloropyrimidine derivative **11** and from various commercially available arylboronic acids under the above described Suzuki-Miyaura cross-coupling conditions. It should be noted that the determined yields were usually based on a single experiment and the reactions were not optimized.

Table 1. Structures and yields of prepared compounds **12**–**28** (the 1st series) and their effect on *in vitro* production of PGE_2 and viability of mouse peritoneal cells.

entry	compd	Ar Scheme 1	yield [%]	inhibition of PGE_2		viability [%][b]
				remaining production (%)[a]	IC_{50} (μM)	
1	control untreat	NA	NA	2.58±1.96[c]	NA	100.00±1.99[c]
2	control LPS	NA	NA	100.00±4.41	NA	NA
3	**1**		NA	12.25±4.48*	**4.83**[c] /3.70-6.32/	102.00±0.89
4	**12**		68	18.60±1.37*	ND	85.00±14.4
5	**13**		76	18.09±2.63*	ND	104.10±1.90
6	**14**		63	26.35±0.12*	ND	104.65±1.30
7	**15**		83	12.28±0.50*	**9.05** /5.53-14.80/	71.17±1.91*
8	**16**		81	16.36±3.77*	ND	105.15±0.49
9	**17**		74	10.07±2.62*	**6.58** /3.90-11.11/	102.58±0.67
10	**18**		71	0.33±0.17*	**0.031** /0.018-0.056/	105.40±0.23

11	**19**		85	30.55±9.77*	ND	99.92±1.02
12	**20**		77	25.60±0.11*	ND	103.99±0.92
13	**21**		57	15.35±0.34*	ND	45.16±1.25*
14	**22**		30	34.62±1.24*	ND	35.59±0.84*
15	**23**		68	32.51 ±2.48*	ND	36.68±2.42*
16	**24**		77	2.69±0.24*	**1.09** /0.26-4.67/	102.91±0.32
17	**25**		28	56.11±12.69*	ND	99.25±1.61
18	**26**		50	64.32±6.14*	ND	97.63±1.21
19	**27**		26	53.70±7.34	ND	101.00±0.46
20	**28**		71	7.92±1.05	**2.70** /1.32-5.55/	88.87±0.83

[a] Effects recorded for 50 µM concentration of compounds and expressed in % of control LPS response; [b] Cell viability expressed in % of control untreated cells; [c] ± S.E.M. Results represent means of three to four experiments. Statistical significance: * $P < 0.001$, not significant in other values. NA: not applicable. ND: not determined.

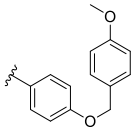
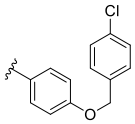
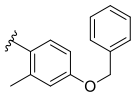
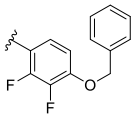
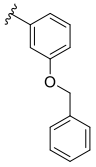
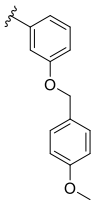
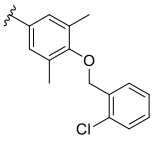
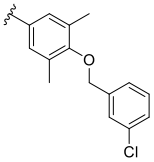
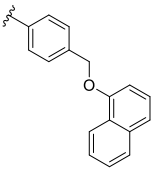
Structure–activity relationship study and structural optimization. All prepared polysubstituted pyrimidines were evaluated for their ability to inhibit *in vitro* PGE_2 production using C57BL6 mouse peritoneal cells. The effects of the pyrimidines on the PGE_2 production are expressed as a percentage change (remaining production of PGE_2) relative to the response of LPS stimulated cells (positive control, 100%, Table 1 and 2, entry 2) or unstimulated cells (negative control, 2.78%, Table 1 and 2, entry 1).

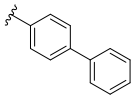
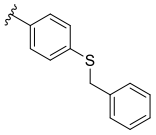
The first series of tested compounds (**12**–**28**, Table 1, entries 4–20) are direct analogues of preclinical candidate **1** (Figure 1, Table 1, entry 3). It was found that majority of new derivatives exhibited similar potency to inhibit PGE_2 production compared to parent compound **1** (with 12% remaining production of PGE_2). Moreover, the compounds did not affect the viability of the mouse peritoneal cells, with the exception of compounds **21–23** (Table 1, entries 13–15) which decreased the viability of the cells significantly.

However, compound **18** (Table 1, entry 10), the derivative bearing 4-(benzyloxy)phenyl moiety in the C4 position of pyrimidine, excelled in the first series of compounds as the most potent inhibitor of PGE_2 production. Compound **18** almost completely (less than 1% of remaining production of PGE_2) inhibited PGE_2 production with no apparent toxicity. This promising result encouraged us to design and synthesize another series of polysubstituted pyrimidines derived from compound **18** and bearing various benzyloxyphenyl-like moieties in the C4 position of the pyrimidine ring.

In vitro evaluation of the second series of compounds (**29**–**39**, Table 2, entries 3–13) revealed compound **32** (Table 2, entry 6), the difluorinated derivative of compound **18**, as the most potent inhibitor of PGE_2 production from the entire SAR study. Compound **32** exhibited a remarkable inhibitory activity with only about 0.04% of remaining production of PGE_2 *in vitro*.

Table 2. Structures of prepared compounds **29**–**39** (the 2nd series) and their effect on *in vitro* production of PGE_2 and viability of mouse peritoneal cells.

entry	compd	Ar Scheme 1	yield [%]	inhibition of PGE_2		viability [%][b]
				remaining production (%)[a]	IC_{50} (μM)	
1	control untreat	NA	NA	2.78±1.02[c]	NA	100.00±2.83[c]
2	control LPS	NA	NA	100.00±3.15	NA	NA
3	**29**		78	4.01±1.53*	**1.64**[c] /1.38-1.94/	104.24±0.35
4	**30**		71	0.73±0.53*	**0.131** /0.073-0.235/	101.25±0.84
5	**31**		85	51.13±0.83*	ND	103.20±0.52
6	**32**		70	0.04±0.20*	**0.012** /0.010-0.017/	103.66±0.44
7	**33**		98	11.74±2.27*	**7.03** /4.27-11.56/	101.58±0.74
8	**34**		99	20.44±1.30*	ND	103.57±0.70
9	**35**		97	7.12±1.95*	**1.65** /1.17-2.33/	103.57±0.32
10	**36**		94	5.54±0.41*	**1.31** /0.97-1.77/	102.82±0.46
11	**37**		36	26.29±5.33*	ND	104.82±0.44

12	**38**		89	2.95±1.86*	**0.45** /0.24-0.85/	101.83±0.21
13	**39**		40	12.86±1.04*	**9.84** /7.18-13.48/	97.00±0.60

[a] Effects recorded for 50 μM concentration of compounds and expressed in % of control LPS response; [b] Cell viability expressed in % of control untreated cells; [c] ± S.E.M. Results represent means of three to four experiments. Statistical significance: * P < 0.001, not significant in other values. NA: not applicable. ND: not determined.

