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Truxillic acid derivatives act as peroxisome proliferator-activated receptor γ activators

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ABSTRACT

In previous studies, we identified a truxillic acid derivative as selective activator of the peroxisome proliferator-activated receptor γ, which is a member of the nuclear receptor family and acts as ligand-activated transcription factor of genes involved in glucose metabolism. Herein we present the structure–activity relationships of 16 truxillic acid derivatives, investigated by a cell-based reporter gene assay guided by molecular docking analysis.

Peroxisome proliferator-activated receptors (PPARs) belong to the superfamily of nuclear receptors. By activation through different ligands and binding to their DNA-response elements after formation of heterodimers with the retinoid X receptor (RXR), they increase gene transcription of several target genes playing a pivotal role in lipid and glucose homeostasis.[1] Three isotypes exist (PPARα, PPARβ (δ) and PPARγ) with different expression patterns according to their function and ligand specificity. PPARα is highly expressed in liver, heart, kidney, skeletal muscle, the large intestine and fat body whereas PPARβ (δ) occurs in nearly all tissues. PPARγ has the highest levels in fat body, heart, kidney and liver. PPAR activation leads to increased expression of key enzymes and proteins that are involved in uptake and metabolism of lipids and glucose, for example, apolipoprotein A/C, fatty acid transport proteins and lipoprotein lipase. Naturally, PPARs are activated by unsaturated fatty acids and eicosanoids such as linoleic acid and arachidonic acid.[2]

Due to their central role in glucose and lipid homeostasis PPARs represent an attractive drug target for therapy of type 2 diabetes

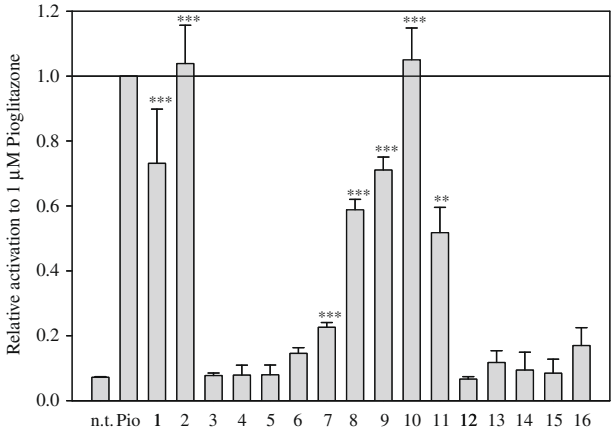

Figure 1. Relative activation of human PPARγ of compounds **1**–**16** compared to 1 µM pioglitazone. Transactivation was determined by a luciferase-based reporter gene assay which was already described elsewhere.[9] Mean values and standard deviation of at least three independent experiments are shown. ***$p < 0.005$; **$p < 0.01$.

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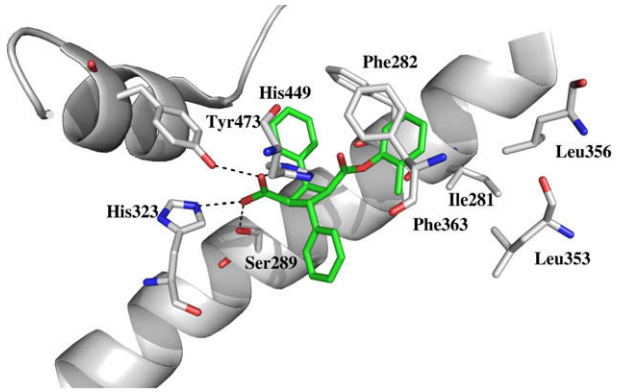
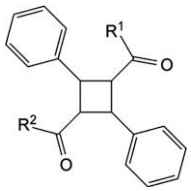


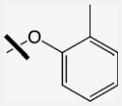
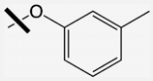
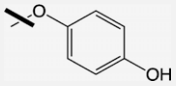
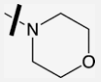
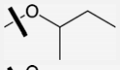
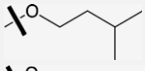
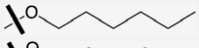
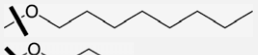
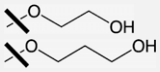
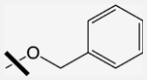
Figure 2. Top-scored binding mode of compound **1** in the ligand binding domain of PPARγ (PDB code: 1fm9)[10] from molecular docking experiments.

