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Identification of pirinixic acid derivatives bearing a 2-aminothiazole moiety combines dual PPARα/γ activation and dual 5-LO/mPGES-1 inhibition

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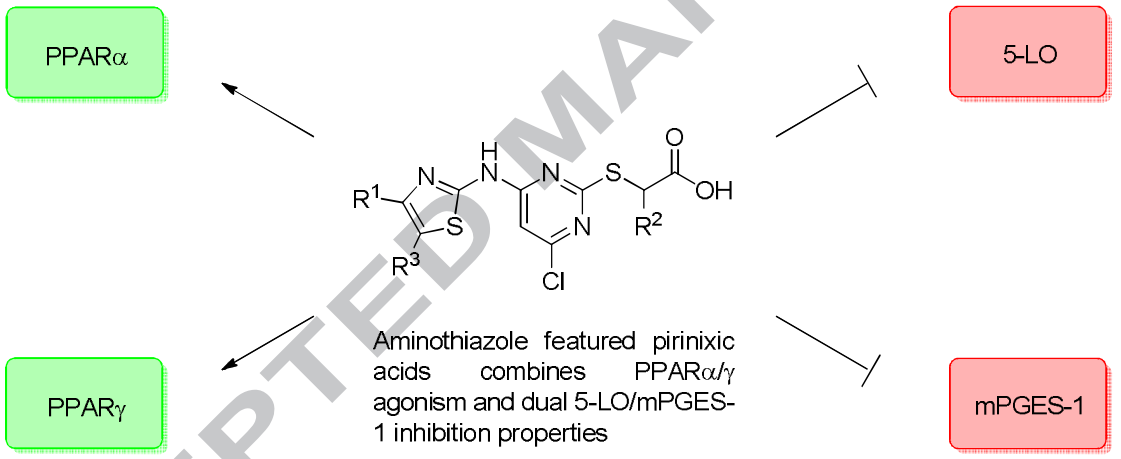
Thomas Hanke[a,†], Christina Lamers[a,†], Roberto Carrasco Gomez[a,b], Gisbert Schneider[b], Oliver Werz[c] and Manfred Schubert-Zsilavecz[a*]

[a] *Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany*
[b] *ETH Zürich, Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland*
[c] *Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, D-07743 Jena, Germany*



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Identification of pirinixic acid derivatives bearing a 2-aminothiazole moiety combines dual PPARα/γ activation and dual 5-LO/mPGES-1 inhibition

Thomas Hanke[a,†], Christina Lamers[a,†], Roberto Carrasco Gomez[a,b], Gisbert Schneider[b], Oliver Werz[c] and Manfred Schubert-Zsilavecz[a*]

[a] Institute of Pharmaceutical Chemistry, Goethe-University Frankfurt, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany
[b] ETH Zürich, Department of Chemistry and Applied Biosciences, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland
[c] Chair of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, Philosophenweg 14, D-07743 Jena, Germany

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ABSTRACT

The concept of dual PPARα/γ activation was originally proposed as a new approach for the treatment of the metabolic syndrome. However, recent results indicated that PPARα as well as PPARγ activation might also be beneficial in the treatment of inflammatory diseases and cancer. We have recently identified aminothiazole-featured pirinixic acids as dual 5-lipoxygenase (5-LO) and microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitors. Here we present the structure-activity relationship of these aminothiazole-featured pirinixic acids as dual PPARα/γ agonists and discuss their advantages with their potential as dual 5-LO/mPGES-1 inhibitors in inflammatory and cancer diseases. Various pirinixic acid derivatives had already been identified as dual PPARα/γ agonists. However, within this series of aminothiazole-featured pirinixic acids we were able to identify the most potent selective PPARγ agonistic pirinixic acid derivative (compound **13**, (2-[(4-chloro-6-{[4-(naphthalen-2-yl)-1,3-thiazol-2-yl]amino}pyrimidin-2-yl)sulfanyl]octanoic acid)). Therefore, docking of **13** on PPARγ was performed to determine the potential binding mode.