Changes in production of PGE_2 may be associated with changes in production of another important mediator of inflammation, nitric oxide (NO), although the crosstalk between them is controversial showing both positive and negative influence.[89,90] Our previously reported data suggested that some pyrimidine derivatives might act as dual inhibitors of PGE_2 and NO production.[80,91] Thus, the ability of studied pyrimidines to inhibit *in vitro* production of NO was also evaluated (data not shown). Mostly insignificant NO suppressive potential was observed in the present set of pyrimidine analogues (including strong PGE_2 inhibitors **18** and **32**) compared to the previous series of compounds,[80] with the exception of compounds **19**, **22**, and **31**, which mildly inhibited NO production. Thus, we have concentrated on the mode of a predominant effect of pyrimidines, i.e. inhibition of PGE_2 production, although some synergistic anti-inflammatory effects *in vivo* can be expected and these will be addressed in our ongoing research.

Several important conclusions can be deducted from the overall SAR study: a) an introduction of 4-(benzyloxy)phenyl moiety at the C4 position of pyrimidine ring was beneficial for the increased inhibition of PGE_2 production (compound **18**, Table 1, entry 10); b) further substitution of the benzyloxy moiety in the *para* position of **18** was well-tolerated (compounds **29** and **30**, Table 2, entries 3 and 4); c) an introduction of the methyl group into *ortho* position of the C4 phenyl ring of **18** resulted in a substantial decrease of biological potency (compound **31**, Table 2, entry 5); d) an introduction of fluorine atoms into the phenyl ring of the 4-(benzyloxy)phenyl moiety in compound **18** resulted in almost one-order of magnitude more potent inhibitor **32** (Table 2, entry 6); e) moving the benzyloxy moiety from *para* to *meta* position of the C4 phenyl ring led to a decreased inhibition of PGE_2 production (compounds **33** and **34**, Table 2, entries 7 and 8); f) a replacement of an oxygen atom in compound **18** for sulfur resulted in a decrease of biological potency (compound **39**, Table 2, entry 13).

Finally, for the set of selected compounds, namely compounds **1**, **15**, **17**, **18**, **24** and **28** (Table 1) and **29**, **30**, **32**, **33**, **35**, **36**, **38** and **39** (Table 2), the IC_{50} values were determined. The data showed that most potent compounds **18** (IC_{50} = 0.031 μM) and **32** (IC_{50} = 0.012 μM) were approx. 150-fold and 400-fold more potent inhibitors of PGE_2 production, respectively, compared to the parent compound **1** (IC_{50} = 4.83 μM).

Evaluation of the mechanism of action. The mechanism of the PGE_2-inhibitory effects of pyrimidines was studied using the most potent compound **32** at first. Applied at the concentration of 20 μM, **32** did not influence the activity of COX-1, whereas it significantly suppressed (by 47%, P < 0.001) the activity of COX-2 (Figure 2A). Interestingly, other compounds from this study (including **1**, **18**, and **28**) did not exhibit any noticeable activity against COX-1/2 enzymes (data not shown). On the other hand, compound **32** (at 20 μM) virtually completely inhibited (by 98%) the activity of human mPGES-1 (Figure 2A). It was inhibited in a dose-dependent manner (Figure 2B), the IC_{50} estimate being 0.066 μM, with 0.047– 0.082 μM 95% limits of confidence. The data indicate that **32** is a dual inhibitor of COX-2 and mPGES-1, although its interaction with mPGES-1 can be considered as the major mechanism underlying the inhibition of PGE_2 production.

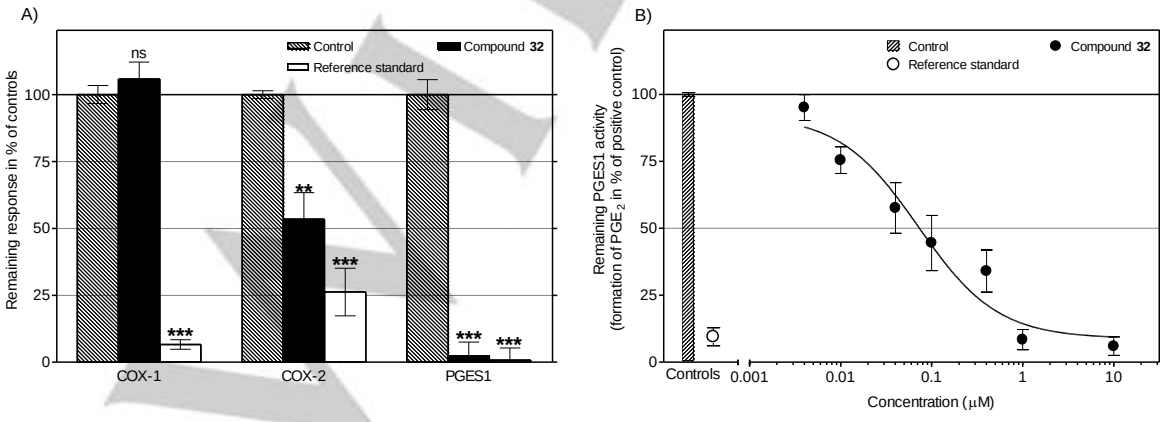


Figure 2. A) Interaction of compound **32** (20 μM) with COX-1/2 and human mPGES-1. The effects are compared to those of reference standards, *i.e.* indomethacin (5 μM) for COX enzymes and CAY10678 (2 μM) for mPGES-1. The bars are means ± S.E.M. obtained from two COX and six mPGES-1 experiments, each run in duplicate. Statistical significance against the control groups: ** P < 0.01, *** P < 0.001. B) Inhibition of mPGES-1 activity is dependent on the concentration of **32**. The curve represents amalgamated effects of two experiments, each done in duplicate. The points are means ± S.E.M.