mellitus and dyslipidemia. Thus, there are several synthetic activators available which are in clinical use for the treatment of such diseases. For example, thiazolidinediones like pioglitazone and rosiglitazone as activators of PPARγ are used as an effective therapy for type 2 diabetes mellitus.[3] Synthetic PPARα activators include drugs like gemfibrozil, fenofibrate and bezafibrate. Their primary clinical use is to treat atherogenic dyslipidemia.[3] Besides synthetic drugs many herbs are traditionally used for the treatment of metabolic disorders and many herbal ingredients have now been identified as PPAR activators,[4] especially for PPARγ, for example, carnosol and carnosic acid[5] as well as several terpenoids and flavonoids.[4]

For some herbals there is evidence that they improve diabetic disorders, as was shown in animal studies, but no molecular mechanism that could explain their glucose-lowering effects was elucidated so far. One example for such a plant is *Cynodon dactylon*, whose aqueous extract was shown to have antidiabetic potential in rats by lowering blood glucose level and improving

Table 1
In vitro transactivation activities on human PPARγ for compounds **1–16**



Compounds	R^1	R^2	Transactivation EC_{50} (μM)	Activation compared to 1 μM pioglitazone
1	–OH		10.0 (±0.2)	138%
2	–OH		3.3 (±0.4)	123%
3	–OH		na	
4	–OH		na	
5	–OH		na	
6	–OH		na	
7	–OH		>>10	23% at 10 μM
8	–OH		4.8 (±0.6)	71%
9	–OH		3.6 (±0.1)	66%
10	–OH		8.6 (±3.0)	153%
11	–OH		9.3 (±2.2)	100%
12	–OH		na	
13	–OH		na	
14	–OH	–OH	na	
15			na	
16			na	

EC_{50} values were calculated using SigmaPlot2001 (Systat Software GmbH, Germany) based on the mean values of at least three determinations in a Gal4-hybrid transactivation assay for the respective PPAR subtypes which was already described elsewhere.[9] All substances were inactive at PPARα and δ at 10 μM (data not shown). Standard deviation is given in parentheses (na = not active at 10 μM).

hyperlipidemia.[6] *C. dactylon* contains several flavonoids and sterols that potentially could cause these effects, but it is also known that there is a high amount of substituted truxillic acids in the cell walls of *C. dactylon*.[7]

In previous studies, we have identified a substituted truxillic acid (**1**) as selective PPARγ activator within a virtual screening.[8] We performed NMR studies to elucidate the stereochemistry of the substituents of the cyclobutane ring of **1**. The results were published in the supplemental information of Rupp et al.[8] The structure of compound **1** in Figure 2 shows the stereochemistry found in the NMR experiments. HPLC analysis revealed that **1** is racemic (supp. inf. of Rupp et al.[8]).

Herein, we further characterize the PPARγ structure–activity relationships of substituted truxillic acid derivatives by investigating 15 additional compounds which were ordered from a commercial compound database by in vitro experiments. Furthermore, we explain the structure–activity relationships of compounds **1**–**16** on the basis of the binding mode proposed by molecular docking software (Fig. 2).

All compounds were tested in a cell-based Gal4-hybrid transactivation assay which was published elsewhere.[9] Figure 1 shows the relative activation of 10 μM of compounds **1**–**16** compared to the activity of 1 μM pioglitazone, which was used as a positive control and considered as 100% activation of PPARγ. Compounds **2** and **10** show an activation comparable to pioglitazone, whereas compounds **1**, **7**–**9**, and **11** show moderate but not full activation at 10 μM. Compounds **3**–**6** and **12**–**16** were not considered as active. All substances turned out to be inactive on human PPARα and PPARδ (data not shown). Table 1 shows structure–activity relationships around the ester moieties by comparing the respective EC_{50} values and maximum activation at the highest tested concentrations. **2** turned out to be the most potent compound with an EC_{50} of 3.3 μM with full activation of human PPARγ. **9** showed a similar EC_{50} value (3.6 μM), but did not achieve full activation of the receptor.

We performed molecular docking experiments with compound **1** in order to obtain a potential ligand binding pose that could help to explain structure–activity relationships of truxillic acid derivatives. The GOLD 4.0 software[11] was used to generate the potential binding mode of compound **1** to the PPARγ ligand binding domain co-crystallized with the full agonist farglitazar (PDB code: 1fm9).[10]

The top-scored binding mode (chemscore = 39)[12] is shown in Figure 2. The carboxylic group of compound **1** interacts with the amino acid residues S289, H323, H449, and Y473 (Figs. 2 and 3), which are responsible for activation of PPAR.[13] Ester derivatives **15** and **16** lack an acidic group and are therefore inactive.

