Chem Biol Drug Des 2016; **87:** 467–471

Research Letter

Synthesis of Naphthyl-, Quinolin- and Anthracenyl Analogues of Clofibric Acid as PPARα Agonists

Letizia Giampietro*, Alessandra Ammazzalorso, Isabella Bruno, Simone Carradori, Barbara De Filippis, Marialuigia Fantacuzzi, Antonella Giancristofaro, Cristina Maccallini and Rosa Amoroso*

Dipartimento di Farmacia, Università "G. d'Annunzio" di Chieti, via dei Vestini, 66100, Chieti, Italy
**Corresponding authors: Letizia Giampietro, l.giampietro@unich.it; Rosa Amoroso, ramoroso@unich.it*

PPARα is a ligand activated transcription factor belonging to the nuclear receptor subfamily, involved in fatty acid metabolism in tissues with high oxidative rates such as muscle, heart and liver. PPARα activation is important in steatosis, inflammation and fibrosis in preclinical models of non-alcoholic fatty liver disease identifying a new potential therapeutic area. In this work, three series of clofibric acid analogues conjugated with naphthyl, quinolin, chloroquinolin and anthracenyl scaffolds were synthesized. In an effort to obtain new compounds active as PPARα agonists, these molecules were evaluated for PPARα transactivation activity. Naphthyl and quinolin derivatives showed a good activation of PPARα; noteworthy, optically active naphthyl derivatives activated PPARα better than corresponding parent compound.

Key words: anthracenyl, clofibric acid, naphthyl, peroxisome proliferator-activated receptor agonist, quinolin, transactivation assay

Received 16 July 2015, revised 22 August 2015 and accepted for publication 14 September 2015

Peroxisome proliferator-activated receptors (PPARs) are a subfamily of ligand-activated nuclear hormone receptors that are highly expressed in metabolically active tissues, where regulate genes encoding lipid and glucose metabolism, and overall energy homeostasis. Three PPAR subtypes, commonly designated as PPARα, PPARγ and PPARδ(β), have been identified; these receptors differ in their tissue distributions, selectivity and responsiveness to ligands, thus leading to the regulation of different sets of genes involved in lipid metabolism and energy balance (1,2). PPARα is highly expressed in metabolically active tissues including liver, muscle, intestine and brown adipose tissue; it plays a pivotal role on lipid metabolism by decreasing both serum triglycerides and free fatty acid levels, and increasing high-density lipoprotein level (HDL) (3). PPARα is activated by the fibrate antilipidemic drugs (4). The PPARγ controls fatty acid storage and glucose metabolism by coordinating the expression of genes involved in lipid metabolism, adipogenesis and inflammation (5). This receptor is activated by the thiazolidinedione (TZD) antidiabetic drugs (6). PPARβ/δ regulates fatty acid catabolism, insulin sensitivity and energy homeostasis in muscle and adipose tissue (7). Some phenoxyacetic acid derivatives, L-165041, GW501516, GW0742 and MBX-8025 are very potent and selective activators of PPARβ both *in vitro* and *in vivo*. Although PPARβ agonists are not yet in clinical use, human studies have been performed providing very encouraging findings for the treatment of metabolic disorders in dyslipidemic obese individuals (8–10).

The structures of all PPARs subtypes are similar: an amino terminal activation domain (AF-1), a DNA binding domain (DBD), a ligand binding domain (LBD) and a second carboxy terminal activation domain (AF-2). When a ligand interacts with PPAR, there is a binding to PPAR responsive elements (PPREs) in the DNA after dimerization with another nuclear receptor, the retinoid-X receptor (RXR). The release of corepressors and recruitment of coactivators regulate the transcription of specific genes (1).