Table 3. Direct inhibition of mouse and human mPGES-1 with selected compounds.

		mPGES-1 inhibition[a] IC_{50} (μM) (95% limits of confidence)	
Entry	Comp	mouse	human
1	**1**	12.780 (9.600 - 54.120)	5.065 (4.071 - 6.303)
2	**18**	0.060 (0.045 - 0.307)	0.117 (0.111 - 0.259)
3	**28**	6.280 (1.760 - 15.010)	2.423 (1.341 - 4.378)
4	**30**	0.700 (0.360 - 2.130)	0.248 (0.160 - 0.385)
5	**32**	2.730 (1.760 - 7.400)	0.066 (0.047 - 0.082)

[a] The enzyme activity reflects changes in transformation of PGH2 to of PGE2. Effects of pyrimidine derivatives were recorded for recombinant enzymes as described in Experimental Procedures. The results represent two independent experiments.

In order to verify that mPGES-1 inhibition is the key mechanism of action common to the whole series of pyrimidine analogues studied (since only **32** partially inhibits also COX-2), we decided to evaluate activity in the mPGES-1 assay with other compounds, namely **1**, **18**, **28**, and **30**. Interestingly, previously described inhibitors of human mPGES-1 were not, in general, potent inhibitors of the mouse or rat enzyme, and only recently, dual inhibitors, active against both human and mouse enzymes, have been reported.[92] In order to correlate our data obtained on mouse peritoneal cells (Table 1 and 2) with those obtained with human mPGES-1 (Fig. 2), selected compounds **1**, **18**, **28**, **30**, and **32** were evaluated on both mouse and human enzymes side by side (Table 3).

All tested compounds are potent inhibitors of both mouse and human mPGES-1 (Table 3), though the human enzymes seems to be more susceptible to the inhibition with polysubstituted pyrimidines. Coumpound **18** (IC_{50} = 60 nM) is the most potent inhibitor of the mouse mPGES-1, while compound **32** (IC_{50} = 66 nM) is the most potent inhibitor of the human enzyme. The obtained data support the notion that mPGES-1 inhibition represents the main mechanism of action common to anti-inflammatory pyrimidines studied in this work.

In vivo evaluation of selected compounds in the model of acute inflammation. For the most potent compounds, namely **18**, **30**, and **32**, we carried out the carrageenan-induced rat paw edema experiments to verify whether the compounds are active also *in vivo* when administered orally (gastric intubation). The rat model is preferred to mouse one for its higher accuracy and reproducibility. Since the aqueous solubility of most of the studied polysubstituted pyrimidines is low, the compounds were solubilized using a mixture of DMSO (1.5%) in TWEEN® 80 before dilution in the physiological saline solution. The results are depicted in Figure 3. It was shown that solvent alone did not have any significant effect on the development of edema induced by carrageenan while solubilized compounds **18**, **30**, and **32** reduced the carrageenan-enhanced paw volumes by 36.3%, 20.7%, and 45.5%, respectively. It can be concluded, that the compounds are bioavailable after oral administration. The most potent compound **32** substantially suppresses the development of rat paw edema validating its potential to become a promising candidate for further development as a systemic anti-inflammatory agent.

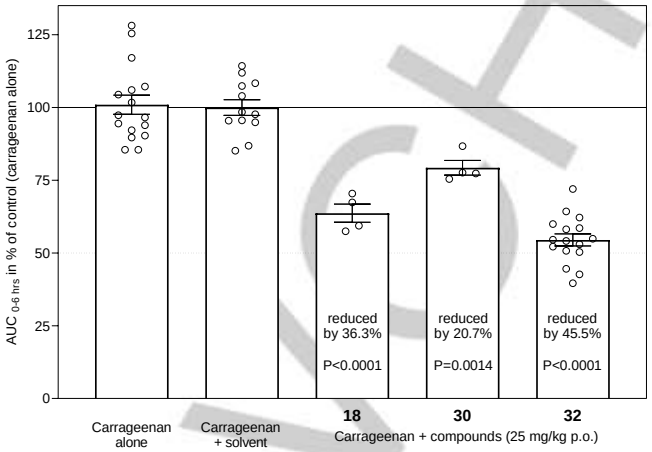


Figure 3. Effect of compounds **18**, **30**, and **32** on the development of the acute carrageenan-induced rat paw edema. P, statistical significance against the positive control group (carrageenan alone). Solvent: DMSO (1.5 wt%)/TWEEN® 80 mixture (100 mg) in sterile 1% saline solution (1 mL). Compound samples: 5 mg of compound in 1 mL of solvent.

Computational Studies. The detailed structure-activity relationship (SAR) of anti-inflammatory pyrimidines was discussed above. In order to elucidate and better understand the binding mode of mPGES-1 inhibitors, we decided to perform molecular docking studies with some of our compounds. High resolution X-ray crystal structure (PDB: 4BPM) of human mPGES-1 with potent inhibitor is available,[93,94] while mouse mPGES-1 structure is so far unavailable. Human mPGES-1 is, however, highly homologous to the mouse enyzme, with sequence identity being 79% and the sequence similarity being 84%. Thus, docking experiments of compounds **1** and **32** were carried out using Glide based on the X-ray crystal structure of human mPGES-1 (PDB: 4BPM) and on homology model of mouse mPGES-1 developed by using the human mPGES-1 structure as template (Figure 4). The molecular docking revealed that both compounds **1** and **32** can favorably bind in conserved region of the active site of human and mouse mPGES-1 enzymes. The conserved region around S127 has mainly hydrophobic pocket surrounded by Y28, I32 (V32 in mouse), G35, L39, Y130, T131 (V131 in mouse), Q134, L135 and A138 (F138 in mouse) for human (mouse) mPGES-1.[95] As shown in Figure 4, the phenyl ring in C6 position of pyrimidine ring stays at bottom of the substrate-binding pocket of mPGES-1 enzymes. 4-Methoxyphenyl (compound **1**) and 4-benzyloxy(3,2-difluoro)phenyl (compound **32**) moiety in C4 position of pyrimidine are involved in a π–π stacking interaction with the side chain of Tyr130 of human subunit A and the side chain of Tyr130 of mouse subunit C, respectively. The computational studies support strong binding of studied derivatives to both mouse and human mPGES-1 enzymes.