According to our model, one of the phenyl moieties and the 2-methylphenylester moiety occupy the left proximal binding pocket.[14] Residues within this hydrophobic region are responsible for selectivity between the PPAR subtypes. This region is larger for PPARγ, therefore compounds with rigid and bulky moieties in this area often lack PPARα activity.[13] One of the phenyl moieties interacts closely with F282. Moreover, the 2-methylphenylester moiety of compound **1** undergoes π–π stacking with F363 (Figs. 2 and 3). Compound **2** occupies the left proximal pocket in a more favorable way, which might provide a structural explanation why compound **2** exhibits a lower EC_{50} than compound **1**. The 3-methylphenylester moiety reaches deeply into the left proximal binding pocket and the 3-methyl substituent interacts favorably with L353, L356, and I281. Compound **1** which carries a methyl substituent in *ortho*-position is not able to interact with any of these residues, which could be a possible explanation for its slightly lower EC_{50} of 10 μM.

The properties of the ester residue are highly important for the structure–activity relationships of the tested truxillic acid derivatives. Neither an aromatic nor an aliphatic hydroxyl group is tolerated in this region, which obviously lacks any polar residues that may undergo favorable interactions with a hydrophilic moiety. This would explain the lack of activity of compounds **3**, **12**, **13** and compound **14**, where the complete ester substituent is missing.

Compounds **4** and **5**, which contain bulky and hydrophobic amide substituents, were also not able to activate the receptor in our transactivation assay. We suspect that the rigid cyclic secondary amide moieties of these compounds cause a sterical clash with F282.

The aliphatic ester residues of compounds **6**–**11** are suitable to interact with the left proximal binding pocket of PPARγ. We observe that the relative activation of compounds **6**–**10** increases with the number of carbon atoms within the aliphatic ester residue (Fig. 4). Compound **11** which contains the most carbon atoms within the ester residue however shows a decreased relative activation. These results lead us to the assumption that the left

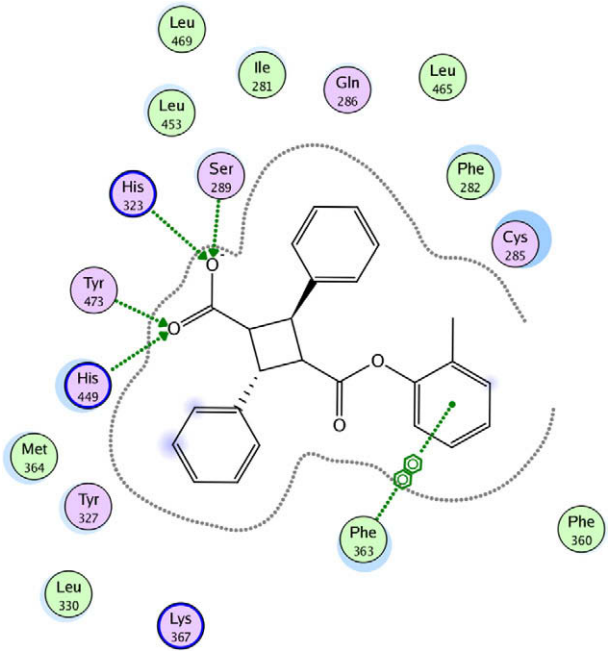


Figure 3. Two-dimensional representation of the residue interaction pattern for compound **1** docked into the LBD of PPARγ; generated using the software MOE (Chemical Computing Group, www.chemcomp.com).

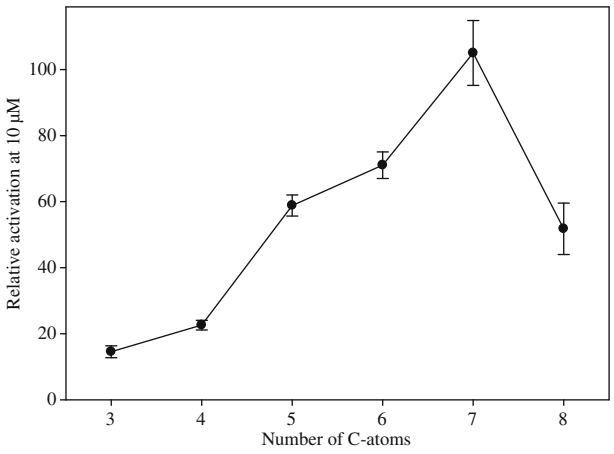


Figure 4. Relation between number of C-atoms in the ester moieties of compounds **6**–**11** to relative activation of PPARγ compared to 1 μM pioglitazone; mean values and standard deviation of at least three independent experiments are shown.

proximal binding pocket is filled optimally with an aliphatic chain with seven carbons whereas an increase in chain length as in compound **11** exceeds the capabilities of the hydrophobic binding site and is therefore less tolerated.

In summary, a series of truxillic acid derivatives was identified as subtype-selective PPARγ activators. The molecular docking experiments suggest that the structure–activity relationships of the tested compounds are mainly influenced by the properties of the ester moieties, which vary between aromatic and aliphatic residues in our study. Compounds with aromatic, hydrophobic ester moieties such as **1** and **2** exhibit the most potent (compound **2**) and full activation of human PPARγ whereas the activity and potency of compounds with aliphatic residues (**6**–**11**) obviously depend on the chain length. Further synthetic efforts, for example considering the substitution pattern of the two phenyl residues, would certainly provide a deeper insight into the structure–activity relationships.

In spite of the fact that the activity of the compounds is rather low compared to known PPARγ agonists, the presented truxillic acid derivatives might provide a starting point for a natural product inspired synthesis program with the aim of improving potency and exploring new chemical space besides existing PPAR agonists. Recently, novel scaffolds are often identified by virtual screening procedures which are increasingly based on natural product libraries.[15,16] These scaffolds might serve as valuable starting points for lead structure optimization and development of potentially safer drugs.

Acknowledgments

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References and notes

1. Desvergne, B.; Wahli, W. *Endocr. Rev.* **1999**, *20*, 649.
2. Willson, T. M.; Wahli, W. *Curr. Opin. Chem. Biol.* **1997**, *1*, 235.
3. Grundy, S. M. *Nat. Rev. Drug Disc.* **2006**, *5*, 295.
4. Huang, T. H.; Kota, B. P.; Razmovski, V.; Roufogalis, B. D. *Basic Clin. Pharmacol. Toxicol.* **2005**, *96*, 3.
5. Rau, O.; Wurglics, M.; Paulke, A.; Zitzkowski, J.; Meindl, N.; Bock, A.; Dingermann, T.; Abdel-Tawab, M.; Schubert-Zsilavecz, M. *Planta Med.* **2006**, *72*, 881.
6. Singh, S. K.; Kesari, A. N.; Gupta, R. K.; Jaiswal, D.; Watal, G. *J. Ethnopharmacol.* **2007**, *114*, 174.
7. Hartley, R. D.; Morrison, W. H., III; Balza, F.; Towers, G. H. N. *Phytochemistry* **1990**, *29*, 3699.
8. Rupp, M.; Schroeter, T.; Steri, R.; Zettl, H.; Proschak, E.; Hansen, K.; Rau, O.; Schwarz, O.; Müller-Kuhrt, L.; Schubert-Zsilavecz, M.; Müller, K. R.; Schneider, G. *ChemMedChem* **2010**, *5*, 191.
9. Zettl, H.; Steri, R.; Lammerhofer, M.; Schubert-Zsilavecz, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4421.
10. Gampe, R. T., Jr.; Montana, V. G.; Lambert, M. H.; Miller, A. B.; Bledsoe, R. K.; Milburn, M. V.; Kliewer, S. A.; Willson, T. M.; Xu, H. E. *Mol. Cell* **2000**, *5*, 545.
11. Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. *Proteins* **2003**, *52*, 609.
12. Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. *J. Comput. Aided Mol. Des.* **1997**, *11*, 425.
13. Xu, H. E.; Lambert, M. H.; Montana, V. G.; Plunket, K. D.; Moore, L. B.; Collins, J. L.; Oplinger, J. A.; Kliewer, S. A.; Gampe, R. T., Jr.; McKee, D. D.; Moore, J. T.; Willson, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13919.
14. Pirard, B. *J. Comput. Aided Mol. Des.* **2003**, *17*, 785.
15. Tanrikulu, Y.; Rau, O.; Schwarz, O.; Proschak, E.; Siems, K.; Müller-Kuhrt, L.; Schubert-Zsilavecz, M.; Schneider, G. *ChemBiochem* **2009**, *10*, 75.
16. Salam, N. K.; Huang, T. H.; Kota, B. P.; Kim, M. S.; Li, Y.; Hibbs, D. E. *Chem. Biol. Drug Des.* **2008**, *71*, 57.