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of the nuclear receptors. They act as ligand-activated transcription factors and regulate various biological processes. Three distinct forms have been identified PPARα (NR1C1), PPARβ/δ (NR1C2) and PPARγ (NR1C3) and each subtype differs in tissue distribution and expression pattern.[1] Several natural and synthetic ligands have been discovered for each subtype. Selective agonists of PPARα, the drug class of fibrates, are used for the treatment of dyslipidemia, and selective PPARγ agonists are used for treatment of type 2 diabetes mellitus. Much effort has been done in the research and development of dual PPARα/γ activators as a new approach for the treatment of the metabolic syndrome (MS). However many of these so-called glitazars (dual PPARα/γ activators) failed in large clinical trials, mainly due to undesired side effects and up to now just one glitazar (Saroglitazar, Lipaglyn™, s. figure 1) was able to enter the market and is approved in India for the therapy of patients suffering from diabetes and dyslipidemia.[2]

Notwithstanding the above, in the last decade much effort has been done to elucidate the complex interaction of the lipid signaling network and the PPARs.[1, 3] Various eicosanoids have been identified as natural PPAR ligands, like 15-keto-PGE_2 or 15d-PGJ_2 as ligands for PPARγ or LTB_4 as ligand for PPARα.[24, 25, 26] Their physiological action, triggered through PPAR activation, is mainly associated with anti-inflammatory effects, which renders PPAR an attractive therapeutic target in inflammation-related diseases.[4, 5] LTB_4 was the first eicosanoid which has been identified to control inflammation via the PPARα pathway.[6] PPARα activation reduces secretion of LTB_4, which demonstrates that LTB_4 has besides its pro-inflammatory action also anti-inflammatory effects mediated through PPARα.[7] Mendez and LaPointe demonstrated that PPARγ activation, mediated by 15d-PGJ_2 or troglitazone, leads to a complete inhibition of IL-1β-mediated induction of microsomal prostaglandin E_2 synthase-1 (mPGES-1).[8]

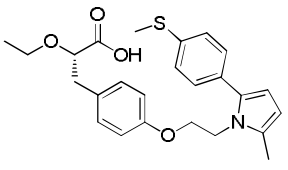


Figure 1: Chemical structure of Saroglitazar (Lipaglyn™)

* Corresponding author. Tel.: +49-69-798 29339; fax: +49-69-798 29332; e-mail: schubert-zsilavecz@pharmchem.uni-frankfurt.de

† Both authors contributed equally to this work.

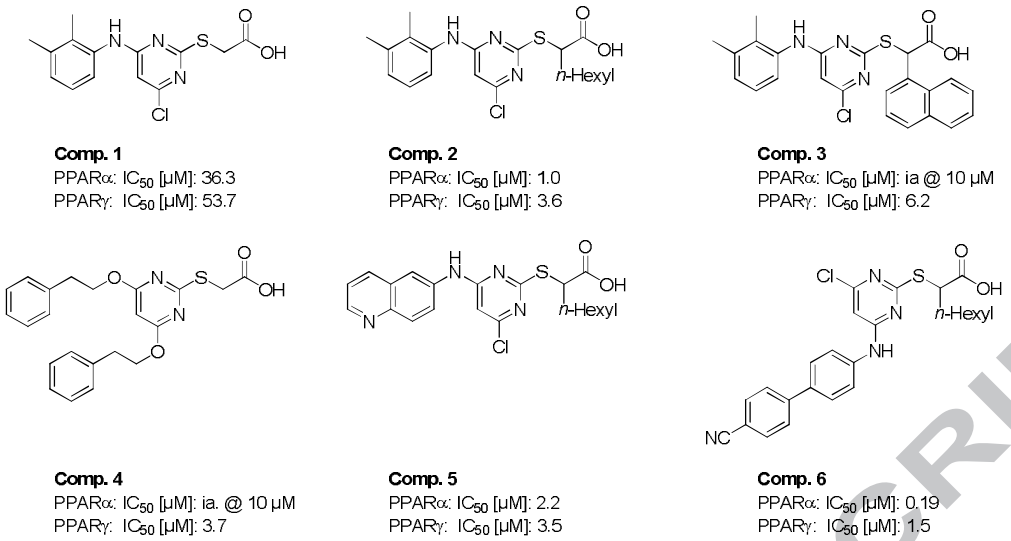
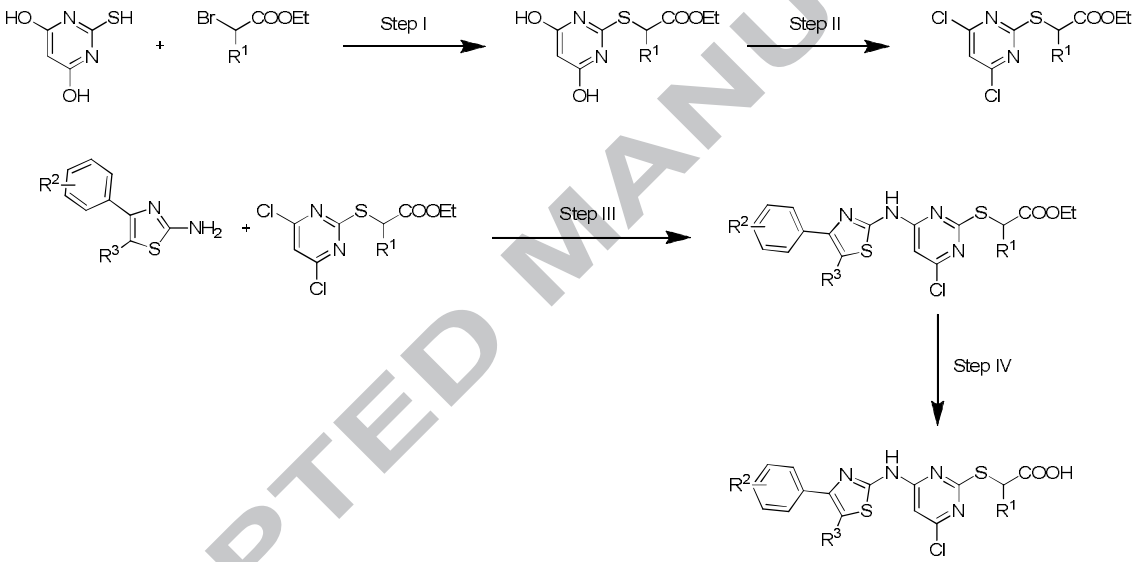