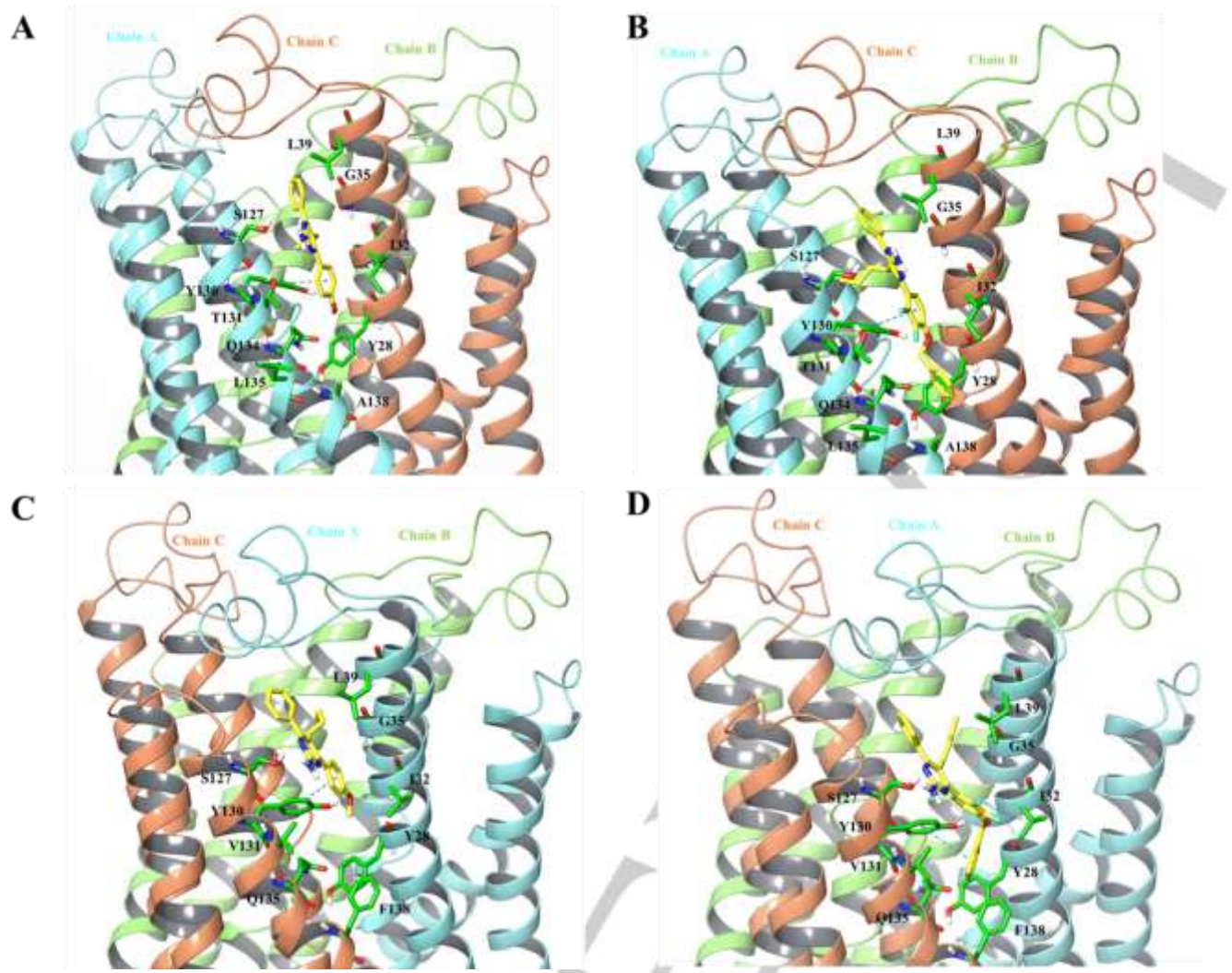


Figure 4. Docked binding pose of A) compound **1** and B) compound **32** in human mPGES-1. Docked binding pose of C) compound **1** and D) compound **32** in modeled mouse mPGES-1. The trimer protein structure of human and modeled mouse mPGES-1 is depicted in ribbon diagram. Human and modeled mouse mPGES-1 interacting residues are represented green stick form and ligand structure in yellow stick form (colored by atom type: C: green or yellow, N: blue, O: red, F: cyan, H: white). The cyan dashed lines indicate π–π interaction. Figure was prepared with Maestro, Schrodinger, LLC.

Conclusion

Compound **1** was identified previously as a potent inhibitor of prostaglandin E_2 (PGE_2) production with confirmed anti-inflammatory properties and is being evaluated as a preclinical candidate for the treatment of ulcerative colitis and rheumatoid arthritis. The goal of the current extensive SAR study was further structural optimization of polysubstituted pyrimidines, identification of more potent anti-inflammatory agents, as well as elucidation of their mechanism of action. Synthesis of 28 new analogues derived from compound **1** bearing substituted and/or modified phenyl moiety in the C4 position of the pyrimidine ring, led to the discovery of several superior inhibitors of PGE_2 production. Firstly, compound **18** (IC_{50} = 31 nM) with the 4-(4-benzyloxy)phenyl arm in C4 position of pyrimidine was found to be a potent inhibitor of PGE_2 production. Subsequently, compound **32**, the difluorinated derivative of compound **18**, was identified as the most potent inhibitor from the whole SAR series. Compound **32** (IC_{50} = 12 nM) was 400-fold more potent inhibitor of PGE_2 production compared to parent compound **1** (IC_{50} = 4.83 μM). Moreover, after oral administration, compounds **18** and **32** significantly (by 36% and 46%, respectively) suppressed the development of carrageenan-induced rat paw edema, confirming *in vivo* potency in the treatment of acute inflammation. An extensive study of mechanism of action revealed that the studied compounds (**1**, **18**, **28**, **30** and **32**) are potent inhibitors of mPGES-1, both mouse and human. Therefore, these inhibitors can be used as tool compounds to study the effects of mPGES-1 inhibition in mice and rats, with potential applications in humans. Moreover, it has been suggested, that the specific inhibition of mPGES-1 is extremely interesting for drug development with respect to the side-effects observed with COX-1/2 inhibitors. The pyrimidines studied did not inhibit COX-1/2 enzymes, only derivative **32** weakly inhibited COX-2. Although the overall mode of anti-inflammatory action of the studied compounds seems to be relatively complex, mPGES-1 evidently is their principal anti-inflammatory target. These exciting data strongly encourage future evaluation of the most potent candidates in various chronic inflammation models (e.g. ulcerative colitis and rheumatoid arthritis) as well as subsequent lead optimization in order to develop novel potent anti-inflammatory and anti-cancer therapeutic agents.

