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A novel class of dual mPGES-1/5-LO inhibitors based on the α-naphthyl pirinixic acid scaffold

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ABSTRACT

Dual inhibition of microsomal prostaglandin E_2 synthase-1 (mPGES-1) and 5-lipoxygenase (5-LO) represents a promising strategy in the development of novel anti-inflammatory drugs targeting the arachidonic acid cascade. Herein, a class of α-naphthyl pirinixic acids is characterized as dual mPGES-1/5-LO inhibitors. Systematic structural variation was focused on the lipophilic backbone of the scaffold and yielded detailed structure-activity relationships (SAR) with compound **16** (IC_{50} mPGES-1 = 0.94 μM; IC_{50} 5-LO = 0.1 μM) showing the most favorable in vitro pharmacological profile.

Represented by the widely used cyclooxygenase (COX)-inhibiting non-steroidal anti-inflammatory drugs (NSAIDs), agents interfering with the arachidonic acid cascade have a long tradition in the treatment of inflammatory diseases. However, especially the long-term use of NSAIDs is under debate because of severe gastrointestinal and cardiovascular side effects.[1] Therefore, the exploration of alternative pharmacological approaches leading to safer anti-inflammatory drugs is of urgent need.

One promising approach to circumvent COX-related side effects while maintaining anti-inflammatory efficacy is the interference with the microsomal prostaglandin E_2 synthase (mPGES)-1. This enzyme catalyzes the transformation of prostaglandin (PG) H_2 to pro-inflammatory PGE_2 and is functionally coupled to COX-2 (see Fig. 1).[2] Because mPGES-1 (as well as COX-2) is mainly induced after inflammatory stimulation, its inhibition would ideally not affect the formation of house-keeping PGs. Recent studies with mPGES-1 inhibitors showed analgesic and anti-inflammatory efficacy in a variety of animal models.[3,4]

Besides PGs, leukotrienes (LTs) are the second major class of lipid mediators derived from arachidonic acid and involved in inflammatory and allergic processes (see Fig. 1). The central step of LT biosynthesis is the initial conversion of arachidonic acid to LTA_4, which is catalyzed by 5-lipoxygenase (5-LO), a non-heme iron dioxygenase. Inhibition of 5-LO has shown to cause several beneficial pharmacological effects, such as suppression of inflammation and allergy-induced bronchoconstriction.[5] However, as many 5-LO inhibitors are lacking selectivity and/or show mechanism-based side effects, zileuton is still the only compound being approved so far.[6]

Based on the fact that COX-inhibiting NSAIDs increase the production of chemotactic LTB_4, a dual approach targeting both pathways of AA metabolism might be superior, in particular with respect to reduced side effects.[7] Indeed, compounds such as licofelone (an inhibitor of mPGES-1, 5-LO and COX-1), which has reached phase III clinical trials, show high anti-inflammatory potency combined with a favorable safety profile.[1,8]

We previously identified novel dual inhibitors of mPGES-1 and 5-LO based on the core structure of pirinixic acid.[9,10] Herein, we present a novel class of α-naphthyl-substituted pirinixic acid derivatives as potent dual inhibitors of mPGES-1 and 5-LO. We focused on the structure–activity relationships (SAR) of the lipophilic backbone of the lead structure (see Fig. 2) and explored a broad variety of aliphatic and especially aromatic residues.

Synthesis of compounds **3**–**24** was performed in a four step reaction (Scheme 1) modified from d'Atri et al. and published previously.[11,12] In brief, commercially available α-bromonaphthyl ethyl acetate was reacted with thiobarbituric acid in DMF/triethylamine (i) and the resulting thioether derivative was chlorinated with $POCl_3$ (ii). The obtained 4,6-dichloro-substituted pyrimidine was refluxed with amine building blocks in EtOH/triethylamine

