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Original article

Pyrrole alkanoic acid derivatives as nuisance inhibitors of microsomal prostaglandin E_2 synthase-1

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ABSTRACT

Microsomal prostaglandin E_2 synthase-1 (mPGES-1) is an enzyme, which is induced during the inflammatory response. Therefore, inhibitors of this enzyme are considered to be potential anti-inflammatory drugs. We have identified 3-(4-dodecanoyl-1,3,5-trimethylpyrrol-2-yl)propionic acid (**12**) as submicromolar inhibitor of mPGES-1. Surprisingly, structural variations made around this lead only resulted in a relatively small change of enzyme inhibitory potency. Such flat structure–activity relationships are reported to be typical for so called nuisance inhibitors, which exert their action not by directly binding to the enzyme, but by forming colloid-like aggregates at micromolar and sometimes submicromolar concentrations, which somehow sequester and inhibit enzyme targets without specificity. Since aggregate-based inhibition is highly sensitive to non-ionic detergents such as Triton X-100, we investigated some of our compounds for inhibition of human recombinant mPGES-1 also in presence of this detergent. The pyrrole derivatives **12**, **67** and **81**, which exhibited IC_{50} values in absence of Triton X-100 in the range of 0.1 and 1 μM, were virtually inactive at the highest test concentration of 10 μM when 0.1% of the detergent was added. In the same way, the published mPGES-1 inhibitor 2-[(4-{[(1,1′-biphenyl)-4-ylmethyl]amino}-6-chloropyrimidin-2-yl)thio]octanoic acid (Cay10589) (**6**) totally lost its activity under these conditions. Therefore, these compounds have to be judged as nuisance inhibitors of the enzyme. In contrast, the known indole derivative 3-[3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-isopropylindol-2-yl]-2,2-dimethylpropionic acid (MK-886) (**2**) showed a considerable activity (75% inhibition at 10 μM) also in the presence of Triton X-100.

1. Introduction

Prostaglandins and thromboxanes comprise a family of lipid mediators, which are involved in physiological as well as in pathophysiological processes [1]. Their synthesis is initiated by a phospholipase A_2, which liberates arachidonic acid (AA) from phospholipid membranes. Arachidonic acid, in turn, is converted to prostaglandin H_2 (PGH_2) by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2). PGH_2 is an unstable intermediate, which is further metabolized to different products in dependence of the downstream enzymes present in the cells. In platelets, its main reaction product is thromboxane B_2 formed via thromboxane synthase. In endothelic cells PGH_2 is converted to prostaglandin I_2 (PGI_2, prostacyclin) by prostacyclin synthase. In many other cells, however, the predominant lipid mediator produced is prostaglandin E_2 (PGE_2). The isomerization of PGH_2 to PGE_2 is catalyzed by three different enzymes [2–5]: the cytosolic prostaglandin E_2 synthase (cPGES), and two membrane bound PGES, namely, the microsomal prostaglandin synthase-1 and -2 (mPGES-1 and PGES-2). cPGES and mPGES-2 are constitutively expressed enzymes with cPGES coupling through COX-1 [2] and mPGES-2 coupling through COX-1 and COX-2 [6]. In contrast, mPGES-1 is an inducible enzyme primarily coupled to COX-2 [7–10]. Strong inducers of the enzyme are proinflammatory stimuli such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) leading to a highly elevated PGE_2 formation during inflammation [9–11]. Mice deficient in mPGES-1 exhibited a reduced production of inflammatory PGE_2 and showed a lower inflammatory response in the collagen induced arthritis model [12–16]. Furthermore, pain reactions were impaired [14–16] and lipopolysaccharides were not able to produce fever in such knock-out mice [17]. Gastrointestinal toxicity usually elicited by nonsteroidal anti-inflammatory drugs (NSAIDs) inhibiting COX-1 [13] as well as side effects typically

Abbreviations: mPGES-1, microsomal prostaglandin E_2 synthase-1; mPGES-2, microsomal prostaglandin E_2 synthase-2; cPGES, cytosolic prostaglandin E_2 synthase; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; PGH_2, prostaglandin H_2; PGE_2, prostaglandin E_2; DMSO, dimethyl sulfoxide; HPLC, high performance liquid chromatography; UV, ultra violet; MS, mass spectrometry; EI, electron ionization; ESI, electrospray ionization; APCI, atmospheric pressure chemical ionization.

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for COX-2 inhibitors such as changes in thrombogenesis and blood pressure could not be seen in these animals [18,19]. Therefore, inhibitors of mPGES-1 are expected to be novel analgetic, antipyretic and anti-inflammatory drugs producing less adverse reactions than the COX inhibitors presently applied [20–22].

In the past years several inhibitors of mPGES-1 have been published [21,22]. One of the first compounds reported to possess inhibitory activities against the enzyme were the sulfonamide derivative **1** (NS-398) [10] and the indolylpropionic acid **2** (MK-886) (Fig. 1) [8]. While **1** inhibits mPGES-1 with a relative low potency (IC_{50}: 10–20 μM), with a published IC_{50} of 1.6 μM **2** is a modest inhibitor of the enzyme. Just as well, the IC_{50} values of the carboxylic acid derivatives **5** and **6** lie in the lower micromolar range [23–25]. Structural refinement of **2** led to **3**, which exhibited a nanomolar IC_{50} [26]. Further inhibitors of mPGES-1 with nanomolar activity against mPGES-1 are the indole-2-carboxylic acid **4** [27], the imidazole derivatives **7**–**9** [28–31] and the sulfonamides **10** and **11** [32–34]. Although compounds with high *in vitro* potency have been found, actually no mPGES-1 inhibitor is reported undergoing clinical evaluation.

As shown in Fig. 1, several of the described mPGES-1 inhibitors are lipophilic carboxylic acid derivatives. For this reason, we started our search for new inhibitors of the enzyme by testing compounds with such structural elements already synthesized in our group for other purposes. During this screening we found that the 4-acylpyrrol-2-ylpropionic acid **12** (Fig. 1) [35,36] effectively reduced enzyme activity [37] with an IC_{50} of 0.80 μM. This paper reports the effects of the structural variation of this lead compound on its mPGES-1 inhibitory properties.

2. Results and discussion

2.1. Chemistry

Quite a few of the pyrroles evaluated for mPGES-1 inhibition have already been published (**13**–**16**,**18**,**19**,**31**,**34**) [35,36,38,39]. For the synthesis of numerous of the dodecanoyl-substituted target compounds (**35**,**43**–**45**,**55**–**67**,**75**) similar synthetic routes were applied as for the preparation of their known corresponding octadecanoyl and octadecyl derivatives, respectively [35,36,38,39].

The benzoyl and 2-naphthoyl substituted pyrrolylpropionic acid derivatives **28** and **30** were synthesized starting from methyl 3-(1,3,5-trimethylpyrrol-2-yl)propionate according to the procedure published for the preparation of **12** [35]. The remaining compounds of this series (**17**,**20**,**26**,**27**,**29**,**32**–**33**) were prepared from ethyl 1,3,5-trimethylpyrrol-2-carboxylate in an analogous manner as shown in Scheme 1 for the synthesis of **26**.

The pyrrolylbutanoic acid **42** was synthesized as outlined in Scheme 2. 1,3,5-Trimethylpyrrole-2-carboxylic acid ethylester (**21**) was acylated in position 4 with dodecanoyl chloride/$AlCl_3$. Saponification of the ester group of obtained intermediate **36** by KOH followed by thermal decarboxylation and formylation by the Vilsmeier–Haak method yielded the carbaldehyde **37**, which was converted to the methoxyvinyl substituted pyrrole **38** by Wittig-reaction with (methoxymethyl)triphenylphosphonium chloride [40]. From this compound acetaldehyde **39** was obtained by treatment with chlorotrimethylsilane/NaI. Horner–Wadsworth–Emmons reaction of **39** with triethyl phosphonoacetate gave the (*E*)-but-2-enoic ester **40**. Catalytic hydrogenation and final ester hydrolysis with KOH led to the target compound **42**.

The preparation of the three 1-phenyl-substitued acid derivatives **49**, **52** and **54** started from the carboxylic acid ester **46** (Scheme 3). Introduction of the phenyl ring in position 1 of the pyrrole was accomplished by Cu(II)acetate catalyzed reaction with phenylboronic acid [41]. Friedel–Crafts acylation of obtained compound **47** with dodecanoyl chloride/$AlCl_3$ followed by alkaline ester hydrolysis yielded the test compound **49**. For synthesis of the corresponding acetic and propionic acid derivatives **52** and **54** first the carboxylic acid group of **49** was removed by heating. The acetic acid side chain was introduced into the obtained intermediate **50** by copper-assisted coupling with ethyl diazoacetate and subsequent ester hydrolysis with KOH. Treatment of **50** with methyl acrylate/boron trifluoride etherate followed by saponification led to the propionic acid target compound.

The synthesis of pyrrole propionic acid derivative **74**, in which the propionic acid chain is flanked by a phenyl and a benzyl residue,

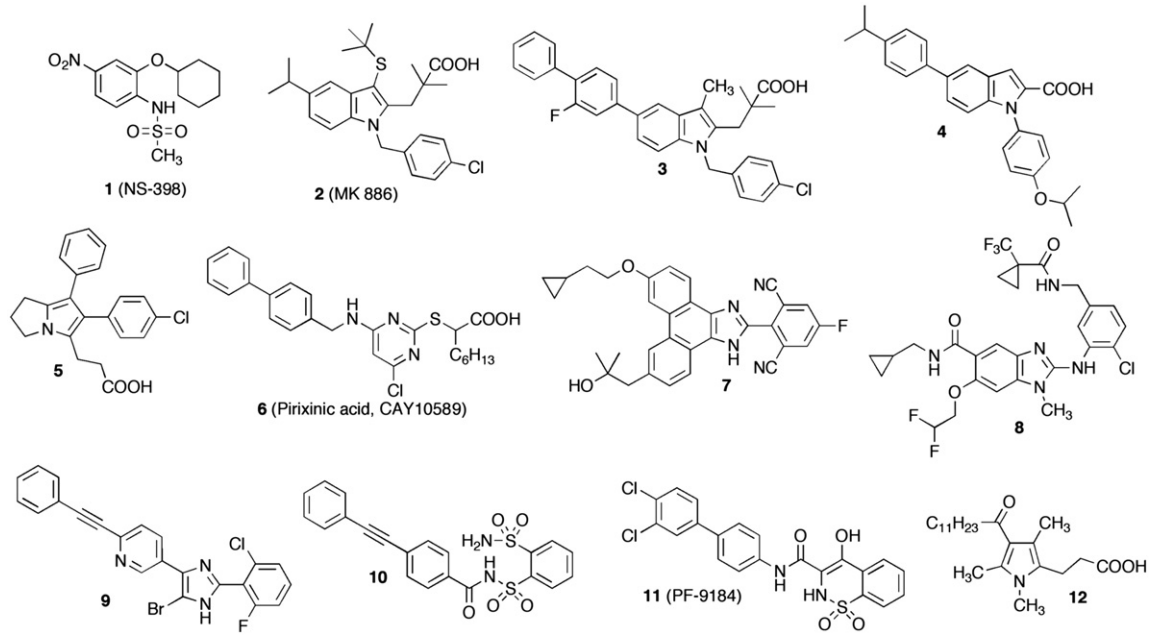
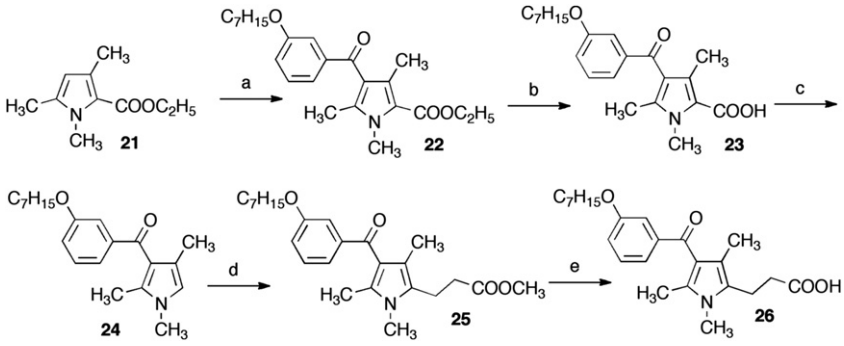


Fig. 1. Structures of inhibitors of mPGES-1 activity.