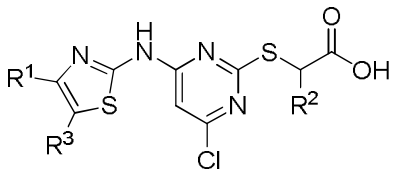
Figure 2: Various pirinixic acid derivatives with PPARα and PPARγ activity.



Scheme 1: Synthesis of pirinixic acid derivatives; Reagents and Conditions: (Step I) 2-Bromo-(R^1)-ethyl acetate (1.2 equiv), thiobarbituric acid (1 equiv), TEA (1.5 equiv), DMF, 90 °C, 3 h, 21–79%; (Step II) $POCl_3$ (18 equiv), *N*,*N*-diethylaniline (1 equiv), 90°C, 6 h, 86–94%; (Step III) $Pd_2(dba)_3$ (0.02 equiv), Xantphos (0.06 equiv), Na_2CO_3 (1.4 equiv), toluene/water, 90 °C, 18 h, 19–66%; (Step IV) LiOH*H_2O (5 equiv), THF/water, 45 °C, 24–48 h, 7–89%.

Dual inhibition of mPGES-1 and 5-lipoxygenase (5-LO) is considered as a new approach for the treatment of cancer, besides its anti-inflammatory action.[9] In addition Avis et al. have shown that exposure of breast cancer cells to a 5-LO inhibitor up-regulated both PPARs expression (PPARα and γ), and exposure of these cells to PPAR agonists, especially PPARγ agonists, led to potent growth inhibition of respective cancer cells.[10] The positive effects of PPARγ activation in lung cancer have been described before.[27, 28] Moreover, the combination of a PPARγ agonist and a 5-LO inhibitor have superadditive effects on growth inhibition and induction of apoptosis in lung cancer cell lines, which is superior over a 5-LO inhibitor or PPARγ agonist alone.[11] These results encourage the research for compounds which are able to interfere within the eicosanoid pathway as well as with PPARs.

Our lead compound pirinixic acid (compound **1**) was first synthesized by Wyeth as anti-hypercholesterolemic agent in 1974.[12] Several attempts have been made in our working group to optimize this lead structure (s. figure 2). Introduction of bulky lipophilic residues in α-position to the carboxylic acid such as an alkyl chain (YS121, compound **2**) led to dual PPARα/γ activators or in case of a naphthyl residue to selective PPARγ activators (compound **3**).[13, 14] Further optimization was done by focusing on the lipophilic backbone and introduction of diphenethoxy-residues (compound **4**),[15] or replacement of the xylidine-moiety by a quinolone (compound **5**)[16] or by a biphenyl-moiety (compound **6**),[17] respectively. Recently we have identified a new class of aminothiazole-featured pirinixic acid derivatives as dual 5-LO/mPGES-1 inhibitors, which exerts anti-inflammatory properties *in vitro* and *in vivo*.[18] Within this work we aimed to reveal the structure-activity relationship of these aminothiazole-