In the last years, the development of new compounds that activate PPARα has been an important target to better understand structure–activity relationships (SARs) for obtaining new hypolipidemic drugs with lower adverse events. A lot of PPARα-selective agonists have been reported previously (11,12). The common structure is composed of an aromatic ring and carboxylic acid with or without various spacers. The aromatic ring of the ligands interacts with the hydrophobic binding pocket and the acidic moiety forms hydrogen bonds with amino acids on the AF-2 region in a roughly Y-shaped ligand binding site (13,14).

In the past years, we have synthesized different series of fibrate derivatives with the aim of obtaining new hypolipidemic compounds active as PPARα agonists (15–17). In this field, stilbenes and chalcones and some of their synthetic derivatives have shown to activate PPARα or to

 doi: 10.1111/cbdd.12677

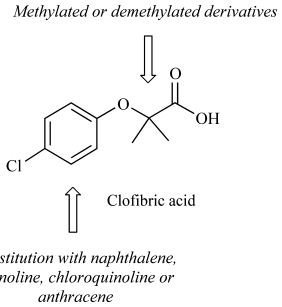


Figure 1: Chemical modifications of clofibric acid.

lower plasma lipid levels (18,19). Therefore, our research group has previously synthesized chiral analogues of clofibrate with different biological activity related to stereochemistry (20,21).

The present work describes the synthesis of methylated and demethylated derivatives of clofibric acid (the active metabolite of clofibrate), where the aromatic centre is substituted by naphthalene, quinoline, chloroquinoline or anthracene scaffolds (Figure 1). The new compounds were *in vitro* evaluated for their PPAR agonistic activity using the reporter gene assay.

All compounds (Figure 2) were easily obtained in good yields by standard synthetic procedures. Esters **13**–**16** and *rac*-**17**–**20** were obtained by S_N2 reaction of phenols **9**–**12** with ethyl 2-bromo-2-methylpropanoate or ethyl 2-bromopropanoate, in the presence of sodium metal in absolute EtOH, under nitrogen atmosphere at reflux. The basic hydrolysis of **13**–**16** and *rac*-**17**–**20** with 1% alcoholic solution of KOH gave the acids **1**, **3**, **5**, **7** and *rac*-**2**, *rac*-**4**, *rac*-**6**, *rac*-**8** (Scheme 1). The phenols 1-naphthol (**9**), quinolin-2-ol (**10**) and 6-chloroquinolin-2-ol (**11**) are commercially available; conversely, 1-anthrol (**12**) was synthesized by the reaction of 1-anthrylamine in the presence of $NaHSO_3$ and EtOH at reflux (Scheme 2).

The clofibric acid chiral analogues (*R*)-**2** and (*S*)-**2** were synthesized by using (*S*)-4-isopropyl-2-oxazolidinone as chiral auxiliary because of its well-established capability of optical resolution of the α-aryloxyacetic acids (22). The acid *rac*-**2** was chlorinated with $SOCl_2$ to obtain the acyl chloride *rac*-**21** that was used to *N*-acylate the chiral auxiliary. The equimolar diastereomeric mixture of chiral imides **22** were obtained in good yield and separated on silica gel. The cleavage with lithium hydroperoxyde led to both dextrorotatory and levorotatory enantiomers of optical active acid **2** (Scheme 3). The enantiomeric purity of (*R*)-**2** and (*S*)-**2** was evaluated by a capillary electrophoretic run, by using *β*-cyclodextrin as a chiral selector. Analyses showed enantiomeric excesses up to 98% for the two tested compounds. As reported elsewhere, the compounds (*R*)-**2** and (*S*)-**2** are levorotatory and dextrorotatory