Scheme 1. Reagents and conditions: (a) 3-heptyloxybenzoyl chloride, $AlCl_3$, CH_2Cl_2, 0 °C, 30 min, (b) aq. KOH, ethanol, reflux, 2 h, (c) 160–170 °C, 20 min, (d) methyl acrylate, BF_3–Et_2O, CH_2Cl_2, room temp., 5 d, (d) aq. KOH, ethanol, reflux, 30 min.

is outlined in Scheme 4. The nitrile, amide, hydrazide and sulfonylamide derivatives of the lead **12** (**78**–**82**) were prepared as shown in Scheme 5.

2.2. Biological evaluation

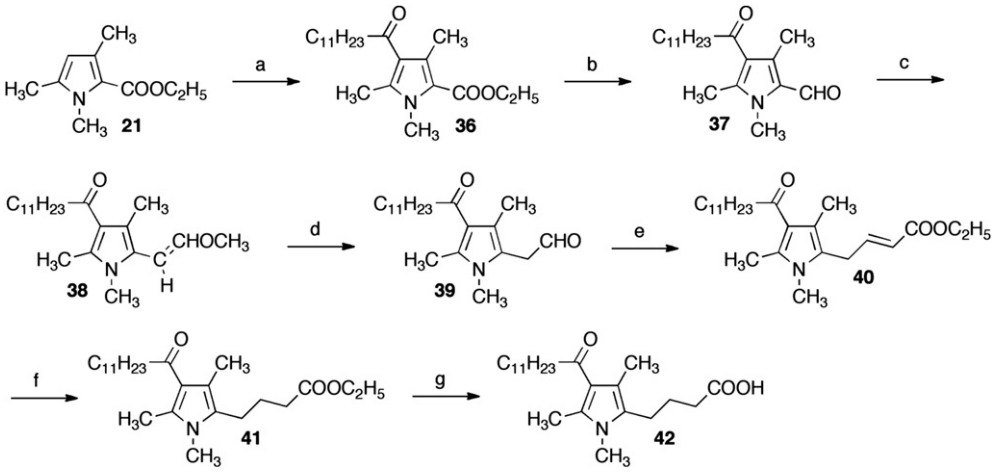
All test assays applied for mPGES-1 inhibitor screening are basing on a method described by Thorén and Jakobson using prostaglandin H_2 (PGH_2) as substrate [10]. One critical factor in these assays is the chemical instability of PGH_2, which degrades in aqueous solutions non-enzymatically into the enzyme product PGE_2 and into PGD_2 with a half life of about 10 min at room temperature [42]. To minimize non-enzymatic PGE_2 formation, short incubation times (30 s–5 min) and low temperatures (0–20 °C) are generally applied [10,20,22,24–34,37,43–49]. At the end of the enzyme reaction unreacted PGH_2 is converted to $PGF_{2\alpha}$ by addition of $SnCl_2$ or $FeCl_2$. The enzyme product is quantified by radioimmunoassay (RIA), enzyme immunoassay (EIA), coupled spectrophotometric detection, HPLC with UV or radiometric detection, or homogenous time resolved fluorescence detection (HTRF). As enzyme sources microsomes from IL-1β stimulated A549 cells or microsomal preparations of mPGES-1 expressed in Chinese hamster ovary (CHO) cells, Sf9 insect cells, and *Escherichia coli* cells, respectively, are used.

For evaluation of our compounds, the formation of PGE_2 from PGH_2 was monitored by online solid phase extraction HPLC with UV-detection [37]. The enzyme reactions were performed at 0 °C for 5 min applying A549 cell microsomes, initially. With this assay, for the lead compound **12** an IC_{50} of 0.80 μM was determined, while compound **1** (NS-398) used as reference exhibited an IC_{50} of 20 μM.

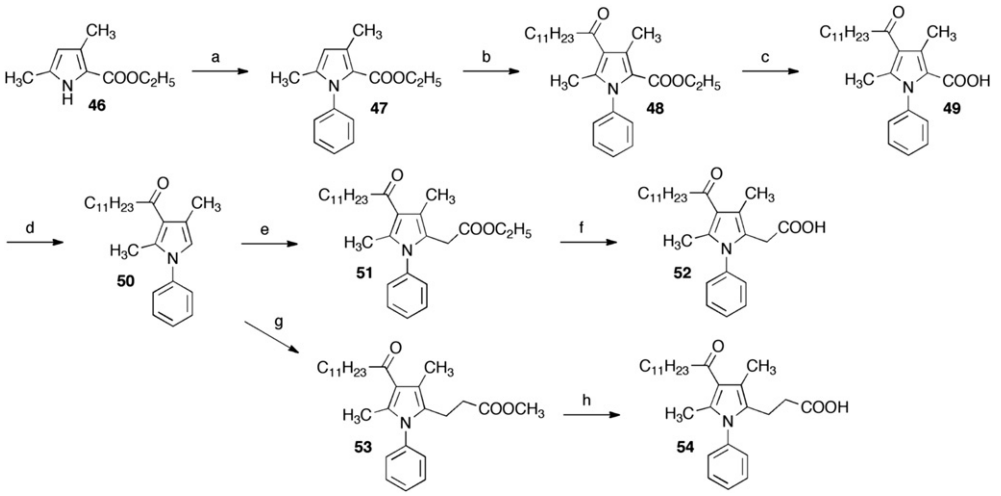
The structural modifications of the lead **12** comprised variation of the dodecanoyl residue, substitution of the pyrrole methyl groups by hydrogen, phenyl and benzyl, elongation and shortening of the propionic acid moiety, and bioisosteric replacement of the carboxylic acid group. The results of the variation of the acyl residue are shown in Table 1. Increasing as well as decreasing the length of the dodecanoyl substituent led to a successive reduction of mPGES-1 inhibitory potency. Constraining the acyl chain by introduction of an oxyphenyl moiety (**20**,**26**,**27**) did not drastically change activity. Replacement of these alkoxybenzoyl residues by less bulky benzoyl, phenylbenzoyl, naphthoyl and 3-phenylpropanoyl substituents (**28**–**32**), respectively, resulted in a loss of mPGES-1 inhibition at 3.3 μM.

Surprisingly, the other structural variations named above did not lead to a significant alteration of enzyme inhibition. The inhibition values of most of the compounds evaluated lay in the range of 30–70% at a concentration of 3.3 μM.

In our opinion, one possible reason for the observed flat structure–activity relationships could have been an impaired solubility of the test compounds at the assay temperature of 0 °C. Since most of these substances are very lipophilic, the concern existed that they precipitate in the assay buffer. Therefore, we decided to re-evaluate the compounds applying an assay temperature of 20 °C. Since at this time of our investigations microsomal preparations of human recombinant mPGES-1 were commercially available, we used this enzyme source for further studies. Applying the altered



Scheme 2. Reagents and conditions: (a) dodecanoyl chloride, $AlCl_3$, 0 °C, 30 min, (b) 1. aq. KOH, ethanol, reflux, 1 h, 2. 160–170 °C, 20 min, 3. DMF, $POCl_3$, toluene, room temp., 2 h, (c) (methoxymethyl)triphenylphosphonium chloride, K-*tert*-butylate, THF, microwave, 80 °C, 60 min, (d) chlorotrimethylsilane, NaI, acetonitrile, room temp., 20 min, (e) triethyl phosphonoacetate, NaH, THF, room temp., 1 h, (f) H_2, Pd/C, THF, ethanol, room temp., 1 h, (g) aq. KOH, ethanol, reflux, 15 min.



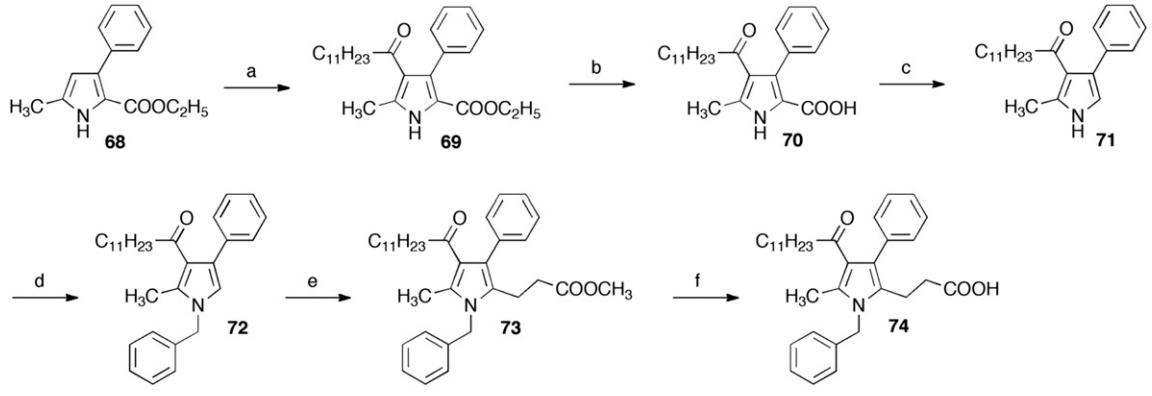
Scheme 3. Reagents and conditions: (a) phenylboronic acid, Cu(II)acetate, triethyl amine, CH_2Cl_2, room temp., 2 d, (b) dodecanoyl chloride, $AlCl_3$, 0 °C, 2 h, (c) aq. KOH, ethanol, reflux, 15 min, (d) 160–170 °C, 20 min, (e) ethyl diazoacetate, Cu(o), toluene, 115–120 °C, (f) aq. KOH, ethanol, reflux, 30 min, (g) methyl acrylate, BF_3–Et_2O, CH_2Cl_2, room temp., 3 d, (h) aq. KOH, ethanol, reflux, 30 min.

conditions, for the lead compound **12** an IC_{50} of 0.67 μM was measured. The published mPGES-1 inhibitors **2** (MK-886) and **6** (pirixinic acid derivative Cay10589) tested in parallel for comparison exhibited IC_{50} values of 1.3 μM and 0.65 μM, respectively. Because the inhibition values seemed to be slightly higher than in the first assay, all test compounds here were evaluated at a concentration of 1 μM.