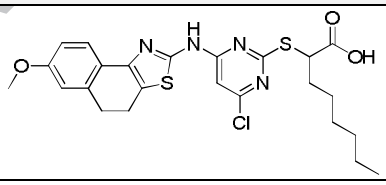
Compound				5-LO IC_{50} [µM][a]		mPGES-1 IC_{50} [µM][a]	PPAR EC_{50} [µM] ± SEM (rel. activation compared to control means ± SEM)		
				cell-based	cell-free		α	β	γ
α-substituted pirinixic acid derivates									
	R^1	R^2	R^3						
7	4-chlorophenyl	*n*-hexyl	-H	0.9±0.2	0.8±0.3	0.7±0.1	6.6±0.9 (57±9%)	ia @10	6.4±0.2 (90±3%)
8	4-chlorophenyl	*n*-butyl	-H	0.9±0.1	3.8±1.0	1.2±0.2	6.5±0.3 (57±2%)	ia @10	7.6±1.1 (136±35%)
9	4-chlorophenyl	*n*-ethyl	-H	3.6±0.8	6.6±1.4	1.4±0.3	3.6±0.4 (51±7%)	ia @10	3.9±0.04 (95±2%)
10	4-chlorophenyl	-H	-H	>10	>10	2.3±0.2	r.a. @10 24±7%	ia @10	r.a. @10 63±13%
4-substituted 2-aminothiazoles									
11	phenyl	*n*-hexyl	-H	0.6±0.03	2.0±0.04	0.8±0.1	r.a. @6 55.36± 1.87%	ia @10	5.7±2.0 (108±24%)
12	4-methylphenyl	*n*-hexyl	-H	0.2±0.04	3.0±0.7	0.7±0.2	8.2±0.07 (125±3%)	ia @10	7.2±1.6 (139±48%)
13	2-naphthyl	*n*-hexyl	-H	0.2±0.1	0.3±0.1	0.4±0.1	r.a. @6 37±8%	ia @10	1.3±0.1 (78±3%)
14	3,4-difluorophenyl	*n*-hexyl	-H	1.5±0.04	2.3±0.7	1.6±0.2	5.7±0.04 (70±1%)	ia @10	2.9±0.7 (94±19%)
15	2,4-difluorophenyl	*n*-hexyl	-H	1.5±0.1	2.5±0.8	1.8±0.1	r.a. @10 18±8%	ia @10	3.6±0.6 (145±31%)
16	4-nitrophenyl	*n*-hexyl	-H	1.4±0.1	1.8±0.4	5.0±1.5	2.9±0.1 (89.4%)	ia @10	3.4±0.7 (130±23%)
17	5,6,7,8-tetrahydro-2-naphthyl	*n*-hexyl	-H	0.4±0.1	2.3±0.8	0.4±0.1	r.a. @3 52.94± 7.6%	ia @3	4.1±0.4 (107±13%)
18	4-benzoic acid	*n*-hexyl	-H	>10	>10	>10	ia @10	ia @10	ia @10
4,5-disubstituted 2-aminothiazoles									
19	phenyl	*n*-hexyl	$-CH_3$	0.6±0.02	1.9±0.2	0.7±0.2	r.a. @6 23±5%	ia @10	2.3±0.1 (99±3%)
20	4-bromophenyl	*n*-hexyl	$-CH_3$	0.2±0.02	1.6±0.1	1.3±0.1	3.9±0.3 (102±14%)	ia @3	3.8±0.2 (143.5± 10.3%)
cyclized 2-aminothiazoles									
21				0.2±0.03	1.9±0.1	1.9±0.1	5.4±0.5 (229.4± 23.9%)	ia @3	3.7±0.1 (86±5.3%)

Table 1: IC_{50} values of aminothiazole featured pirinixic acid derivatives regarding 5-LO (cell-based and cell-free) and mPGES-1 (recently published in[18]) and EC_{50} values regarding PPARα/β/γ; [a]Data are expressed as means ± SEM of single determinations obtained in at least three independent experiments; ia: inactive at given concentration; r.a.: remaining activity at given concentration.

featured pirinixic acids on PPARα and PPARγ. We were able to identify one derivative (compound **13**) which was slightly superior as PPARγ agonist compared to the previous reported compound **6.** In contrast, **13** shows no PPARα activation which motivated us to prepare a docking pose of compound **13** on PPARγ to predict the possible binding mode.

The syntheses of the presented compounds (**7**–**17** and **19**–**21**) have been described previously.[18] Compounds **7**–**17** and **19**–**21** were synthesized in a four step reaction (s. Scheme 1). For compound **18** the corresponding 2-aminothiazole derivative was prepared according to Scheme 2.