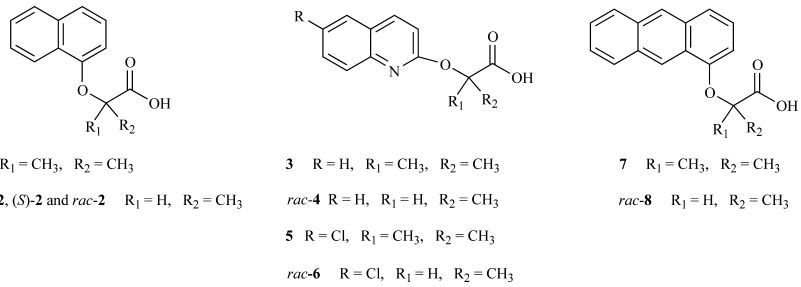
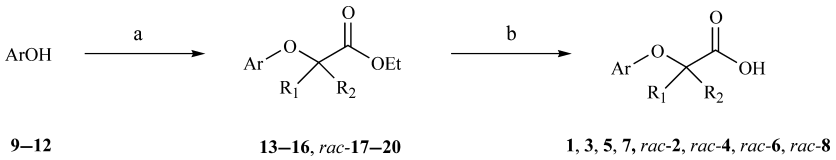
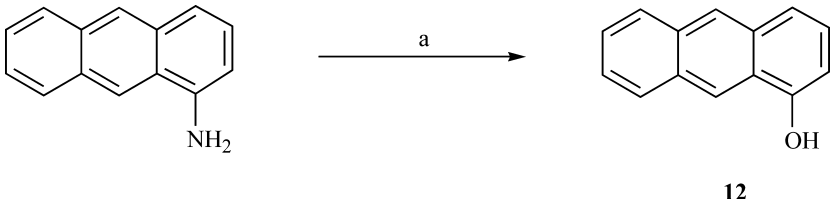


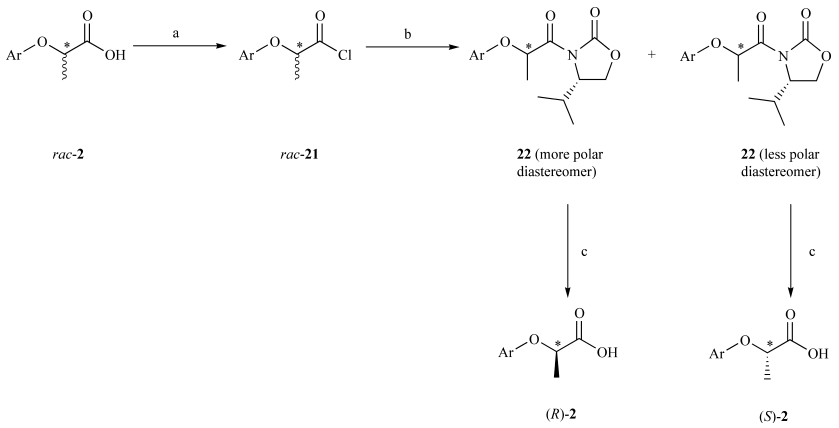
Figure 2: New compounds.



Scheme 1: Reagents and conditions: (a) ethyl 2-bromo-2-methylpropanoate or ethyl 2-bromopropanoate, Na, EtOH, N_2, reflux; (b) KOH, EtOH, reflux.

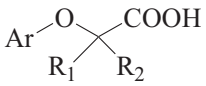


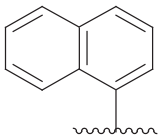
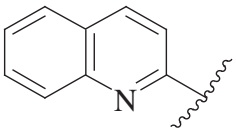
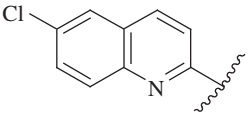
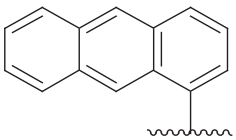
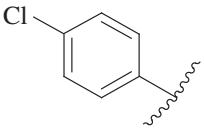
Scheme 2: Reagents and conditions: (a) $NaHSO_3$, H_2O, EtOH, reflux.



Scheme 3: Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, 40 °C; (b) BuLi, (*S*)-4-isopropyl-2-oxazolidinone, THF, −78 °C, (c) LiOOH, THF/H_2O, rt.