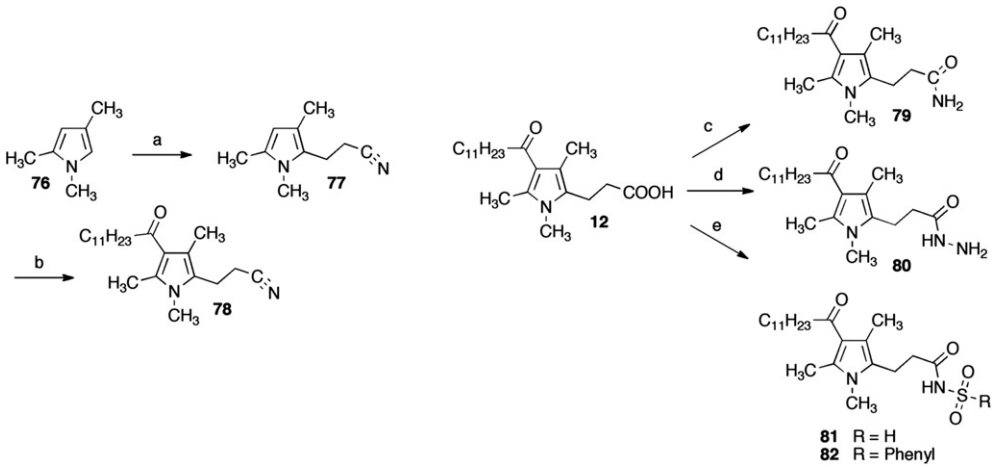
Comparing the results obtained for the acyl derivatives listed in Table 1, one striking difference could be noted. In the assay with the enzyme from A549 cells performed at 0 °C elongation of the dodecanoyl residue of **12** led to a successive decrease of mPGES-1 inhibitory potency, while in the assay with the recombinant enzyme carried out at 20 °C such an enlargement of the acyl chain length led to a significant enhancement of activity of the compounds (Table 1). A reason for this result could lie in solubility problems of the very lipophilic tetradecanoyl, hexadecanoyl and octadecanoyl derivatives **17**–**19** in water at 0 °C.

Table 2 highlights the effects of the substitution of the methyl substituents in pyrrole 1, 3 and 5 position of **12** and of the variation of the alkanoic acid chain length on inhibition of human recombinant mPGES-1. Furthermore, the consequence of the replacement of the dodecanoyl moiety of the carboxylic acid derivative **34** by a dodecyl residue (**75**) is displayed. The inhibition data obtained with this second assay again demonstrated that most structural variations made around the lead **12** only resulted in a small change of activity. In the majority of cases the inhibition value of the compounds against human recombinant mPGES-1 lay within 30–70% at 1 μM. The only compounds showing a greater divergence from this range were the three derivatives, which are unsubstituted in pyrrole 3 and 5 position (**64**–**66**), and two derivatives with a phenyl residue standing vicinal to the acid moiety (**49** and **67**). Compounds **64**–**66** were inactive, while **49** and **67** showed a nearly total inhibition of the enzyme at 1 μM.

Since the replacement of the carboxylic acid group of **12** by bioisosteric residues like nitrile, amide, hydrazide, and sulfonylamide (**78**–**82**) also did not lead to a drastical change of activity (Table 3), we started looking for an explanation for these unordinary results. During these investigations we came across an excellent publication of Brian K. Shoichet, in which it was described that flat structure–activity relationships are unusual with classic, well behaved drugs but common among so called "nuisance" inhibitors [50]. These nuisance inhibitors exert their action not by directly binding to the enzyme, but by forming colloid-like aggregates at micromolar and sometimes submicromolar concentrations, which somehow sequester and inhibit enzyme targets without specificity. One characteristic of aggregate-based inhibition is its high sensitivity to non-ionic detergents such as Triton X-100. Thus, molecules that inhibit an enzyme in the absence and not



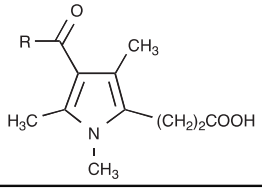
Scheme 4. Reagents and conditions: (a) *N*,*N*-dimethyldodecanamide, $POCl_3$, toluene, reflux, 10 h, (b) aq. KOH, ethanol, reflux, 30 min, (c) 160–170 °C, 20 min, (d) benzyl bromide, tetrabutylammonium bromide, aq. NaOH (50%), diethyl ether, CH_2Cl_2, reflux, 3 h, (e) methyl acrylate, BF_3–Et_2O, CH_2Cl_2, room temp., 3 d, (f) aq. KOH, ethanol, reflux, 30 min.



Scheme 5. Reagents and conditions: (a) acrylnitrile, BF_3—Et_2O, room temp., 1 h, (b) dodecanoyl chloride, $AlCl_3$, CH_2Cl_2, 0 °C—room temp., 1.5 h, (c) *N,N′*-carbonyldiimidazole, NH_3 conc., CH_2Cl_2, 0 °C, 1 h/room temp., 20 min, (c) *N,N′*-carbonyldiimidazole, hydrazine hydrate, CH_2Cl_2, room temp., 16 h, (d) methanesulfonamide or benzenesulfonamide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide—HCl (EDC—HCl), 4-dimethylaminopyridine, triethylamine, CH_2Cl_2, room temp., overnight.

in the presence of Triton X-100 are likely to inhibit the enzyme not in a "true" manner [50–52]. Therefore, we investigated some of the compounds described above also in presence of 0.1% Triton X-100. This detergent was not reported to be used in mPGES-1 screening assays until now. Only the chemical company Cayman had added this substance to the assay buffer, when testing the activity of microsomal preparations of recombinantly produced enzyme [53]. Table 4 shows the inhibition data of the selected compounds obtained without and with the detergent. The pyrrolylpropionic acid **12**, the phenylpyrrolylpropionic acid **67**, and the methylsulfonylamide derivative **81**, which exhibited IC_{50} values in absence of Triton X-100 in the range of 0.1 and 1 μM, were virtually inactive at the highest test concentration of 10 μM in presence of 0.1% of the detergent. Just so, the published mPGES-1 inhibitor **6** [25] totally lost its activity when Triton X-100 was added. Therefore, these compounds have to be judged as nuisance inhibitors of the enzyme. In contrast, the indole derivative **2** [8,26] showed a considerable

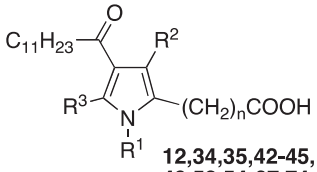
Table 1
Inhibition of mPGES-1 activity.



Comp.	R	Inhibition of mPGES-1 from A549 cells at 3.3 μM [%][a]	Inhibition of human recombinant mPGES-1 at 1 μM [%][b]
12	$C_{11}H_{23}$	74 ± 8 ($n = 5$)	54 ± 8% ($n = 7$)
13	C_3H_7	n.a.[c]	n.a.[e]
14	C_6H_{13}	n.a.[c]	n.a.[e]
15	C_7H_{15}	55	n.a.[e]
16	C_9H_{19}	62	n.a.[e]
17	$C_{13}H_{27}$	63	91
18	$C_{15}H_{31}$	35	97
19	$C_{17}H_{35}$	n.a.[c]	97
20	2-Octyloxyphenyl	23	n.a.[e]
26	3-Heptyloxyphenyl	67	n.a.[e]
27	4-Hexyloxyphenyl	64	32
28	Phenyl	n.a.[c]	n.a.[e]
29	Naphthalene-1-yl	n.a.[c]	n.a.[e]
30	Naphthalene-2-yl	n.a.[c]	n.a.[e]
31	2-Phenylethyl	n.a.[c]	n.a.[e]
32	4-Phenylphenyl	n.a.[c]	n.a.[e]
33	4-Phenylbenzyl	n.t.[d]	n.a.[e]

[a] Incubation at 0 °C, values are the means of at least two independent determinations.
[b] Incubation at 20 °C, values are the means of at least two independent determinations.
[c] n.a.: not active at 3.3 μM.
[d] n.t.: not tested.
[e] n.a.: not active at 1 μM.

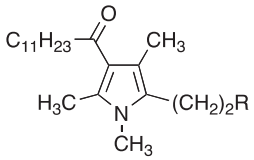
Table 2
Inhibition of mPGES-1 activity.



Comp.	R^1	R^2	R^3	*n*	Inhibition of human recombinant mPGES-1 at 1 μM [%]
12	CH_3	CH_3	CH_3	2	54 ± 8 ($n = 7$)
34	CH_3	CH_3	CH_3	0	59
35	CH_3	CH_3	CH_3	1	22
42	CH_3	CH_3	CH_3	3	70
43	H	CH_3	CH_3	0	62
44	H	CH_3	CH_3	1	17
45	H	CH_3	CH_3	2	38
49	Phenyl	CH_3	CH_3	0	99
52	Phenyl	CH_3	CH_3	1	23
54	Phenyl	CH_3	CH_3	2	65
55	Benzyl	CH_3	CH_3	0	65
56	Benzyl	CH_3	CH_3	1	35
57	Benzyl	CH_3	CH_3	2	62
58	4-CH_3—Benzyl	CH_3	CH_3	2	52
59	4-Cl—Benzyl	CH_3	CH_3	2	78
60	4-OCH_3—Benzyl	CH_3	CH_3	2	72
61	4-OH—Benzyl	CH_3	CH_3	2	53
62	4-COOH—Benzyl	CH_3	CH_3	2	27
63	4-$CONH_2$—Benzyl	CH_3	CH_3	2	28
64	CH_3	H	H	0	n.a.
65	CH_3	H	H	1	n.a.
66	CH_3	H	H	2	n.a.
67	CH_3	Phenyl	CH_3	2	90
74	Benzyl	Phenyl	CH_3	2	64
75					37

Values are the means of at least two independent determinations.

Table 3
Inhibition of mPGES-1 activity.



Comp.	R	Inhibition of human recombinant mPGES-1 at 1 μM [%]
12	COOH	54 ± 8 ($n = 7$)
78	CN	22
79	$CONH_2$	39
80	$CONHNH_2$	35
81	$CONHSO_2CH_3$	52
82	$CONHSO_2$Phenyl	77

Values are the means of at least two independent determinations.

activity (75% inhibition at 10 μM) in the presence of Triton X-100, making it likely that this compound is a true inhibitor of mPGES-1.

Because in all screening assays for mPGES-1 inhibitors microsomal preparations were used, it is imaginable that the nuisance compounds not only lead to a reduced mPGES-1 activity by forming aggregates that physically interact with the enzyme, but also by disturbing the structure of the microsomes. Beside the prevention of aggregate formation, therefore, the effect of Triton X-100 here could also result from a stabilization of the microsomes against the test compounds leading to a preservation of enzyme activity.

As a result of the present investigation it appears to be recommendable that the activity of published mPGES-1 inhibitors should be re-evaluated with Triton X-100 present in the incubation buffer, particularly in those cases, in which flat structure–activity relationships have been found.

3. Conclusion

The *in vitro* assays commonly used for the evaluation of mPGES-1 inhibitors can lead to false positive results, especially when the test compounds are lipophilic carboxylic acid derivatives. These pitfalls can be avoided by addition of the detergent Triton X-100 to the assay composition.

4. Experimental section

4.1. Chemistry

4.1.1. General

Column chromatography was performed on silica gel 60, particle size 0.040–0.063 mm, from Merck or Macherey–Nagel. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. ^{1}H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were recorded on a Varian Mercury Plus 400 spectrometer. Electron ionization (EI) mass spectra were obtained on a Finnigan GCQ apparatus. The high resolution mass spectra were recorded on a Bruker micrOTOF-Q II spectrometer applying electro spray chemical ionization (ESI) or atmospheric pressure chemical ionization (APCI). Purity of the target compound was determined by reversed phase HPLC on a Phenomenex Aqua RP 18 column (100 × 4.6 mm, 3 μ) or a Nucleosil 100 RP 18 column (125 × 3 mm, 3 μ), eluting with CH_3CN/H_2O containing 0.1% H_3PO_4 at a flow rate of 0.7 and 0.5 mL/min, respectively. UV-absorbance was measured at 254 nm. The purity of all target compounds was greater than 95%.