Experimental Section

Experimental details are given in the Supporting information. All protocols were approved by the institutional ethics committee.

Acknowledgements

This work was supported by the subvention for development of research organization (Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, RVO: 61388963), by the Technology Agency of the Czech Republic (TE01020028 and IT4Innovations National Supercomputing Center – LM2015070). We are grateful to Vladimír Král (Faculty of Chemical Engineering, University of Chemistry and Technology, Prague) for help with selection and preparation of suitable formulation used for the in vivo experiments.

Abbreviations used. AA, arachidonic acid; COX, cyclooxygenase; GI, gastrointestinal; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; NBS, *N*-bromosuccinimide; NO, nitric oxid, NSAIDs, non-steroidal anti-inflammatory drugs; PGE_2, prostaglandin E_2; mPGES-1, microsomal prostaglandin E_2 synthase; t-NSAIDs, traditional non-steroidal anti-inflammatory drugs.

Keywords: pyrimidines • Suzuki Miyaura reaction • mPGES-1 • prostaglandin E_2 • anti-inflammatory

[1] C. Pereira-Leite, C. Nunes, S. K. Jamal, I. M. Cuccovia, S. Reis, *Med. Res. Rev.* **2017**, *37*, 802–859.
[2] D. L. Simmons, *Pharmacol. Rev.* **2004**, *56*, 387–437.
[3] A. L. Blobaum, L. J. Marnett, *J. Med. Chem.* **2007**, *50*, 1425–1441.
[4] W. L. Smith, Y. Urade, P. J. Jakobsson, *Chem. Rev.* **2011**, *111*, 5821–5865.
[5] B. J. R. Whittle, *Fundam. Clin. Pharmacol.* **2003**, *17*, 301–313.
[6] G. Coruzzi, N. Venturi, S. Spaggiari, *Acta Biomed. l'Ateneo Parm.* **2007**, *78*, 96–110.
[7] P. N. P. Rao, E. E. Knaus, T. P. Road, L. Jolla, *J. Pharm. Pharm. Sci.* **2008**, *11*, 81–110.
[8] J.-M. Dogné, C. T. Supuran, D. Pratico, *J. Med. Chem.* **2005**, *48*, 2251–2257.
[9] A. S. Kalgutkar, A. B. Marnett, B. C. Crews, R. P. Remmel, L. J. Marnett, *J. Med. Chem.* **2000**, *43*, 2860–2870.
[10] A. S. Kalgutkar, B. C. Crews, S. W. Rowlinson, A. B. Marnett, K. R. Kozak, R. P. Remmel, L. J. Marnett, *Proc. Natl. Acad. Sci.* **2000**, *97*, 925–930.
[11] A. Palomer, F. Cabré, J. Pascual, J. Campos, M. A. Trujillo, A. Entrena, M. A. Gallo, L. García, D. Mauleón, A. Espinosa, *J. Med. Chem.* **2002**, *45*, 1402–1411.
[12] A. S. Kalgutkar, B. C. Crews, S. Saleh, D. Prudhomme, L. J. Marnett, *Bioorg. Med. Chem.* **2005**, *13*, 6810–6822.
[13] N. Yamakawa, S. Suemasu, M. Matoyama, A. Kimoto, M. Takeda, K. I. Tanaka, T. Ishihara, T. Katsu, Y. Okamoto, M. Otsuka, T. Mizushima, *J. Med. Chem.* **2010**, *53*, 7879–7882.
[14] N. Yamakawa, S. Suemasu, Y. Okamoto, K. I. Tanaka, T. Ishihara, T. Asano, K. Miyata, M. Otsuka, T. Mizushima, *J. Med. Chem.* **2012**, *55*, 5143–5150.
[15] S. K. Suthar, M. Sharma, *Med. Res. Rev.* **2015**, *35*, 341–407.
[16] S. Fiorucci, L. Santucci, E. Distrutti, *Dig. Liver Dis.* **2007**, *39*, 1043–1051.
[17] L. M. Lichtenberger, J. J. Romero, E. J. Dial, J. E. Moore, *Inflammopharmacology* **2009**, *17*, 1–5.
[18] L. M. Lichtenberger, J. J. Romero, E. J. Dial, *Br. J. Pharmacol.* **2009**, *157*, 252–257.
[19] L. Huang, G. G. MacKenzie, N. Ouyang, Y. Sun, G. Xie, F. Johnson, D. Komninou, B. Rigas, *Br. J. Pharmacol.* **2011**, *162*, 1521–1533.
[20] A. H. Abadi, G. H. Hegazy, A. A. El-Zaher, *Bioorg. Med. Chem.* **2005**, *13*, 5759–5765.
[21] U. K. Bandarage, L. Chen, X. Fang, D. S. Garvey, A. Glavin, D. R. Janero, L. G. Letts, G. J. Mercer, J. K. Saha, J. D. Schroeder, M. J. Shumway, S. W. Tam, *J. Med. Chem.* **2000**, *43*, 4005–4016.
[22] C. Cena, P. Tosco, E. Marini, L. Lazzarato, M. Piccinini, C. Ramondetti, E. Lupino, R. Fruttero, A. Gasco, *ChemMedChem* **2011**, *6*, 523–530.
[23] C. Cena, M. L. Lolli, L. Lazzarato, E. Guaita, G. Morini, G. Coruzzi, S. P. McElroy, I. L. Megson, R. Fruttero, A. Gasco, *J. Med. Chem.* **2003**, *46* (5), 747–754.
[24] K. Chegaev, L. Lazzarato, P. Tosco, C. Cena, E. Marini, B. Rolando, P. A. Carrupt, R. Fruttero, A. Gasco, *J. Med. Chem.* **2007**, *50*, 1449–1457.