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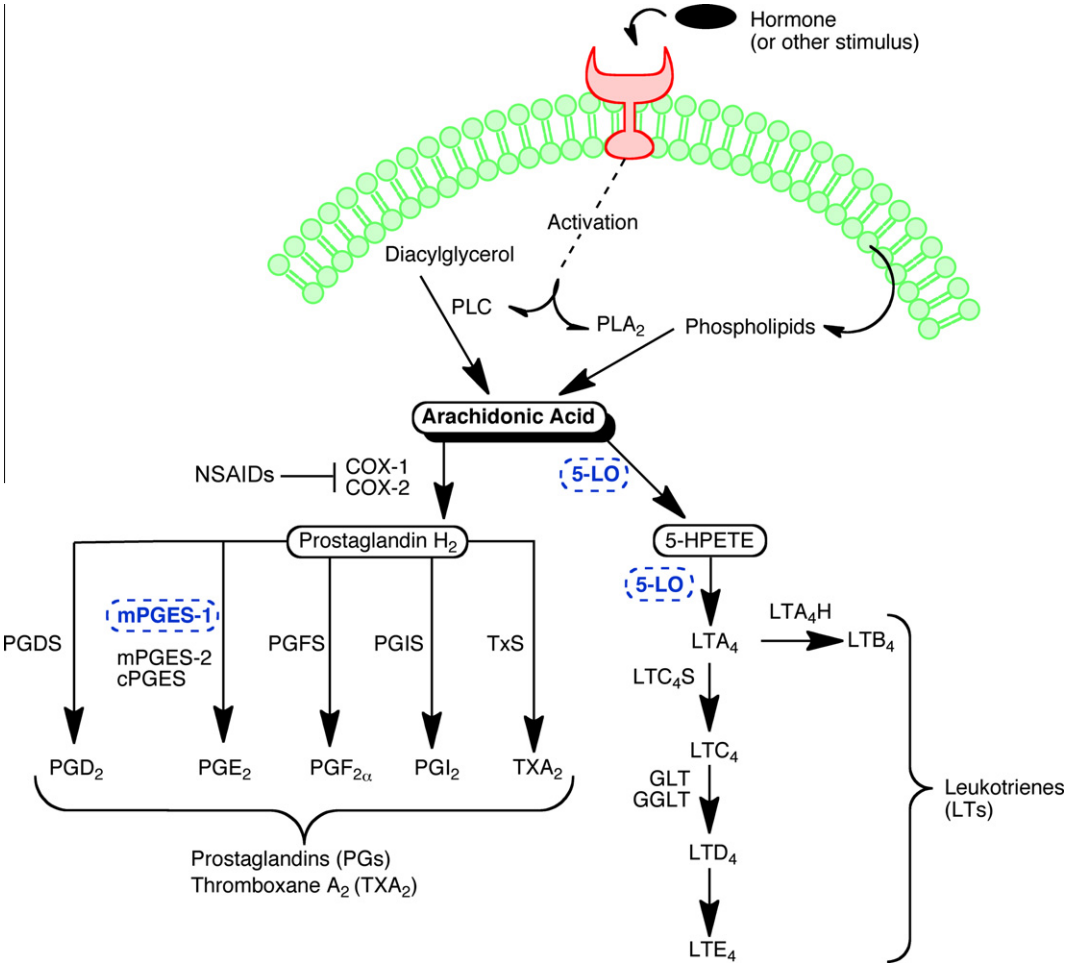


Figure 1. Schematic illustration of the arachidonic acid cascade. Targeted enzymes (mPGES-1 and 5-LO) are highlighted.

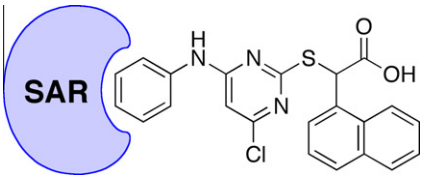
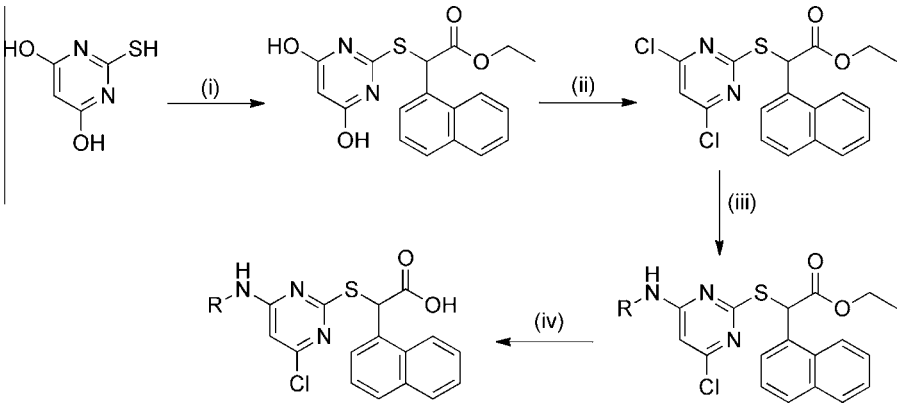


Figure 2. General structure of the presented series of α-naphthyl pirinixic acids.

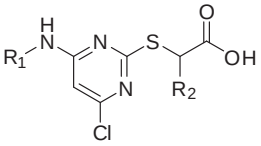
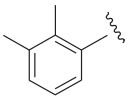
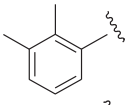
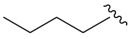
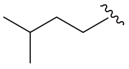
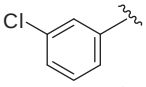
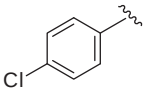
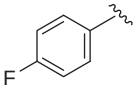
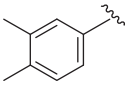
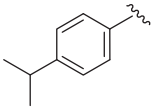
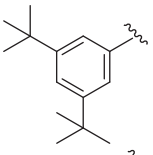
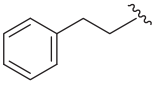
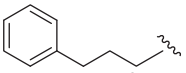
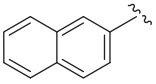
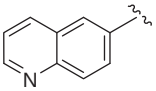
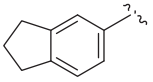
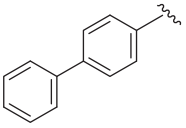
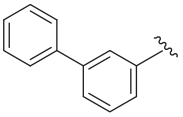
resulting in a nucleophilic substitution of one chlorine (iii). Finally, ester hydrolysis with KOH in EtOH (iv) gave the desired carboxylic acids.

Inhibition of mPGES-1 activity (transformation of PGH_2 to PGE_2) was assessed in a cell-free assay using the mitochondrial fraction of IL-1β-stimulated A549 lung epithelial adenocarcinoma cells (that overexpress mPGES-1) and 20 μM PGH_2 as substrate.[8] Unfortunately, a cell-based test system that allows selective analysis of



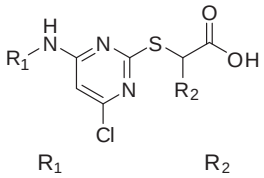
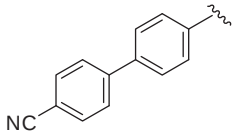
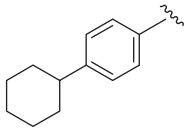
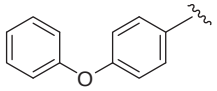
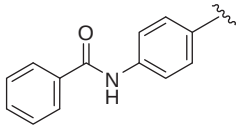
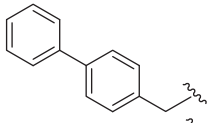
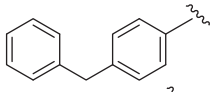
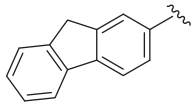
Scheme 1. Reagents and conditions: (i) α-bromonaphtyl ethyl acetate, triethylamine, DMF, rt–80 °C, 24 h; (ii) $POCl_3$ *N*,*N*-diethylaniline, reflux, 3.5 h; (iii) R-NH_2, triethylamine, ethanol, reflux, 4–96 h; (iv) KOH, ethanol, rt–80 °C, 1–24 h.

Table 1
Inhibition of mPGES-1 and 5-LO by α -naphthyl pirinixic acid derivatives

Compound			mPGES-1 IC_{50} [μM] or r.a. @ 10 μM (%)	5-LO IC_{50} [μM] or r.a. @ 10 μM (%)	
				PMNL	Purified 5-LO
	R_1	R_2			
1		H	Inactive	Inactive	Inactive
2		1-Naphthyl	5.1	5.0	n.d.
3		1-Naphthyl	90.2% (±5.8)	2.4	70.6% (±15.2)
4		1-Naphthyl	65.3% (±3.0)	2.5	54.8% (±8.2)
5		1-Naphthyl	45.5% (±11.7)	0.7	5.0
6		1-Naphthyl	5.1	0.8	8.9
7		1-Naphthyl	49.9% (±4.5)	3.1	67.3% (±2.9)
8		1-Naphthyl	5.7	0.7	8.5
9		1-Naphthyl	35.8% (±8.8)	0.26	5.8
10		1-Naphthyl	0.9	0.26	1.9
11		1-Naphthyl	42.3% (±7.8)	2.5	44.2% (±12.3)
12		1-Naphthyl	6.1	1.8	68.9% (±3.5)
13		1-Naphthyl	1.9	0.5	2.9
14		1-Naphthyl	49.1% (±11.0)	4.4	19.0% (±2.5)
15		1-Naphthyl	36.7% (±4.4)	0.5	4.2
16		1-Naphthyl	0.94	0.1	2.3
17		1-Naphthyl	1.9	0.5	5.0

(*continued on next page*)

Table 1 (*continued*)

Compound			mPGES-1 IC_{50} [μM] or r.a. @ 10 μM (%)	5-LO IC_{50} [μM] or r.a. @ 10 μM (%)	
				PMNL	Purified 5-LO
	R_1	R_2			
18		1-Naphthyl	1.6	0.17	2.3
19		1-Naphthyl	1.45	0.4	2.0
20		1-Naphthyl	0.94	0.4	3.1
21		1-Naphthyl	38.8% (±1.6)	0.6	5.2
22		1-Naphthyl	0.88	0.7	3.0
23		1-Naphthyl	1.5	0.3	2.2
24		1-Naphthyl	1.55	0.24	1.6