Table 4
Inhibition of human recombinant mPGES-1 activity in absence and in presence of Triton X-100.

Comp.	Inhibition of human recombinant mPGES-1 IC_{50} [μM][a]	Inhibition of human recombinant mPGES-1 in presence of 0.1% Triton X-100 at 10 μM
12	0.67 ± 0.34	n.a.[b]
67	0.14 ± 0.03	n.a.[b]
81	0.60 ± 0.26	n.a.[b]
2 (MK-866)	1.3 ± 0.42	75 ± 13[c]
6 (Cay10589)	0.65 ± 0.20	n.a.[b]

[a] Mean ± standard deviation, $n = 4$ (in case of **12**: $n = 5$).
[b] n.a.: not active, $n = 3$.
[c] Mean ± standard deviation, $n = 5$.

4.1.2. Ethyl 4-(3-heptyloxybenzoyl)-1,3,5-trimethylpyrrole-2-carboxylate (**22**)

A mixture of 3-heptyloxybenzoic acid [54] (141 mg, 0.47 mmol) and thionyl chloride (5 mL) was heated under reflux for 2 h. The remaining thionyl chloride was distilled off under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL) and treated at 0 °C with ethyl 1,3,5-trimethylpyrrole-2-carboxylate (**21**) [55] (125 mg, 0.69 mmol) followed by anhydrous $AlCl_3$ (118 mg, 0.88 mmol). After stirring at 0 °C for 30 min, the reaction mixture was diluted with water and extracted exhaustively with diethyl ether. The combined organic layers were washed with half-saturated brine and saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated. Chromatography on silica gel (hexane/ethyl acetate, 9:1) yielded **22** as an oil (144 mg, 46%). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, $J = 6.7$ Hz, 3H), 1.24–1.40 (m, 9H), 1.40–1.52 (m, 2H), 1.73–1.85 (m, 2H), 2.17 (s, 3H), 2.20 (s, 3H), 3.82 (s, 3H), 3.99 (t, $J = 6.6$ Hz, 2H), 4.31 (q, $J = 7.1$ Hz, 2H), 7.03–7.11 (m, 1H), 7.27–7.36 ppm (m, 3H), MS (EI) m/z (%): 399 (100) M^+, 284 (89).

4.1.3. 4-(3-heptyloxybenzoyl)-1,3,5-Trimethylpyrrole-2-carboxylic acid (**23**)

A solution of **22** (128 mg, 0.32 mmol) in ethanol (5 mL) was treated with 10% aqueous KOH (15 mL). The resulting mixture was refluxed for 2 h, cooled, diluted with water, acidified with dilute HCl, and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute HCl, dried (Na_2SO_4) and evaporated to yield **23** as an oil (112 mg, 94%). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, $J = 6.8$ Hz, 3H), 1.16–1.40 (m, 6H), 1.40–1.54 (m, 2H), 1.71–1.86 (m, 2H), 2.19–2.27 (m, 6H), 3.84 (s, 3H), 3.95–4.04 (m, 2H), 7.06–7.11 (m, 1H), 7.22–7.35 ppm (m, 3H), MS (EI) m/z (%): 371 (24) M^+, 326 (100).

4.1.4. (3-Heptyloxyphenyl)(1,2,4-trimethylpyrrol-3-yl)methanone (**24**)

Compound **23** (103 mg, 0.28 mmol) was heated at 160–170 °C for 20 min. Chromatography on silica gel (hexane/ethyl acetate, 9:1) yielded **24** as an oil (65 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 0.89 (t, $J = 6.8$ Hz, 3H), 1.22–1.40 (m, 6H), 1.40–1.50 (m, 2H), 1.71–1.86 (m, 2H), 1.92 (s, 3H), 2.17 (s, 3H), 3.48 (s, 3H), 3.96 (t, $J = 6.6$ Hz, 2H), 6.32 (s, 1H), 7.00–7.05 (m, 1H), 7.21–7.32 ppm (m, 3H), MS (EI) m/z (%): 327 (93) M^+, 326 (100).

4.1.5. Methyl 3-[4-(3-heptyloxybenzoyl)-1,3,5-trimethylpyrrol-2-yl] propionate (**25**)

A solution of **24** (62 mg, 0.19 mmol) in dry CH_2Cl_2 (5 mL) was treated with methyl acrylate (17 μL, 0.19 mmol) and boron trifluoride diethyl etherate (12 μL). After being stirred at room temperature for 5 d, the mixture was diluted with water and extracted exhaustively with diethyl ether. The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was

chromatographed on silica gel (hexane/ethyl acetate, 9:1) to yield **25** as a solid (33 mg, 42%), mp 54–55 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 6.9 Hz, 3H), 1.20–1.39 (m, 6H), 1.39–1.50 (m, 2H), 1.71–1.83 (m, 2H), 1.92 (s, 3H) 2.15 (s, 3H), 2.44–2.51 (m, 2H), 2.86–2.94 (m, 2H), 3.44 (s, 3H), 3.67 (s, 3H), 3.96 (t, J = 6.6 Hz, 2H), 7.00–7.05 (m, 1H), 7.22–7.31 ppm (m, 3H), MS (EI) m/z (%): 413 (32) M^+, 340 (100).

4.1.6. 3-[4-(3-Heptyloxybenzoyl)-1,3,5-trimethylpyrrol-2-yl] propionic acid (**26**)

A solution of **25** (31 mg, 0.08 mmol) in ethanol (4 mL) was treated with 10% aqueous KOH (1.3 mL). The resulting mixture was refluxed for 30 min, cooled, diluted with water, acidified with dilute HCl, and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute HCl, dried (Na_2SO_4) and evaporated to a few mL. By addition of petroleum ether, **26** precipitated (26 mg, 88%), mp 105 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H), 1.17–1.38 (m, 6H), 1.38–1.50 (m, 2H), 1.69–1.81 (m, 2H), 1.91 (s, 3H) 2.13 (s, 3H), 2.51 (t, J = 7.9 Hz, 2H), 2.90 (t, J = 7.9 Hz, 2H), 3.43 (s, 3H), 3.96 (t, J = 6.6 Hz, 2H), 6.99–7.04 (m, 1H), 7.18–7.30 ppm (m, 3H), ^{13}C NMR ($CDCl_3$): δ 10.9, 12.3, 14.1, 19.4, 22.6, 29.0, 31.8, 26.0, 29.2, 30.4, 33.9, 68.2, 114.1, 116.0, 118.4, 120.7, 121.9, 127.3, 129.0, 134.1, 142.6, 159.1, 177.7, 194.1 ppm, MS (EI) m/z (%): 399 (75) M^+, 340 (100), HRMS-ESI m/z $[M + Na]^+$ calcd for $C_{24}H_{33}NO_4$: 422.2302, found: 422.2301.

4.1.7. Ethyl 4-dodecanoyl-1,3,5-trimethylpyrrole-2-carboxylate (**36**)

A solution of ethyl 1,3,5-trimethylpyrrole-2-carboxylate (**21**) [55] (1.05 g, 5.79 mmol) in CH_2Cl_2 (30 mL) was treated with dodecanoyl chloride (1.5 mL, 6.5 mmol) and cooled to 0 °C. Then anhydrous $AlCl_3$ (865 mg, 6.48 mmol) was added in portions and the mixture was stirred at 0 °C for 30 min. After addition of half-saturated brine, the reaction mixture was exhaustively extracted with diethyl ether. The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 9:1) to give **36** as a solid (1.49 g, 71%), mp 37–39 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 6.9 Hz, 3H), 1.18–1.41 (m, 19H), 1.59–1.71 (m, 2H), 2.40 (s, 3H), 2.48 (s, 3H), 2.69–2.75 (m, 2H), 3.74 (s, 3H), 4.28–4.35 ppm (m, 2H), MS (EI) m/z (%): 363 (20) M^+, 208 (100).

4.1.8. 4-Dodecanoyl-1,3,5-trimethylpyrrole-2-carbaldehyde (**37**)

A mixture of **36** (508 mg, 1.51 mmol), ethanol (20 mL) and 10% aqueous KOH (20 mL) was heated under reflux for 1 h, cooled, diluted with water, acidified with 10% aqueous HCl and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute aqueous HCl, dried (Na_2SO_4) and concentrated. The dry residue was heated at 160–170 °C for 20 min. The reaction was performed a second time. A solution of obtained intermediate (694 mg, 2.38 mmol) in toluene (4 mL) was added dropwise to a mixture of dry *N*,*N*-dimethylformamide (553 μL, 7.14 mmol) and $POCl_3$ (222 μL, 2.38 mmol) that had been stirred before at 0 °C for 30 min. After stirring at room temperature for 2 h, a solution of sodium acetate (1 g) in water (4 mL) was added and the mixture was heated under reflux for 15 min. Then the reaction mixture was cooled, diluted with water, and extracted exhaustively with diethyl ether/CH_2Cl_2 (3:1). The combined organic phases were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (CH_2Cl_2/ethyl acetate, 9:1) to yield **37** as a solid (452 mg, 47%), mp 60–62 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H), 1.16–1.44 (m, 16H), 1.62–1.75 (m, 2H), 2.47 (s, 3H), 2.52 (s, 3H), 2.68–2.76 (m, 2H), 3.87 (s, 3H), 9.78 ppm (s, 1H), MS (EI) m/z (%): 319 (28) M^+, 164 (100).

4.1.9. (E)- and (Z)-1-[5-(2-Methoxyvinyl)-1,2,4-trimethylpyrrol-3-yl]dodecan-1-one (**38**)

In a microwave tube (10 mL), a solution of **37** (423 mg, 1.38 mmol) in dry THF (1 mL) was treated with (methoxymethyl) triphenylphosphonium chloride (497 mg, 1.45 mmol) and potassium-*tert*-butylate (170 mg, 1.52 mmol). The reaction mixture was then stirred and irradiated in a microwave oven at 90 W and 80 °C for 60 min. After cooling and addition of water, the reaction mixture was exhaustively extracted with ethyl acetate. The organic phases were dried (Na_2SO_4) and concentrated. Silica gel chromatography (hexane/ethyl acetate, 9:1) gave an oily product, which was a mixture of the *E* and *Z*-isomers in a ratio of 6:4 (372 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.17–1.41 (m, 16H), 1.58–1.74 (m, 2H), 2.17 (s, 1.2H), 2.25 (s, 1.8H), 2.47 (s, 3H), 2.68–2.75 (m, 2H), 3.36–3.41 (m, 3H), 3.68–3.73 (m, 3H), 5.06 (d, J = 6.7 Hz, 0.4H, *Z*-isomer), 5.43 (d, J = 12.8 Hz, 0.6H, *E*-isomer), 6.17 (d, J = 6.7 Hz, 0.4H, *Z*-isomer), 6.52 ppm (d, J = 12.8 Hz, 0.6H, *E*-isomer), MS (EI) m/z (%): 347 (49) M^+, 192 (100).