[25] S. Consalvi, G. Poce, R. Ragno, M. Sabatino, C. La Motta, S. Sartini, V. Calderone, A. Martelli, C. Ghelardini, L. Di Cesare Mannelli, M. Biava, *ChemMedChem* **2016**, *11*, 1804–1811.
[26] Z. Huang, C. A. Velázquez, K. R. A. Abdellatif, M. A. Chowdhury, J. A. Reisz, J. F. Dumond, S. B. King, E. E. Knaus, *J. Med. Chem.* **2011**, *54*, 1356–1364.
[27] M. L. Lolli, C. Cena, C. Medana, L. Lazzarato, G. Morini, G. Coruzzi, S. Manarini, R. Fruttero, A. Gasco, *J. Med. Chem.* **2001**, *44*, 3463–3468.
[28] R. R. Ranatunge, M. Augustyniak, U. K. Bandarage, R. A. Earl, J. L. Ellis, D. S. Garvey, D. R. Janero, L. G. Letts, A. M. Martino, M. G. Murty, S. K. Richardson, J. D. Schroeder, M. J. Shumway, S. W. Tam, A. M. Trocha, D. V. Young, *J. Med. Chem.* **2004**, *47*, 2180–2193.
[29] R. R. Ranatunge, M. E. Augustyniak, V. Dhawan, J. L. Ellis, D. S. Garvey, D. R. Janero, L. G. Letts, S. K. Richardson, M. J. Shumway, A. M. Trocha, D. V. Young, I. S. Zemtseva, *Bioorg. Med. Chem.* **2006**, *14*, 2589–2599.
[30] I. T. Schiefer, S. Abdul-Hay, H. Wang, M. Vanni, Z. Qin, G. R. J. Thatcher, *J. Med. Chem.* **2011**, *54*, 2293–2306.
[31] C. A. Velázquez, P. N. P. Rao, E. E. Knaus, *J. Med. Chem.* **2005**, *48*, 4061–4067.
[32] C. A Velázquez, P. N. Praveen Rao, M. L. Citro, L. K. Keefer, E. E. Knaus, *Bioorg. Med. Chem.* **2007**, *15*, 4767–4774.
[33] C. A. Velázquez, Q.-H. Chen, M. L. Citro, L. K. Keefer, E. E. Knaus, *J. Med. Chem.* **2008**, *51*, 1954–1961.
[34] J. Kaur, A. Bhardwaj, Z. Huang, E. E. Knaus, *ChemMedChem* **2012**, *7*, 144–150.
[35] D. Basudhar, G. Bharadwaj, R. Y. Cheng, S. Jain, S. Shi, J. L. Heinecke, R. J. Holland, L. A. Ridnour, V. M. Caceres, R. C. Spadari-Bratfisch, N. Paolocci, C. A. Valázquez-Martínez, D. A. Wink, K. M. Miranda, *J. Med. Chem.* **2013**, *56*, 7804–7820.
[36] L. Lazzarato, K. Chegaev, E. Marini, B. Rolando, E. Borretto, S. Guglielmo, S. Joseph, A. Di Stilo, R. Fruttero, A. Gasco, *J. Med. Chem.* **2011**, *54*, 5478–5484.
[37] J. Sidhapuriwala, L. Li, A. Sparatore, M. Bhatia, P. K. Moore, *Eur. J. Pharmacol.* **2007**, *569*, 149–154.
[38] M. Magierowski, K. Magierowska, M. Surmiak, M. Hubalewska-Mazgaj, S. Kwiecien, J. L. Wallace, T. Brzozowski, *J. Physiol. Pharmacol.* **2017**, *68*, 749–756.
[39] A. E. Dief, D. K. Mostafa, G. M. Sharara, T. H. Zeitoun, *Eur. Rev. Med. Pharmacol. Sci.* **2015**, *19*, 1537–1546.
[40] M. V. Chan, J. L. Wallace, *AJP Gastrointest. Liver Physiol.* **2013**, *305*, G467–G473.
[41] S. Fiorucci, S. Orlandi, A. Mencarelli, G. Caliendo, V. Santagada, E. Distrutti, L. Santucci, G. Cirino, J. L. Wallace, *Br. J. Pharmacol.* **2007**, *150*, 996–1002.
[42] J. L. Wallace, G. Caliendo, V. Santagada, G. Cirino, *Br. J. Pharmacol.* **2010**, *159*, 1236–1246.
[43] S. Jain, S. Tran, M. A. M. El Gendy, K. Kashfi, P. Jurasz, C. A. Velázquez-Martínez, *J. Med. Chem.* **2012**, *55*, 688–696.
[44] B. Samuelsson, R. Morgenstern, P. Jakobsson, *Pharmacol. Rev.* **2007**, *59*, 207–224.

[45] J. Bauer, B. Waltenberger, S. M. Noha, D. Schuster, J. M. Rollinger, J. Boustie, M. Chollet, H. Stuppner, O. Werz, *ChemMedChem* **2012**, *7*, 2077–2081.

[46] F. Rörsch, I. Wobst, H. Zettl, M. Schubert-Zsilavecz, S. Grösch, G. Geisslinger, G. Schneider, E. Proschak, *J. Med. Chem.* **2010**, *53*, 911–915.

[47] F. Rörsch, E. La Buscató, K. Deckmann, G. Schneider, M. Schubert-Zsilavecz, G. Geisslinger, E. Proschak, S. Grösch, *J. Med. Chem.* **2012**, *55*, 3792–3803.

[48] A. Koeberle, E. M. Haberl, A. Rossi, C. Pergola, F. Dehm, H. Northoff, R. Troschuetz, L. Sautebin, O. Werz, *Bioorg. Med. Chem.* **2009**, *17*, 7924–7932.

[49] G. Lauro, P. Tortorella, A. Bertamino, C. Ostacolo, A. Koeberle, K. Fischer, I. Bruno, S. Terracciano, I. M. Gomez-Monterrey, M. Tauro, F. Loiodice, E. Novellino, R. Riccio, O. Werz, P. Campiglia, G. Bifulco, *ChemMedChem* **2016**, *11*, 612–619.

[50] S. He, C. Li, Y. Liu, L. Lai, *J. Med. Chem.* **2013**, *56*, 3296–3309.

[51] J. G. Luz, S. Antonysamy, S. L. Kuklish, B. Condon, M. R. Lee, D. Allison, X. P. Yu, S. Chandrasekhar, R. Backer, A. Zhang, M. Russell, S. S. Chang, A. Harvey, A. V. Sloan, M. J. Fisher, *J. Med. Chem.* **2015**, *58*, 4727–4737.

[52] K. M. Partridge, S. Antonysamy, S. N. Bhattachar, S. Chandrasekhar, M. J. Fisher, A. Fretland, K. Gooding, A. Harvey, N. E. Hughes, S. L. Kuklish, J. G. Luz, P. R. Manninen, J. E. McGee, D. R. Mudra, A. Navarro, B. H. Norman, S. J. Quimby, M. A. Schiffler, A. V. Sloan, A. M. Warshawsky, J. M. Weller, J. S. York, X.-P. Yu. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 1478–1483.

[53] A. J. Liedtke, P. R. W. E. F. Keck, F. Lehmann, A. Koeberle, O. Werz, S. A. Laufer, *J. Med. Chem.* **2009**, *52*, 4968–4972.

[54] R. W. Friesen, J. A. Mancini, *J. Med. Chem.* **2008**, *51*, 4059–4067.

[55] A. Kats, T. Båge, P. Georgsson, J. Jönsson, H. C. Quezada, A. Gustafsson, L. Jansson, C. Lindberg, K. Näsström, T. Yucel-Lindberg, *FASEB J.* **2013**, *27*, 2328–2341.

[56] A. Wiegard, W. Hanekamp, K. Griessbach, J. Fabian, M. Lehr, *Eur. J. Med. Chem.* **2012**, *48*, 153–163.

[57] S. Chandrasekhar, A. K. Harvey, X.-P. Yu, M. G. Chambers, J. L. Oskins, C. Lin, T. W. Seng, S. J. Thibodeaux, B. H. Norman, N. E. Hughes, M. A. Schiffler, M. J. Fisher, *J. Pharmacol. Exp. Ther.* **2016**, *356*, 635–644.

[58] A. Koeberle, O. Werz, *Biochem. Pharmacol.* **2015**, *98*, 1–15.

[59] S. Di Micco, S. Terracciano, V. Cantone, K. Fischer, A. Koeberle, A. Foglia, R. Riccio, O. Werz, I. Bruno, G. Bifulco, *Eur. J. Med. Chem.* **2018**, *143*, 1419–1427.

[60] K. Ding, Z. Zhou, S. Zhou, Y. Yuan, K. Kim, T. Zhang, X. Zheng, F. Zheng, C.-G. Zhan, *Bioorg. Med. Chem. Lett.* **2018**, *28*, 858–862.

[61] S. L. Kuklish, S. Antonysamy, S. N. Bhattachar, S. Chandrasekhar, M. J. Fisher, A. J. Fretland, K. Gooding, A. Harvey, N. E. Hughes, J. G. Luz, P. R. Manninen, J. E. McGee, A. Navarro, B. H. Norman, K. M. Partridge, S. J. Quimby, M. A. Schiffler, A. V. Sloan, A. M. Warshawsky, J. S. York, X.-P. Yu, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4824–4828.

[62] A. Koeberle, E. Muñoz, G. B. Appendino, A. Minassi, S. Pace, A. Rossi, C. Weinigel, D. Barz, L. Sautebin, D. Caprioglio, J. A. Collado, O. Werz, *J. Med. Chem.* **2014**, *57*, 5638–5648.

[63] D. L. Flynn, H. Capiris, W. J. Cetenko, D. T. Connor, R. D. Dyer, C. R. Kostlan, D. E. Nies, D. J. Schrier, J. C. Sircar, *J. Med. Chem.* **1990**, *33*, 2070–2072.

[64] M. Inagaki, T. Tsuri, H. Jyoyama, T. Ono, K. Yamada, M. Kobayashi, Y. Hori, A. Arimura, K. Yasui, K. Ohno, S. Kakudo, K. Koizumi, R. Suzuki, M. Kato, S. Kawai, S. Matsamuto, *J. Med. Chem.* **2000**, *43*, 2040–2048.

[65] J. M. Janusz, P. A. Young, J. M. Ridgeway, M. W. Scherz, K. Enzweiler, L. I. Wu, L. Gan, R. Darolia, R. S. Matthews, D. Hennes, D. E. Kellstein, S. A. Green, J. L. Tullich, T. Rosario-Jansen, I. J. Magrisso, K. R. Wehmeyer, D. L. Kuhlenbeck, T. H. Eichhold, R. L. M. Dobson, S. P. Sirko, R. W. Farmer, *J. Med. Chem.* **1998**, *41*, 1112–1123.

[66] D. Lokwani, R. Azad, A. Sarkate, P. Reddanna, D. Shinde, *Bioorg. Med. Chem.* **2015**, *23*, 4533–4543.

[67] M. Zheng, Z. Zhang, W. Zhu, H. Liu, X. Luo, K. Chen, H. Jiang, *Bioorg. Med. Chem.* **2006**, *14*, 3428–3437.

[68] M. Elkady, R. Nieß, A. M. Schaible, J. Bauer, S. Luderer, G. Ambrosi, O. Werz, S. A. Laufer, *J. Med. Chem.* **2012**, *55*, 8958–8962.

[69] T. Hanke, F. Dehm, S. Liening, S. D. Popella, J. MacZewsky, M. Pillong, J. Kunze, C. Weinigel, D. Barz, A. Kaiser, M. Wurglick, M. Lämmerhofer, G. Schneider, L. Sautebin, M. Schubert-Zsilavecz, O. Werz, *J. Med. Chem.* **2013**, *56*, 9031–9044.

[70] A. Koeberle, H. Zettl, C. Greiner, M. Wurglics, M. Schubert-Zsilavecz, O. Werz, *J. Med. Chem.* **2008**, *51*, 8068–8076.

[71] F. Bruno, S. Errico, S. Pace, M. B. Nawrozkij, A. S. Mkrtchyan, F. Guida, R. Maisto, A. Olgaç, M. D'Amico, S. Maione, M. De Rosa, E. Banoglu, O. Werz, A. Fiorentino, R. Filosa, *Eur. J. Med. Chem.* **2018**, *155*, 946–960.

[72] S.-Y. Cheung, M. Werner, L. Esposito, F. Troisi, V. Cantone, S. Liening, S. König, J. Gerstmeier, A. Koeberle, R. Bilancia, R. Rizza, A. Rossi, F. Roviezzo, V. Temml, D. Schuster, H. Stuppner, M. Schubert-Zsilavecz, O. Werz, T. Hanke, S. Pace, *Eur. J. Med. Chem.* **2018**, 156, 815–830.

[73] G. Lauro, S. Terracciano, V. Cantone, D. Ruggiero, K. Fischer, S. Pace, O. Werz, I. Bruno, G. Bifulco, *ChemMedChem* **2020**, *15*, 481–489.

[74] M. Bertinaria, M. A. A. G. Shaikh, C. Buccellati, C. Cena, B. Rolando, L. Lazzarato, R. Fruttero, A. Gasco, M. Hoxha, V. Capra, A. Sala, G. E. Rovati, *ChemMedChem* **2012**, *7*, 1647–1660.

[75] G. Palermo, A. D. Favia, M. Convertino, M. De Vivo, *ChemMedChem* **2016**, *11*, 1252–1258.

[76] R. Scarpelli, O. Sasso, D. Piomelli, *ChemMedChem* **2016**, *11*, 1242–1251.

[77] P. Jansa, A. Holý, Z. Zídek, E. Kmoníčková, Z. Janeba, Pyrimidine Compounds Inhibiting the Formation of Nitric Oxide and Prostaglandin E2, Method of Production Thereof and Use Thereof. 2012, WO2012/116666.

[78] P. Jansa, A. Holý, M. Dračínský, V. Kolman, Z. Janeba, P. Kostecká, E. Kmoníčková, Z. Zídek, *Med. Chem. Res.* **2014**, *23*, 4482–4490.

[79] P. Jansa, A. Holý, M. Dračínský, V. Kolman, Z. Janeba, E. Kmoníčková, Z. Zídek, *Med. Chem. Res.* **2015**, *24*, 2154–2166.

[80] V. Kolman, P. Jansa, F. Kalčic, Z. Janeba, Z. Zídek, *Nitric Oxide* **2017**, *67*, 53–57.

[81] V. Kolman, F. Kalčic, P. Jansa, Z. Zídek, Z. Janeba, *Eur. J. Med. Chem.* **2018**, *156*, 295–301.

[82] M. C. Carreno, J. L. Garcia Ruano, G. Sanz, M. A. Toledo, A. Urbano, *J. Org. Chem.* **1995**, *60*, 5328–5331.

[83] W. Li, D. P. Nelson, M. S. Jensen, R. S. Hoerrner, D. Cai, R. D. Larsen, P. J. Reider, *J. Org. Chem.* **2002**, *67*, 5394–5397.

[84] K. Harada, K. Makino, N. Shima, H. Okuyama, T. Esumi, M. Kubo, H. Hioki, Y. Asakawa, Y. Fukuyama, *Tetrahedron* **2013**, *69*, 6959–6968.

[85] O. Saku, M. Saki, M. Kurokawa, K. Ikeda, T. Takizawa, N. Uesaka, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1090–1093.

[86] D. Bogdal, M. Warzala, *Tetrahedron* **2000**, *56*, 8769–8773.

[87] Y. Yamaguchi, I. Akimoto, K. Motegi, T. Yoshimura, K. Wada, N. Nishizono, K. Oda, *Chem. Pharm. Bull.* **2013**, *61*, 997–1001.

[88] J. H. Musser, U. Chakraborty, K. Bailey, S. Sciortino, C. Whyzmuzis, D. Amin, C. A. Sutherland, *J. Med. Chem.* **1987**, *30*, 62–67.

[89] K. Raddassi, J. F. Petit, G. Lemaire, *Cell. Immunol.* **1993**, *149*, 50–64.

[90] M. G. Schwacha, S. D. Somers, *J. Leuko. Biol.* **1998**, *63*, 51–58.

[91] Z.Zídek, M. Kverka, A. Dusilová, E. Kmoníčková, P. Jansa, *Nitric oxide* **2016**, *57*, 48–56.

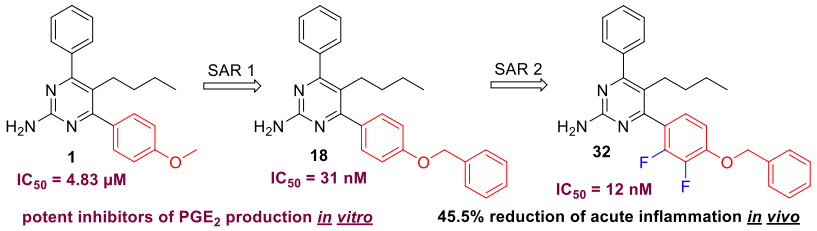
[92] K. Ding, Z. Zhou, S. Hou, Y. Yuan, S. Zhou, X. Zheng, J. Chen, C. Loftin, F. Zheng, C.-G. Zhan, *Sci. Rep.* **2018**, *8*, 5205.

[93] T. Sjögren, J. Nord, M. Ek, P. Johansson, G. Liu, S. Geschwindner, *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 3806–3811.

[94] D. Li, N. Howe, A. Dukkipati, S. T. Shah, B. D. Bax, C. Edge, A. Bridges, P. Hardwicke, O. M. Singh, G. Giblin, A. Pautsch, R. Pfau, G. Schnapp, M. Wang, V. Olieric, M. Caffrey, *Cryst. Growth Des.* **2014**, *14*, 2034–2047.

[95] A. Hamza, X. Zhao, M. Tong, H. H. Tai, C. G. Zhan, *Bioorg. Med. Chem*. **2011**, *19*, 6077–6086.

Entry for the Table of Contents



Anti-inflammatory drugs belong to the mostly used therapeutic agents, but both traditional NSAIDs and coxibs suffer from serious side effects. Thus, development of anti-inflammatory drugs with novel mechanisms of actions is desirable and inhibitors of mPGES-1 may be good approach. We designed and synthesized potent mPGES-1 inhibitors based on polysubstituted pyrimidines, which showed strong anti-inflammatory effects *in vivo*.