The final compounds were tested in a PPAR transactivation assay as described previously.[17] Parental compound of this series of aminothiazole-featured pirinixic acid derivatives is compound **7** with well-balanced moderate activity on PPARα and PPARγ (s. Table 1). First investigations focused on the α-position by shortening the *n*-alkyl chain. As expected shortening to an *n*-butyl residue (compound **8**) was slightly less active regarding PPARγ and the unsubstituted derivative (compound **10**) was dramatically less active at least for PPARα. These results are in accordance to our previously data on the analysis of the variation of the α-position.[13] However, an interesting feature is, that the *n*-ethyl derivative (compound **9**) was more potent for PPARα and PPARγ than parental compound **7**. In a second step we investigated the influence of the *p*-chlorophenyl residue of the aminothiazole moiety. Diminishing compound **7** to a phenyl residue (compound **11)** slightly increases the activity for PPARγ, whereas the 4-tolyl derivative (compound **12**) was again less potent on both receptors. Increasing the lipophilic backbone by replacement of the *p*-chlorophenyl moiety with a 2-naphthyl moiety enhances the activity on PPARγ about a factor of five, whereas for PPARα compound **13** was less active than parental compound **7**. Fluorinated derivatives (compound **14** and **15**) enhance the activity mainly for PPARγ but did not reach the potency of the 2-naphthyl moiety. An interesting feature is migration of one fluorine from position 3 to 2 (compound **15**) that completely diminished the activity regarding PPARα. Introducing a nitro group in *p*-position (compound **16**) enhanced the activity on both receptors round about a factor 2. Exchange of the most potent PPARγ moiety, the 2-naphthyl residue (in compound **13**) by a 5,6,7,8-tetrahydro-2-naphthyl moiety (compound **17**) was again more potent than parental compound **7** but did not reach the potency of **13** regarding PPARγ. To evaluate the concept of fatty acids and fatty acid analogs as ligands for PPAR we introduced a second carboxylic acid moiety in compound **18** yielding dicarboxylic acids. And indeed, this dicarboxylic acid (compound **18**) totally lost activity for PPARα as well as for PPARγ which is in accordance with the model of PPAR agonists presented previously.[17] In a last step we investigated the substitution pattern at the aminothiazole by introducing a methyl-group on position 5 or by a cyclized aminothiazole moiety. The introduction of the methyl group on position 5 (compound **19**) enhanced the activity on PPARγ in comparison to compound **11**, whereas the activity on PPARα was slightly impaired. Enlargement of the lipophilic backbone by introducing a *p*-bromo-substituent (compound **20**), restored the activity on PPARα and was just slightly less active on PPARγ in comparison to **19**. Further enlargement of the lipophilic backbone by introducing the 7-methoxy-4,5-dihydronaphtho[1,2-*d*]thiazole moiety (compound **21**) was also well tolerated by both receptors. The rigidity of the latter moiety implied that less flexible compounds are also accepted by both receptors, which is in accordance with the result of the quite large binding pocket of PPARs (>1300 Å^3).[20, 21] An interesting feature of the SAR is the fact that all the *p*-chlorophenyl compounds (**7**, **8** and **9**) act as partial agonists on PPARα with a maximal activation of about 50% in comparison to the PPARα ligand GW7647. Likewise, we were able to identify a superagonist on PPARα (compound **21**), which leads to a maximal activation of about 230% (compared to GW7647).

The structure-activity relationship of the presented compounds regarding 5-LO and mPGES-1 have been described previously,[18] except of compound **18**, which is inactive on 5-LO as well as on mPGES-1 up to 10 μM. In summary it can be concluded that most of the compounds have a lower IC_{50} regarding 5-LO and mPGES-1 than the corresponding EC_{50} for PPARα and PPARγ. Starting from parental compound **7** the difference between the IC_{50} (5-LO, mPGES-1) and EC_{50} (PPARs) values is about one magnitude, which was an encouraging result for us to obtain some selectivity between these targets. However, we were not able to completely diminish the PPAR activity of the presented compounds. In contrast, the most potent dual 5-LO/mPGES-1 inhibitor (compound **13**) is also the most potent PPARγ agonist, though less active regarding PPARα. Nevertheless, as mentioned above these dual inhibitory properties in case of pro-inflammatory mediators (PGE_2 and LTs) and activation of anti-inflammatory pathways through PPAR agonism could enhance the anti-inflammatory efficiency of the presented compounds. Together, the compounds can be categorized into at least four different groups.

I. Selective mPGES-1 inhibitors

The most selective compound is the α-unsubstituted derivative compound **10**, which has just minor activity on PPARγ at 10 μM, and was not able to inhibit the LT production, nor is PPARα agonism conferred.

II. Dual 5-LO/mPGES-1 inhibitors

The most selective compound featuring dual 5-LO/mPGES-1 inhibition is compound **12**, which is about 36- to 41-fold less active as PPAR agonist compared to LT inhibition at the cellular level, and about 10-12-fold less potent PPAR agonist versus mPGES-1 inhibition.