IC_{50} values were calculated based on the mean values of at least three determinations. Reference compounds were MK-886 for inhibition of mPGES-1 and BWA4C for inhibition of 5-LO (both 3 μM); r.a.: remaining activity.

interference with endogenous mPGES-1 activity in the cell (i.e., the transformation of PGH_2 to PGE_2) is not available. The inhibition of 5-LO product formation was analyzed in a cell-based assay using polymorphonuclear leukocytes (PMNL) as well as in a cell-free assay using purified human recombinant 5-LO enzyme.[10,13] The latter assay was chosen because a given test compound may suppress 5-LO product synthesis in intact cells without inhibiting 5-LO directly, for example by interference with co-factors regulating 5-LO in the cell or with other enzymes involved in LT biosynthesis (e.g., 5-lipoxygenase activating protein, LTA_4 hydrolase, LTC_4 synthase). On the other hand many compounds inhibit 5-LO in cell-free assays but fail in intact cells for several reasons.[6] Therefore, we performed the cell-based assay and the cell-free assay side by side.

Pirinixic acid itself (compound **1**) is inactive on both mPGES-1 and 5-LO.[9] As we have shown previously, the introduction of a naphthyl residue in α-position of the carboxylic acid leads to a well-balanced dual mPGES-1/5-LO inhibitor (**2**; IC_{50} mPGES-1 = 5.1 μM, IC_{50} 5-LO = 5.0 μM in PMNL).[10] Based on the promising in vitro pharmacology of compound **2**, we replaced the 2,3-dimethylphenyl residue by a broad variety of aliphatic and aromatic substructures (see Table 1). Introduction of aliphatic *n*-butyl (**3**) and isobutyl (**4**) residues caused a complete loss of activity on mPGES-1. Among the phenyl-substituted compounds **5**–**10**, the 3,5-di-*tert*-butylphenyl-subsituted **10** showed clearly the most favorable pharmacological profile with nanomolar activity on mPGES-1 (IC_{50} = 0.9 μM) and 5-LO (in PMNL IC_{50} = 0.26 μM). In contrast, introduction of alkyl spacers between the central pyrimidine and an unsubstituted phenyl residue as in phenethyl-substituted **11** and phenpropyl-substituted **12** led to diminished activities on both enzymes. Next, we examined bicyclic substituents such as 2-naphthyl (**13**), 6-quinolinyl (**14**) and 5-indanyl (**15**). The 2-naphthyl-subsituted **13** was a potent dual mPGES-1/5-LO inhibitor, whereas the 5-indanyl substituted compound (**15**) displayed some selectivity for 5-LO. The introduction of an additional hydrogen-bond acceptor by using 6-quinolinyl (**14**) instead of 2-naphthyl clearly diminished activity on both mPGES-1 and 5-LO. The most potent dual mPGES-1 and 5-LO inhibitors were obtained among the series substituted with biphenyl (and analogue) residues (**16**–**24**). In regard to 5-LO inhibition, SARs for these compounds are very flat with all IC_{50} values (PMNL) in the triple digit nanomolar range. Nanomolar inhibition of mPGES-1 was achieved by introduction of 4-biphenyl (**16**; IC_{50} = 0.94 μM), 4-phenoxyphenyl (**20**; IC_{50} = 0.94 μM) and 4-phenylbenzyl (**22**; IC_{50} = 0.88 μM) residues. Interestingly, connection of the two phenyl residues by an amide bond (**21**) clearly diminished mPGES-1 inhibition. Regarding all presented derivatives, 4-phenylbenzyl-substituted compound **16** showed the most favorable pharmacological profile with mPGES-1 IC_{50} = 0.94 μM and 5-LO IC_{50} = 0.1 μM (in PMNL).

In summary, we were able to optimize the initial α-naphthyl-substituted pirinixic acid **2** towards potent, nanomolar dual mPGES-1/5-LO inhibitors by introducing larger aromatic substituents to the lipophilic backbone of the lead structure.

Depending on the residues, we obtained compounds covering the range of a slight preference for the inhibition of cellular leukotriene biosynthesis to equipotent dual mPGES-1/5-LO inhibitors. SAR studies revealed that lipophilic aromatic substructures are generally well tolerated whereas the introduction of polar atoms or groups was detrimental for the activity on both enzymes. With their high potency and interesting in vitro pharmacological profile, α-naphthyl pirinixic acid derivatives are highly attractive candidates for further exploration in pharmacological assays and might be an appealing alternative to the established NSAIDs circumventing COX-related side effects.

Acknowledgements

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