4.1.10. 2-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)acetaldehyde (**39**)

To a solution of **38** (361 mg, 1.04 mmol) in acetonitrile (29 mL) were added NaI (183 mg, 1.22 mmol) and chlorotrimethylsilane (160 μL, 0.75 mmol). The mixture was stirred at room temperature for 20 min, diluted with 0.5 M aqueous $Na_2S_2O_3$ solution (114 mL) and extracted exhaustively with ethyl acetate. The combined organic layers were dried (Na_2SO_4) and evaporated. Chromatography on silica gel (hexane/ethyl acetate, 8:2) yielded **39** as solid (116 mg, 33%), mp 73–74 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.18–1.38 (m, 16H), 1.61–1.72 (m, 2H), 2.23 (s, 3H), 2.47 (s, 3H), 2.67–2.75 (m, 2H), 3.36 (s, 3H), 3.67 (d, J = 2.3 Hz, 2H), 9.58 ppm (t, J = 2.3 Hz, 1H), MS (EI) m/z (%): 334 (5) M^+, 304 (100).

4.1.11. (E)-Ethyl 4-(4-dodecanoyl-1,3,5-trimethylpyrrol-2-yl)but-2-enoate (**40**)

A suspension of sodium hydride (60% in mineral oil) (16 mg, 0.39 mmol) in dry THF (1 mL) was treated at 0 °C with a solution of triethyl phosphonoacetate (85 μL, 0.43 mmol) in dry THF (1 mL). The mixture was stirred at 0 °C for 30 min. Then a solution of **39** (111 mg, 0.33 mmol) in dry THF (1 mL) was added at this temperature and stirring was continued at room temperature for 1 h. After addition of water, the reaction mixture was extracted exhaustively with diethyl ether. The combined organic phases were washed with brine, dried (Na_2SO_4) and evaporated. Chromatography on silica gel (hexane/ethyl acetate, 9:1) yielded **40** as a solid (56 mg, 42%), mp 47–48 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H), 1.19–1.39 (m, 19H), 1.62–1.73 (m, 2H), 2.17 (s, 3H), 2.47 (s, 3H), 2.67–2.74 (m, 2H), 3.34 (s, 3H), 3.45 (dd, J = 5.4 Hz and 1.7 Hz, 2H), 4.17 (q, J = 7.1 Hz, 2H), 5.61 (dt, J = 15.6 Hz and 1.8 Hz, 1H), 6.97 ppm (dt, J = 15.6 Hz and 5.5 Hz, 1H), MS (EI) m/z (%): 404 (14) M^+, 220 (100).

4.1.12. Ethyl 4-(4-dodecanoyl-1,3,5-trimethylpyrrol-2-yl)butanoate (**41**)

A solution of **40** (56 mg, 0.14 mmol) in THF/ethanol (1:1) (5 mL) was treated with a small amount of palladium (10%) on charcoal and stirred under a balloon filled with H_2 at room temperature for 1 h. The reaction mixture was filtered through Celite. The residue was washed with THF and the combined filtrates were evaporated to dryness. Silica gel chromatography (hexane/ethyl acetate, 9:1) yielded **41** as an oil (45 mg, 80%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.19–1.39 (m, 19H), 1.62–1.72 (m, 2H), 1.72–1.82 (m, 2H), 2.19 (s, 3H), 2.33 (t, J = 7.1 Hz, 2H), 2.46 (s, 3H), 2.55–2.62 (m, 2H), 2.67–2.73 (m, 2H), 3.43 (s, 3H), 4.12 ppm (q, J = 7.1 Hz, 2H), MS (EI) m/z (%): 406 (14) M^+, 250 (100).

*4.1.13. 4-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)butanoic acid (**42**)*

A solution of **41** (45 mg, 0.11 mmol) in ethanol (5 mL) was treated with 10% aqueous KOH (1.7 mL). The resulting mixture was refluxed for 15 min, cooled, diluted with water, acidified with dilute HCl, and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute HCl, dried (Na_2SO_4) and evaporated to yield **42** as an oil (36 mg, 91%). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.18–1.40 (m, 16H), 1.59–1.72 (m, 2H), 1.74–1.85 (m, 2H), 2.19 (s, 3H), 2.39 (t, $J = 7.0$ Hz, 2H), 2.47 (s, 3H), 2.61 (t, $J = 7.5$ Hz, 2H), 2.67–2.75 (m, 2H), 3.42 ppm (s, 3H), ^{13}C NMR ($CDCl_3$): δ 12.3, 12.6, 14.1, 22.7, 29.3, 29.6, 31.9, 23.2, 24.5, 25.1, 30.4, 32.7, 42.2, 115.1, 120.6, 128.5, 135.9, 178.2, 199.2 ppm, MS (EI) *m*/*z* (%): 378 (8) M^+, 164 (100), HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{23}H_{39}NO_3$: 400.2822, found: 400.2825.

*4.1.14. Ethyl 3,5-dimethyl-1-phenylpyrrole-2-carboxylate (**47**)*

To a solution of ethyl 3,5-dimethylpyrrole-2-carboxylate (**46**) (899 mg, 5.37 mmol) in dry CH_2Cl_2 (5 mL) was added phenylboronic acid (1.42 g, 11.7 mmol), Cu(II)acetate (1.53 g, 8.41 mmol) and triethylamine (1.5 mL, 11 mmol). The mixture was stirred at room temperature for 2 d, filtered through Celite, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate, a) 9.5:0.5, b) 9:1) to yield **47** as a solid (274 mg, 21%), mp 41 °C. ^{1}H NMR ($CDCl_3$): δ 1.05 (t, $J = 7.1$ Hz, 3H), 1.96 (s, 3H), 2.38 (s, 3H), 4.04 (q, $J = 7.1$ Hz, 2H), 5.91 (s, 1H), 7.14–7.20 (m, 2H), 7.35–7.46 ppm (m, 3H), MS (EI) *m*/*z* (%): 243 (100) M^+, 170 (63).

*4.1.15. Ethyl 4-dodecanoyl-3,5-dimethyl-1-phenylpyrrole-2-carboxylate (**48**)*

A solution of **47** (631 mg, 2.59 mmol) in dry CH_2Cl_2 (20 mL) was treated with dodecanoyl chloride (0.69 mL, 2.93 mmol) and cooled to 0 °C. Then anhydrous $AlCl_3$ (438 mg, 3.28 mmol) was added in portions and the mixture was stirred at 0 °C for 2 h. After addition of half-saturated brine, the reaction mixture was exhaustively extracted with CH_2Cl_2/diethyl ether (3:1). The combined organic layers were washed with saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (hexane/ethyl acetate, 9.5:0.5) to give **48** as a solid (481 mg, 44%), mp 53–54 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 0.96 (t, $J = 7.1$ Hz, 3H), 1.18–1.44 (m, 16H), 1.66–1.75 (m, 2H), 2.18 (s, 3H), 2.60 (s, 3H), 2.75–2.81 (m, 2H), 4.01 (q, $J = 7.1$ Hz, 2H), 7.12–7.17 (m, 2H), 7.41–7.48 ppm (m, 3H), MS (EI) *m*/*z* (%): 426 (7) M^+, 285 (100).

*4.1.16. 4-Dodecanoyl-3,5-dimethyl-1-phenylpyrrole-2-carboxylic acid (**49**)*

A solution of **48** (430 mg, 1.01 mmol) in ethanol (5 mL) was treated with 20% aqueous KOH (2 mL). The resulting mixture was refluxed for 15 min, cooled, diluted with water, acidified with dilute HCl, and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute HCl, dried (Na_2SO_4) and evaporated to yield **49** as a solid (282 mg, 70%), mp 95–96 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.16–1.44 (m, 16H), 1.62–1.77 (m, 2H), 2.15 (s, 3H), 2.58 (s, 3H), 2.77 (t, $J = 7.4$ Hz, 2H), 7.08–7.16 (m, 2H), 7.38–7.46 ppm (m, 3H), MS (EI) *m*/*z* (%): 397 (2) M^+, 198 (100), HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{35}NO_3$: 420.2509, found: 420.2519.

*4.1.17. 1-(2,4-Dimethyl-1-phenylpyrrol-3-yl)dodecan-1-one (**50**)*

Compound **49** (258 mg, 0.68 mmol) was heated at 160–170 °C for 20 min to yield **50** as an oil (175 mg, 74%). The product was used for the further reactions without purification. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.17–1.42 (m, 16H), 1.67–1.76 (m, 2H), 2.32 (d, $J = 1.0$ Hz, 3H), 2.39 (s, 3H), 2.73–2.79 (m, 2H), 6.48–6.50 (m, 1H), 7.22–7.27 (m, 2H), 7.36–7.49 ppm (m, 3H), MS (EI) *m*/*z* (%): 354 (4) M^+, 198 (100).

*4.1.18. Ethyl 2-(4-dodecanoyl-3,5-dimethyl-1-phenylpyrrol-2-yl) acetate (**51**)*

A solution of **50** (164 mg, 0.46 mmol) in dry toluene (5 mL) was treated with a solution of ethyl diazoacetate (63 mg, 0.55 mmol) and some copper powder and heated at 115–120 °C until the evolution of nitrogen had ceased. After cooling, the mixture was directly chromatographed on silica gel (hexane/ethyl acetate, a) 9.75:0.25, b) 9.5:0.5) to yield **51** as an oil (57 mg, 28%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.16 (t, $J = 7.1$ Hz, 3H), 1.18–1.42 (m, 16H), 1.63–1.76 (m, 2H), 2.23 (s, 3H), 2.26 (s, 3H), 2.73–2.80 (m, 2H), 3.34 (s, 2H), 4.01 (q, $J = 7.1$ Hz, 2H), 7.09–7.23 (m, 2H), 7.42–7.51 ppm (m, 3H), MS (EI) *m*/*z* (%): 440 (17) M^+, 284 (100).

*4.1.19. 2-(4-Dodecanoyl-3,5-dimethyl-1-phenylpyrrol-2-yl)acetic acid (**52**)*

A mixture of **51** (50 mg, 0.11 mmol), ethanol (5 mL) and 10% aqueous KOH (2 mL) was heated under reflux for 30 min, cooled, diluted with water, acidified with dilute HCl and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute aqueous HCl, dried (Na_2SO_4) and concentrated. The product was purified by RP-HPLC (acetonitrile/H_2O/formic acid, 9:1:0.04). The eluates were concentrated under reduced pressure until most of the acetonitrile was distilled off. The remaining solvent was removed by freeze drying yielding **52** as a solid (27 mg, 64%), mp 87 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.18–1.46 (m, 16H), 1.61–1.78 (m, 2H), 2.23 (s, 3H), 2.28 (s, 3H), 2.76 (t, $J = 7.4$ Hz, 2H), 3.39 (s, 2H), 7.13–7.21 (m, 2H), 7.43–7.53 ppm (m, 3H), ^{13}C NMR ($CDCl_3$): δ 12.3, 13.7, 14.1, 22.7, 29.3, 29.6, 29.7, 31.9, 24.4, 30.2, 42.8, 117.8, 121.8, 122.2, 128.6, 129.0, 129.5, 135.9, 137.0, 175.7, 198.7 ppm, MS (EI) *m*/*z* (%): 412 (8) M^+, 226 (100), HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{26}H_{37}NO_3$: 434.2666, found: 434.2664.

*4.1.20. Methyl 3-(4-dodecanoyl-3,5-dimethyl-1-phenylpyrrol-2-yl) propionate (**53**)*

A solution of **50** (175 mg, 0.49 mmol) in dry CH_2Cl_2 (5 mL) was treated with methyl acrylate (46 μL, 0.50 mmol) and boron trifluoride diethyl etherate (63 μL). After being stirred at room temperature for 3 d, the mixture was diluted with water and extracted exhaustively with diethyl ether. The combined organic layers were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9.5:0.5) to yield **53** as an oil (68 mg, 31%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 6.8$ Hz, 3H), 1.18–1.43 (m, 16H), 1.62–1.78 (m, 2H), 2.14–2.22 (m, 5H), 2.28 (s, 3H), 2.66–2.78 (m, 4H), 3.58 (s, 3H), 7.15–7.20 (m, 2H), 7.43–7.53 ppm (m, 3H), MS (EI) *m*/*z* (%): 440 (19) M^+, 284 (100).

*4.1.21. 3-(4-Dodecanoyl-3,5-dimethyl-1-phenylpyrrol-2-yl) propionic acid (**54**)*

A mixture of **53** (53 mg, 0.12 mmol), ethanol (7.5 mL) and 10% aqueous KOH (2.5 mL) was heated under reflux for 30 min, cooled, diluted with water, acidified with dilute HCl and extracted exhaustively with diethyl ether. The combined organic phases were washed with dilute aqueous HCl, dried (Na_2SO_4), and concentrated to a few mL. By addition of petroleum ether, **54** precipitated (50 mg, 99%), mp 74–76 °C. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, $J = 6.8$ Hz, 3H), 1.18–1.43 (m, 16H), 1.61–1.76 (m, 2H), 2.15–2.24 (m, 5H), 2.26 (s, 3H), 2.64–2.78 (m, 4H), 7.14–7.20 (m, 2H), 7.43–7.53 ppm (m, 3H), ^{13}C NMR ($CDCl_3$): δ 12.1, 13.5, 14.1, 19.5, 22.7, 29.3, 29.6, 29.7, 31.9, 24.5, 33.9, 42.8, 115.6, 121.7, 128.0, 128.4, 128.9, 129.5, 135.3, 137.5, 177.5, 199.1 ppm, MS (EI) *m*/*z* (%): 426 (14) M^+, 270 (100), HRMS-ESI *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{39}NO_3$: 448.2822, found: 448.2821.

*4.1.22. Ethyl 4-dodecanoyl-5-methyl-3-phenylpyrrole-2-carboxylate (**69**)*

A solution of ethyl 5-methyl-3-phenylpyrrole-2-carboxylate [56] (**68**) (900 mg, 3.93 mmol) in toluene (15 mL) was added dropwise to a refluxing solution of *N,N*-dimethyldodecanamide (955 μL, 4.2 mmol) and $POCl_3$ (416 μL, 4.53 mmol) in toluene (20 mL). After stirring under reflux for 10 h, a solution of sodium acetate (6 g) in water (24 mL) was added and the mixture was further heated under reflux for 15 min. Then the reaction mixture was cooled, diluted with water, and extracted exhaustively with diethyl ether and CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to yield **69** as a solid (299 mg, 19%), mp 56–58 °C. ^{1}H NMR ($CDCl_3$): δ 0.80–0.91 (m, 3H), 0.91–1.44 (m, 21H), 1.96–2.09 (m, 2H), 2.55 (s, 3H), 4.09 (q, J = 7.1 Hz, 2H), 7.27–7.41 (m, 5H), 9.34 ppm (s, broad, 1H), MS (EI) *m/z* (%): 411 (11) M^+, 271 (100).

*4.1.23. 4-Dodecanoyl-5-methyl-3-phenylpyrrole-2-carboxylic acid (**70**)*

Compound **69** (457 mg, 1.11 mmol) was saponified according to the procedure described for the synthesis of **54**. Recrystallization from petroleum ether yielded **70** (292 mg, 69%), mp 130–131 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 0.91–1.45 (m, 18H), 1.93–2.03 (m, 2H), 2.52 (s, 3H), 7.27–7.44 ppm (m, 5H), MS (EI) *m/z* (%): 384 (3) M^+, 243 (100).

*4.1.24. 1-(2-Methyl-4-phenylpyrrol-3-yl)dodecan-1-one (**71**)*

Compound **70** (263 mg, 0.68 mmol) was heated at 160–170 °C for 20 min. Chromatography on silica gel (hexane/ethyl acetate, 9:1) yielded **71** as a solid (178 mg, 76%), mp 57 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 0.94–1.37 (m, 16H), 1.37–1.55 (m, 2H), 2.22–2.34 (m, 2H), 2.50 (s, 3H), 6.55 (s, 1H), 7.26–7.41 (m, 5H), 8.25 ppm (s, broad, 1H), MS (EI) *m/z* (%): 339 (21) M^+, 184 (100).

*4.1.25. 1-(1-Benzyl-2-methyl-4-phenylpyrrol-3-yl)dodecan-1-one (**72**)*

A mixture of **71** (171 mg, 0.50 mmol), benzyl bromide (94 mg, 0.55 mmol), tetrabutylammonium bromide (81 mg, 0.25 mmol), diethyl ether (5 mL), CH_2Cl_2 (2.5 mL), and 50% aqueous NaOH (2.5 mL) was refluxed for 3 h with vigorous stirring. The reaction mixture was cooled and the organic phase separated. The aqueous layer was extracted exhaustively with diethyl ether/CH_2Cl_2 (3:1), and the combined organic phases were dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to yield **72** as an oil (184 mg, 86%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 0.95–1.36 (m, 16H), 1.39–1.53 (m, 2H), 2.26–2.34 (m, 2H), 2.40 (s, 3H), 5.06 (s, 2H), 6.54 (s, 1H), 7.04–7.12 (m, 2H), 7.23–7.40 ppm (m, 8H), MS (EI) *m/z* (%): 430 (20) M^+, 289 (100).

*4.1.26. Methyl 3-(1-benzyl-4-dodecanoyl-5-methyl-3-phenylpyrrol-2-yl)propionate (**73**)*

Compound **72** (173 mg, 0.40 mmol) was reacted with methyl acrylate as described for the preparation of **53**. Chromatography on silica gel (hexane/ethyl acetate, 9:1) yielded **73** as an oil (88 mg, 42%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 0.92–1.33 (m, 16H), 1.33–1.49 (m, 2H), 2.00–2.12 (m, 2H), 2.13–2.24 (m, 2H), 2.42 (s, 3H), 2.66–2.78 (m, 2H), 3.53 (s, 3H), 5.15 (s, 2H), 6.94 (d, J = 7.2 Hz, 2H), 7.20–7.44 ppm (m, 8H), MS (ESI) *m/z* $[M + Na]^+$ 538.4.

*4.1.27. 3-(1-Benzyl-4-dodecanoyl-5-methyl-3-phenylpyrrol-2-yl) propionic acid (**74**)*

Compound **73** (87 mg, 0.17 mmol) was saponified according to the procedure described for the synthesis of **54** to yield **74** as a solid (55 mg, 65%), mp 121–123 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 6.9 Hz, 3H), 0.91–1.34 (m, 16H), 1.34–1.46 (m, 2H), 2.03–2.12 (m, 2H), 2.12–2.22 (m, 2H), 2.42 (s, 3H), 2.66–2.74 (m, 2H), 5.13 (s, 2H), 6.93 (d, J = 7.2 Hz, 2H), 7.22–7.39 ppm (m, 8H), ^{13}C NMR ($CDCl_3$): δ 11.8, 14.1, 19.6, 22.7, 29.2, 29.3, 29.4, 29.6, 31.9, 24.8, 34.3, 42.5, 46.8, 121.9, 123.1, 125.5, 127.0, 127.6, 127.8, 128.3, 129.0, 130.3, 134.5, 136.6, 136.9, 177.4, 200.6 ppm, HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{33}H_{43}NO_3$: 524.3135, found: 524.3137.

*4.1.28. 3-(1,3,5-Trimethylpyrrol-2-yl)propanenitrile (**77**)*

A mixture of 1,2,4-trimethylpyrrole (**76**) (2.0 g, 18.3 mmol), acrylnitrile (2 mL), and boron trifluoride diethyl etherate (1 mL) was stirred at room temperature for 1 h. After addition of diethyl ether, the organic phase was washed with water, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel to yield **77** as a solid (747 mg, 25%), mp 56 °C. ^{1}H NMR ($CDCl_3$): δ 2.02 (s, 3H), 2.17 (s, 3H), 2.45 (t, J = 7 Hz, 2H), 2.93 (t, J = 7 Hz, 2H), 3.41 (s, 3H), 5.69 ppm (s, 1H), MS (CI) *m/z* (%): 163 (100) $(M + 1)^+$, 122 (22).

*4.1.29. 3-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)propanenitrile (**78**)*

Compound **78** was prepared from **77** (700 mg, 5.54 mmol) in a similar manner as described for the synthesis of **36**. Chromatography on silica gel yielded **79** as a solid (507 mg, 27%), mp 75–76 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 7 Hz, 3H), 1.14–1.51 (m, 16H), 1.67 (quint, J = 7 Hz, 2H), 2.19 (s, 3H), 2.45 (s, 3H), 2.45 (t, J = 7 Hz, 2H), 2.69 (t, J = 7 Hz, 2H), 2.96 (t, J = 7 Hz, 2H), 3.41 ppm (s, 3H). MS (EI) *m/z* (%): 344 (27) M^+, 189 (100).

*4.1.30. 3-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)propanamide (**79**)*

A solution of **12** (150 mg, 0.41 mmol) and *N,N′*-carbonyldiimidazole (200 mg) in dry CH_2Cl_2 (8 mL) was stirred at room temperature for 1 h. After cooling in an ice bath, conc. NH_3 (8 mL) was added and the mixture was stirred with ice cooling for 1 h and at room temperature for 20 min. Then water and NaCl were added, and the reaction mixture was extracted with diethyl ether and with CH_2Cl_2. The combined organic layers were washed with dilute aqueous NaOH, dried (Na_2SO_4) and evaporated. Chromatography on silica gel ($CHCl_3$/THF, 3:1) yielded **79** as a solid (26 mg, 17%), mp 136–137 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.19–1.43 (m, 16H), 1.65 (quint, J = 7 Hz, 2H), 2.21 (s, 3H), 2.34 (t, J = 8 Hz, 2H), 2.46 (s, 3H), 2.70 (t, J = 7 Hz, 2H), 2.93 (t, J = 8 Hz, 2H), 3.43 (s, 3H), 5.25–5.35 ppm (m, broad, 2H). MS (EI) *m/z* (%): 362 (2) M^+, 304 (100).

*4.1.31. 3-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)propanehydrazide (**80**)*

A solution of **12** (150 mg, 0.41 mmol) and *N,N′*-carbonyldiimidazole (200 mg) in dry CH_2Cl_2 (8 mL) was stirred at room temperature for 1 h. After addition of hydrazine hydrate (0.25 mL), the mixture was stirred at room temperature for 16 h. Then water and NaCl were added and the mixture was extracted exhaustively with diethyl ether. The combined organic layers were dried (Na_2SO_4) and evaporated. The product was precipitated from THF/petroleum ether and subsequently dissolved again in diethyl ether/CH_2Cl_2 (5:1). The organic solution was washed with dilute aqueous HCl, dried (Na_2SO_4), and concentrated to a few mL. After addition of petroleum ether, **80** precipitated (96 mg, 62%), mp 96–98 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, J = 7 Hz, 3H), 1.19–1.43 (m, 16H), 1.65 (quint, J = 7 Hz, 2H), 2.19 (s, 3H), 2.23 (t, J = 8 Hz, 2H), 2.45 (s, 3H), 2.69 (t, J = 7 Hz, 2H), 2.93 (t, J = 8 Hz, 2H), 3.43 (s, 3H), 6.64 ppm (s, 1H). MS (EI) *m/z* (%): 377 (23) M^+, 304 (100).

4.1.32. 3-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)-N-(methanesulfonyl)propanamide (**81**)

A mixture of **12** (100 mg, 0.27 mmol), methanesulfonyl chloride (67 mg, 0.70 mmol), 4-dimethylaminopyridine (10 mg, 0.08 mmol), 3-(3-dimethylaminopropyl)-1-ethyl carbodiimide–hydrochloride (EDC–HCl) (48 mg), triethylamine (0.07 mL) and CH_2Cl_2 (15 mL) was stirred at room temperature overnight. After addition of CH_2Cl_2, the organic solution was washed with water, dried (Na_2SO_4) and evaporated. Chromatography on silica gel with ethyl acetate followed by reprecipitation from CH_2Cl_2/petroleum ether (1:5) yielded **81** as a solid (58 mg, 49%), mp 133 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, $J = 7$ Hz, 3H), 1.22–1.43 (m, 12H), 1.56–1.67 (m, 6H), 2.20 (s, 3H), 2.46 (s, 3H), 2.47 (t, $J = 7$ Hz, 2H), 2.69 (t, $J = 7$ Hz, 2H), 2.96 (t, $J = 7$ Hz, 2H), 3.28 (s, 3H), 3.44 ppm (s, 3H). MS (EI) *m/z* (%): 440 (10) M^+, 304 (100).

4.1.33. 3-(4-Dodecanoyl-1,3,5-trimethylpyrrol-2-yl)-N-(benzenesulfonyl)propanamide (**82**)

Compound **12** (364 mg, 1 mmol) was reacted with benzenesulfonamide according to the procedure described for the synthesis of **81**. Chromatography on silica gel (petroleum ether/ethyl acetate, 7:3) yielded **82** as a solid (99 mg, 20%), mp 56–57 °C. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, $J = 7$ Hz, 3H), 1.15–1.33 (m, 16H), 1.66 (quint, $J = 7$ Hz, 2H), 2.08 (s, 3H), 2.40 (t, $J = 7$ Hz, 2H), 2.43 (s, 3H), 2.68 (t, $J = 7$ Hz, 2H), 2.85 (t, $J = 7$ Hz, 2H), 3.32 (s, 3H), 4.90 (s, broad, 1H), 7.55–7.63 (m, 2H), 7.68 (t, $J = 8$ Hz, 1H), 8.05 ppm (d, $J = 8$ Hz, 2H). MS (EI) *m/z* (%): 502 (2) M^+, 77 (100).

4.2. Inhibition assays for mPGES-1 applying recombinant human enzyme

4.2.1. Test assay without Triton X-100

To 2 μL of a DMSO solution of a test compound or to 2 μL of DMSO in case of the controls was added at room temperature 186 μL Tris-buffer (50 mM, pH 7.2 at 20 °C), in which 2.5 mM reduced glutathione had been dissolved immediately before. After 5 min, 4 μL of an about 1:200 dilution of commercially available human microsomal prostaglandin E_2 synthase-1 solution (Cayman Chemicals, Catalog No. 10007939, Protein concentration: 46 mg/mL, activity: 5500 U/mL) in phosphate buffer (50 mM sodium phosphate, 100 mM NaCl, 20% (v/v) glycerol, pH 7.2 at 20 °C) was added and pre-incubation at room temperature was continued for 5 min. Then the enzyme reaction was started by addition of 8 μL of a PGH_2 solution in acetone (0.28 mM) and carried out for 5 min at room temperature. The reaction was terminated by addition of 100 μL of a solution of $FeCl_2$ (60 mM in 0.05 M HCl) followed by 100 μL of a solution of the internal standard PGE_1 (20 μM PGE_1 in acetonitrile containing 0.1% trifluoroacetic acid (v/v)). An aliquot of this solution (225 μL) was injected onto the HPLC system. To determine the amount of non-enzymatic production of PGE_2 from PGH_2, reference incubations were carried out in the same way applying 2 μL of DMSO, 186 μL Tris-buffer (50 mM, pH 7.2 at 20 °C) containing glutathione (2.5 mM), 4 μL of phosphate buffer (50 mM sodium phosphate, 100 mM NaCl, 20% (v/v) glycerol, pH 7.2 at 20 °C) and 8 μL of a PGH_2 solution in acetone (0.28 mM).

The online solid phase extraction (SPE) LC–UV analysis was performed at a detection wavelength of 195 nm as described previously [37] applying a LiChrospher 100-RP18 EC 5 μm (40 mm × 4 mm) trap column and a Kromasil 100-RP18 5 μm (250 mm × 3 mm) analytical column (CS-Chromatographie, Germany), which was protected by a RP18 (4 mm × 3 mm) SecurityGuard Cartridge (Phenomenex, Germany).

The peak areas of PGE_2 formed in the incubations with the enzyme in absence of test compounds averaged 50–70% of the area of the internal standard PGE_1. The ratio of enzymatic to non-enzymatic production of PGE_2 usually was about 2.5–3.

For calculation of enzyme inhibition the peak ratio of the enzyme product PGE_2 formed enzymatically and the internal standard PGE_1 obtained in presence of a test compound was compared with the mean level of this peak ratio determined in absence of test compounds (=control tests). The IC_{50} values were calculated with the aid of Probit transformation.

The reference inhibitors **2** (3-[3-(*tert*-butylthio)-1-(4-chlorobenzyl)-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid, MK-886) and **6** (2-[(4-{[(1,1′-biphenyl)-4-ylmethyl]amino}-6-chloropyrimidin-2-yl)thio]octanoic acid, Cay10589) were purchased from Cayman Chemicals.

4.2.2. Test assay with Triton X-100

The assay was carried out in the same way applying 186 μL Tris/glutathione-buffer, which contained 0.108% Triton X-100 (w/v). The final concentration of Triton X-100 was 0.10% (w/v). For the enzymatic reactions the commercial available enzyme solution was diluted only 1:100 with phosphate buffer. The peak areas of PGE_2 formed in the incubations with the enzyme in absence of test compounds averaged 40–50% of the area of the internal standard PGE_1. The ratio of enzymatic to non-enzymatic production of PGE_2 here was about 1.

Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.12.009.

References

[1] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875.
[2] T. Tanioka, Y. Nakatani, N. Semmyo, M. Murakami, I. Kudo, Molecular identification of cytosolic prostaglandin E_2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E_2 biosynthesis, J. Biol. Chem. 275 (2000) 32775–32782.
[3] P.J. Jakobsson, S. Thorén, R. Morgenstern, B. Samuelsson, Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target, Proc. Natl. Acad. Sci. USA 96 (1999) 7220–7225.
[4] K. Watanabe, K. Kurihara, T. Suzuki, Purification and characterization of membrane-bound prostaglandin E synthase from bovine heart, Biochim. Biophys. Acta 1439 (1999) 406–414.
[5] N. Tanikawa, Y. Ohmiya, H. Ohkubo, K. Hashimoto, K. Kangawa, M. Kojima, S. Ito, K. Watanabe, Identification and characterization of a novel type of membrane-associated prostaglandin E synthase, Biochem. Biophys. Res. Commun. 291 (2002) 884–889.
[6] M. Murakami, K. Nakashima, D. Kamei, S. Masuda, Y. Ishikawa, T. Ishii, Y. Ohmiya, K. Watanabe, I. Kudo, Cellular prostaglandin E_2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2, J. Biol. Chem. 278 (2003) 37937–37947.
[7] M. Murakami, H. Naraba, T. Tanioka, N. Semmyo, Y. Nakatani, F. Kojima, T. Ikeda, M. Fueki, A. Ueno, S. Oh, I. Kudo, Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E_2 synthase that acts in concert with cyclooxygenase-2, J. Biol. Chem. 275 (2000) 32783–32792.
[8] J.A. Mancini, K. Blood, J. Guay, R. Gordon, D. Claveau, C.C. Chan, D. Riendeau, Cloning, expression, and up-regulation of inducible rat prostaglandin E synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis, J. Biol. Chem. 276 (2001) 4469–4475.
[9] F. Kojima, H. Naraba, Y. Sasaki, R. Okamoto, T. Koshino, S. Kawai, Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells, J. Rheumatol. 29 (2002) 1836–1842.
[10] S. Thorén, P.J. Jakobsson, Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C_4, Eur. J. Biochem. 267 (2000) 6428–6434.
[11] D.O. Stichtenoth, S. Thorén, H. Bian, M. Peters-Golden, P.J. Jakobsson, L.J. Crofford, Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells, J. Immunol. 167 (2001) 469–474.
[12] S. Uematsu, M. Matsumoto, K. Takeda, S. Akira, Lipopolysaccharide-dependent prostaglandin E_2 production is regulated by the glutathione-dependent

prostaglandin E_2 synthase gene induced by the Toll-like receptor 4/MyD88/NF-IL6 pathway, J. Immunol. 168 (2002) 5811–5816.
[13] L. Boulet, M. Ouellet, K.P. Bateman, D. Ethier, M.D. Percival, D. Riendeau, J.A. Mancini, N. Methot, Deletion of microsomal prostaglandin E_2 (PGE_2) synthase-1 reduces inducible and basal PGE_2 production and alters the gastric prostanoid profile, J. Biol. Chem. 279 (2004) 23229–23237.
[14] D. Kamei, K. Yamakawa, Y. Takegoshi, M. Mikami-Nakanishi, Y. Nakatani, S. Oh-Ishi, H. Yasui, Y. Azuma, N. Hirasawa, K. Ohuchi, H. Kawaguchi, Y. Ishikawa, T. Ishii, S. Uematsu, S. Akira, M. Murakami, I. Kudo, Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1, J. Biol. Chem. 279 (2004) 33684–33695.
[15] C.E. Trebino, J.L. Stock, C.P. Gibbons, B.M. Naiman, T.S. Wachtmann, J.P. Umland, K. Pandher, J.M. Lapointe, S. Saha, M.L. Roach, D. Carter, N.A. Thomas, B.A. Durtschi, J.D. McNeish, J.E. Hambor, P.J. Jakobsson, T.J. Carty, J.R. Perez, L.P. Audoly, Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase, Proc. Natl. Acad. Sci. USA 100 (2003) 9044–9049.
[16] T. Mabuchi, H. Kojima, T. Abe, K. Takagi, M. Sakurai, Y. Ohmiya, S. Uematsu, S. Akira, K. Watanabe, S. Ito, Membrane-associated prostaglandin E synthase-1 is required for neuropathic pain, Neuroreport 15 (2004) 1395–1398.
[17] D. Engblom, S. Saha, L. Engström, M. Westman, L.P. Audoly, P.J. Jakobsson, A. Blomqvist, Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis, Nat. Neurosci. 6 (2003) 1137–1138.
[18] Y. Cheng, M. Wang, Y. Yu, J. Lawson, C.D. Funk, G.A. Fitzgerald, Cyclooxygenases, microsomal prostaglandin E synthase-1, and cardiovascular function, J. Clin. Invest. 116 (2006) 1391–1399.
[19] M. Wang, A.M. Zukas, Y. Hui, E. Ricciotti, E. Pure, G.A. Fitzgerald, Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis, Proc. Natl. Acad. Sci. USA 103 (2006) 14507–14512.
[20] B. Samuelsson, R. Morgenstern, P.J. Jakobsson, Membrane prostaglandin E synthase-1: a novel therapeutic target, Pharmacol. Rev. 59 (2007) 207–224.
[21] S.M. Jachak, PGE synthase inhibitors as an alternative to COX-2 inhibitors, Curr. Opin. Investig. Drugs 8 (2007) 411–415.
[22] R.W. Friesen, J.A. Mancini, Microsomal prostaglandin E_2 synthase-1 (mPGES-1): a novel anti-inflammatory therapeutic target, J. Med. Chem. 51 (2008) 4059–4067.
[23] G. Dannhardt, L. Steindl, M. Lehr, Substituted Pyrrolizine Compounds and Their Use as Pharmaceuticals EP397175 (1990).
[24] A.J. Liedtke, P.R. Keck, F. Lehmann, A. Koeberle, O. Werz, S.A. Laufer, Arylpyrrolizines as inhibitors of microsomal prostaglandin E_2 synthase-1 (mPGES-1) or as dual inhibitors of mPGES-1 and 5-lipoxygenase (5-LOX), J. Med. Chem. 52 (2009) 4968–4972.
[25] A. Koeberle, H. Zettl, C. Greiner, M. Wurglics, M. Schubert-Zsilavecz, O. Werz, Pirinixic acid derivatives as novel dual inhibitors of microsomal prostaglandin E_2 synthase-1 and 5-lipoxygenase, J. Med. Chem. 51 (2008) 8068–8076.
[26] D. Riendeau, R. Aspiotis, D. Ethier, Y. Gareau, E.L. Grimm, J. Guay, S. Guiral, H. Juteau, J.A. Mancini, N. Methot, J. Rubin, R.W. Friesen, Inhibitors of the inducible microsomal prostaglandin E_2 synthase (mPGES-1) derived from MK-886, Bioorg. Med. Chem. Lett. 15 (2005) 3352–3355.
[27] K. Olofsson, E. Suna, B. Pelcman, V. Ozola, M. Katkevics, I. Kalvins, Indoles Useful in the Treatment of Inflammation WO2005123673 (2005).
[28] A. Giroux, L. Boulet, C. Brideau, A. Chau, D. Claveau, B. Cote, D. Ethier, R. Frenette, M. Gagnon, J. Guay, S. Guiral, J. Mancini, E. Martins, F. Masse, N. Methot, D. Riendeau, J. Rubin, D. Xu, H. Yu, Y. Ducharme, R.W. Friesen, Discovery of disubstituted phenanthrene imidazoles as potent, selective and orally active mPGES-1 inhibitors, Bioorg. Med. Chem. Lett. 19 (2009) 5837–5841.
[29] T.Y. Wu, H. Juteau, Y. Ducharme, R.W. Friesen, S. Guiral, L. Dufresne, H. Poirier, M. Salem, D. Riendeau, J. Mancini, C. Brideau, Biarylimidazoles as inhibitors of microsomal prostaglandin E_2 synthase-1, Bioorg. Med. Chem. Lett. 20 (2010) 6978–6982.
[30] D. Chianelli, V. Molteni, P.A. Albaugh, H.S. Choi, J. Loren, Z. Wang, P. Mishra, Preparation of Phenylimidazole Derivatives for Use as mPGES-1 Inhibitors WO2010127152 (2010).
[31] R. Pfau, K. Arndt, H. Doods, K. Klinder, R. Kuelzer, D. Lubriks, J. Mack, B. Pelcman, H. Priepke, R. Roenn, D. Stenkamp, E. Suna, 3*H*-Imidazo[4,5-*c*]pyridine-6-Carboxamides as Anti-inflammatory Agents WO2010100249 (2010).
[32] J. Bylund, M. Ek, J. Holenz, M.H. Johansson, A. Kers, K. Naerhi, G. Nordvall, L. Oehberg, D. Sohn, J. Viklund, S. Von Berg, Preparation of Bis(sulfonylamino) Derivatives as Inhibitors of Microsomal Prostaglandin E Synthase-1 WO2009064250 (2010).
[33] J. Wang, D. Limburg, J. Carter, G. Mbalaviele, J. Gierse, M. Vazquez, Selective inducible microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitors derived from an oxicam template, Bioorg. Med. Chem. Lett. 20 (2010) 1604–1609.
[34] G. Mbalaviele, A.M. Pauley, A.F. Shaffer, B.S. Zweifel, S. Mathialagan, S.J. Mnich, O.V. Nemirovskiy, J. Carter, J.K. Gierse, J.L. Wang, M.L. Vazquez, W.M. Moore, J.L. Masferrer, Distinction of microsomal prostaglandin E synthase-1 (mPGES-1) inhibition from cyclooxygenase-2 inhibition in cells using a novel, selective mPGES-1 inhibitor, Biochem. Pharmacol. 79 (2010) 1445–1454.
[35] M. Lehr, Structure–activity relationship studies on (4-acylpyrrol-2-yl)alkanoic acids as inhibitors of the cytosolic phospholipase A_2: variation of the alkanoic acid substituent, the acyl chain and the position of the pyrrole nitrogen, Eur. J. Med. Chem. 32 (1997) 805–814.
[36] M. Lehr, Acylpyrrole-alkanoic Acids and Indole-2-alkanoic Acids Plus Their Derivatives for Use as Inhibitors of Phospholipase A_2 WO9513266 (1995).
[37] J. Fabian, Determination of prostaglandin E_2 by on-line solid-phase extraction-liquid chromatography with ultraviolet detection for microsomal prostaglandin E_2 synthase-1 inhibitor screening, J. Chromatogr. B 875 (2008) 557–561.
[38] M. Lehr, 3-(3,5-Dimethyl-4-octadecanoylpyrrol-2-yl)propionic acids as inhibitors of 85 kDa cytosolic phospholipase A_2, Arch. Pharm. Pharm. Med. Chem. 329 (1996) 483–488.
[39] M. Lehr, Structure–activity relationships of (4-acylpyrrol-2-yl)alkanoic acids as inhibitors of the cytosolic phospholipase A_2: variation of the substituents in position 1, 3, and 5, J. Med. Chem. 40 (1997) 3381–3392.
[40] D.J. Kempf, S.L. Condon, Synthesis of rigid, heterocyclic dipeptide analogues, J. Org. Chem. 55 (1990) 1390–1394.
[41] T. Purkarthofer, K. Gruber, M.H. Fechter, H. Griengl, Asymmetric biocatalytic hydrocyanation of pyrrole carboxaldehydes, Tetrahedron 61 (2005) 7661–7668.
[42] D.H. Nugteren, E. Christ-Hazelhof, Chemical and enzymic conversions of the prostaglandin endoperoxide PGH_2, Adv. Prostaglandin Thromboxane Res. 6 (1980) 129–137.
[43] F. Masse, S. Guiral, L.J. Fortin, E. Cauchon, D. Ethier, J. Guay, C. Brideau, An automated multistep high-throughput screening assay for the identification of lead inhibitors of the inducible enzyme mPGES-1, J. Biomol. Screen. 10 (2005) 599–605.
[44] M. David Percival, Continuous spectrophotometric assay amenable to 96-well format for prostaglandin E synthase activity, Anal. Biochem. 313 (2003) 307–310.
[45] A. Bruno, L. Di Francesco, I. Coletta, G. Mangano, M.A. Alisi, L. Polenzani, C. Milanese, P. Anzellotti, E. Ricciotti, M. Dovizio, A. Di Francesco, S. Tacconelli, M.L. Capone, P. Patrignani, Effects of AF3442 [*N*-(9-ethyl-9*H*-carbazol-3-yl)-2-(trifluoro)], a novel inhibitor of human microsomal prostaglandin E synthase-1, on prostanoid biosynthesis in human monocytes in vitro, Biochem. Pharmacol. 79 (2010) 974–981.
[46] B. Cote, L. Boulet, C. Brideau, D. Claveau, D. Ethier, R. Frenette, M. Gagnon, A. Giroux, J. Guay, S. Guiral, J. Mancini, E. Martins, F. Masse, N. Methot, D. Riendeau, J. Rubin, D. Xu, H. Yu, Y. Ducharme, R.W. Friesen, Substituted phenanthrene imidazoles as potent, selective, and orally active mPGES-1 inhibitors, Bioorg. Med. Chem. Lett. 17 (2007) 6816–6820.
[47] A. Koeberle, U. Siemoneit, U. Bühring, H. Northoff, S. Laufer, W. Albrecht, O. Werz, Licofelone suppresses prostaglandin E_2 formation by interference with the inducible microsomal prostaglandin E_2 synthase-1, J. Pharmacol. Exp. Ther. 326 (2008) 975–982.
[48] A. Koeberle, E.M. Haberl, A. Rossi, C. Pergola, F. Dehm, H. Northoff, R. Troschuetz, L. Sautebin, O. Werz, Discovery of benzo[g]indol-3-carboxylates as potent inhibitors of microsomal prostaglandin E_2 synthase-1, Bioorg. Med. Chem. 17 (2009) 7924–7932.
[49] M. Ouellet, J.P. Falgueyret, P.H. Ear, A. Pen, J.A. Mancini, D. Riendeau, M.D. Percival, Purification and characterization of recombinant microsomal prostaglandin E synthase-1, Protein Expr. Purif. 26 (2002) 489–495.
[50] B.K. Shoichet, Screening in a spirit haunted world, Drug Discov. Today 11 (2006) 607–615.
[51] B.Y. Feng, B.K. Shoichet, A detergent-based assay for the detection of promiscuous inhibitors, Nat. Protoc. 1 (2006) 550–553.
[52] A. Jadhav, R.S. Ferreira, C. Klumpp, B.T. Mott, C.P. Austin, J. Inglese, C.J. Thomas, D.J. Maloney, B.K. Shoichet, A. Simeonov, Quantitative analyses of aggregation, autofluorescence, and reactivity artifacts in a screen for inhibitors of a thiol protease, J. Med. Chem. 53 (2010) 37–51.
[53] Cayman Chemical Product Information Prostaglandin E synthase-1 (microsomal) (human recombinant) Catalog No. 10007939. Procedure for determination of specific activity: one unit is defined as the amount of enzyme required to produce 1 nmol of PGE_2 per minute at 25 °C in 100 mM sodium phosphate pH 7.2, containing 2.5 mM GSH, 1 mM EDTA, 5 mM Triton X-100, and 40 μM PGH_2.
[54] S.A. Kotlyar, E.I. Klimova, N.G. Lukyanenko, Crown-ether catalyzed alkylation of hydroxybenzoic acids, Zh. Org. Khim. 23 (1987) 667–668.
[55] J.A. Ganske, R.K. Pandey, M.J. Postich, K.M. Snow, K.M. Smith, Some mercuration reactions of substituted pyrroles, J. Org. Chem. 54 (1989) 4801–4807.
[56] S. Mataka, K. Takahashi, Y. Tsuda, M. Tashiro, Preparation of ethyl 3,5-disubstituted pyrrole-2-carboxylates from 1,3-diketones and ethyl glycinate hydrochloride, Synthesis 2 (1982) 157–159.