III. Dual 5-LO/mPGES-1 inhibitors and PPARγ agonists

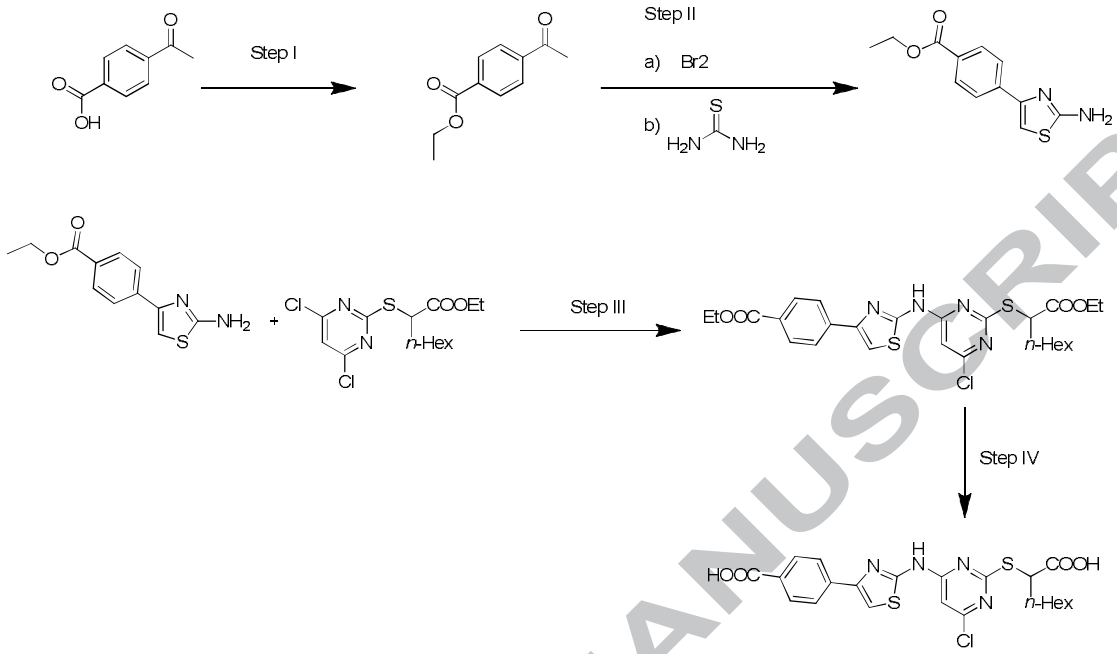
Compounds **13**, **15** and **19** possess most selectivity between PPARα and PPARγ, besides their dual 5-LO/mPGES-1 inhibition, while **13** is the most potent compound on all three targets.

IV. Dual 5-LO/mPGES-1 inhibitors and PPARα/γ agonist

Compounds **9** and **16** have IC_{50} and EC_{50} values in a similar range on all four targets. However, **9** seems to have more druglikeness features, due to a smaller molecular weight (441 vs 508) and the lack of the metabolically prone *n*-hexyl residue.

Because compound **13** was even slightly more potent for PPARγ than the previously reported compound **6** and additionally, it was selective for PPARγ with no PPARα activity in contrast to **6**, we were encouraged to predict the possible binding mode. We used the recently published crystal structure of PPARγ (PDB ID: 3VSO)[22] for molecular docking simulations, because this crystal structure has a high resolution of 2.00 Å and a ligand with a similar motif, a α-substituted carboxylic acid derivative which is closely related to our α-substituted pirinixic acid derivatives. As a result of the synthesis of the compounds (s. Scheme 1, Step I) all presented compounds are in racemic form. In our previous work[17] we have shown that the absolute configuration in α-position has a strong impact on the activity of the compounds on PPARα. However, the impact of the absolute configuration on PPARγ was less distinctive. Thus, for our most

potent compound (compound **13**), which has negligible activity on PPARα, we have compared both enantiomers (s. Figure 1 SI) and it seems that the (*S*)-enantiomer would be better tolerated by PPARγ. The possible binding mode of **13** is in accordance with our previous results[14], as well as with the control compound MEKT21 [(2*R*)-2-benzyl-3-[4-propoxy-3-({[4-(pyrimidin- 2-yl)benzoyl]amino}methyl)phenyl]propanoic acid] in 3VSO[22]. The carboxylic acid head group interacts with two tyrosines (Tyr327 and Tyr473) as well as with



Scheme 2: Synthesis of dicarboxylic pirinixic acid derivative (comp. **18**); Reagents and Conditions: (Step I) Acetyl benzoic acid (1 equiv), EtOH (21 equiv), H_2SO_4 (0.2 equiv), reflux, 18 h; (Step II) a) Ethyl 4-acetylbenzoate (1 equiv), Br_2 (1.05 equiv), $CHCl_3$, RT, 3 h; b) α-bromo-ketone (1 equiv), thiourea (1.5 equiv), MeOH, 3 h, RT; (Step III) $Pd_2(dba)_3$ (0.02 equiv), Xantphos (0.06 equiv), Na_2CO_3 (1.4 equiv), toluene/water, 90 °C, 18 h; (Step IV) LiOH*H_2O (5 equiv), THF/water, 45 °C, 24 h.