Table 1: *In vitro* transactivation activity of new clofibric acid analogues



Compound	Ar	R_1	R_2	clogP[a]	PPARα (E%)[b]	PPARγ (E%)[b]	PPARδ (E%)[b]
1		CH_3	CH_3	3.269 ± 0.262	37	23	31
rac-**2**		H	CH_3	2.929 ± 0.255	50	36	29
(*R*)-**2**		H	CH_3	2.929 ± 0.255	106	35	12
(*S*)-**2**		H	CH_3	2.929 ± 0.255	56	12	33
3		CH_3	CH_3	2.458 ± 0.305	43	32	32
rac-**4**		H	CH_3	2.108 ± 0.293	31	23	23
5		CH_3	CH_3	3.104 ± 0.334	37	22	11
rac-**6**		H	CH_3	2.754 ± 0.324	50	35	22
7		CH_3	CH_3	4.499 ± 0.263	12	14	34
rac-**8**		H	CH_3	4.150 ± 0.256	12	24	15
Clofibric acid		CH_3	CH_3	2.724 ± 0.272	100	–	–
Rosiglitazone				–	–	100	–
L-165 041				–	–	–	100

[a]clogP were calculated by using ACD/Labs Extension for CS ChemDraw (version 5.0).
[b]Compounds were tested in at least three separate experiments at 150 μM. Efficacy values were calculated as percentage of the maximum obtained fold induction with the clofibric acid for PPARα, rosiglitazone for PPARγ and L-165 041 for PPARδ.

respectively (23). All compounds were purified by column chromatography on silica gel or by crystallization and fully characterized by IR, ^{1}H and ^{13}C NMR spectroscopy.

To investigate PPARα agonistic potency and subtype selectivity, all compounds were evaluated for human PPARα, PPARγ and PPARδ functional activity by a cell-based transactivation assay in eukaryotic cells (24). In this method, we utilized firefly luciferase reporter gene that provides a good assay sensitivity, dynamic range when quantifying nuclear receptor activity and optimal correlation with *in vivo* activity. In this study, we used clofibric acid, rosiglitazone and L-165 041, as reference compounds for PPARα, PPARγ and PPARδ respectively; results are expressed as efficacy (E%) relative to positive control. These results and the clogP calculated with ACD/Labs Extension for CS ChemDraw (version 5.0) were reported in Table 1.

All naphthyl derivatives (**1**, *rac*-**2**, (*R*)-**2** and (*S*)-**2**) showed an activation of PPARα higher than anthracenyl analogues (**7** and *rac*-**8**) but similar to quinolin and 6-chloroquinolin derivatives (**3**, *rac*-**4**, **5** and *rac*-**6**). Probably, the activity is influenced by the lipophilicity; indeed, these compounds have clogP values similar to clofibric acid (**1** clogP = 3.269 ± 0.262; *rac*-**2**, (*R*)-**2** and (*S*)-**2** clogP = 2.929 ± 0.255; **3** clogP = 2.458 ± 0.305; *rac*-**4** clogP = 2.108 ± 0.293; **5** clogP = 3.104 ± 0.334; *rac*-**6** clogP = 2.754 ± 0.324; clofibric acid clogP = 2.724 ± 0.272). Furthermore, naphthyl, quinolin and 6-chloroquinolin analogues possess a less hindered aromatic scaffold that probably fit better the hydrophobic pocket of the PPARα receptor, whereas the anthracenyl scaffold of compounds **7** and *rac*-**8** probably accesses in an alternative hydrophobic site with displacement of carboxylic head. When one of the methyls in α to the carboxylic group was deleted, generating a chiral center (*rac*-**2**, *rac*-**4**, *rac*-**6** and *rac*-**8**), it resulted in a different agonistic activity as compared to ramificated compound (**1**, **3**, **5** and **7**), showing that the α-C to the carboxylic function plays an important role in the modulation of activity of the test compounds.

To better understand how the chirality influences the PPARα activity, we have selected the acid *rac*-**2** and we have synthesized its optically active derivatives (*R*)-**2** and (*S*)-**2**; they showed a good activation of PPARα, with efficacies of 106% and 56% respectively; (*R*)-**2** and (*S*)-**2**, the more efficacious of our series of compounds, were also selected for the determination of EC_{50} values and tested in at least three separate experiments at five concentrations ranging from 1 to 150 μM. Both compounds, with EC_{50} = 6.9 ± 0.6 μM for (*R*)-**2** and EC_{50} = 11.6 ± 0.9 μM for (*S*)-**2** showed a better activation of the PPARα than clofibric acid (EC_{50} = 55 ± 3.9 μM). On the basis these results, we hypothesize that compounds with (*R*)-stereochemistry fit very well into the receptor Y-shaped pocket; however, a rationalization of the results will require further investigations based on molecular modelling studies. At last, none of tested compounds was able to exhibit full agonist activity on PPARγ and PPARδ, showing lower potency than rosiglitazone and L-165 041.

In conclusion, three series of clofibric acid analogues were synthesized and *in vitro* evaluated for human PPARs transactivation assay. Compounds with naphthyl, quinolin and 6-chloroquinolin scaffold showed a better increase in the transcriptional activity of receptor than the anthracenyl derivatives. The best results were obtained with compounds (*R*)-**2** and (S)-**2** that showed EC_{50} values of 6.9 ± 0.6 μM and 11.6 ± 0.9 μM respectively.

Acknowledgments

This study was supported by University "G. d'Annunzio" of Chieti local grants.

Conflict of interest

The authors declare no conflict of interest.

References

1. Berger J., Moller D.E. (2002) The mechanisms of action of PPARs. Annu Rev Med;53:409–435.
2. Varga T., Czimmerer Z., Nagy L. (2011) PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. Biochim Biophys Acta;1812:07–1022.
3. Pawlak M., Lefebvre P., Staels B. (2015) Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. J Hepatol;62:720–733.
4. Staels B., Maes M., Zambon A. (2008) Fibrates and future PPARalpha agonists in the treatment of cardiovascular disease. Nat Clin Pract Cardiovasc Med;5:542–553.
5. Takada I., Makishima M. (2015) PPARγ ligands and their therapeutic applications: a patent review (2008–2014). Expert Opin Ther Pat;25:175–191.
6. Desai N.C., Pandit U.P., Dodiya A. (2015) Thiazolidinedione compounds: a patent review (2010 present). Expert Opin Ther Pat;25:479–488.
7. Kahremany S., Livne A., Gruzman A., Senderowitz H., Sasson S. (2015) Activation of PPARδ: from computer modelling to biological effects. Br J Pharmacol;172:754–770.
8. Neels J.G., Grimaldi P.A. (2014) Physiological functions of peroxisome proliferator-activated receptor β. Physiol Rev;94:795–858.
9. Chen W., Gao R., Xie X., Zheng Z., Li H., Li S., Dong F., Wang L. (2015) A metabolomic study of the PPARδ agonist GW501516 for enhancing running endurance in Kunming mice. Sci Rep;5:09884.
10. Coll T., Barroso E., Alvarez-Guardia D., Serrano L., Salvadò L., Merlos M., Palomer X., Vazquez-Carrera

M. (2009) Peroxisome proliferator-activated receptor (PPAR) beta/delta: a new potential therapeutic target for the treatment of metabolic syndrome. Curr Mol Pharmacol;2:46–55.
11. Nissen S.E., Nicholls S.J., Wolski K., Howey D.C., McErlean E., Wang M.D., Gomez E.V., Russo J.M. (2007) Effects of a potent and selective PPAR-α agonist in patients with atherogenic dyslipidemia or hypercholesterolemia: two randomized controlled trials. J Am Med Assoc;297:1362–1373.
12. Nomura M., Yumoto K., Shinozaki T., Isogai S., Takano Y., Murakami K. (2012) Discovery of cyclic amine-substituted benzoic acids as PPARα agonists. Bioorg Med Chem Lett;22:334–338.
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. (2001) Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proc Natl Acad Sci USA;98:13919.
14. Dhoke G.V., Gangwal R.P., Sangamwar A.T. (2012) A combined ligand and structure based approach to design potent PPAR-alpha agonists. J Mol Struct;1028:22–30.
15. Giampietro L., Ammazzalorso A., Giancristofaro A., Lannutti F., Bettoni G., De Filippis B., Fantacuzzi M., Maccallini C., Petruzzelli M., Morgano A., Moschetta A., Amoroso R. (2009) Synthesis and biological evaluation of 2-heteroarylthioalkanoic acid analogues of clofibric acid as Peroxisome Proliferator-Activated Receptor α agonists. J Med Chem;52:6224–6232.
16. De Filippis B., Giampietro L., Giancristofaro A., Ammazzalorso A., Fantacuzzi M., Maccallini C., Petruzzelli M., Amoroso R. (2011) Synthesis and biological evaluation of gemfibrozil chiral analogues as potential PPARα agonists. Lett Drug Des Discov;8:154–158.
17. Giampietro L., D'Angelo A., Giancristofaro A., Ammazzalorso A., De Filippis B., Fantacuzzi M., Linciano P., Maccallini C., Amoroso R. (2012) Synthesis and structure–activity relationships of fibrate-based analogues inside PPARs. Bioorg Med Chem Lett;22:7662–7666.
18. De Filippis B., Giancristofaro A., Ammazzalorso A., D'Angelo A., Fantacuzzi M., Giampietro L., Maccallini C., Petruzzelli M., Amoroso R. (2011) Discovery of gemfibrozil analogues that activate PPARα and enhance the expression of gene CPT1A involved in fatty acids catabolism. Eur J Med Chem;46:5218–5224.
19. Giampietro L., D'Angelo A., Giancristofaro A., Ammazzalorso A., De Filippis B., Di Matteo M., Fantacuzzi M., Linciano P., Maccallini C., Amoroso R. (2014) Effect of stilbene and chalcone scaffolds incorporation in clofibric acid on PPARα agonistic activity. Med Chem; 10:59–65.
20. Amoroso R., Bettoni G., Tricca M.L. (1998) Synthesis of (*R*)-aryloxy acids by SN2 reaction from diastereomerically enriched alfa-bromo esters. Synlett;4:363–364.
21. Bettoni G., Loiodice F., Tortorella V., Conte-Camerino D., Mambrini M., Ferrannini E., Bryant S.H. (1987) Stereospecificity of the chloride ion channel: the action of chiral clofibric acid analogs. J Med Chem;30:1267–1270.
22. Amoroso R., Bettoni G., Tricca M.L., Loiodice F., Ferorelli S. (1998) Direct resolution of α-monoalkyl-α-aryloxyacetic acids via ester or imide derivatives. Il Farmaco;53:73–79.
23. Ammazzalorso A., Bettoni G., De Filippis B., Fantacuzzi M., Giampietro L., Giancristofaro A., Maccallini C., Re N., Amoroso R., Coletti C. (2008) Synthesis of 2-aryloxypropanoic acids analogues of clofibric acid and assignment of the absolute configuration by ^{1}H NMR spectroscopy and DFT calculations. Tetrahedron Asymmetry;19:89–997.
24. Matthews J.M., Chen X., Cryan E., Hlasta D.J., Rybczynski P.J., Strauss K., Tang Y., Xu J.Z., Yang M., Zhou L., Demarest K.T. (2007) Design and synthesis of indane-ureido-thioisobutyric acids: a novel class of PPARα agonists. Bioorg Med Chem Lett;17:6773–6778.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and methods.