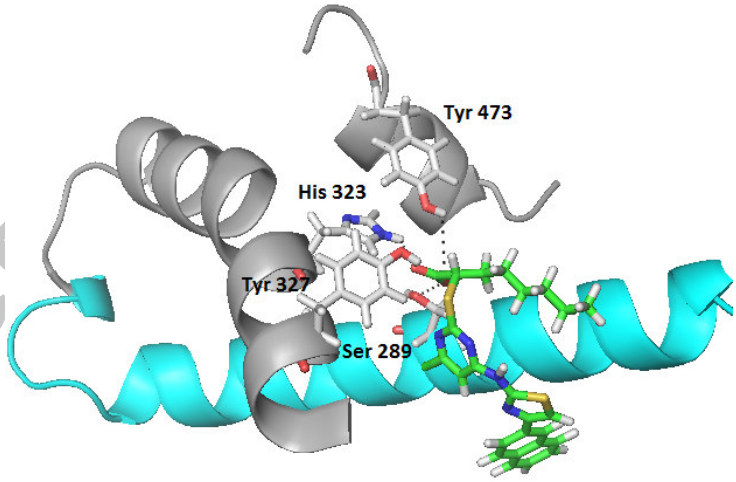


Figure 3: Potential binding mode of compound **13** on PPARγ (PDB ID: 3VSO[22]). Amino acid which interact with the carboxylic acid are Ser289, His323, Tyr327 and Tyr473. Helix 3 is marked in blue.

Ser289 and His323 (s. Figure 3). Compound **13** has a U-shaped binding mode from the *n*-hexyl residue to the thiazole moiety, whereas the 2-naphthyl residue is wriggled around helix 3. A remarkable feature of the SAR is the fact, that compound **13** has negligible activity on PPARα, whereas it was the most potent derivative on PPARγ. Therefore, an alignment of the docking mode of compound **13** in the ligand binding domain of PPARγ (PDB ID: 3VSO[22]) was performed with the ligand binding domain of PPARα (PDB ID: 3KDT[29]). Interestingly distinct differences are identifiable. The 2-naphthyl-moiety of compound **13** seems to be too big to fit into the ligand binding domain of PPARα whereas it was well tolerated in the PPARγ subpocket (s. Figure 4). Mainly responsible for the differences in these subpockets is helix 2´ on the entrance of the ligand binding pocket which is shifted towards the 2-naphthyl-moiety (s. Figure 5).

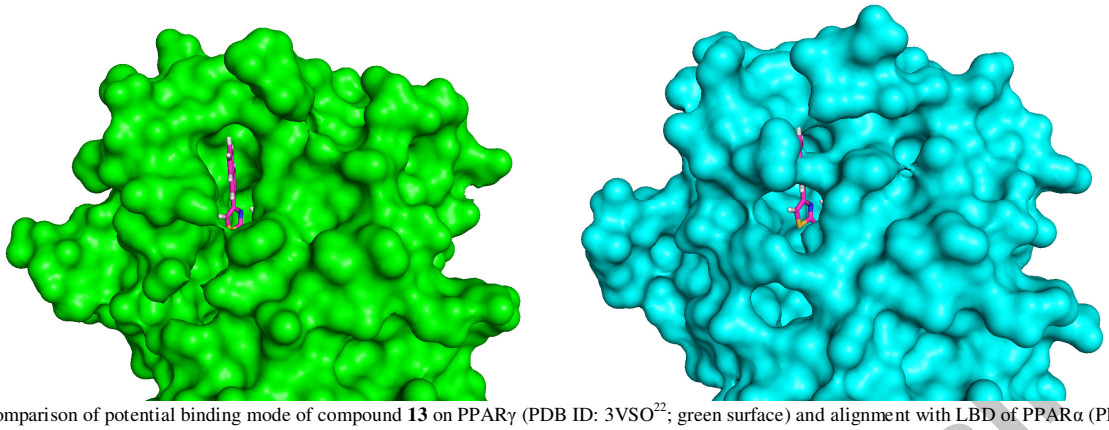


Figure 4: Comparison of potential binding mode of compound **13** on PPARγ (PDB ID: 3VSO[22]; green surface) and alignment with LBD of PPARα (PDB ID: 3KDT[29]; cyan surface).

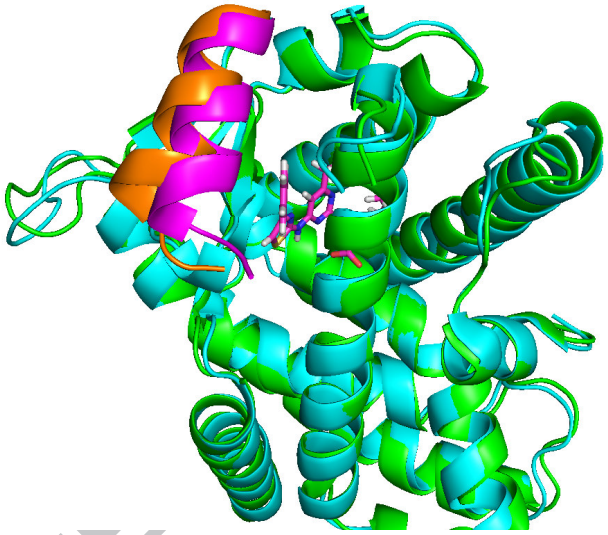


Figure 5: Alignment of PPARγ LBD (PDB ID: 3VSO[22]; green surface) with PPARα LBD (PDB ID: 3KDT[29]; cyan surface).The varying orientation of helix 2´ is highlighted in different colours. The helix 2´ of PPARα (in magenta) is directed to the 2-naphthyl-moiety of compound **13**, whereas helix 2´ of PPARγ (in orange) is targeted away, so that the bulky 2-naphthyl-moiety is better tolerated from PPARγ than from PPARα.

Particularly three amino acids in PPARα could be identified (L247, E251 and V255) which are directed to the 2-naphthyl-moiety of compound **13** and therefore reduce the space in the lipophilic backbone (s. Figure 2 SI). This feature explains on the one hand why our previous compound **6** was well tolerated on PPARα, on the other hand it could be used to explain the differences between the different classes of our compounds and how they interact in the LBD. The biphenyl-moiety in compound **6** is smaller than the bulkier 4-(2´-naphthyl)-thiazole-2-yl moiety in compound **13** and that explain, why compound **6** fits perfectly into the LBD of PPARα and not compound **13**. Additionally compounds with a long unbranched lipophilic backbone like in compound **6**, **7**, **12** or **16** are about equal potent on both enzymes, whereas more branched compounds like **13**, **14** or **15** are better tolerated from PPARγ. Thus, we can conclude that the selectivity of the presented compounds on the PPARα or PPARγ subtype depends on the space of the lipophilic backbone. PPARδ activity is not induced at 10 µM for all compounds of this series. M453 (M417) (V444 in PPARα, L453 in PPARγ) present in the PPARδ LBD seems to hinder binding of compounds with bulkier lipophilic substituents in alpha position of the carbocylic acid.[30, 31]

In conclusion, within this work we identified PPAR agonistic activity of a set of aminothiazole-based pirinixic acid derivatives supporting their suitability as anti-inflammatory or anti-cancer drugs. Even though anti-inflammatory and anti-proliferative properties of PPAR agonism have been reported over a decade ago[23], suitable clinical studies are still needed to validate this concept. Nevertheless an increasing demand has emerged for design of PPAR agonists to elaborate the PPAR effects in inflammation and inflammation-related diseases[4]. In our previous work we have shown, that aminothiazole-based pirinixic acid derivatives were highly potent in dual 5-LO and mPGES-1 inhibition. The interference within several pathways at once might have superadditive effects[11], and to the best of our knowledge no such compounds that combine this dual PPARα/γ agonism and dual 5-LO/mPGES-1 inhibition have been described before. Here, we have identified several compounds with distinct pharmacological profiles on the presented targets. The most

potent derivative regarding PPARγ (compound **13**) has also shown anti-inflammatory efficacy *in vivo*. Compound **13** was able to reduce the PGE_2 and LTC_4 levels *in vitro* and *in vivo*. Additionally, we have seen a reduction of the vascular permeability and an inhibition of neutrophil infiltration in a zymosan-induced peritonitis model in mice.[18] Whether the PPARγ agonism contributes to these anti-inflammatory effects need to be further elucidated. Finally, our broad *in vitro* pharmacological characterization of these aminothiazole featured pirinixic acids provides the opportunity to examine their potential in further *in vitro* and *in vivo* models of inflammation and especially cancer diseases, e.g. lung cancer.

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Supplementary Material

Supplementary material, including synthetic procedure analytical data and assay descriptions, can be found online: