Synthesis and biological evaluation of regulators of peroxisome proliferator-activated receptors

Dissertation for the degree of Ph.D.

Calin Constantin Ciocoiu

School of Pharmacy
Faculty of Mathematics and Natural Sciences

UNIVERSITY OF OSLO

2010
Acknowledgements

I carried out my doctoral studies between July 2006 and September 2010 at the Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, Norway, under the supervision of Associate Professor Trond Vidar Hansen and co-supervisor Associate Professor Pål Rongved.

Birkeland Innovasjon is gratefully acknowledged for the financial support.

Firstly, I sincerely thank my supervisor associate professor Trond Vidar Hansen for the opportunity to be a part of his research group. His knowledge, help, and guidance have been very important for me during these years.

Furthermore, I would like to thank Professors Arild Rustan and G Hege Thorensen for their help with the biological assays. I am also grateful to Ph.D. student Nataša Nicolić for her guidance and availability. Professor Ingebrigt Sylte and Dr.scient. Aina Westrheim Ravna are gratefully acknowledged for the molecular modeling studies.

I would also like to express my deep gratitude to Professor Emeritus Arne Jørgen Aasen for the very helpful scientific discussions, precious advices, and for proofreading my thesis.

All members of the group of Medicinal Chemistry I’ve had the joy to work with are greatly acknowledged for their scientific and social contribution: Hany, Øyvind A., Kristin, Alexander, Yasser, Yang, Anders, and Martin.

A special thank goes to my chemistry book, Øyvind, for the long discussions, late in the evening, for his great help, and for his incredible patience with my numberless questions.

I am wordlessly grateful to Cezarina, my second mum, without whose guidance, psychological support and daily help, this work would not have been achieved. I also thank very much the third member of the Romanian triangle, Mioara, for her NMR help, kindness and good food.
Finally, I thank my husband Simen for his love and patience when listening to my endless complaints, for his encouragement and his belief in me. And, of course, to my dog Ava who was so nice to not eat my memory stick.
Abstract

The powerful and selective PPARδ agonist, GW 501516 (18), was used as a lead compound for new compounds with potential agonistic effects. The compounds were biologically evaluated using the oleic acid oxidation assay and the luciferase-based transient transfection assay.

Modification of the lead compound by replacing the thiazole ring with a 1,4-disubstituted 1,2,3-triazole ring and by conducting structure-activity relationship (SAR) studies led to three series of new agonists. Triazole 52e of the first series increased the oxidation of oleic acid exhibiting an EC50-value of 0.85 nM. Compound 52e showed dual PPARα/δ agonistic effects at 10 μM concentration. Acid 55a of the second series was 600 times less potent than the lead compound GW 501516 (18) regarding to the oxidation of oleic acid, but it proved to be a medium effective PPARα agonist at 10 μM concentration. Compounds 69a-69c of the third series induced oxidation of oleic acid with nanomolar potencies and exhibited dual PPARα/δ agonistic effects at 10 μM concentration.

Further SAR studies led to compound 62e with high potency both in the oleic acid oxidation assay and the luciferase-based transient transfection assay. Moreover, the thiazole 62e showed a high selectivity towards PPARδ.

The influence of a fluorine atom on the acidic moiety of our most potent compounds was investigated. This modification led to three new dual PPARα/δ at 10 μM concentration.

Finally, we prepared and biologically evaluated using the oleic acid oxidation assay two known PPARδ antagonists, GSK 0660 (42) and GSK 3787 (43), and a potentially new PPARδ antagonist (103). Compound 103 showed initial promising biological effects.
Graphical abstract

Triazole-based analogues of GW 501516 (18). First series

Triazole-based analogues of GW 501516 (18). Second series

Thiazole-based analogues of GW 501516 (18)

Triazole-based analogues of GW 501516 (18). Third series

69a, EC<sub>50</sub> = 11.6 nM
α-Fluorinated thiazole- and triazole-based analogues of GW 5101516 (18)

New potential antagonist of PPARδ
### List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>DBD</td>
<td>DNA-binding domain</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified Eagle’s medium</td>
</tr>
<tr>
<td>DPBS</td>
<td>Dulbecco's phosphate buffered saline</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>concentration of a compound where 50% of its maximal effect is observed</td>
</tr>
<tr>
<td>FCS</td>
<td>fetal calf serum</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HEPES</td>
<td>2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>concentration of a compound that inhibits 50% of a given biological process</td>
</tr>
<tr>
<td>LBD</td>
<td>ligand-binding domain</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>MS</td>
<td>metabolic syndrome</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PDB</td>
<td>protein data bank</td>
</tr>
<tr>
<td>PG</td>
<td>prostaglandin</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PPRE</td>
<td>peroxisome proliferator response element</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid</td>
</tr>
<tr>
<td>SAR</td>
<td>structure activity relationship</td>
</tr>
<tr>
<td>VLDL</td>
<td>very low-density lipoprotein</td>
</tr>
</tbody>
</table>
# Table of contents

1. **INTRODUCTION**  

1.1 Obesity, diabetes and the metabolic syndrome  

1.2 Peroxisome proliferator activated receptors  

1.3 Molecules targeting the peroxisome proliferator-activated receptors  
  1.3.1 Natural ligands of the peroxisome proliferator-activated receptors  
  1.3.2 Synthetic ligands of peroxisome proliferator-activated receptors  
    1.3.2.1 Peroxisome proliferator-activated receptors α agonists  
    1.3.2.2 Peroxisome proliferator-activated receptors δ agonists  
    1.3.2.3 Peroxisome proliferator-activated receptors γ agonists  
    1.3.2.4 Dual peroxisome proliferator-activated receptor agonists  
      1.3.2.4.1 Dual PPARα/δ agonists  
      1.3.2.4.2 Dual PPARα/γ agonists  
      1.3.2.4.3 Dual PPARγ/δ agonists  
  1.3.3 Peroxisome proliferator-activated receptors antagonists  

1.4 “Click chemistry” in drug discovery  

1.5 Aim of the study  

2. **RESULTS AND DISCUSSIONS**  

2.1 PPAR agonists  
  2.1.1 Triazole-based analogues of GW 501516 (18). First series (Paper I)  
    2.1.1.1 Synthesis  
    2.1.1.2 Biological evaluation  
    2.1.1.3 Results and Discussion  
    2.1.1.4 Conclusions  
  2.1.2 Triazole-based analogues of GW 501516 (18). Second series  
    2.1.2.1 Synthesis  
    2.1.2.2 Biological evaluation  
    2.1.2.3 Results and Discussion  
    2.1.2.4 Conclusions  
  2.1.3 Thiazole based-analogues of GW 501516 (18) (Paper II)  
    2.1.3.1 Synthesis
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.3.2</td>
<td>Biological evaluation</td>
<td>34</td>
</tr>
<tr>
<td>2.1.3.3</td>
<td>Results and Discussions</td>
<td>34</td>
</tr>
<tr>
<td>2.1.3.4</td>
<td>Molecular modelling</td>
<td>38</td>
</tr>
<tr>
<td>2.1.3.5</td>
<td>Conclusions</td>
<td>39</td>
</tr>
<tr>
<td>2.1.4</td>
<td>Triazole-based analogues of GW 501516 (18). Third series</td>
<td>40</td>
</tr>
<tr>
<td>2.1.4.1</td>
<td>Synthesis</td>
<td>40</td>
</tr>
<tr>
<td>2.1.4.2</td>
<td>Biological evaluation</td>
<td>41</td>
</tr>
<tr>
<td>2.1.4.3</td>
<td>Results and Discussion</td>
<td>42</td>
</tr>
<tr>
<td>2.1.4.4</td>
<td>Conclusions</td>
<td>44</td>
</tr>
<tr>
<td>2.1.5</td>
<td>α-Mono fluorinated thiazole- and triazole-based analogues of GW 5101516 (18) (Paper III)</td>
<td>44</td>
</tr>
<tr>
<td>2.1.5.1</td>
<td>Synthesis</td>
<td>45</td>
</tr>
<tr>
<td>2.1.5.2</td>
<td>Biological evaluation</td>
<td>47</td>
</tr>
<tr>
<td>2.1.5.3</td>
<td>Results and Discussion</td>
<td>47</td>
</tr>
<tr>
<td>2.1.5.4</td>
<td>Conclusions</td>
<td>52</td>
</tr>
<tr>
<td>2.2</td>
<td>Preparation and biological evaluation of SRT 1720 (85)</td>
<td>52</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Synthesis</td>
<td>52</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Synthesis of one triazole-based SRT 1720 analogue (91)</td>
<td>54</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Biological evaluation of SRT 1720 (85) and the analogue 91</td>
<td>55</td>
</tr>
<tr>
<td>2.3</td>
<td>Synthesis and biological evaluation of antagonists of peroxisome proliferator-activated receptor δ</td>
<td>56</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Preparation of GSK 0660 (42)</td>
<td>56</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Preparation of GSK 3787 (43)</td>
<td>56</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Synthesis of a potential new antagonist of PPARδ (103) (Paper IV)</td>
<td>57</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Biological evaluation of GSK 0660 (42), GSK 3787 (43) and 103</td>
<td>58</td>
</tr>
<tr>
<td>2.3.5</td>
<td>Molecular modelling</td>
<td>59</td>
</tr>
<tr>
<td>3.</td>
<td>Summary</td>
<td>61</td>
</tr>
<tr>
<td>4.</td>
<td>Conclusions and future perspectives</td>
<td>63</td>
</tr>
<tr>
<td>5.</td>
<td>APPENDIX</td>
<td>64</td>
</tr>
<tr>
<td>5.1</td>
<td>Experimental: synthesis</td>
<td>65</td>
</tr>
<tr>
<td>5.1.1</td>
<td>Triazole-based analogues of GW 501516 (18). Second series</td>
<td>65</td>
</tr>
<tr>
<td>5.1.2</td>
<td>Triazole-based analogues of GW 501516 (18). Third series</td>
<td>70</td>
</tr>
<tr>
<td>5.1.3</td>
<td>Preparation and biological evaluation of SRT 1720 (85)</td>
<td>74</td>
</tr>
<tr>
<td>5.1.4</td>
<td>Synthesis of triazole-based analogue of SRT 1720 (91).</td>
<td>74</td>
</tr>
<tr>
<td>5.1.5</td>
<td>Preparation of GSK 0660 (42)</td>
<td>76</td>
</tr>
</tbody>
</table>
5.2 Experimental: biology

5.2.1 Measurement of oleic acid oxidation

5.2.1.1 Human myotubes culture

5.2.1.2 Substrate oxidation assay

5.2.1.3 Protein measuring

5.2.1.4 Cell associated radioactivity

5.2.2 Luciferase-based transient transfection assay

5.3 Molecular modelling

References

List of errata

List of papers
1. INTRODUCTION

1.1 Obesity, diabetes and the metabolic syndrome

Obesity and diabetes mellitus are two major health problems affecting the global population. Once considered problems of the developed countries, they have spread to the developing countries as well.\textsuperscript{1,2} According to the World Health Organization, in 2005 approximately 1.6 billion adults were overweight and at least 400 million adults were obese. It is foreseen that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese.\textsuperscript{1} Furthermore, in 2005 it was reported that 1.1 million people died from diabetes mellitus, but the real number is believed to be much higher due to the fact that the cause of death is often recorded as heart disease or kidney failure. By 2030 the number of deaths provoked by diabetes is likely to double.\textsuperscript{2}

The high prevalence of the two conditions drew attention to a set of metabolic disturbances which, in 1998, was defined by the World Health Organization as the metabolic syndrome (MS). From the clinical point of view, MS is described by three out of the five following metabolic derangements: (1) high serum triglyceride level; (2) low serum high-density lipoprotein (HDL) cholesterol level; (3) hypertension; (4) elevated fasting blood glucose; or (5) increased waist circumference.\textsuperscript{3} Furthermore, in recent years MS is increasingly seen as a major factor for both myocardial infarction and stroke, having a great impact on morbidity and mortality.\textsuperscript{3}

Given the gravity of the metabolic syndrome and of the associated conditions, discovering ways to treat them has become of main importance. The discovery of the crucial role of peroxisome proliferator activated receptors (PPARs) in the regulation of the lipid and glucose metabolism has increased the interest in the development of synthetic ligands as potential tools for treating type 2 diabetes and the metabolic syndrome.\textsuperscript{4}
1.2 Peroxisome proliferator activated receptors

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins belonging to the nuclear hormone receptor superfamily. Three isotypes have been identified and characterized: PPARα, PPARδ*, and PPARγ. PPARs are involved in gene expression by interacting with specific DNA response elements (PPRE).5

The overall structure of the isotypes consists of two domains having a central role in biological activity of PPARs: a part called ligand-binding domain (LBD) required for heterodimerization, interaction with transcriptional cofactors and with ligands, and a part which binds to DNA called DNA-binding domain (DBD). The ligand-binding pocket (LBD) is a very large Y-shaped cavity within the protein with a total volume of 1300 to 1400 Å³ and it consists of three branches. The main branch has a polar part at the entrance and a hydrophobic interior. The two smaller ones are represented by arm I, a polar cavity which is involved in hydrogen-bond interactions with the ligand, and arm II which is mainly hydrophobic. The two arms are almost 12 Å in length. Several experimental 3D structures have revealed that the binding site entrance is very flexible suggesting that it can allow rather large ligands to enter the binding pocket without major changes of the structure of the LBD. Sequence comparison shows that DBDs are highly conserved, while LBDs have a slightly lower level of conservation (80%) with large sequence variation regarding the amino acids that line the ligand-binding pocket which is reflected in the fact that each receptor subtype is pharmacologically distinct (Figure 1.1).6

PPARα is expressed in tissues with high catabolic rates of fatty acids and high peroxisomal activity such as liver, kidney, heart, muscle, adipose tissue. The major role of PPARα is the regulation of energy homeostasis. In the liver especially, PPARα activates fatty acid catabolism, stimulates gluconeogenesis and ketone body synthesis, and is also involved in the control of lipoprotein assembly. PPARα also stimulates heme synthesis and cholesterol catabolism. Furthermore, it attenuates inflammatory responses and participates in the control of amino acid metabolism and urea synthesis. Increased fatty acid oxidation by activated PPARα lowers circulating triglyceride levels, liver and muscle steatosis, and reduces adiposity, which improves insulin sensitivity. In addition, PPARα has demonstrated

* Note: PPARδ is sometimes named PPARβ/δ.
significant anti-inflammatory activities that seem to play a role in its protective actions within the cardiovascular system.\textsuperscript{7}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{A) Structural superposition of PPAR\(\alpha\) (white), PPAR\(\delta\) (magenta) and PPAR\(\gamma\) (cyan). B) 3D structure of PPAR\(\alpha\). C) 3D structure of PPAR\(\delta\). The binding site cavity is shown in white. D) 3D structure of PPAR\(\gamma\).\textsuperscript{6}}
\end{figure}

PPAR\(\gamma\) is expressed in all tissues, including heart, muscle, colon, kidney, pancreas, large intestine and white adipose tissue. It has been reported as an important factor in adipose tissue differentiation and in maintaining adipocyte specific functions, such as lipid storage in the white adipose tissue. In addition, PPAR\(\gamma\) is involved in glucose metabolism by improving
insulin sensitivity. PPARγ activation seems to limit inflammation, playing a possible role in limiting atherosclerosis and/or diabetes.7

PPARδ is overly expressed, but especially in brain, adipose tissue, and muscle. It is involved in the control of energy homeostasis by stimulating genes involved in fatty acid catabolism. In addition, PPARδ has an important role in the control of cell proliferation, differentiation, and survival and is involved in tissue repair.7 Activation of PPARδ was reported to decrease hepatic glucose output (gluconeogenesis) and to inhibit free fatty acids (FFA) release from adipocytes.8

1.3 Molecules targeting the peroxisome proliferator-activated receptors

1.3.1 Natural ligands of the peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) were initially thought to be orphan of natural ligands, but shortly it was discovered that they respond to endogenous fatty acids and eicosanoids.9-12

PPARα binds to different natural ligands such as leukotrienes and prostaglandins. Besides that, PPARα is activated by a variety of long fatty acids, such as palmitic acid (1) and oleic acid (2), and polyunsaturated fatty acids (PUFAs), like linoleic acid (3), linolenic acid (4), arachidonic acid (5), and docosahexaenoic acid (6) (Figure 1.2).10,12,13

![Figure 1.2. Structures of PPARα natural ligands](image)

PPARδ is activated by fatty acids such as 1 and 2 as well as by polyunsaturated fatty acids (PUFAs) 3 to 6, and also by prostaglandins such as PGA1(7) and PGD2 (8) (Figure 1.3).10,12-14
PPARγ was shown to be weakly activated by PUFAs 3-6 and prostaglandins 7 and 8 (Figures 1.2, 1.3). Recently, a prostaglandin derivative, 15d-PGJ\(_2\) (9), and an oxidized phospholipid, hexadecyl azelaoyl phosphatidylcholine (10) (Figure 1.4) were discovered to activate PPARγ at 20–50 nM concentrations.9

1.3.2 Synthetic ligands of peroxisome proliferator-activated receptors

Typically, a synthetic PPAR agonist is composed of an acidic head, a moiety recurrent in the structure of natural ligands as well, which is carried by an aromatic ring attached to a linker varying in length and with high flexibility. The linker is connected to a hydrophobic tail group which can be an aromatic or a heterocyclic ring (Figure 1.5). An agonist acts on the receptor through different types of chemical features: aromatic rings, hydrogen bond donors/acceptors, polar groups, and hydrophobic moieties.15
1.3.2.1 Peroxisome proliferator-activated receptors α agonists

A group of molecules which were extensively studied for their PPARα activation properties is represented by fibrate class of drugs. These molecules showed good effects in decreasing the low density lipoprotein (LDL) fraction rich in cholesterol and the very low density lipoprotein (VLDL) fraction rich in triglycerides. In addition, they increased the high density lipoprotein (HDL) cholesterol fraction.\textsuperscript{16}

Fibrates marketed as drugs are represented by: benzafibrate (11),\textsuperscript{17} ciprofibrate (12),\textsuperscript{18} gemfibrozile (13),\textsuperscript{16,19} clofibrate (14),\textsuperscript{18} and fenofibrate (15).\textsuperscript{17} Structurally, the dimethyl α-substituted carbon atom to the carboxylic moiety is common to all the presented fibrates. Phenoxyphenyl was preferred as the carrier of the acidic moiety; beside that, two atoms seemed to be the preferred length between carboxyl group and benzene ring in most of the chemical structures of the fibrates (Figure 1.6).

These structural features are recurrent at the compounds published in 2003 by Nomura et al.\textsuperscript{20} Following an extensive activity relationship study (SAR), they discovered that compound 16 possessed high potency against PPARα (EC\textsubscript{50} = 0.06 μM). Nomura underlined that introduction of a substituent in the α-position to the carboxyl group was important for PPARα transactivation activity and subtype selectivity: ethyl group was preferred, since bulkier substituents led to decreased potency. Additionally, the distance between the carboxyl group and the benzene ring was important: the preferred distance appeared to correspond to two carbon atoms. Besides that, the authors noticed a strong enantio-dependency of the transactivation activity towards PPARα with the (S)-enantiomer (16) being the more potent one with EC\textsubscript{50}-value of 0.06 μM (Figure 1.6).\textsuperscript{20}

![Figure 1.5: General structure of a PPAR agonist](image-url)
1.3.2.2 Peroxisome proliferator-activated receptors δ agonists

Synthetic selective and potent agonists for PPARδ were reported rather recently. In early research efforts, all published ligands had either low affinity for PPARδ, or lacked subtype selectivity.

In 2003, following the screening of existing libraries containing lipophilic carboxylic acids, a group from GlaxoSmithKline discovered compound 17 (Figure 1.7) as having nanomolar activity against PPARδ. SAR studies were conducted to the different parts of the molecule: acidic moiety (A), aromatic ring (B), linker (C) and cyclic tail (D) (Figure 1.7). At the acidic moiety level (A), replacement of the propanoic acid with the isosteric oxyacetic acid proved to be useful in order to block β-oxidation. The methyl group in the ortho-position of the aromatic ring (B) enhanced PPARδ activity; this was supported by crystallographic studies showing that small substituents could be well hosted by the lipophilic pocket of PPARδ LBD. A thiazole ring and a sulphur atom were preferred as parts of the linker (C). The addition of a fluorine atom in ortho-position to the CF₃ group maintained the activity and the selectivity toward PPARδ. These modifications led to the discovery of two very potent selective PPARδ agonists: GW 501516 (18) and GW 0742 (19) (Figure 1.7). Compounds 18 and 19 exhibited EC₅₀-values towards PPARδ of 1 nM, towards PPARα of 1.1 μM, and towards PPARγ of 0.85 μM and 2 μM, respectively.²¹
1.3.2.3 Peroxisome proliferator-activated receptors $\gamma$ agonists

Molecules acting as agonist on PPAR$\gamma$ exhibit biological effects especially related to the glycemic control by increasing insulin sensitivity.$^{16}$ Lately, studies revealed the effects of rosiglitazone (20) and pioglitazone (21) (Figure 1.8), compounds belonging to the thiazolidinedione class of drugs well known for their PPAR$\gamma$ agonist action, on reduction of LDL and elevation of HDL levels.$^{22,23}$

Thieme et al.$^{24}$ reported in 2010 new PPAR$\gamma$ agonists based on pirinixic acid (22) (Figure 1.8) which was known for its moderately dual PPAR$\alpha$/y agonistic effects. The authors stressed the importance of the substitution alpha to the carboxylic moiety of 22 for increasing the selectivity towards the $\gamma$ subtype. $\alpha$-Substitution increased the activation of PPAR$\alpha$ as well, but it was the size of the substituent that dictated the preference for PPAR$\gamma$. According to crystallographic data, the LBD of PPAR$\gamma$ was observed to be larger than the one of PPAR$\alpha$. Hence, bulkier aromatic residues can be better accommodated. Bulky substitutes appeared to be important as well at the disubstituted amine level: replacement of the 2,3-dimethylphenyl residue with less bulkier aliphatic linear chains led to a total loss of the PPAR$\gamma$ activity. These modifications led to compound 23 (Figure 1.8) reported as the most potent out of the published compounds.$^{24}$

Figure 1.7. Structures of the first selective and potent PPAR$\delta$ agonists.
1.3.2.4 Dual peroxisome proliferator-activated receptor agonists

PPAR dual agonists are envisioned as a new class of molecules that should combine the therapeutic benefits without the side effects of the selective modulators.

1.3.2.4.1 Dual PPARα/δ agonists

Dual PPARα/δ agonists are expected to display properties like decreasing hyperlipidemia, insulin resistance and reducing risk of atherogenesis. Even if some agonists have been synthesized there is no available information on their clinical activities.

Kasuga et al. and Shen et al. developed new dual PPARα/δ agonists (Figure 1.9: compounds 24 and 25, compounds 26 and 27) from a selective PPARα agonist and a selective PPARδ agonist, respectively. Structurally, experimental data showed the importance of the substitution at the α-position to the carboxylic group for PPARα activation. The connector of the aromatic ring which carries the acidic head to the linker should be a conformationally flexible group of atoms or atom. It was noticed that the methylene group and sulphur atom were preferred to oxygen atom. Like in the case of PPARγ/δ dual agonists, the presence of lipophilic groups or atoms (F, Cl, CF₃, OCF₃) proved to be important for PPAR activation (Figure 1.9). Kasuga noticed an enantio-dependency of the transactivation activity towards PPARα and PPARδ. The (S)-enantiomer proved to be the more potent stereoisomer ((R)-24: EC₅₀α = 150 nM, EC₅₀δ = 840 nM, and (S)-24: EC₅₀α = 12 nM, EC₅₀δ = 23 nM).
1.3.2.4.2 Dual PPARα/γ agonists

Simultaneous activation of PPARα and PPARγ should alter the tissues distribution of free fatty acids by stimulating their uptake and utilisation in adipose tissue, liver and skeletal muscle. Published data confirmed their ability to reduce triglycerides, raise HDL levels and improve insulin sensitivity.4,8,30,31

Molecules that act as dual PPARα/γ agonists prove to be structurally diverse: glitazars (28), thiazolidinediones (29), azole acids (30)33 and pirinixic acids (31)34 (Figure 1.10).

Zettl et al.34 reported in 2009 modifications of pirinixic acid (22) (Figure 1.8) leading to new dual PPARα/γ agonists. Compound 22 was chosen due to its known moderately dual PPARα/γ agonistic effects. In order to increase the activation towards both subtypes, different substituents were introduced at the α-position to the carboxylic moiety. Out of a series of aliphatic substituents, n-hexyl activated both receptors in a balanced way. Substitution of the 2,3-dimethylphenyl residue with bulkier moieties (4-phenyl-benzonitrile) seemed to increase the activation of the two receptors even further (compound 31: EC50α = 0.19 μM, EC50γ = 1.5 μM). As in the case of dual PPARα/δ agonists, a strong enantio-dependency was noticed with the (R)-enantiomer being the more potent one ((R)-31: EC50α = 0.03 μM, EC50γ = 1.1 μM, and (S)-31: EC50α = 2.2 μM, EC50γ = 3.4 μM).34
1.3.2.4.3 Dual PPARγ/δ agonists

Dual PPARγ/δ agonists are not fully biologically understood due to the relatively small number of compounds reported and that the physiological role of PPARδ is not totally clarified. Still, it was envisioned they should improve dyslipidemia in diabetes mellitus type 2 and influence the development of atherosclerosis by stimulating reverse cholesterol transport. Moreover, given the effect of PPARδ on the fatty acid oxidation, it is believed that the weight gain observed in the case of using PPAR γ modulators could be avoided.35

Three different groups from Lilly Research Laboratories, GlaxoSmithKline, and Bayer Pharmaceuticals Corporation reported five potent PPARδ/γ dual agonists (Figure 1.11).35-39 Linear or branched aliphatic acid residues with three and four carbon atoms were preferred as acidic moiety as observed for compounds 32-35. Benzene rings with aliphatic ortho-substituents to the acidic head are recurrent chemical features: ethyl group (compounds 32-34) is thought to play a protective role against of β-oxidation of the acidic moiety by steric hindrance.35,36 Aliphatic chains carrying a stereocenter were used as linkers: substitution of a hydrogen atom with a methyl group, creating a chiral carbon atom (compounds 32-34), proved to be important for enhancing PPARγ activity.35 Generally, as hydrophobic tail, aromatic rings bearing lipophilic atoms or group of atoms (Cl, CH3, C2H5, CF3) were desirable for enhancing dual PPARγ/δ activity as observed for compounds 32-36 (Figure 1.11).35,36

Figure 1.10. Structures of different PPARα/γ dual agonists.
1.3.2.5 Pan peroxisome proliferator-activated receptors agonists

A pan PPAR agonist has to modulate all three PPAR isoforms and to exhibit balanced activation on each subtype for the biological responses to be equilibrated.\textsuperscript{15} Kasuga\textit{ et al.}\textsuperscript{40} reported a potent pan agonist, compound 37 (Figure 1.12), developed from a substituted \(\alpha\)-phenylpropanoic acid scaffold that was successfully used to discover new selective and dual agonists. The bulky substituent from the 4-position of benzene ring B proved to be important for enhancing the activation of all PPAR isoforms. The adamantyl moiety was shown to be the best adapted by the hydrophobic pocket of PPAR ligand-binding domain. As in the case of dual agonists, the (\(S\))-enantiomer proved to be more potent than the (\(R\))-enantiomer ((\(S\))-37: \(EC_{50}\alpha = 61\) nM, \(EC_{50}\delta = 120\) nM, \(EC_{50}\gamma = 43\) nM, and (\(R\))-37: \(EC_{50}\alpha = 500\) nM, \(EC_{50}\delta = 870\) nM, \(EC_{50}\gamma = 83\) nM).

Out of the glitazars class of drugs, sodelglitazar (38) and indeglitazar (39), showed pan PPAR agonistic effects (Figure 1.12).\textsuperscript{41,42} Common structural features with compound 37 can be noticed: \(\alpha\)-substitution to the carboxylic group (compound 38) and lipophilic substituents on the hydrophobic tail.
1.3.3 Peroxisome proliferator-activated receptors antagonists

The search for synthetic ligands acting on the peroxisome proliferator-activated receptors focused essentially on developing agonists. These compounds proved to be interesting due to the metabolic pathways they trigger.

Lately, scientists became interested in molecules exhibiting antagonistic effects on PPARs due to their potential both as a pharmacological tool for proving new information about PPARs. Moreover, they exhibited unexpected pharmacological activity in several animal disease models.43

GW 6471 (40) (Figure 1.12) was reported in 2002 by a group from GlaxoSmithKline to be a potent antagonist towards PPARα with an IC50-value of 0.24 μM.44 GW 6471 (40) manifested its antagonistic effects by blocking PPARα to assume the biologically active conformation.43 One of the applications of the antagonist was a tool in elucidating the role of PPARα in cardiomyocyte differentiation in the heart during embryonic development.45

PPARγ antagonists showed their potential in possible treatments of obesity46 and of breast cancer.47 GW 9662 (41) (Figure 1.12) proved to be a selective full antagonist of PPARγ with an IC50-value of 3.3 nM. The molecule binds covalently to the cysteine residues in the ligand-binding pocket blocking PPARγ to interact with other ligands.48
The development of molecules as antagonist for PPARδ started recently. In 2008 and 2010, a group of researchers from GlaxoSmithKline reported two antagonist with a high selectivity for PPARδ: GSK 0660 (42) and GSK 3787 (43) (Figure 1.12). GSK 0660 (42) was identified via a high-throughput screening of the GSK compound collection and it was reported as the first selective antagonist ligand for PPARδ. This compound proved to be a potent antagonist towards the δ-subtype with an IC50-value in an antagonist assay of 300 nM. Since GSK 0660 (42) lacked oral bioavailability, Shearer et al. continued their screening. Later they identified GSK 3787 (43) that proved to be a powerful antagonist towards δ-subtype. Compound 43 blocked the LBD of PPARδ by binding covalently to the Cys249 within. The molecule showed good oral pharmacokinetic properties.

Elikkottil et al. published in 2009 a review commenting on the possibility of using PPARδ antagonists as potential anticancer agents of the liver, lung and colorectal malignancies.

Figure 1.12. Chemical structure of antagonists.
1.4 “Click chemistry” in drug discovery

Drug discovery is an effervescent field demanding new and better methodologies to generate collections of compounds for screening. A computational study performed by the group of Bohacek estimated a number of \(10^{63}\) “drug-like” compounds.\(^5\) Given the very large number of structures, it is likely that the synthetic chemistry has to move from the classical and tedious syntheses to faster ones in order to obtain new drug candidates.

“Click chemistry”, a concept conceived by Sharpless in 2001, encompasses simple, efficient, selective, and versatile chemical transformations.\(^5\) A chemical reaction can be classified as a “click reaction” if it fulfills certain criteria: “A click reaction must be of wide scope, giving consistently high yields with a variety of starting materials. It must be easy to perform, be insensitive to oxygen or water, and use only readily available reagents. Reaction work-up and product isolation must be simple, without requiring chromatographic purification”\(^5\)

There have been identified some classes of reactions which can be named “click reactions”:

- cycloaddition reactions: 1,3-dipolar cycloadditions and hetero-Diels-Alder reactions
- nucleophilic ring-opening reactions of strained heterocyclic electrophiles (epoxides, aziridines)
- carbonyl chemistry of non-aldol type: formation of oxime ethers, hydrazones and aromatic heterocycles
- addition to carbon-carbon multiple bonds: epoxidations, dihydroxylation, aziridination and some Michael addition reactions.\(^5\)

Huisgen’s 1,3-dipolar cycloadditions of alkynes and azides affords a mixture of approximately 1:1 of 1,4- and 1,5-disubstituted 1,2,3-triazoles requiring heating and long reaction times.\(^5\)

The mechanism proposed for the Huisgen cycloaddition involves a concerted mechanism with two \(\sigma\)-bonds formed simultaneously (Figure 1.1).\(^5,6\)
In 2002, two groups reported the use of copper (I) salts in Huisgen cycloaddition. Cu(I)-catalyzed reaction between azide and alkyne yields exclusively the 1,4-dibsubstituted regioisomer.\textsuperscript{57,58} This modification turned Huisgen’s reaction into a good example of “click chemistry” reaction type: readily available starting materials, no use of protecting groups, highly diverse, reliable, biocompatible, complete and selective conversion.\textsuperscript{59}

Copper-(I)-catalyzed Huisgen’s 1,3-dipolar cycloaddition proved to be an useful tool in natural products modifications and \textit{in situ} chemistry.\textsuperscript{60-62} Synthesis of neoglycoconjugates, fucosyltransferase inhibitors and development of HIV protease inhibitors are few examples of the use of Huisgen’s reaction.\textsuperscript{53}

\section*{1.5 Aim of the study}

As previously mentioned, obesity and diabetes mellitus are two major health problems affecting the global population both in developed and developing countries. The crucial role of peroxisome proliferator activated receptors (PPARs) in the regulation of the lipid and glucose metabolism has indicated them as potential targets for treating type 2 diabetes and the metabolic syndrome.

The aims of this study have been:

I. To discover new selective and dual PPAR agonists by:
a. synthesizing 1,4-disubstituted 1,2,3-triazole analogues of GW 501516 (18) and submitting them to the biological testing using the oleic acid oxidation assay and the luciferase-based transient transfection assay, along with the synthesis of GW 501516 (18) as a standard for the biological assays.

b. synthesizing thiazole-based analogues of GW 501516 (18) and submitting them to the biological testing along with the molecular modelling of a selected analogue.

c. synthesizing mono fluorinated thiazole- and triazole-based analogues of GW 501516 (18) and submitting them to the biological testing.

II. To prepare and to biologically evaluate a new potential antagonist of PPARδ.
2. RESULTS AND DISCUSSIONS

2.1 PPAR agonists

The aim was to synthesize new molecules with potential agonistic effects towards PPARs using GW 501516 (18) as a lead compound (Figure 2.1). Compound 18 was found interesting since it has been reported to be a both potent and selective agonist of the PPARδ receptor.21 Furthermore, experimental biological data revealed that treatment with GW 501516 (18) led to an increase of the level of plasma HDL-cholesterol and a reduction of the level of plasma triglyceride.63

2.1.1 Triazole-based analogues of GW 501516 (18). First series (Paper I)

Our interest focused on modifications of the thiazole moiety and of the R1 and R2 of the benzene ring B (Figure 2.1). Modifications of the thiazole linker were reported by Shen et al.27,28 They synthesized several PPARα/δ dual agonists where the 1,2,4-thiadiazole moiety replaced the methylthiazole ring in 18. Based on Grimm’s bioisosteric rule64,65 we envisioned that 1,2,3-triazoles and the methylthiazole group in GW 501516 (18) might be bioisosteric groups. Recently, triazoles have been used to mimic amides, in modifications of natural products, in library syntheses and in situ library screening.60-62,66,67

Figure 2.1. Structures of GW 501516 (18) and prepared triazoles.
2.1.1.1 Synthesis

The synthetic approach towards potential agonists 52a-52j (Table 2.1) started with the synthesis of the azides 45a-45j from the anilines 44a-44j using standard diazotization conditions (Scheme 2.1).67

\[
\text{NH}_2 \quad \overset{a}{\longrightarrow} \quad \text{N}_3
\]

44a-44j

\[
\begin{align*}
45a & : R_1 = H, R_2 = H, (36\%) \\
45b & : R_1 = H, R_2 = \text{CH}_3, (41\%) \\
45c & : R_1 = F, R_2 = \text{CH}_3, (70\%) \\
45d & : R_1 = H, R_2 = \text{CF}_3, (55\%) \\
45e & : R_1 = F, R_2 = \text{CF}_3, (46\%) \\
45f & : R_1 = H, R_2 = \text{OCH}_3, (11\%) \\
45g & : R_1 = F, R_2 = \text{OCH}_3, (49\%) \\
45h & : R_1 = H, R_2 = \text{OCF}_3, (57\%) \\
45i & : R_1 = \text{Cl}, R_2 = \text{OCF}_3, (77\%) \\
45j & : R_1 = F, R_2 = \text{OCF}_3, (49\%)
\end{align*}
\]

Scheme 2.1. (a) NaNO₂, NaN₃, H₂O, HCl.

Para-mercaptophenol 48 was prepared according to a literature procedure which consisted in bromination of phenol 46 followed by the reaction with NaSCN to give 47 in 98% yield. Thiocyanate 47 was reduced with LiAlH₄ to the thiol 48 in 97% yield. (Scheme 2.2).68

\[
\begin{align*}
\text{OH} & \quad \overset{a}{\longrightarrow} \quad \text{OH} \quad \overset{b}{\longrightarrow} \quad \text{OH} \\
46 & \quad 98\% & \quad 97\% & \quad 48
\end{align*}
\]

Scheme 2.2. (a) Br₂, NaBr, NaSCN, MeOH; (b) LiAlH₄, THF.

Thiol 48 was alkylated with propargyl bromide affording the phenolic sulphide 49. Alkylation of phenol 49 with ethyl bromoacetate produced terminal alkyne 50 in 50% yield over the two steps (Scheme 2.3).68
Scheme 2.3. (a) Propargyl bromide, Cs$_2$CO$_3$, CH$_3$CN; (b) Ethyl bromoacetate, Cs$_2$CO$_3$, CH$_3$CN.

Reaction of terminal alkyne 50 with azides 45a-45j in the presence of catalytic amounts of copper(I) as described by Sharpless _et al._$^{57}$ yielded triazole-based esters 51a-51j. Basic aqueous hydrolysis of esters 51a-51j afforded the target compounds 52a-52j (Scheme 2.4). The yields over the two reaction steps to 1,2,3-triazoles 52a-52j were 28-63%.

Scheme 2.4. (a) Sodium ascorbate, CuSO$_4$, tert-BuOH, H$_2$O; (b) LiOH, THF, H$_2$O.

2.1.1.2 Biological evaluation

GW 501516 (18) and 1,4-disubstituted 1,2,3-triazoles 52a-52j, at five different concentrations, were exposed for 96 h to fully differentiated human skeletal muscle cells.
cultured in 96-well plates. After this period of time, the level of oxidation of oleic acid (\((9Z)\)-octadec-9-enoic acid) was measured by detection of the accumulation of $^{14}$C-labeled oxidized oleic acid. The results from the oxidation of $^{14}$C-labeled oleic acid assay of the triazoles 52a-52j are compiled in Figure 2.2. The EC$_{50}$-values for the five triazoles 52d, 52e, 52i, 52j, and 52h were obtained from Figure 3 (Table 2.1). Compounds 52e and 53i were also tested against the three peroxisome proliferator activated receptors (PPAR$\alpha$, PPAR$\delta$ and PPAR$\gamma$) in a luciferase-based transient transfection assay (Figures 2.4).

Table 2.1. Substitution pattern (see Figure 2.1) and EC$_{50}$-values of prepared compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>EC$_{50}$(nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>52a</td>
<td>H</td>
<td>H</td>
<td>n.d.$^b$</td>
</tr>
<tr>
<td>52b</td>
<td>H</td>
<td>CH$_3$</td>
<td>n.d.</td>
</tr>
<tr>
<td>52c</td>
<td>F</td>
<td>CH$_3$</td>
<td>n.d.</td>
</tr>
<tr>
<td>52d</td>
<td>H</td>
<td>CF$_3$</td>
<td>1.80</td>
</tr>
<tr>
<td>52e</td>
<td>F</td>
<td>CF$_3$</td>
<td>0.85</td>
</tr>
<tr>
<td>52f</td>
<td>H</td>
<td>OCH$_3$</td>
<td>n.d.</td>
</tr>
<tr>
<td>52g</td>
<td>F</td>
<td>OCH$_3$</td>
<td>n.d.</td>
</tr>
<tr>
<td>52h</td>
<td>H</td>
<td>OCF$_3$</td>
<td>10.0</td>
</tr>
<tr>
<td>52i</td>
<td>Cl</td>
<td>OCF$_3$</td>
<td>12.0</td>
</tr>
<tr>
<td>52j</td>
<td>F</td>
<td>OCF$_3$</td>
<td>1.4</td>
</tr>
<tr>
<td>GW 501516 (18)</td>
<td>-</td>
<td>-</td>
<td>0.07</td>
</tr>
</tbody>
</table>

$^a$ Results of three replicates

$^b$ n.d., not determined: EC$_{50}$-value was not calculated when the curve didn’t flatten

2.1.1.3 Results and Discussion
The results showed increased oxidation of oleic acid when the cells were treated with 1,4-disubstituted 1,2,3-triazoles 52a-52j and GW 501516 (18) (Table 2.1, Figures 2.2 and 2.3). Compound 18 proved to be more potent than triazoles 52a-52j with an EC$_{50}$-value of 0.07 nM.
(Table 2.1). The most potent triazoles were 52d, 52e and 52j with EC\textsubscript{50}-values of 1.8 nM, 0.85 nM, and 1.4 nM, respectively (Table 2.1, Figures 2.2A-C, Figure 2.4A).

![Figure 2.2](image-url)

**Figure 2.2.** Oxidation of oleic acid in human myotubes in the presence of 1,4-disubstituted 1,2,3-triazoles 52d, 52e and 52j at five different concentrations. GW 501516 (18) was used as positive control.

Triazoles 52h and 52i exhibited a more than 100 times lower potency compared to lead GW 501516 (18) with EC\textsubscript{50}-values of 10 nM and 12 nM, respectively (Table 2.1, Figures 2.2A,B, Figure 2.4B).

![Figure 2.3](image-url)

**Figure 2.3.** Oxidation of oleic acid in human myotubes in the presence of 1,4-disubstituted 1,2,3-triazoles 52a-52e and 52f-52i at different concentrations. GW 501516 (18) was used as positive control.
Figure 2.4. Non-linear fit curves of oxidation of oleic acid in human myotubes in the presence of different concentrations of 1,4-disubstituted 1,2,3-triazoles 52d, 52e, 52j (A); 52i and 52h (B). GW 501516 (18) was used as positive control.

The effects of triazoles 52e and 52i, at 10 μM concentration, exercised on the three different peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) in a luciferase-based transient transfection system were investigated as well. Triazole 52e activated both PPARα and PPARδ with similar efficacy as the known PPARα agonist (2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid (EHA), but with an enhanced efficacy compared to GW 501516 (18) (Figure 2.5). Triazole 52i seemed to be as effective at this concentration as EHA towards PPARα, while having lower efficacy towards PPARδ as compound 18 (Figure 2.5). Compounds 52e and 52i did not appear to activate PPARγ (Figure 2.5).
2.1.1.4 Conclusions

The replacement of the thiazole ring of GW 501516 (18) with 1,4-disubstituted 1,2,3-triazoles led to two new analogues of 18 that, at 10 μM concentration, showed dual PPARα/δ agonistic effects.

Recently, dual agonists received an increased interest since they could be an useful tool in the treatment of several diseases such as metabolic disorders, type 2 diabetes and cardiovascular diseases. Dual activation of PPARα and PPARδ could combine the decrease of the levels of triglycerides in blood, due to PPARα, with the enhancement of both the fatty acid metabolism and the HDL cholesterol level, due to PPARδ. A few potent PPARα/δ dual agonists have been reported in the literature.8,28,29
2.1.2 Triazole-based analogues of GW 501516 (18). Second series

Since efficient synthesis was achieved for triazoles 52a-52i, we wanted to prepare a second series of triazole based analogues of GW 501516 (18). Modifications were performed at the level of benzene ring A by substituting the methyl moiety with a hydrogen atom and inserting different substituents on benzene ring B (Figure 2.6).

Figure 2.6. Structures of the prepared analogues 55a-55j

2.1.2.1 Synthesis

The first step in the 1,4-disubstituted 1,2,3-triazoles 55a-55i synthesis selective alkylation, using a one pot procedure, of the thiol-, and the phenolic group of compound 53 with propargyl bromide and with ethyl bromoacetate, respectively, to the terminal alkyne 54 in 55% yield (Scheme 2.5).68

Scheme 2.5. (a) Propargyl bromide, Cs₂CO₃, CH₃CN; (b) Ethyl bromoacetate, Cs₂CO₃, CH₃CN.
A convenient one pot procedure was used to synthesize final compounds 55a-55i: the copper(I) catalyzed reaction between terminal alkyne 54 and azides 45a-45i was followed by basic aqueous hydrolysis. Total yields of the one pot procedure were 2-23% (Scheme 2.6). This synthetic approach proved to be about as efficient the one applied for the syntheses of the triazoles 52a-52i (2-17%).

Scheme 2.6. (a) Sodium ascorbate, CuSO₄, tert-BuOH, H₂O; (b) LiOH, THF, H₂O.

2.1.2.2 Biological evaluation

GW 501516 (18) and the 1,4-disubstituted 1,2,3-triazoles 55a-55i were biologically evaluated as agonists as described in subchapter 2.1.1.2. The results from the oxidation of ¹⁴C-labeled oleic assay of the triazoles 55a-55i are compiled in Figure 2.7. Compound 55i was tested against the three peroxisome proliferator activated receptors (PPARα, PPARδ and PPARγ) in a luciferase-based transient transfection assay (Figure 2.8, Table 2.2).
2.1.2.3 Results and Discussion
Triazoles 55a-55i increased the oxidation of oleic acid in the cultured cells, but they proved to be less potent than GW 501516 (18) and compounds 52a-52i. The most potent triazole 55i exhibited a 600 times lower potency than the lead compound GW 501516 (18) (Table 2.2, Figure 2.7) and a 6 times lower potency than the analogue bearing an ortho-methyl substituent on benzene ring A, compound 52i (EC50 = 12.0 nM).

Table 2.2. Substitution pattern (see Figure 2.1) and EC50-values of prepared compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
<th>EC50(nM)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>55a</td>
<td>H</td>
<td>H</td>
<td>n.d.</td>
</tr>
<tr>
<td>55b</td>
<td>H</td>
<td>CH3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55c</td>
<td>F</td>
<td>CH3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55d</td>
<td>H</td>
<td>CF3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55e</td>
<td>F</td>
<td>CF3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55f</td>
<td>H</td>
<td>OCH3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55g</td>
<td>F</td>
<td>OCH3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55h</td>
<td>H</td>
<td>OCF3</td>
<td>n.d.</td>
</tr>
<tr>
<td>55i</td>
<td>Cl</td>
<td>OCF3</td>
<td>76.2</td>
</tr>
<tr>
<td>GW 501516 (18)</td>
<td>-</td>
<td>-</td>
<td>0.12</td>
</tr>
</tbody>
</table>

aResults of three replicates
b n.d., not determined

Experimental data from the luciferase-based transient transfection assay showed that, at 10 μM concentration, compound 55i was medium effective towards PPARα (Figure 2.8A). The efficacy of the triazole 55i towards PPARδ was lower than that of the positive control: only 40% of the activation induced by GW 501516 (18) was observed (Figure 2.8B). No activation of PPARγ was noticed at 10 μM concentration (Figure 2.8C). Compound 55i was as well less effective than 52i against all PPAR subtypes.
Figure 2.7. Oxidation of oleic acid in human myotubes in the presence of 1,4-disubstituted 1,2,3-triazoles 55a-55i at different concentrations. GW 501516 (18) was used as positive control.

Figure 2.8. Activation of the ligand-binding domain of PPARα (A), PPARδ (B), PPARγ (C) by compound 55i in a luciferase-based transfection assay. Positive controls: PPARα: EHA ((2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid); PPARδ: GW 501516 (18); PPARγ: Rosiglitazone (BRL).
These results revealed the importance of the ortho-methyl substituent for the activation of PPARs. Sznaidman et al.\textsuperscript{21} also reported a reduction of the efficacy for their thiazole-based agonists when an ortho-methyl group on a corresponding benzene ring (A) was replaced by a hydrogen atom.

2.1.2.4 Conclusions
Substitution of the methyl group with a hydrogen atom led to a significant decrease of the potency compared to the lead compound as well as to the analogues bearing an ortho-methyl group on the benzene ring A. A significant reduction of the efficacy in luciferase-based transient transfection was also observed.

2.1.3 Thiazole based-analogues of GW 501516 (18) (Paper II)
The goal was to perform structural modifications at the alpha-carbon atom to the carboxylic acid moiety and at the ortho-position of the benzene ring A of GW 501516 (18) (Figure 2.9). As of today, only few analogues of GW 501516 (18) with modifications at the alpha-carbon atom to the carboxylic acid moiety have been reported.\textsuperscript{28,29}

![Figure 2.9. Structures of GW 501516 (18) and prepared analogues 62a-62k.](image-url)
2.1.3.1 Synthesis

Compounds 57a-57e were synthesized using a one pot procedure comprising bromination of the phenols 56a-56e followed by treatment with NaSCN (Scheme 2.7).68

![Scheme 2.7](image)

\[
57a: R_1 = \text{Ethyl, (70%)} \\
57b: R_1 = \text{Isopropyl, (81%)} \\
57c: R_1 = \text{tert-Butyl, (68%)} \\
57d: R_1 = \text{Cyclopentyl, (83%)} \\
57e: R_1 = \text{Cyclohexyl, (61%)}
\]

Scheme 2.7. (a) Br₂, NaBr, NaSCN, MeOH.

Thiocyanates 57a and 57b were reduced with LiAlH₄ to mercaptophenols 58a and 58b in 71-88% yield (Scheme 2.8). 4-Mercapto-2-methylphenol (48), 58a and 58b, respectively, were reacted with 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (59) in the presence of Cs₂CO₃ at r.t. affording 60a-60c in 69-96% yield. Sulphur-substituted para-mercaptophenols 60a-60c were then treated with the corresponding ethyl 2-bromoesters in the presence of Cs₂CO₃ to yield esters 61a-61h (Scheme 2.9).

![Scheme 2.8](image)

\[
58a: R_1 = \text{Ethyl, 71%} \\
58b: R_1 = \text{Isopropyl, 88%}
\]

Scheme 2.8. (a) LiAlH₄, THF;
Scheme 2.9. (a) Cs$_2$CO$_3$, CH$_3$CN; (b) Ethyl 2-bromoesters, Cs$_2$CO$_3$, CH$_3$CN.

Basic aqueous hydrolysis of thiazole-based esters 61a-61h afforded the final compounds 62a-62h in 35-66% yields (Scheme 2.10). The hydrolysis of the esters bearing two methyl groups on alpha-carbon to the carboxylic moiety (61b, 61e and 61h) was performed using an aqueous solution of (CH$_3$)$_3$COK. Only the racemates of 62a, 62d and 62g were prepared.

Scheme 2.10. (a) (i) LiOH, THF, H$_2$O; (ii) (CH$_3$)$_3$COK, THF, H$_2$O, reflux.
Since the treatment of thiocyanates 57c-57e with LiAlH₄ afforded large quantities of the corresponding disulphide dimers, a different approach was used in order to synthesize compounds 62i-62k.

First, an alkylation reaction with ethyl 2-bromoacetate in the presence of Cs₂CO₃ of compounds 57c-57e was performed, followed by the reduction with NaBH₄ and 1,4-dithioerythritol to afford 64a-64c in 61-77% yield (Scheme 2.11).

Scheme 2.11. (a) Ethyl 2-bromoacetate, Cs₂CO₃, CH₃CN; (b) NaBH₄, 1,4-dithioerythritol, EtOH.

Oxygen-substituted para-mercaptophenols 64a-64c were alkylated with 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (59) to produce esters 61i-61k which after basic aqueous hydrolysis afforded acids 62i-62k in 23-41% yield over the two steps (Scheme 2.12).

Scheme 2.12. (a) Cs₂CO₃, CH₃CN; (b) LiOH, THF, H₂O.
2.1.3.2 Biological evaluation

GW 501516 (18) and compounds 62a-62k were biologically evaluated as described in chapter 2.1.1.2. The EC50-values for compounds 62a-62k are presented in Table 2.3.

2.1.3.3 Results and Discussions

The lead compound GW 501516 (18) was highly potent with EC = 0.10 nM in the human skeletal muscle cell assay. Substitution of the ortho-methyl group attached to the benzene ring A of GW 501516 (18) with ortho-substituents increasing in size, from methyl, ethyl, isopropyl, tert-butyl, cyclopentyl to cyclohexyl, led to reduction in potency (62c: EC50 = 4.15 nM, 62f: EC50 = 4.15 nM, 62i: EC50 = 5.51 nM, 62j: EC50 = 16.60 nM, 62k: EC50 = 17.30 nM) (Table 2.3). A similar pattern was noticed for compounds (±)-62d and (±)-62g, and 62b, 62e and 62h, respectively ((±)-62d: EC50 = 0.36 nM and (±)-62g: EC50 = 5.79 nM; 62b: EC50 = 0.24 nM, 62e: EC50 = 0.54 nM and 62h: EC50 = 9.11 nM) (Table 2.3). On contrary, when the ortho-methyl group of benzene ring A of compound (±)-62a was replaced by an ethyl group leading to (±)-62d, an increase in potency was observed ((±)-62a: EC50 = 0.65 nM, (±)-62d: EC50 = 0.36 nM). These substituents may be too bulky to interact optimally with the ligand-binding domain of PPARδ. Crystallographic studies have indicated the presence of a lipophilic pocket in the PPARδ ligand-binding domain which could accommodate only small substituents at the ortho-position of the aromatic ring.21,73

Substitution of the hydrogen atoms attached to the alpha-carbon atom to the carboxylic acid moiety with one or two methyl groups generally led to a decrease in potency. Introduction of one methyl group alpha to the carboxylic acid moiety of GW 501516 (18) afforded compound (±)-62a which showed reduced potency ((±)-62a: EC50 = 0.65 nM). Substitution of one hydrogen atom of alpha-carbon atom to the carboxylic acid moiety of 62c with a methyl group afforded racemate 62d that exhibited a slightly reduced potency (62c: EC50 = 0.31 nM, (±)-62d: EC50 = 0.36 nM). Methyl substitution of the second hydrogen atom in (±)-62d afforded compound 62e that showed a significant decline of the potency ((±)-62d: EC50 = 0.36 nM, 62e: EC50 = 0.54 nM). A similar pattern of decreased potency manifested when the hydrogen atoms of the alpha-carbon atom to the carboxylic acid moiety of 62f (EC50 = 4.15 nM) were replaced by one methyl group, affording compound (±)-62g (EC50 = 5.79 nM), or two methyl groups, affording 62h (EC50 = 9.11 nM). Introduction of a second methyl group on the alpha-carbon atom to the carboxylic acid moiety of (±)-62a led to compound 62b
which, interestingly, showed a significant increase in potency ((±)-62a: EC$_{50}$ = 0.65 nM, 62b: EC$_{50}$ = 0.24 nM).

**Table 2.3.** Substitution pattern (see **Figure 2.9** and EC$_{50}$-values of tested compounds in the oleic acid oxidation assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>R$_1$</th>
<th>R$_2$</th>
<th>R$_3$</th>
<th>EC$_{50}$ (nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-62a</td>
<td>Methyl</td>
<td>Methyl</td>
<td>H</td>
<td>0.65</td>
</tr>
<tr>
<td>62b</td>
<td>Methyl</td>
<td>Methyl</td>
<td>Methyl</td>
<td>0.24</td>
</tr>
<tr>
<td>62c</td>
<td>Ethyl</td>
<td>H</td>
<td>H</td>
<td>0.31</td>
</tr>
<tr>
<td>(±)-62d</td>
<td>Ethyl</td>
<td>Methyl</td>
<td>H</td>
<td>0.36</td>
</tr>
<tr>
<td>62e</td>
<td>Ethyl</td>
<td>Methyl</td>
<td>Methyl</td>
<td>0.54</td>
</tr>
<tr>
<td>62f</td>
<td>Isopropyl</td>
<td>H</td>
<td>H</td>
<td>4.15</td>
</tr>
<tr>
<td>(±)-62g</td>
<td>Isopropyl</td>
<td>Methyl</td>
<td>H</td>
<td>5.79</td>
</tr>
<tr>
<td>62h</td>
<td>Isopropyl</td>
<td>Methyl</td>
<td>Methyl</td>
<td>9.11</td>
</tr>
<tr>
<td>62i</td>
<td>tert-Butyl</td>
<td>H</td>
<td>H</td>
<td>5.51</td>
</tr>
<tr>
<td>62j</td>
<td>Cyclopentyl</td>
<td>H</td>
<td>H</td>
<td>16.60</td>
</tr>
<tr>
<td>62k</td>
<td>Cyclohexyl</td>
<td>H</td>
<td>H</td>
<td>17.30</td>
</tr>
<tr>
<td>GW 501516 (18)</td>
<td>Methyl</td>
<td>H</td>
<td>H</td>
<td>0.10</td>
</tr>
</tbody>
</table>

$^a$Results of three replicates

The effects compounds 62a-62k exhibited on all of the peroxisome proliferator-activated receptors (PPAR$_{\alpha}$, PPAR$_{\delta}$ and PPAR$_{\gamma}$) in a luciferase-based transient transfection system was also investigated. Compounds 62a-62e, 62g and 62h showed a higher activation of both PPAR$_{\alpha}$ as well as PPAR$_{\delta}$ at 10 μM concentrations than the positive controls (**Figures 2.10-2.11**). Compounds 62i and 62j activated only the PPAR$_{\delta}$ receptor with the efficacy comparable to the lead compound 18 at 10 μM (**Figure 2.11**). The prepare compounds were less effective than the positive control towards PPAR$_{\gamma}$ (**Figure 2.12**).
To evaluate more completely the agonistic effects of compound 62e, the EC_{50}-values were determined against all three PPARs using the luciferase-based transient transfection assay. The EC_{50}-value for 62e against PPARδ was determined to be 5.0 nM, being in the same range as the reported EC_{50}-value of 1.0 nM for GW 501516 (18). The EC_{50}-value against the PPARα receptor was determined to be 750 nM, showing a moderate potency of 62e toward PPARα. No activity was observed for compound 62e against PPARγ (EC_{50} > 5000 nM). A much higher selectivity (> 1000) against PPARδ than PPARγ is to be noticed.

Figure 2.10. Activation of the ligand-binding domain of PPARα by compounds 62a-62k. Positive control: EHA ((2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid.)
Figure 2.11. Activation of the ligand-binding domain of PPARδ by compounds 60a-60k. Positive control: GW 501516 (18).

Figure 2.12. Activation of the ligand-binding domain of PPARγ by compounds 62a-62k. Positive control: Rosiglitazone (BRL).
2.1.3.4 Molecular modelling

To gain more information on the binding of **62e** with the ligand-binding domain of the PPARδ receptor, molecular modelling studies were performed.

According to the reported X-ray crystallographic structure, in the active receptor conformation helix H12 from the LBD structure folds into lid-like conformation closing the binding cavity, while in the inactive state takes an open conformation and the binding site is more accessible. In the activated receptor conformation of PPARδ, the amino acids His323, His449 and Tyr473 are essential for agonist interactions.

Agonist **62e** was docked into an activated receptor conformation of PPARδ. The docking indicated that **62e** was well accommodated in the activated receptor conformation, with a binding mode similar to that of the full PPARδ agonist 2-{2,3-dimethyl-4-[2-propargyloxy-4-((4-trifluoromethylphenoxy)methyl)phenylthio]phenoxy}acetic acid (65, Figure 2.13) (Figure 2.14B). The docking of **62e** revealed key interactions with amino acids Arg284, Cys285, His323, His449 and Tyr473 (Figure 2.14A). The acidic group of **62e** interacted with His323, His449, Tyr473. The trifluoromethyl group had contact with Arg284. Moreover, the calculated interaction energy of the **62e**-PPARδ complex was -14.9 kcal/mol indicating a significant affinity of **62e** for PPARδ LBD. The docking mode supports the observation that compound **62e** is a PPARδ agonist.

In the series of tested compounds, the potency decreased with increasing size of the substituent in the R1-position. In the docked complex of **62e** the ethyl group in R1 points in the direction of Thr289, Ile326 and Phe327. A larger substituent R1 will produce severe steric interactions with these residues and this may explain the decrease in potency when the size of the substituent is increased to isopropyl, tert-butyl, cyclopentyl or cyclohexyl groups.

![Figure 2.13](image-url) Structure of the agonist 2-{2,3-dimethyl-4-[2-propargyloxy-4-((4-trifluoromethylphenoxy)methyl)phenylthio]phenoxy}acetic acid.
Figure 2.14. **A.** 62e docked into PPARδ. Colour coding: red O, blue N, grey H, yellow C in 62e, white C in PPARδ. Colouring of the Cα traces of PPARδ is blue via white to red from N-terminal to C-terminal. **B.** The docked complex of 62e (purple) superimposed on the X-ray structure complex of the agonist 2-{2,3-dimethyl-4-[2-prop-2-ynyloxy-4-((4-trifluoromethyl)phenoxymethyl)phenylthio]phenoxy}acetic acid (green) (65) (Figure 2.13) (PDB, entry code: 3GZ9).

### 2.1.3.5 Conclusions

Modifications of the structure of the lead compound GW 501516 (18) afforded eleven compounds of which 62a-62d, 62g and 62h displayed dual agonistic effects at 10 μM against both PPARα and PPARδ. EC$_{50}$-values for PPARδ, along with the modelling of the docking of
compound 62e into the ligand-binding domain of PPARδ, supported that compound 62e is a strong agonist and selective towards PPARδ.

2.1.4 Triazole-based analogues of GW 501516 (18). Third series

Our goal was to synthesize new molecules as potential PPAR agonists by combining the ring carrying the acid moiety (A) of the most potent thiazole-based agonists of the series 62c-62e (Table 2.3) with the tail (B) of the most potent triazole-based agonist 52e (Table 2.1) (Figure 2.15).

![Figure 2.15. Structures of prepared agonists 69a-69c.](image)

2.1.4.1 Synthesis

Our synthetic effort to produce compounds 69a-69c started with the alkylation of the sulphur atom of phenol 58a with propargyl bromide in the presence of Cs2CO3 to produce compound 68 which reacted with 4-azido-2-fluoro-1-trifluoromethylbenzene (45e) under copper (I) catalysis using the methods described by Sharpless and coworkers’ conditions57 to afford compound 67 in 45% yield over two steps (Scheme 2.13).
Scheme 2.13. (a) Propargyl bromide, Cs_{2}CO_{3}, CH_{3}CN; (b) 4-Azido-2-fluoro-1-trifluoromethylbenzene (45e), sodium ascorbate, CuSO_{4}, \textit{tert}-BuOH, H_{2}O.

Alkylation of compound 67 afforded esters 68a-68c which were hydrolyzed to acids 69a-69c (Scheme 2.14). Ester 68c was hydrolyzed using an aqueous solution of (CH_{3})_{3}COK. Only the racemate of 69b was prepared.

Scheme 2.14. (a) Ethyl 2-bromoesters, Cs_{2}CO_{3}, CH_{3}CN; (b) (i) LiOH, THF, H_{2}O; (ii) (CH_{3})_{3}COK, THF, H_{2}O, reflux.

2.1.4.2 Biological evaluation

GW 501516 (18) and compounds 69a-69c (Table 2.4) were biologically evaluated as agonists as described in \textbf{subchapter 2.1.1.2}. The EC_{50}-values for triazoles 69a-69c were obtained from \textbf{Figure 2.17} and are presented in Table 2.4.
Table 2.4. Substitution pattern (see Figure 2.15) and EC₅₀-values of prepared compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>EC₅₀(nM)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>69a</td>
<td>H</td>
<td>H</td>
<td>11.6</td>
</tr>
<tr>
<td>(±)-69b</td>
<td>Methyl</td>
<td>H</td>
<td>34.8</td>
</tr>
<tr>
<td>69c</td>
<td>Methyl</td>
<td>Methyl</td>
<td>n.d.ᵇ</td>
</tr>
<tr>
<td>GW 501516 (18)</td>
<td>-</td>
<td>-</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Results of three experiments
ᵇ n.d., not determined

2.1.4.3 Results and Discussion

Oleic acid oxidation assay showed that the ability of the compounds 69a-69c to induce β-oxidation was lower than that of GW 501516 (18). The most potent triazole had 1/300ᵇ of the potency of the positive control 18 (Table 2.4). Substitution of the methyl group on the benzene ring A compound 52e (Table 2.1) with an ethyl group led to compound 69a which exhibited a decreased potency (52e: EC₅₀ = 0.85 nM, 69a: EC₅₀ = 11.6 nM). Replacement of a hydrogen atom alpha to the carboxylic group of compound 69a with one methyl group afforded racemic 69b which exhibited an EC₅₀ value of 34.8 nM. Introduction of a second methyl group yielded agonist 69c. Both compounds exhibited diminished efficacy as observed in Figure 2.16.

Figure 2.16. Oxidation of oleic acid in human myotubes in the presence of compounds 69a-69c at different concentrations. GW 501516 (18) was used as positive control.
Figure 2.17. Non-linear fit curves of oxidation of oleic acid in human myotubes in the presence of different concentrations of compounds 69a and (±)-69b. GW 501516 (18) was used as positive control.

Activation of the ligand-binding domain of PPARα, PPARδ, PPARγ in a luciferase-based transient transfection system by the triazoles 69a-69c at 10 μM concentration was also investigated (Figure 2.18).

Figure 2.18. Activation of the ligand-binding domain of PPARα (A), PPARδ (B), PPARγ (C) of triazoles 52e and 69a-69c. Positive controls for PPARα, PPARδ and PPARδ: EHA ((2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid), GW 501516 (18), and Rosiglitazone (BRL), respectively.
Ethyl substitution on 52e (Table 2.1), leading to 69a, appeared to maintain the efficacy towards PPARα, but decreased the activation of PPARδ (Figure 2.18A, B). Substitution of a hydrogen atom from the alpha-carbon atom to the carboxylic group in 69a with a methyl group, affording racemate 69b, seemed to significantly increase the activation of PPARα (Figure 2.18A). A second replacement with a methyl group to the corresponding dimethyl derivative, compound 69c, improved even more the activation of PPARα (Figure 2.18A): this effect might be explained by the structural similarities with the fibrate class of drugs. Both compounds were as effective as compound 69a and GW 501516 (18) towards PPARδ (Figure 2.18B). Even if methyl substituents seemed to have beneficial effects against PPARγ, no significant activation was observed (Figure 2.18C).

2.1.4.4 Conclusions
These synthetic efforts furnished two new dual PPARα/δ agonists at 10 μM concentration. Ethyl substitution of the methyl group decreased the efficacy towards PPARα and PPARδ. Introduction of the methyl groups on the alpha-carbon atom to the carboxylic group led to a significant increase of the efficacy towards PPARα, while the activation of PPARδ was maintained.

2.1.5 α-Mono fluorinated thiazole- and triazole-based analogues of GW 5101516 (18) (Paper III)
Fluorine atom has found a wide range of applications in medicinal chemistry: from tooth paste, antidepressants, anti-inflammatory agents, antimalarial drugs, to antipsychotics, antiviral agents, steroids, and general anaesthetics.76 Fluorine atom substitution has a strong influence on the physical and chemical properties of organic compounds: the high electronegativity of fluorine can modify electron distribution in the molecule, affecting its lipophilicity, absorption, distribution, pharmacodynamic and pharmacokinetic properties.77,78 Additionally, fluorine atom is able to participate in hydrogen bonding interactions. Therefore, bioisosteric substitution of hydrogen atoms by fluorine atoms is an important strategy for modification of known drugs.78
The interest was to introduce the F atom on the *alpha*-carbon to the carboxylic group of some of the thiazole- (compounds 62c and 62f) and triazole-based agonists (compounds 52e and 69a) described above (Figure 2.19). We envisioned that the fluorine atom would lower the pKa-value of the carboxylic group. Hopefully, this would improve the affinity to the ligand-binding domain of PPARs. Only the racemates of 71a-71c and 74a-74b were prepared.

### 2.1.5.1 Synthesis

The syntheses of racemates 71a-71c started with the alkylation of sulphur-substituted phenols 60a-60c with ethyl 2-bromo-2-fluoroacetate affording phenoxyesters (±)-70a-70c in 52-89% yield. Hydrolysis of esters 70a-70c yielded carboxylic acids (±)-71a-71c (Scheme 2.15).

![Scheme 2.15](image)

**Scheme 2.15.** (a) Ethyl 2-bromo-2-fluoroacetate , Cs₂CO₃, CH₃CN; (b) LiOH, THF, H₂O.
The syntheses of acids (±)-74a and (±)-74b started by reacting compound 54 with 4-azido-2-fluoro-1-trifluoromethylbenzene (45e) in the presence of catalytic amounts of copper (I) to yield the intermediate triazole 72 in 75% yield (Scheme 2.16).

Scheme 2.16. (a) Sodium ascorbate, CuSO₄, tert-BuOH, H₂O.

Triazoles 67 and 72 were then alkylated with ethyl 2-bromo-2-fluoroacetate to esters (±)-73a and (±)-73b which were hydrolysed to acids (±)-74a and (±)-74b in 25-36% overall yield (Scheme 2.17).

Scheme 2.17. (a) Ethyl 2-bromo-2-fluoroacetate, Cs₂CO₃, CH₃CN. (b) LiOH, THF, H₂O.
2.1.5.2 Biological evaluation

GW 501516 (18) and racemates 71a-71c, 74a and 74b were evaluated as agonists as described in the subchapter 2.1.1.2. The EC$_{50}$-values for the thiazole based compounds (±)-71a-71c and (±)-74a were obtained from Figure 2.22 and are presented in Table 2.5 for compounds (±)-71a-71c, and in Table 2.6 for compound (±)-74a.

Table 2.5. Substitution pattern (see Figure 2.19) and EC$_{50}$-values of prepared compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_1$</th>
<th>EC$_{50}$ (nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-71a</td>
<td>CH$_3$</td>
<td>3.7</td>
</tr>
<tr>
<td>(±)-71b</td>
<td>Ethyl</td>
<td>5.04</td>
</tr>
<tr>
<td>(±)-71c</td>
<td>Isopropyl</td>
<td>31.4</td>
</tr>
<tr>
<td>GW 501516 (18)</td>
<td>-</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^a$ Results of three replicates

Table 2.6. Substitution pattern (see Figure 2.19) and EC$_{50}$-values of prepared compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_1$</th>
<th>EC$_{50}$ (nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(±)-74a</td>
<td>CH$_3$</td>
<td>20.2</td>
</tr>
<tr>
<td>(±)-74b</td>
<td>Ethyl</td>
<td>n.d.$^b$</td>
</tr>
<tr>
<td>GW 501516 (18)</td>
<td>-</td>
<td>0.03</td>
</tr>
</tbody>
</table>

$^a$ Results of three replicates

$^b$ n.d., not determined

2.1.5.3 Results and Discussion

All thiazole-based racemates increased the oxidation of oleic acid (Table 2.5; Figure 2.20), but they showed a reduced potency compared to GW 501516 (18). Introduction of a fluorine atom on the alpha-carbon to the carboxylic group led to a decline of the potency compared to the non-fluorinated analogues (GW 501516 (18): EC$_{50}$ = 0.03 nM and (±)-71a: EC$_{50}$ = 3.7
nM; 62c: EC$_{50}$ = 0.31 nM and (±)-71b: EC$_{50}$ = 5.04 nM; 62f: EC$_{50}$ = 4.15 nM and (±)-71c: EC$_{50}$ = 31.4 nM). In the series of compounds (±)-71a-71c, it seemed that increasing the size of the ortho-substituent on A ring led to a decrease in potency ((±)-71a: EC$_{50}$ = 3.7, (±)-71b: EC$_{50}$ = 5.04, (±)-71c: EC$_{50}$ = 31.4 nM). Similar patterns were observed in our previous findings regarding compounds 62f (EC$_{50}$ = 4.15 nM), 62i (EC$_{50}$ = 5.51 nM) 62j (EC$_{50}$ = 16.60 nM) and 62k (EC$_{50}$ = 17.30 nM) (Table 2.3).

The triazole-based racemates showed ability to induce oxidation of oleic acid (Table 2.6; Figure 2.21), but they were less potent than GW 501516 (18) as well. The introduction of a fluorine atom on the alpha-carbon to the carboxylic group led to a reduction of the potency compared to non-fluorinated analogues. Compound (±)-74a (EC$_{50}$ = 20.2 nM) exhibited a 20 times lower potency than its non-fluorinated analogue, compound 52e (EC$_{50}$ = 0.85 nM).

Figure 2.20. Oxidation of oleic acid in human myotubes in the presence of racemates 71a-71c at different concentrations. GW 501516 (18) was used as positive control.
Figure 2.21. Oxidation of oleic acid in human myotubes in the presence of racemates 74a and 74b at different concentrations. GW 501516 (18) was used as positive control.

Figure 2.22. Non-linear fit curves of oxidation of oleic acid in human myotubes in the presence of different concentrations of racemates 71a-71c and 74a. GW 501516 (18) was used as positive control.

The effects of racemates 71a-71c, 74a and 74b on the three peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) in a luciferase-based transient transfection system at 10 μM concentration were investigated.

The introduction of a fluorine atom on the alpha-carbon to the carboxylic group of GW 501516 (18) afforded racemate 71a that activated both PPARα and PPARδ higher than the
positive controls at 10 μM concentration (Figures 2.23, 2.24). The introduction of a fluorine atom on the alpha-carbon to the carboxylic group of 62c (Table 2.3) afforded racemate 71b which seemed to activate less both PPARα and PPARδ than non-fluorinated analogue 62c (Figures 2.23, 2.24). The introduction of a fluorine atom on the alpha-carbon to the carboxylic group of 62f (Table 2.3) afforded racemate 71c that showed a higher activation of PPARα, but it was less effective against PPARδ (Figures 2.23, 2.24). Compared to the positive controls, racemates 71b and 71c seemed to be more effective than EHA, and almost as effective as GW 501516 (18) at 10 μM concentration. No significant activation was observed against PPARγ (Figure 2.25).

Introduction of a fluorine atom on alpha to the carboxylic group of triazole-based agonists 52e and 69a afforded racemates 74a and 74b, respectively. This modification, unfortunately, led to a significant decrease of the efficacy towards all three receptors (Figures 2.23-2.25).

Figure 2.23. Activation of the ligand-binding domain of PPARα by the compounds (±)-71a, 62c, (±)-71b, 62f, (±)-71c, 52s, (±)-74a, 69a and (±)-74b. Positive control: EHA ((2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid.)
Figure 2.24. Activation of the ligand-binding domain of PPARδ by the compounds (±)-71a, 62c, (±)-71b, 62f, (±)-71c, 52s, (±)-74a, 69a and (±)-74b. Positive control: GW 501516 (18).

Figure 2.25. Activation of the ligand-binding domain of PPARγ by the compounds (±)-71a, 62c, (±)-71b, 62f, (±)-71c, 52s, (±)-74a, 69a and (±)-74b. Positive control: Rosiglitazone (BRL).
2.1.5.4 Conclusions
Bioisosteric substitution of hydrogen atom on alpha-carbon atom to the carboxylic group with the fluorine atom led to three racemates exhibiting dual PPARα/δ agonistic effects. Compounds (±)-71a-71c activated α and δ subtypes with same or higher efficiencies than the positive controls at 10 μM concentration. Modification of the triazole-based agonists led to a significant decline of the activity towards all three receptors.

2.2 Preparation and biological evaluation of SRT 1720 (85)

Sirtuins (SIRT1, SIRT2, SIRT3) constitute a class of proteins with important metabolic roles, such as protein deacetylation. In Zucker fa/fa rats activation of SIRT1 improved insulin sensitivity in adipose tissue, skeletal muscle and liver and thus being a possible target for treatment of type 2 diabetes.

SIRT1 activator SRT 1720 (85) was reported in 2007 by a group from Sirtris Pharmaceuticals Inc. as a result of a high-throughput assay. It showed a high selectivity and strong activation toward SIRT1. Given the biological response of SIRT1 activation by SRT 1720 (85), we decided to investigate this molecule using the oleic acid oxidation essay.

2.2.1 Synthesis
The synthesis of SRT 1720 was achieved according to a literature procedure of which first step was the reaction of thiazole 75 with 2-bromo-2'-nitroacetophenone (76) to afford compound 77. Ester 77 was converted to alcohol 78 which reacted with methanesulfonyl chloride to yield compound 79 (Scheme 2.18).
Scheme 2.18. (a) Methyl ethyl ketone, reflux; (b) NaOH, H_2O, THF; (c) Isobutyl chloroformate, (d) NaBH_4, N-methylmorpholine, THF; (e) Methanesulfonyl chloride, Et_3N, CH_2Cl_2.

Scheme 2.19. (a) Et_3N, CH_3CN; (b) H_2, Pd/C, MeOH.

Compound 79 reacted with tert-butyl piperazine-1-carboxylate (80) through a nucleophilic substitution to afford compound 81 which was then hydrogenated to amine 82 in 46% yield over the two steps (Scheme 2.19).

The next step was the amide formation by the nucleophilic attack of amine 82 on 2-quinoxaloyl chloride (83) under basic conditions affording compound 84. The treatment with TFA of the compound 84 produced its TFA salt which was neutralized with NaHCO_3, extracted, and treated with HCl to afford compound SRT 1720 (85) (Scheme 2.20).
2.2.2 Synthesis of one triazole-based SRT 1720 analogue (91)

The synthetic approach towards the triazole SRT 1720 analogue (91) started with the syntheses of azide 87 from 2-nitroaniline (86), using standard diazotization conditions (Scheme 2.21), and of terminal alkyne 88 by alkylation of tert-butyl piperazine-1-carboxylate (80) with propargyl bromide. The next step was reaction of compound 88 with 1-azido-2-nitrobenzene (87) as previously described to the triazole 89 in 48% yield over the two steps (Scheme 2.22).

Scheme 2.20. (a) DMAP, DMF; (b) TFA, CH₂Cl₂; (c) NaHCO₃, H₂O, extraction; (d) HCl, H₂O, CH₃CN.

Scheme 2.21. (a) NaNO₂, NaN₃, H₂O, HCl
Scheme 2.22. (a) Propargyl bromide, Et₃N, CH₃CN; (b) 1-Azido-2-nitrobenzene (87), sodium ascorbate, CuSO₄, tert-BuOH, H₂O.

Triazole 89 was hydrogenated to amine 90 which reacted with 2-quinoxaloyl chloride (83) under basic conditions to afford compound 91 in 25% overall yield (Scheme 2.23).

Scheme 2.23. (a) H₂, Pd/C, MeOH; (b) 2-Quinoxaloyl chloride (83), DMAP, DMF.

2.2.3 Biological evaluation of SRT 1720 (85) and the analogue 91
The results of the biological testing of SRT 1720 (85) and compound 91 are not yet available.
2.3 Synthesis and biological evaluation of antagonists of peroxisome proliferator-activated receptor δ

The aim was to evaluate antagonists GSK 0660 (42) and GSK 3787 (43) in the oleic acid oxidation assay. Hence, we needed to prepare compounds 42 and 43. An analogue of GSK 3787 (100) was also synthesized and biologically evaluated the oleic acid oxidation assay.

2.3.1 Preparation of GSK 0660 (42)

The synthesis of GSK 0660 (42) was performed by a nucleophilic attack of disubstituted amine 92 on sulfone 93 (Scheme 2.24).

Scheme 2.24. (a) Pyridine, diethyl ether.

2.3.2 Preparation of GSK 3787 (43).

The synthesis of GSK 3787 (43) was conducted according to a literature procedure. Compound 94 reacted with tert-butyl 3-bromopropylcarbamate (95) through a nucleophilic attack to afford compound 96 which was oxidized with Oxone™ to the sulphone 97 in 80% yield over the two steps (Scheme 2.25).
Sulphone 97 was deprotected to compound 98 which reacted with 4-chlorobenzoyl chloride through a nucleophilic substitution to produce GSK 3787 (43) in 77% yield over the two steps (Scheme 2.26).

2.3.3 Synthesis of a potential new antagonist of PPARδ (103) (Paper IV)

The synthesis of the GSK 3787 analogue (103) started by a Hantzsch synthesis of thiazole 101 which was obtained in 75% yield from compound 99 and ketoester 101. Thiazole-Based ester 101 was then hydrolyzed to acid 102 in 92% yield (Scheme 2.27).
Scheme 2.27. (a) EtOH, reflux; (b) LiOH, H₂O, THF, reflux.

The last step of the synthesis of 103 was the reaction of amine 98 with acid 102 to amide 103 in the presence of N,N’-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole (Scheme 2.28).

Scheme 2.28. (a) N,N’-Dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, Et₃N, CH₂Cl₂.

2.3.4 Biological evaluation of GSK 0660 (42), GSK 3787 (43) and 103

The antagonists GSK 0660 (42), GSK 3787 (43) and compound 103, at three concentrations, were evaluated as described in the subchapter 2.1.1.2. The result of one replicate is presented in Figure 2.26.
Experimental data showed that all three molecules inhibited oleic acid oxidation. Based on these preliminary results, we decided to submit compound 103 for luciferase-based transient transfection assay. The results are not yet available.

### 2.3.5 Molecular modelling

Compound 103 was docked into the LBD of PPARδ. These studies revealed that compound 103 has a high affinity for PPARδ with a calculated value of interaction energy of -17.1 kcal/mol. Moreover, the molecular modelling studies indicated that compound 103 interacted with the following amino acids located in the LBD: Arg284, Cys285, His323, His449 and Tyr473 (Figure 2.27). An interaction between Cys285 and the pyridinium sulphone of 103 was also observed.

According to Shearer et al.⁴⁹, agonist GSK 3787 (43) manifested its antagonistic activity by covalently binding to Cys285* from the LBD of PPARδ.⁴⁹ Hashimoto et al.⁷⁴ stated that essential for the biological activity of all PPARs is the folding of helix H12. Tyr473, located in the C-terminal part of helix H12, appears to play a significant role for the folding of H12 in the active conformation. Hashimoto et al.⁷⁴ stressed also that the interaction with Tyr473 is crucial for antagonist effects to occur. Moreover, there are two possible modes of actions for a

---

* In SWISS-PROT database Cy285 is numbered as Cys249
PPAR antagonist: either the antagonist inhibits the folding or the antagonist induces misfolding of H12.

Hence, two interactions are noteworthy for the potential antagonistic activity of 103. First, the interaction between 103 and Cys285 is similar to what Shearer et al. observed in their PPARδ binding studies with antagonist 43. Second, an interaction between Tyr473 and the para-substituted trifluoromethyl phenyl group and 103 was observed from the docking studies. These observations render support for the potential antagonistic activity of 103.

**Figure 2.27.** Compound 103 docked into PPARδ. Colour coding: red - oxygen, blue - nitrogen, grey - hydrogen, green - fluorine, yellow - carbon in 103, white - carbon in PPARδ. Colouring of the Ca traces of PPARα is blue via white to red from N-terminal to C-terminal.
3. Summary

1) Substitution of 4-methyl-thiazole with 1,4-disubstituted 1,2,3-triazole

Data revealed of loss in potency in the oleic acid oxidation assay.

Data from luciferase-based transient transfection assay showed that the activation of PPARδ was retained together with an increase of PPARα activation. No changes in PPARγ activation were observed.

2) Modifications of ortho-substituent of the benzene ring A

Biological data showed a significant reduction of the potency in the oleic acid oxidation assay when methyl group was replaced by a hydrogen atom. Data from luciferase-based transient transfection assay revealed reduced activation of PPARδ. The activation of PPARα was maintained. No changes in PPARγ activation were observed.

Increasing size of the ortho-substituent on ring A decreased the potency in the oleic acid oxidation assay. Biological data from luciferase-based transient transfection assay showed that activation of PPARα was dependent of the size of the substituent: an ethyl group increased activation, while larger groups led to lower activation. Meanwhile, the activation of PPARδ was maintained or increased. The activation of PPARγ was not influenced.

3) Methyl group introduction on the alpha-carbon to the carboxylic moiety

Substitution of a hydrogen atom with a methyl group led generally to a decrease in potency in the oleic acid oxidation assay; introduction of a second methyl group decreased potency even further.

Biological results from the luciferase-based transient transfection assay showed that substitution of hydrogen atoms with methyl groups led to a reduction of activation of PPARδ. However, an increase in the activation of PPARα was observed. Even if introduction of methyl groups increased the activation of PPARγ, no compound was more effective than the positive control.
4) Fluorine atom introduction on the $\alpha$-carbon to the carboxylic moiety

Introduction of a fluorine atom generally led to a reduction of the oxidation oleic acid.

Biological data from the luciferase-based transient transfection assay showed that introduction of a fluorine atom generally retained activation of PPAR$\delta$. Activation of PPAR$\alpha$ generally increased. No changes in PPAR$\gamma$ activation were observed.

In the case of 1,4-disubstituted 1,2,3-triazole-based agonists, introduction of a fluorine atom led to a significant reduction of the activation of PPAR$\alpha$ and PPAR$\delta$. No changes in PPAR$\gamma$ activation were observed.

5) Selective PPAR$\delta$ antagonists

An analogue (105) of the recently reported selective PPAR$\delta$ antagonist GSK 3787 (100) was prepared. Initial biological evaluation using the oleic acid oxidation assay showed promising antagonistic effects.
4. Conclusions and future perspectives

Conclusions:

The present study focused on the syntheses of 1,4-disubstituted-1,2,3-triazole and thiazole-based analogues of GW 501516 (18), as well as their biological activities. In total, 38 agonistic analogues of 18 have been prepared. All analogues exhibited agonistic activities in the oleic acid oxidation assay. Some of these analogues showed promising dual PPARα/δ agonistic effects. The thiazole-based compound 62e proved to be a selective and powerful agonist towards PPARδ with an EC$_{50}$-value of 5 nM.

Additionally, a new potential PPARδ antagonist has been prepared and the initial biological data are promising. The biological testing of the potential antagonist is still ongoing.

Future perspectives:

Given the spread of metabolic syndrome among the current global population, development of new treatment strategies is of main importance. Based on initial medicinal chemistry efforts, clinical and literature data, the PPARs appear to be an important biological target for the treatment of metabolic syndrome. Therefore it is likely that compounds with either agonistic or antagonistic effects towards the PPARs will be of importance in future clinical use.
5. APPENDIX
5.1 Experimental: synthesis

All reagents and solvents were used as purchased without further purification. Melting points are uncorrected. Analytical TLC was performed using silica gel 60 F$_{254}$ on aluminium sheets (Merck). Flash chromatography was performed on silica gel 60 (40-60 μm, Fluka). NMR spectra were recorded on a Bruker Avance DPX-300 MHz spectrometer for $^1$H NMR and 75 MHz for $^{13}$C NMR. Coupling constants ($J$) are reported in Hz, and chemical shifts are reported in parts per million (ppm, δ) relative to CDCl$_3$ (7.24 ppm for $^1$H and 77.00 ppm for $^{13}$C) and DMSO-d$_6$ (2.50 ppm for $^1$H and 39.51 ppm for $^{13}$C). High resolution mass spectra were performed with aVG Prospec and with a Micromass Q-TOF-2$^\text{TM}$. The LC/MS analyses were performed on an Agilent Technologies 1200 Series (Eclipse XDB-C18, 5 μm, 4.6 x 150 mm), coupled with an Agilent 6310 ion trap.

5.1.1 Triazole-based analogues of GW 501516 (18). Second series

Ethyl 2-[4-(prop-2-ynylthio)phenoxy]acetate (54)

To a solution of $p$-mercaptophenol (53) (630 mg, 5 mmol) in dry CH$_3$CN (20 mL) was added Cs$_2$CO$_3$ (1.6 g, 4.9 mmol). To this mixture was added dropwise a solution of propargyl bromide (385 μL, 4.3 mmol) in dry CH$_3$CN (25 mL). The mixture was stirred for 2 h at r.t., and then an additional quantity of Cs$_2$CO$_3$ (2.4 g, 7.4 mmol) was added. To this mixture was added dropwise a solution of ethyl bromoacetate (776 μL, 7 mmol) in dry CH$_3$CN (5 mL). The mixture was stirred for 4 h under argon at r.t., followed by dilution with water and extraction with ethyl acetate (3x100 mL). The combined organic layers were dried over MgSO$_4$ and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) to obtain the title compound as yellow oil in 46% yield (579 mg, 2.32 mmol). $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.42 (d, $J = 8.9$ Hz, 2H), 6.83 (d, $J = 8.9$ Hz, 2H), 4.57 (s, 2H), 4.23 (q, $J = 7.1$ Hz, 2H), 3.46 (d, $J = 2.6$ Hz, 2H), 2.19 (t, $J = 2.6$ Hz, 1H), 1.25 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 168.51, 157.72, 133.88, 126.32, 115.21, 80.01, 71.58, 65.31, 61.31, 24.16, 14.04.
2-{4-[(1-Phenyl-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55a)

Sodium ascorbate (120 mg, 0.6 mmol, 20 mol %) and CuSO₄ (48 mg, 0.3 mmol, 10 mol %) were added to a solution of the alkyne 54 (750 mg, 3 mmol) and azidobenzene (45a) (358 mg, 3 mmol) in 10 mL tert-BuOH/H₂O (1:1). The mixture was stirred at r.t. over night. The formed precipitate was filtered off, washed with aqueous NH₃ (3.5%, 2x50 mL), brine, dissolved in a mixture of THF (30 mL) and H₂O (15 mL) and cooled to 0°C. To this mixture 750 μL of an aqueous solution 2.0 M LiOH were added slowly. The reaction mixture was stirred until TLC indicated complete hydrolysis and then diluted with 100 mL H₂O. The reaction mixture was washed with hexane (3x50 mL). The remaining aqueous phase was acidified with 0.1 M HCl and extracted with diethyl ether, dried over MgSO₄, and concentrated. The residue was recrystallized from CH₂Cl₂ giving 55a as a colourless solid in 54% yield (553 mg, 1.6 mmol). Mp 182-183°C. ¹H NMR (300 MHz, DMSO-d₆): δ = 13.01 (bs, 1H), 8.58 (s, 1H), 7.85 (d, J = 9.3 Hz, 2H), 7.62 – 7.53 (m, 2H), 7.50 – 7.43 (m, 1H), 7.36 (d, J = 8.9 Hz, 2H), 6.89 (d, J = 8.9 Hz, 2H), 4.66 (s, 2H), 4.25 (s, 2H). ¹³C NMR (75 MHz, DMSO-d₆): δ = 169.93, 156.97, 144.91, 136.46, 132.14, 129.78, 128.49, 125.95, 121.20, 119.85, 115.18, 64.43, 29.15. MS (ESI) m/z 340.1 [M-H]⁻; HRMS calcd for C₁₇H₁₅N₃O₃S [M]: 341.0834; found 341.0830.

2-{4-[(1-p-tolyl-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55b)

The title compound was prepared in 60% yield (213 mg, 0.6 mmol) as a colourless solid from 54 (250 mg, 1 mmol) and 1-azido-4-methylbenzene (133 mg, 1 mmol) (45b) according to the general procedure described for 55a. Mp 148-149°C. ¹H NMR (300 MHz, DMSO-d₆): δ = 8.51 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 1.9 Hz, 2H), 7.34 (d, J = 2.3 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 4.66 (s, 2H), 4.24 (s, 2H), 2.36 (s, 3H).³¹C NMR (75 MHz, DMSO-d₆): δ = 170.04, 157.07, 144.86, 138.22, 134.34, 132.21, 130.22, 126.11, 121.19, 119.85, 115.29, 64.55, 29.26, 20.57. MS (ESI) m/z 354.1 [M-H]⁻; HRMS calcd for C₁₈H₁₇N₃O₃S [M]⁺: 355.0991; found 355.0983.
2-{4-[(1-(3-Fluoro-4-methylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55c)

The title compound was prepared in 60% yield (213 mg, 0.6 mmol) as a colourless solid from 54 (250 mg, 1 mmol) and 1-azido-4-methylbenzene (133 mg, 1mmol) (45c) according to the general procedure described for 55a. Mp 129-130°C. $^1$H NMR (200 MHz, DMSO-$d_6$): $\delta$ = 13.01 (bs, 1H), 8.60 (s, 1H), 7.73 (dd, $J$ = 10.6, 2.1 Hz, 1H), 7.63 (dd, $J$ = 8.3, 2.2 Hz, 1H), 7.56 – 7.41 (m, 1H), 7.36 (d, $J$ = 8.9 Hz, 2H), 6.88 (d, $J$ = 8.9 Hz, 2H), 4.66 (s, 2H), 4.24 (s, 2H), 2.28 (d, $J$ = 1.7 Hz, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 170.02, 160.61 (d, $J$ = 244.3 Hz), 157.07, 145.11, 135.57 (d, $J$ = 10.4 Hz), 124.67 (d, $J$ = 17.2 Hz), 132.67 (d, $J$ = 6.0 Hz), 132.20, 126.01, 121.35, 115.45 (d, $J$ = 3.4 Hz), 115.28, 107.05 (d, $J$ = 27.4 Hz), 64.52, 29.21, 13.82 (d, $J$ = 2.9 Hz). MS (ESI) $m/z$ 372.4 [M-H]; HRMS calcd for C$_{18}$H$_{16}$FN$_3$O$_3$S [M]: 373.0896; found 373.0889.

2-{4-[(1-(4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55d)

The title compound was prepared in 65% yield (265 mg, 0.65 mmol) as a colourless solid from 54 (250 mg, 1 mmol) and 1-azido-4-(trifluoromethyl)benzene (187 mg, 1mmol) (45d) according to the general procedure described for 55a. Mp 157-158°C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 13.00 (bs, 1H), 8.74 (s, 1H), 8.11 (d, $J$ = 8.5 Hz, 2H), 7.95 (d, $J$ = 8.7 Hz, 2H), 7.36 (d, $J$ = 8.8 Hz, 2H), 6.89 (d, $J$ = 8.8 Hz, 2H), 4.66 (s, 2H), 4.26 (s, 2H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 170.03, 157.12, 145.50, 139.31 (distorted q, $J$ = 1.2 Hz), 132.34, 128.59 (q, $J$ = 32.5 Hz), 127.17 (q, $J$ = 3.8 Hz), 125.90, 123.80 (q, $J$ = 272.2 Hz), 121.57, 120.38, 115.28, 64.52, 29.21. MS (ESI) $m/z$ 408.1 [M-H]; HRMS calcd for C$_{18}$H$_{14}$F$_3$N$_3$O$_3$S [M]: 409.0708; found 409.0701.
2-{4-[(1-(3-Fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55e)

The title compound was prepared in 67% yield (286 mg, 0.67 mmol) as a colourless solid from 54 (250 mg, 1 mmol) and 4-azido-2-fluoro-1 trifluoromethylbenzene (205 mg, 1 mmol) (45e) according to the general procedure described for 55a. Mp 152-153°C. 1H NMR (300 MHz, DMSO-d6): δ = 13.04 (bs, 1H), 8.81 (s, 1H), 8.16 (dd, J = 11.5, 0.3 Hz, 1H), 8.05 – 7.94 (m, 2H), 7.36 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 4.65 (s, 2H), 4.26 (s, 2H). 13C NMR (75 MHz, DMSO-d6): δ = 169.99, 159.40 (dq, J = 256.6, 2.5 Hz), 157.13, 145.76, 140.96 (d, J = 10.8 Hz), 132.31, 129.16 (qd, J = 4.4, 1.8 Hz), 125.78, 122.29 (qd, J = 271.9, 1.1 Hz), 121.77, 115.76 (d, J = 3.7 Hz), 115.53 (qd, J = 32.9, 12.3 Hz), 115.29, 108.77 (d, J = 25.8 Hz), 64.53, 29.14. MS (ESI) m/z 426.0 [M-H]−; HRMS calcd for C18H13F4N3O3S [M]+: 427.0614; found 427.0605.

2-{4-[(1-(4-Methoxyphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55f)

The title compound was prepared in 26% yield (135 mg, 0.36 mmol) as a colourless solid from 54 (350 mg, 1.4 mmol) and 1-azido-4-methoxybenzene (208 mg, 1.4 mmol) (45f) according to the general procedure described for 55a. Mp 159-160°C. 1H NMR (300 MHz, DMSO-d6): δ = 8.46 (s, 1H), 7.74 (d, J = 9.1 Hz, 2H), 7.35 (d, J = 8.9 Hz, 2H), 7.11 (d, J = 9.1 Hz, 2H), 6.88 (d, J = 8.9 Hz, 2H), 4.66 (s, 2H), 4.23 (s, 2H), 3.82 (s, 3H). 13C NMR (75 MHz, DMSO-d6): δ = 170.04, 159.20, 157.05, 144.72, 132.19, 130.01, 126.12, 121.29, 115.28, 114.87, 64.53, 55.56, 29.24. MS (ESI) m/z 370.1 [M-H]−; HRMS calcd for C18H17N3O4S [M]+: 371.0940; found 371.0932.

2-{4-[(1-(3-Fluoro-4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55g)

The title compound was prepared in 34% yield (477 mg, 1.22 mmol) as a colourless solid from 54 (900 mg, 3.6 mmol) and 4-azido-2-fluoro-1 methoxybenzene (590 mg, 3.6 mmol) (45g) according to the general procedure described for 55a. Mp 143-144°C. 1H NMR (300 MHz, DMSO-d6): δ = 13.02 (bs, 1H), 8.81 (s, 1H), 8.16 (dd, J = 11.5, 0.3 Hz, 1H), 8.05 – 7.94 (m, 2H), 7.36 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 4.65 (s, 2H), 4.26 (s, 2H). 13C NMR (75 MHz, DMSO-d6): δ = 169.99, 159.40 (dq, J = 256.6, 2.5 Hz), 157.13, 145.76, 140.96 (d, J = 10.8 Hz), 132.31, 129.16 (qd, J = 4.4, 1.8 Hz), 125.78, 122.29 (qd, J = 271.9, 1.1 Hz), 121.77, 115.76 (d, J = 3.7 Hz), 115.53 (qd, J = 32.9, 12.3 Hz), 115.29, 108.77 (d, J = 25.8 Hz), 64.53, 29.14. MS (ESI) m/z 426.0 [M-H]−; HRMS calcd for C18H13F4N3O3S [M]+: 427.0614; found 427.0605.
MHz, DMSO-\textit{d}_6): \delta = 13.03 (bs, 1H), 8.55 (b, 1H), 7.81 (dd, \textit{J} = 12.1, 2.6 Hz, 1H), 7.70 – 7.62 (m, 1H), 7.41 – 7.31 (m, 3H), 6.88 (d, \textit{J} = 8.9 Hz, 2H), 4.66 (s, 2H), 4.23 (s, 2H), 3.90 (s, 3H). 13C NMR (75 MHz, DMSO-\textit{d}_6): \delta = 170.03, 157.06, 151.26 (d, \textit{J} = 245.7 Hz), 147.18 (d, \textit{J} = 10.3 Hz), 144.97, 132.18, 129.57 (d, \textit{J} = 9.0 Hz), 126.05, 121.40, 116.29 (d, \textit{J} = 3.6 Hz), 115.28, 114.48 (d, \textit{J} = 2.5 Hz), 108.71 (d, \textit{J} = 22.9 Hz), 64.53, 56.36, 29.22. MS (ESI) \textit{m/z} 388.1 [M-H]; HRMS calcd for C\textsubscript{18}H\textsubscript{16}FN\textsubscript{3}O\textsubscript{4}S [M] \textsuperscript{+}: 389.0846; found 389.0856.

2-{4-[(1-(4-trifluoromethoxyphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55h)

The title compound was prepared in 85% yield (718 mg, 1.7 mmol) as a colourless solid from 54 (500 mg, 2 mmol) and 1-azido-4-(trifluoromethoxy)benzene (406 mg, 2 mmol) (45h) according to the general procedure described for 55a. Mp 140-141°C. 1H NMR (200 MHz, DMSO-\textit{d}_6): \delta = 12.99 (bs, 1H), 8.63 (s, 1H), 7.99 (d, \textit{J} = 9.1 Hz, 2H), 7.60 (d, \textit{J} = 8.3 Hz, 2H), 7.36 (d, \textit{J} = 8.9 Hz, 2H), 6.88 (d, \textit{J} = 8.9 Hz, 2H), 4.66 (s, 2H), 4.25 (s, 2H). 13C NMR (75 MHz, DMSO-\textit{d}_6): \delta = 170.04, 157.11, 147.78 (distorted q, \textit{J} = 1.9 Hz), 145.27, 135.42, 132.32, 125.94, 122.59, 121.93, 121.60, 120.00 (q, \textit{J} = 257.0 Hz), 115.28, 64.52, 29.22. MS (ESI) \textit{m/z} 424.0 [M-H]; HRMS calcd for C\textsubscript{18}H\textsubscript{14}F\textsubscript{3}N\textsubscript{3}O\textsubscript{4}S [M] \textsuperscript{+}: 425.0657; found 425.0674.

2-{4-[(1-(3-Chloro-4-trifluoromethoxyphenyl)-1H-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55i)

The title compound was prepared in 85% yield (390 mg, 0.85 mmol) as a white colourless from 54 (250 mg, 1 mmol) and 4-azido-2-chloro-1-(trifluoromethoxy)benzene (237 mg, 1 mmol) (45i) according to the general procedure described for 55a. Mp 116-117°C. 1H NMR (200 MHz, DMSO-\textit{d}_6): \delta = 12.83 (bs, 1H), 8.75 (s, 1H), 8.29 (d, \textit{J} = 2.6 Hz, 1H), 8.02 (dd, \textit{J} = 9.0, 2.6 Hz, 1H), 7.79 (dd, \textit{J} = 9.0, 1.3 Hz, 1H), 7.36 (d, \textit{J} = 8.9 Hz, 2H), 6.88 (d, \textit{J} = 8.9 Hz, 2H), 4.66 (s, 2H), 4.25 (s, 2H). 13C NMR (75 MHz, DMSO-\textit{d}_6): \delta = 170.02, 157.12, 145.52, 143.47 (q, \textit{J} = 1.8 Hz), 136.04, 132.29, 127.44, 125.91, 124.42, 122.24, 121.79, 120.28, 120.00 (q, \textit{J} = 259.1 Hz), 115.29, 64.52, 29.20. MS (ESI) \textit{m/z} 458.0 [M-H]; HRMS calcd for C\textsubscript{18}H\textsubscript{13}ClF\textsubscript{3}N\textsubscript{3}O\textsubscript{4}S [M] \textsuperscript{+}: 459.0267; found 459.0258.
5.1.2 Triazole-based analogues of GW 501516 (18). Third series

2-Ethyl-4-(prop-2-ynylthio)phenol (66)

To a solution of 2-ethyl-4-mercaptophenol (58a) (400 mg, 2.6 mmol) in dry CH$_3$CN (20 mL), Cs$_2$CO$_3$ (847 mg, 2.6 mmol) was added. To this mixture, a solution of propargyl bromide (216 μL, 2.3 mmol) in dry CH$_3$CN (5 mL) was added dropwise. The mixture was stirred for 3 h under argon at r.t., then diluted with water and extracted with ethyl acetate (3x100 mL). The organic layers were combined, dried over MgSO$_4$ and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) as eluent to give 66 as yellow oil in 56% yield (280 mg, 1.46 mmol). $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.34 (d, $J = 2.3$ Hz, 1H), 7.24 (dd, $J = 8.3$, 2.3 Hz, 1H), 6.71 (d, $J = 8.3$ Hz, 1H), 5.45 (s, 1H), 3.49 (d, $J = 2.6$ Hz, 2H), 2.61 (q, $J = 7.5$ Hz, 2H), 2.25 (t, $J = 2.6$ Hz, 1H), 1.21 (t, $J = 7.5$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ = 153.42, 133.89, 131.50, 130.88, 124.65, 115.74, 80.29, 71.64, 24.42, 22.64, 13.57.

2-Ethyl-4-[[1-(3-fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl]methylthio} phenol (67)

To a solution of 66 (339 mg, 1.76 mmol) and 4-azido-2-fluoro-1-trifluoromethylbenzene (45e) (361 mg, 1.76 mmol) in 20 mL t-BuOH/H$_2$O (1:1), sodium ascorbate (70 mg, 20 mol %) and copper sulphate (28 mg, 10 mol %) were added. The mixture was stirred at r.t. overnight. The formed precipitate was filtered off, washed with aqueous NH$_3$ (3.5%, 2x50 mL) and cold water. The precipitate was purified by column chromatography on silica gel with hexane/ethyl acetate (2:1) as eluent to give 67 as a yellow solid in 81% yield (567 mg, 1.43 mmol). Mp 97-98°C. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.77 (s, 1H), 7.75-7.68 (m, 1H), 7.09 (d, $J = 2.2$ Hz, 1H), 7.04 (dd, $J = 8.2$, 2.3 Hz, 1H), 6.67 (d, $J = 8.2$ Hz, 1H), 4.13 (s, 2H), 2.54 (q, $J = 7.5$ Hz, 2H), 1.11 (t, $J = 7.5$ Hz, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$) δ = 160.16 (dq, $J = 259.1$, 2.0 Hz), 154.34, 147.04, 140.52 (dd, $J = 9.9$, 0.4 Hz), 133.97, 131.53, 131.45, 128.79 (qd, $J = 4.6$, 2.5 Hz), 123.57,
Ethyl 2-{4-[1-(3-fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]-2-ethylphenoxy}acetate (68a)

To a solution of 67 (150 mg, 0.38 mmol) in dry CH$_3$CN (20 mL) was added Cs$_2$CO$_3$ (150 mg, 0.46 mmol). To this mixture was added dropwise a solution of ethyl 2-bromoacetate (60 μL, 0.46 mmol) in dry CH$_3$CN (3 mL). The mixture was stirred overnight at r.t. under argon, then diluted with water and extracted with ethyl acetate (3x100 mL). The organic layers were combined, dried over MgSO$_4$, and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) to give 68a as a white solid in 82% yield (151 mg, 0.31 mmol). Mp 69-70°C. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.75-7.68 (m, 1H), 7.65-7.59 (m, 2H), 7.54 (dd, $J$ = 8.5, 0.4 Hz, 1H), 7.15 (d, $J$ = 2.1 Hz, 1H), 7.12 (dd, $J$ = 8.3, 2.3 Hz, 1H), 6.58 (d, $J$ = 8.3 Hz, 1H), 4.59 (s, 2H), 4.20 (q, $J$ = 7.1 Hz, 2H), 4.14 (s, 2H), 2.59 (q, $J$ = 7.5 Hz, 2H), 1.24 (t, $J$ = 7.1 Hz, 3H), 1.11 (t, $J$ = 7.5 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 168.63, 160.11 (dq, $J$ = 258.6, 1.9 Hz), 155.43, 146.56, 140.69 (d, $J$ = 10.2 Hz), 134.16, 133.33, 130.64, 128.67 (qd, $J$ = 4.6, 2.5 Hz), 125.81, 121.95 (qd, $J$ = 272.3, 1.2 Hz), 119.80, 117.99 (qd, $J$ = 33.7, 12.6 Hz), 114.92 (d, $J$ = 3.9 Hz), 111.56, 108.81 (d, $J$ = 25.6 Hz), 65.21, 61.21, 30.41, 23.03, 13.99, 13.82.

Ethyl 2-{4-[1-(3-fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]-2-ethylphenoxy}propanoate (68b)

The title compound was prepared in 87% yield (164 mg, 0.85 mmol) as a light yellow oil from 67 (150 mg, 0.38 mmol) and ethyl 2-bromopropanoate (70 μL, 0.46 mmol) according to the general procedure described for 68a. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.74 - 7.66 (m, 1H), 7.66 – 7.58 (m, 2H), 7.53 (dd, $J$ = 8.5, 0.7 Hz, 1H), 7.14 (d, $J$ = 2.3 Hz, 1H), 7.08 (dd, $J$ = 8.4, 2.4 Hz, 1H), 6.55 (d, $J$ = 8.4 Hz, 1H), 4.69 (q, $J$ = 6.8 Hz, 1H), 4.18 – 4.09 (m, 4H), 2.58 (q, $J$ = 7.5 Hz, 2H), 1.58 (d, $J$ = 6.8 Hz, 3H), 1.18 (t, $J$ = 7.1 Hz, 3H), 1.11 (t, $J$ = 7.5 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 171.78, 160.07 (qd, $J$ = 258.5, 2.0 Hz), 155.19, 146.52,
Ethyl 2-{4-[(1-(3-fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]-2-ethylphenoxy}-2-methylpropanoate (68c)

The title compound was prepared in 95% yield (185 mg, 0.36 mmol) as a light yellow oil from 67 (150 mg, 0.38 mmol) and ethyl 2-bromo-2-methylpropanoate (68 μL, 0.46 mmol) according to the general procedure described for 68a. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.75-7.68 (m, 1H), 7.65 – 7.58 (m, 2H), 7.54 (dd, J = 8.5, 0.7 Hz, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.04 (dd, J = 8.5, 2.4 Hz, 1H), 6.52 (d, J = 8.5 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 4.15 (s, 2H), 2.55 (q, J = 7.5 Hz, 2H), 1.56 (s, 6H), 1.18 (t, J = 7.1 Hz, 3H), 1.10 (t, J = 7.5 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 174.05, 160.16 (dq, J = 258.8, 2.0 Hz), 153.21, 146.67, 140.73 (d, J = 272.3, 1.2 Hz), 119.81, 118.07 (qd, J = 33.8, 12.7 Hz), 116.30, 114.95 (d, J = 3.9 Hz), 108.85 (d, J = 25.5 Hz), 78.92, 61.39, 30.36, 25.33, 23.45, 13.96, 13.93.

2-{4-[(1-(3-Fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]-2-ethylphenoxy}acetic acid (69a)

To a stirred solution of 68a (151 mg, 0.31 mmol) in THF (10 mL) and H$_2$O (5 mL) at 0 °C was added slowly 215 μL of 2.0 M LiOH. The reaction mixture was stirred until TLC indicated completion of the reaction. The mixture was diluted with 50 mL H$_2$O, acidified with 0.1 M HCl, extracted with diethyl ether (3x50 mL), dried over MgSO$_4$, and concentrated. The residue was recrystallized from CH$_2$Cl$_2$ to give 69a as a colourless solid in 78% yield (110 mg, 0.24 mmol). Mp 154-155°C. $^1$H NMR (300 MHz, DMSO-$d_6$): δ = 13.00 (bs, 1H), 8.79 (s, 1H), 8.15 (d, J = 11.3 Hz, 1H), 8.05 – 7.94 (m, 2H), 7.22 (dd, J = 8.4, 2.4 Hz, 1H), 7.18 (d, J = 2.2 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 4.68 (s, 2H), 4.25 (s, 2H), 2.55 (q, J = 7.5 Hz, 2H), 1.09 (t, J = 7.5 Hz, 3H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): δ = 170.13, 159.39 (qd, J = 254.5, 2.1 Hz), 154.95, 145.80, 140.96 (dd, J = 10.6, 0.3 Hz), 132.86, 131.82, 129.61, 129.15 (qd, J
= 4.5, 1.8 Hz), 125.44, 122.28 (qd, J = 272.0, 0.7 Hz), 121.79, 115.93 (qd, J = 32.9, 12.2 Hz), 115.71 (d, J = 3.6 Hz), 112.13, 108.72 (d, J = 25.8 Hz), 64.71, 29.18, 22.64, 13.95. MS (ESI) m/z 454.4 [M-H]⁻; HRMS calcd for C₂₀H₁₇F₄N₃O₃S [M]: 455.0927; found 455.0917.

2-{4-[(1-(3-Fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]-2-ethylphenoxy}propanoic acid (69b)

The title compound was prepared in 30% yield (47 mg, 0.1 mmol) as a colourless solid from 68b (64 mg, 0.33 mmol) according to the general procedure described for 69a. Mp 152-153°C. ¹H NMR (300 MHz, DMSO-d₆): δ = 12.99 (bs, 1H), 8.78 (s, 1H), 8.13 (d, J = 12.0 Hz, 1H), 8.01 – 7.93 (m, 2H), 7.21 (dd, J = 8.4, 2.4 Hz, 1H), 7.18 (d, J = 2.2 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 4.79 (q, J = 6.7 Hz, 1H), 4.25 (s, 2H), 2.54 (q, J = 7.5 Hz, 2H), 1.50 (d, J = 6.7 Hz, 3H), 1.09 (t, J = 7.5 Hz, 3H). ¹³C NMR (75 MHz, DMSO-d₆): δ = 173.04, 159.43 (dq, J = 254.6, 2.1 Hz), 154.69, 145.81, 140.98 (d, J = 10.6 Hz), 133.08, 131.91, 129.60, 129.12 (qd, J = 4.5, 1.9 Hz), 125.44, 122.30 (qd, J = 272.0, 0.9 Hz), 121.78, 115.97 (qd, J = 33.0, 12.3 Hz), 115.67 (d, J = 3.5 Hz), 112.47, 108.68 (d, J = 25.8 Hz), 71.67, 29.22, 22.77, 18.29, 13.89. MS (ESI) m/z 468.1 [M-H]⁻; HRMS calcd for C₂₁H₁₉F₄N₃O₃S [M]: 469.1083; found 469.1077.

2-{4-[(1-(3-Fluoro-4-trifluoromethylphenyl)-1H-1,2,3-triazol-4-yl)methylthio]-2-ethylphenoxy}-2-methylpropanoic acid (69c)

To a stirred solution of 68c (146 mg, 0.3 mmol) in THF (10 mL) and H₂O (5 mL) was added 1 mL aqueous solution of 2.0 M t-BuOK. The reaction mixture was refluxed for 24 h. After the completion, the mixture was diluted with 50 mL H₂O, acidified with 0.1 M HCl, extracted with diethyl ether (3x50 mL), dried over MgSO₄, and concentrated. The residue was recrystallized from CH₂Cl₂ to give 69c as a colourless solid in 83% yield (146 mg, 0.30 mmol). Mp 155-156°C. ¹H NMR (300 MHz, DMSO-d₆): δ = 13.01 (bs, 1H), 8.78 (s, 1H), 8.14 (d, J = 11.8 Hz, 1H), 8.03 – 7.92 (m, 2H), 7.21 – 7.15 (m, 2H), 6.64 (d, J = 9.2 Hz, 1H), 4.26 (s, 2H), 2.51 (q, J = 7.5 Hz, 2H), 1.50 (s, 6H), 1.07 (t, J = 7.5 Hz, 3H). ¹³C NMR (75 MHz, DMSO-d₆): δ = 175.10, 159.40 (dq, J = 254.4, 2.0 Hz), 152.49, 145.70, 140.96 (dd, J =
10.3, 0.3 Hz), 134.92, 131.80, 129.13 (qd, $J = 4.4, 1.8$ Hz), 128.84, 125.89, 122.28 (qd, $J = 272.5, 0.7$ Hz), 121.82, 116.19, 115.95 (qd, $J = 33.0, 12.3$ Hz), 115.69 (d, $J = 3.7$ Hz), 108.69 (d, $J = 25.8$ Hz), 78.23, 28.94, 25.01, 22.99, 14.01. MS (ESI) $m/z$ 483.1 [M-H]$^-$; HRMS calcd for C$_{22}$H$_{21}$F$_4$N$_3$O$_3$S [M]: 483.1240; found 483.1244.

5.1.3 Preparation and biological evaluation of SRT 1720 (85)

N-(2-(3-(piperazin-1-ylmethyl)imidazo[2,1-b]thiazol-6-yl)phenyl)quinoxaline-2-carboxamide (SRT 1720) (85) was prepared according to the literature procedure.$^{80}$

5.1.4 Synthesis of triazole-based analogue of SRT 1720 (91).

1-Azido-2-nitrobenzene (87)

Amine 86 (705 mg, 5.1 mmol) was suspended in HCl/H$_2$O (1:1) (40 mL) and cooled to 0ºC and an aqueous solution of sodium nitrite (422 mg, 6.1 mmol) was added dropwise. The reaction mixture was stirred at 0ºC for 1 h. To this mixture an aqueous solution of sodium azide (397 mg, 6.1 mmol) was added dropwise. The reaction mixture was stirred at r.t. for another 3 h, and extracted with hexane (3x50 mL). The organic layers were washed with brine (2x100 mL), dried over MgSO$_4$ and concentrated to afford azide 87 brown oil 84% yield (706 mg, 4.3 mmol). $^1$H NMR (200 MHz, CDCl$_3$): $\delta = 7.91$ (dd, $J = 8.2, 1.4$ Hz, 1H), 7.60 (ddd, $J = 8.2, 7.5, 1.5$ Hz, 1H), 7.32 (dd, $J = 8.2, 1.1$ Hz, 1H), 7.28 – 7.18 (m, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta =$142.04, 133.84, 129.95, 123.88, 123.179, 121.46.

tert-Butyl 4-(prop-2-ynyl)piperazine-1-carboxylate (88)

To a stirred solution of tert-butyl piperazine-1-carboxylate (80) (186 mg, 1 mmol) in dry CH$_3$CN (20 mL) was added triethylamine (167 $\mu$L, 1.2 mmol). To this mixture a solution of ethyl 3-bromopropyne (90 $\mu$L, 1.2 mmol) in dry CH$_3$CN (3 mL) was added dropwise. The mixture was stirred over night at r.t. under argon, then diluted with water and extracted with
ethyl acetate (3x100 mL). The organic layers were combined, dried over MgSO₄, and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (2:1) as eluent to give 88 as light yellow oil in 74% yield (166 mg, 0.74 mmol). ¹H NMR (300 MHz, CDCl₃): δ = 3.34 (t, J = 6.0 Hz, 4H), 3.19 (d, J = 2.4 Hz, 2H), 2.38 (t, J = 6.0 Hz, 4H), 2.16 (t, J = 2.4 Hz, 1H), 1.34 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ = 154.38, 79.39, 78.17, 73.31, 51.38, 46.75, 43.18, 28.20.

tert-Butyl 4-[1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl]methylpiperazine-1-carboxylate (89)

Sodium ascorbate (30 mg, 20 mol %) and copper sulphate (12 mg, 10 mol %) were added to a stirred solution of 88 (166 mg, 0.74 mmol) and 1-azido-2-nitrobenzene (87) (121 mg, 0.74 mmol) in 20 mL tert-BuOH/H₂O (1:1). The mixture was stirred at r.t over night and then it was diluted with 50 mL water and extracted with CH₂Cl₂ (3x50 mL). The organic phases were washed with aqueous NH₃ (3.5%, 2x500 mL), brine, dried over MgSO₄, and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (1:3) as eluent to give 89 as brown oil in 65% yield (188 mg, 0.48 mmol). ¹H NMR (300 MHz, CDCl₃): δ = 7.97 (dd, J = 8.1, 1.5 Hz, 1H), 7.75 (s, 1H), 7.71 (td, J = 7.7, 1.5 Hz, 1H), 7.61 (td, J = 7.7, 1.5 Hz, 1H), 7.54 (dd, J = 7.8, 1.5 Hz, 1H), 3.68 (s, 2H), 3.35 (t, J = 6.0 Hz, 4H), 2.41 (t, J = 6.0 Hz, 4H), 1.35 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ = 154.47, 144.57, 144.16, 133.73, 130.62, 130.00, 127.64, 125.33, 124.22, 79.40, 52.87, 52.41, 43.40, 28.17.

tert-Butyl 4-[[1-(2-aminophenyl)-1H-1,2,3-triazol-4-yl]methyl]piperazine-1-carboxylate (90)

To a stirred solution of 89 (129 mg, 0.33 mmol) in MeOH (20 mL) wet Pd/C (70 mg, 10 mol %) was added in portions. The solution was stirred over night under argon at r.t. The solution was filtered and concentrated. The residue was purified by column chromatography on silica gel with CH₂Cl₂/MeOH (16:1) as eluent to give 90 as a colourless solid in 85% yield (100 mg, 0.28 mmol). Mp 143-144°C. ¹H NMR (300 MHz, CDCl₃): δ = 7.75 (s, 1H), 7.23 – 7.14 (m, 2H), 6.85 (dd, J = 8.0, 0.9 Hz, 1H), 6.78 (td, J = 8.1, 1.3 Hz, 1H), 4.59 (bs, 2H), 3.74 (s, 2H), 1.35 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ = 154.47, 144.57, 144.16, 133.73, 130.62, 130.00, 127.64, 125.33, 124.22, 79.40, 52.87, 52.41, 43.40, 28.17.
3.42 (t, J = 5.9 Hz, 4H), 2.48 (d, J = 5.9 Hz, 4H), 1.42 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ = 154.64, 143.95, 140.74, 129.96, 124.06, 123.27, 122.96, 118.14, 117.61, 79.65, 53.20, 52.69, 43.56, 28.35.

tert-Butyl 4-[(1-(2-(quinoxaline-2-carboxamido)phenyl)-1H-1,2,3-triazol-4-yl)methyl]piperazine-1-carboxylate (91)

To a stirred solution of 90 (100 mg, 0.28 mmol) in dry DMF (10 mL) DMAP on resin (187 mg, 0.56 mmol, 3 mmol/1 g resin) and quinoxaline-2-carbonyl chloride (84) (65 mg, 0.34 mmol) were added. The solution was stirred overnight under argon at r.t. The reaction mixture was diluted with 50 mL water, extracted with EtOAc (3x50 mL); the organic phases were washed with brine, dried over MgSO₄ and concentrated. The residue was purified by column chromatography on silica gel with CH₂Cl₂/MeOH (16:1) as eluent to give 91 as a colourless solid in 61% yield (88 mg, 0.17 mmol). Mp 143-144°C. ¹H NMR (300 MHz, CDCl₃): δ = 11.63 (s, 1H), 9.67 (s, 1H), 8.75 (d, J = 8.3 Hz, 1H), 8.31 – 8.08 (m, 2H), 7.94 – 7.79 (m, 3H), 7.56 (t, J = 7.8 Hz, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 3.81 (s, 2H), 3.39 (d, J = 6.0 Hz, 4H), 2.51 (d, J = 6.0 Hz, 4H), 1.40 (s, 9H). ¹³C NMR (75 MHz, CDCl₃): δ = 161.84, 154.64, 145.01, 144.00, 143.54, 142.98, 140.10, 132.09, 131.63, 131.06, 130.26, 130.19, 129.29, 127.18, 124.87, 123.83, 123.77, 123.02, 79.69, 53.30, 52.82, 43.43, 28.36. MS (ESI) m/z 514.4 [M-H]⁻; HRMS calcd for C₂₇H₃₀F₄N₈O₃ [M]⁺: 514.2441; found 514.2448.

5.1.5 Preparation of GSK 0660 (42)

To a stirred solution of 92 (321 mg, 1.5 mmol) in diethyl ether (50 mL) pyridine (160 μL, 2 mmol) and 93 (317 μL, 1.5 mmol) were added. The solution was stirred overnight at r.t. The reaction mixture was diluted with 50 mL water, extracted with EtOAc (3x50 mL); the organic phases were washed with brine, dried over MgSO₄ and concentrated. The residue was purified by column chromatography on silica gel with CH₂Cl₂/MeOH (16:1) as eluent to give 942 as a light green solid in 59% yield (370 mg, 0.88 mmol). ¹H NMR (300 MHz, CDCl₃): δ = 8.24 (s, 1H), 7.42 – 7.33 (m, 3H), 7.26 – 7.15 (m, 2H), 6.98 (dd, J = 8.5, 1.0 Hz, 2H), 6.94 – 6.86 (m, 1H), 6.54 (dd, J = 8.6, 2.4 Hz, 1H), 6.42 (d, J = 2.3 Hz, 1H), 5.65 (s, 1H), 3.97 (s, 3H), 3.49
(s, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 160.64$, 152.54, 145.22, 142.55, 142.37, 131.59, 131.40, 129.42, 129.37, 126.63, 121.41, 118.23, 117.94, 109.72, 100.41, 55.35, 53.01.

5.2 Experimental: biology

5.2.1 Measurement of oleic acid oxidation

The biological assay was performed in collaboration with the group of Dr. G. Hege Thoresen, School of Pharmacy, Department of Pharmaceutical Biosciences, University of Oslo.

5.2.1.1 Human myotube culture

A cell bank of satellite cells was established from muscle biopsy samples of the *musculus vastus lateralis* of six healthy volunteers. The biopsies were obtained with informed consent and approval by the National Committee for Research Ethics (Oslo, Norway). Muscle cell cultures free of fibroblasts were isolated as previously described.82

The cells were cultured on 96-well microplates (CellBIND®) with 100 µL/well of DMEM-Glutamax™ (5.5 mM glucose), 2% FCS, 2% Ultroser G, Penicillin/Streptomycin (P/S), and amphotericin B. Cell confluence at seeding was 60000 cells/well. At 70%–80% confluence (Figure 5.1), the growth medium was replaced by DMEM-Glutamax™ supplemented with 2% FCS, P/S, 1.25 µg/ml amphotericin B, and 25 pM insulin to induce the differentiation of myoblasts into multinucleated myotubes.

![Figure 5.1. Muscle cell culture: 4 days after seeding](image-url)
The cells were cultured in humidified 5% CO₂ atmosphere at 37°C, and the medium was changed every 2–3 days. After three days of differentiation (Figure 5.2), the cells were treated with either different concentrations of agonists or vehicle (DMSO) for 4 days. Experiments were performed 7 days after the onset of differentiation.

**Figure 5.2.** Muscle cell culture: after 3 days of differentiation

### 5.2.1.2 Substrate oxidation assay

The muscle cells were cultured on 96-well CellBIND® microplates as described above. Seven days after the onset of differentiation, growth medium was removed, and substrate, [1-¹⁴C]oleic acid (1 μCi/ml, 100 μM), was given in 50 μL DPBS with 10 mM HEPES and 1 mM L-carnitine (per well). A 96-well UniFilter®-96 GF/B microplate was mounted on top of the CellBIND® plate as described before, and CO₂ was trapped as described before. Briefly, 96-well UniFilter®-96 GF/B microplate was shortly pre-wetted with 20 μL 1 M NaOH (per well). A trapping devise was quickly assembled with a 96-holes silicone gasket fitting between the bottom 96-well plate (CellBIND®) and a 96-well UniFilter®-96 GF/B microplate on top, and incubated for 4 h at 37°C. 40 μL of Optiphase Supermix® was then added to each well on the UniFilter®-96 GF/B microplate, which was further sealed with a translucent plastic adhesive (TopSeal®, and the CO₂ trapped in the filter was counted after 2 days by liquid scintillation in a 1450 MicroBeta Plus scintillation counter (PerkinElmer). The cells were rinsed twice with 150 μL PBS in order to remove the excess ¹⁴C-labeled medium, harvested with 200 μL
0.1 M NaOH, homogenized and used to determine protein content and cell associated radioactivity in each well.

5.2.1.3 Protein measuring
Protein content in each well was determined by the method of Bradford. Briefly, 50 μL of the cell homogenate obtained as described above, and the BSA standards, were transferred to a 96-well microtiter-plate. 200 μL of Bio-Rad Protein Assay Reagent Concentrate, previously diluted 1:5 in distilled water, was added to each well. After 10 min at r.t., absorbance at 595 nM was measured using Wallac Victor™.

5.2.1.4 Cell associated radioactivity
Cell associated radioactivity was measured in 50 μL of the homogenized cell lysate obtained as described in 4.2.1.2. The lysate was transferred to a 96 wells-Isoplate in the same pattern as the original 96-wells plate. First row was used for adding the radioactive trapping medium, 50 μL in four wells. 100 μL of Optiphase Supermix® was added to each well and the plate was sealed with TopSeal®-A. Radioactivity was quantified after 2 h at r.t. using 1450 MicroBeta® Plus scintillation counter.

5.2.2 Luciferase-based transient transfection assay
The biological assay was performed by the group of Prof. Hilde I. Nebb, Faculty of Medicine, Department of Nutrition, University of Oslo.

5.3 Molecular modelling
The molecular modelling was performed by the group of Prof. Ingebrigt Sylte, Medical Pharmacology and Toxicology, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, N-9037 Tromsø, Norway
References

(2) http://www.who.int/mediacentre/factsheets/fs312/en/.
(18) Kesaniemi, Y. A.; Grundy, S. M. *JAMA* 1984, 251, 2241.


(57) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.


(64) Grimm, H. G. *Naturwissenschaften* 1929, *17*, 535.


List of errata

Paper I:

In Scheme 4A it should be: % of EHA control.

In the $^1$H NMR data for compound 2c instead of 7.21 (d, $J = 10.02$ Hz, 2H) it should be
7.20 (dd, $J = 8.4$, 2.2 Hz, 1H), 7.23 (dd, $J = 1.7$ Hz, 1H)

In the $^1$H NMR data for compound 4g instead of 6.93 (t, $J = 9.0$ Hz, 1H) it should be 6.87-6.97 (m, 1H).

In the $^1$H NMR data for compound 4g instead of 6.93 (t, $J = 9.0$ Hz, 1H) it should be 6.97-7.06 (m, 1H).

Instead of [M]$^+$ it should be [M]$^\dagger$.

Paper II:

Compounds 62a, 62d and 62g are racemates.

Instead of 62a, 62d, 62g it should be (±)-62a, (±)-62d, (±)-62g.

Instead of [M]$^+$ it should be [M]$^\dagger$. 
List of papers

I) Synthesis and dual PPARα/δ agonist effects of 1,4-disubstituted 1,2,3-triazole analogues of GW 501516


II) Synthesis, biological evaluation and molecular modeling of analogues of GW 501516


III) Synthesis and biological evaluation of fluorine analogs of GW 501516


IV) Synthesis, molecular modeling and initial biological evaluation of CC 618: a PPARδ antagonist

Calin C. Ciocoiu, Aina W. Ravna, Ingebrigt Sylte, G. Hege Thoresen, Trond Vidar Hansen - manuscript
Synthesis, biological evaluation and molecular modeling of analogues of GW 501516

Calin C. Ciocoiu,a Aina W. Ravna,b Ingebrigt Sylte,b Trond Vidar Hansena

aSchool of Pharmacy, Department of Pharmaceutical Chemistry, University of Oslo, PO BOX 1068, Blindern, N-0316 Oslo, Norway
bMedical Pharmacology and Toxicology, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, N-9037 Tromsø, Norway

Abstract: Eleven analogues of GW 501516 (1) were prepared and subjected to biological testing in a semi-high throughput human skeletal muscle cell assay. The assay testing indicated that all analogues elicited oxidation of oleic acid. Among the most potent agonists, 2e (2-{2-ethyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}-2-methylpropanoic acid), was also subjected to a luciferase-based transfection assay, which showed that this compound is a potent agonist against PPARδ and a moderate agonist against PPARα. Docking of compound 2e into PPARδ revealed that it occupied the agonist binding site and exhibited key hydrogen bonding interactions with His323, His449 and Tyr473.

Keywords: GW 501516; agonists; PPARα; PPARδ; multi well assay, molecular modeling;

1. Introduction

Peroxisome proliferator-activated receptors are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily [1]. So far, three isotypes have been identified and characterized: PPARα, PPARδ and PPARγ. PPARs are involved in expression of genes responsible of the lipid and carbohydrate metabolism by interacting with specific DNA peroxisome proliferator response elements (PPRE) [2]. Agonists acting on the PPARα have been shown to have beneficial effects on lipid metabolism by decreasing both serum triglycerides and free fatty acid levels, but also increasing high-density lipoprotein level (HDL) [3]. PPARγ agonists have the ability to improve glucose tolerance in type 2 diabetic patients [4]. Dyslipidemia and insulin resistance, two major components of the metabolic syndrome and diabetes, are usually treated with either the fibrate or the thiazolidinedione (TZD) classes of drugs that target PPARα and PPARγ, respectively [4, 5, 6]. Several studies have suggested that PPARδ plays an important role in regulating lipid
metabolism and energy homeostasis in muscle and adipose tissues [7-11]. Furthermore, the activation of PPARδ increases HDL levels, attenuates weight gain, and improves insulin sensitivity [7, 9]. As of today, no drugs that target the PPARδ receptor have been approved, and only a few selective and potent ligands that target this receptor have been identified [12, 13]. In 2003, scientists from GlaxoSmithKline reported the compound GW 501516 (1) (Figure 1) to be both highly potent and selective against the PPARδ receptor [13]. When obese Rhesus monkeys were treated with this agonist, an increase in the plasma HDL level, as well as a decrease in the plasma triglyceride level, was observed [14]. Based on these observations, compound 1 is an interesting lead compound for the development of remedies against type-II diabetes and metabolic syndrome. Moreover, recently dual agonists have received attention as potential remedies against several diseases such as metabolic disorders, type-II diabetes and cardiovascular diseases [15]. Among such possible dual agonists it may be an advantage to activate simultaneously both PPARα and PPARδ by a single dual compound to effectively reduce the risk of cardiovascular disease [15]. So far only a few dual agonists of this type have been reported [16]. Herein we report the synthesis, biological evaluation, and molecular modeling studies of analogues of GW 501516 (1) in which structural modifications at the alpha-carbon atom next to the carboxylic acid moiety and at the ortho-position of the benzene ring A have been made (Figure 1). These efforts led to the identification of a moderate agonist against PPARα while retaining potent agonist effects against PPARδ. Additionally, six other analogues displayed dual agonist effects at 10 μM against both PPARα and PPARδ.

2. Chemistry

Compounds 3a-3e were synthesized according to a literature procedure [17]. Thiocyanates 3a and 3b were reduced with LiAlH₄ to mercaptophenols 4a and 4b in 71-88% yield. Reaction between commercially available 4-mercapto-2-methylphenol, 4a and 4b, respectively, with commercially available 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole in the presence of Cs₂CO₃ at ambient temperature afforded 5a-5c in 69-84% yield. Sulfur-substituted para-mercaptophenols 5a-5c were then reacted with the corresponding ethyl 2-bromoesters in the presence of Cs₂CO₃ to yield esters 8a-8h which after basic aqueous hydrolysis afforded acids 2a-2h in 51-80% yield (Scheme 1).
Treatment of thiocyanates 3c-3e with LiAlH₄ afforded large quantities of the corresponding disulfide dimers. Hence, compounds 3c-3e were first reacted with ethyl 2-bromoacetate and then reduced with NaBH₄ and 1,4-dithioerythritol to afford 7a-7c in 61-77% yield [18]. Oxygen-substituted para-mercaptophenols 7a-7c were reacted with 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole to produce esters 8i-8k which after basic aqueous hydrolysis afforded acids 2i-2k in 49-69% yield over the two steps (Scheme 2).

3. Biological evaluation

GW 501516 (1) and compounds 2a-2k at five different concentrations were exposed for 96 hours to fully differentiated human skeletal muscle cells cultured in 96-well plates. After this period of time, the level of oxidation of oleic acid was measured by detection of accumulated ¹⁴C-labeled oxidized oleic acid [19]. The EC₅₀-values for compounds 2a-2k are presented in Table 1. Compounds 2a-2k were also tested against all three peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) in a luciferase-based transient transfection assay (Figures 2-4).

4. Results and Discussion

The results from the oxidation of the oleic acid assay are compiled in Table 1. The lead compound GW 501516 (1) was highly potent with EC = 0.10 nM in the human skeletal muscle cell assay (Table 1). Substituting one hydrogen atom from the alpha-carbon atom next to the carboxylic acid moiety of GW 501516 (1) with a methyl group led to a decrease in potency (2a: EC₅₀ = 0.65 nM) compared to 1. Introduction of two methyl groups afforded agonist 2b that was slightly more potent than 2a (2b: EC₅₀ = 0.24 nM), but exhibited slightly lower potency than the lead compound 1.
When the methyl group from the R1-position of GW 501516 (1) was substituted to an ethyl group, a decrease of the potency was noticed. (2c: EC50 = 0.31 nM). Substituting one hydrogen atom in 2c with a methyl group alpha-carbon atom next to the carboxylic acid moiety retained the potency, as observed for 2d (EC50 = 0.36 nM). Increasing the size of the ortho-substituent in the benzene ring A (R1), by substitution with an iso-propyl, tert-butyl, cyclopentyl or cyclohexyl group, led to a reduction in potency (2f: EC50 = 4.15 nM; 2i: EC50 = 5.51 nM; 2j: EC50 = 16.60 nM; 2k: EC50 = 17.30 nM). These substituents may be too bulky for the ligand-binding domain of PPARδ. Crystallographic studies have shown that a lipophilic pocket in the PPARδ ligand-binding domain can accommodate small substituents at the ortho-position of the aromatic ring [13, 20]. Changing the methyl group in 2b (R1) to an ethyl group led to agonist 2e that exhibited an EC50-value of 0.54 nM. Replacing the ethyl group in 2e with an iso-propyl group afforded agonist 2h (EC50 = 9.11 nM) that was even less potent than 2e. In the series of compounds 2b, 2e and 2h, the potency decreased with increasing size of R1, a trend that was also observed with 2i, 2j and 2k. Replacement of the hydrogen atoms from the alpha-carbon atom to the carboxylic moiety of 2f with one and two methyl groups diminished the potency compared to 2e, as noticed for 2g (EC50 = 5.79 nM) and 2h (EC50 = 9.11 nM).

Next, we investigated the effects compounds 2a-2k exhibited on all of the peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) in a luciferase-based transient transfection system. Compounds 2a-2e, 2g and 2h showed a higher activation of both PPARα as well as PPARδ at 10 μM concentrations than the positive controls (Figures 2-3). Compounds 2i and 2j activated only the PPARδ receptor with the efficacy comparable to the lead compound 1 at 10 μM (Figure 3). No notable activation of PPARγ was observed (Figure 4) at the same concentration of all prepared analogs of 1. To further investigate the agonist effects of compound 2e, the EC50-values were determined against all three PPARs using the aforementioned transfection assay. The EC50-value for 2e against PPARδ was determined to be 5.0 nM, which is slightly lower than the EC50-value of 1.0 nM reported for GW 501516 (1) [13]. Interestingly, the EC50-value against the PPARα receptor was determined to be 750 nM, which is a moderate agonist effect. Compound 2e was found to be inactive against PPARγ (EC50 > 5000 nM).

In order to gain information on the binding of 2e with the ligand-binding domain of the PPARδ receptor, molecular modeling studies were performed. The activation process of PPARs has been extensively studied [21] and X-ray crystallographic structures have been reported for both active and inactive receptor conformations. In the active receptor conformation, the most C-terminal α-helix (helix 12) acts as a lid closing the binding cavity, while in the inactive state the binding site is more accessible from the outside. In the activated receptor conformation of PPARδ, the amino acids
His323, His449 and Tyr473 are essential for agonist interactions [22]. In the present study 2e was docked into an activated receptor conformation of PPARδ, and the docking showed that 2e was well accommodated to the activated receptor conformation, with a binding mode very similar to that of the full PPARδ agonist 2-\{2,3-dimethyl-4-[2-prop-2-ynyloxy-4-(4-trifluoromethylphenoxy)methyl]phenyl-thio\}phenoxy\}acetic acid (PDB entry: 3GZ9) (Figure 5). The docking of 2e revealed key interactions with amino acids Arg284, Cys285, His323, His449 and Tyr473 (Figure 5). As for the full agonist, the acidic group of 2e interacted with His323, His449, Tyr473. The trifluoromethyl group had contact with Arg284. The calculated interaction energy of the 2e-PPARδ complex was -14.9 kcal/mol. The docking mode supports the observation that compound 2e is a PPARδ agonist.

In the series of tested compounds, the potency decreased with increasing size of the substituent in the R1-position. In the docked complex of 2e the ethyl group in R1 points in the direction of Thr289, Ile326 and Phe327. A larger substituent R1 will produce severe steric interactions with these residues and this may explain the decrease in potency when the size of the substituent is increased to iso-propyl, tert-butyl, cyclopentyl or cyclohexyl groups.

[INSERT FIGURE 5]

5. Conclusions

To the best of our knowledge, very few analogues of GW 501516 (1) have been reported in which modifications at the alpha-carbon atom next to the carboxylic acid moiety have been made [16c, 16d, 23]. This moiety is a common feature for the chemical structures of most PPARδ agonists reported [12, 13]. Herein we report that compounds 2a-2d, 2g and 2h displayed dual agonist effects at 10 μM against both PPARα and PPARδ. Docking of compound 2e into the ligand binding domain of PPARδ supported that compound 2e is a potent PPARδ agonist with EC50 = 5 nM. Moderate potency was observed against PPARα for compound 2e (EC50 = 750 nM). Since very few dual PPARα/δ agonists have been reported in the literature [16], further studies are underway focusing on the preparation of potent and dual PPARα/δ agonists based on the results reported herein. These efforts will be reported in due course.
6. Experimental

6.1. General methods

All dry solvents were commercially available. NMR spectra were recorded on a Bruker DPX 300 spectrometer. Coupling constants ($J$) are reported in hertz, and chemical shifts are reported in parts per million (δ) relative to CDCl$_3$ (7.24 ppm for 1H and 77.00 ppm for 13C) or DMSO-$d_6$ (2.50 ppm for $^1$H and 39.51 ppm for $^{13}$C). Melting points were measured using a Barnstead Electrothermal apparatus. Melting points are uncorrected. Flash column chromatography was performed on silica gel 60 (40–63 μm, Fluka). LC/MS analyses were performed on an Agilent Technologies 1200 Series (Eclipse XDB-C18, 5μm 4.6×150mm), coupled with an Agilent 6310 ion trap. According to LC/MS spectra, all final compounds submitted to the biological testing had a purity > 99%.

6.2. 2-Ethyl-4-thiocyanatophenol (3a)

The title compound was prepared as following: to a stirred solution of 2-ethylphenol (2 mmol, 240 μL), sodium thiocyanate (520 mg, 6.4 mmol) and methanol (40 mL) at 0 °C was added a solution of sodium bromide (206 mg, 2 mmol) and bromine (206 μL, 2 mmol) in methanol (60 mL). The mixture was stirred for 3 hours under argon at 0 °C and then diluted with saturated aqueous solution of NaHCO$_3$. The mixture was extracted (CH$_2$Cl$_2$, 3x100 mL), the organic phases were combined, washed with brine, dried (MgSO$_4$), and concentrated. The residue was purified by column chromatography using hexane/ethyl acetate (3:1) as eluent to give 3a as a white solid in 70% yield (252 mg, 1.4 mmol). Mp = 61-62 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.35 (d, $J$ = 2.4 Hz, 1H), 7.26 (dd, $J$ = 8.4, 2.5 Hz, 1H), 6.83 (d, $J$ = 8.4 Hz, 1H), 6.71 (br s, 1H), 2.64 (q, $J$ = 7.5 Hz, 2H), 1.23 (t, $J$ = 7.5 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 156.11, 133.40, 133.06, 131.36, 116.79, 112.84, 111.94, 22.75, 13.35.

6.3. 2-Iso-propyl-4-thiocyanatophenol (3b)

The title compound was prepared in 81% yield (785 mg, 4.07 mmol) as orange oil from 2-iso-propylphenol (5 mmol, 685 μL) following the general procedure. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.34 (d, $J$ = 2.4 Hz, 1H), 7.24 (dd, $J$ = 8.4, 24 Hz, 1H), 6.76 (d, $J$ = 8.4 Hz, 1H), 5.72 (br s, 1H),
3.19 (hept, \( J = 6.8 \) Hz, 1H), 1.21 (d, \( J = 6.9 \) Hz, 6H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 155.18, 137.34, 131.17, 131.01, 117.07, 113.27, 112.37, 27.17, 22.19. \)

6.4. 2-Tert-butyl-4-thiocyanatophenol (3c)

The title compound was prepared in 68% yield (280 mg, 1.35 mmol) as a light yellow solid from 2-tert-butylphenol (301 mg, 2 mmol) following the general procedure. Mp = 77-78 °C. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta = 7.42 (d, J = 2.4 \) Hz, 1H), 7.26 (dd, \( J = 8.3, 2.4 \) Hz, 1H), 6.73 (d, \( J = 8.3 \) Hz, 1H), 6.00 (br s, 1H), 1.38 (s, 9H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 156.89, 138.86, 131.74, 131.55, 118.25, 112.69, 112.40, 34.90, 29.10. \)

6.5. 2-Cyclopentyl-4-thiocyanatophenol (3d)

The title compound was prepared in 83% yield (230 mg, 1.05 mmol) as a yellow oil from 2-cyclopentylphenol (353 mg, 2 mmol) following the general procedure. \(^1\)H (300 MHz, CDCl\(_3\)): \( \delta = 7.40 (d, J = 2.4 \) Hz, 1H), 7.26 (dd, \( J = 8.4, 2.4 \) Hz, 1H), 6.82 (d, \( J = 8.4 \) Hz, 1H), 6.44 (br s, 1H), 3.32 – 3.18 (m, 1H), 2.19 – 1.96 (m, 2H), 1.92 – 1.46 (m, 6H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 156.11, 135.13, 131.50, 131.13, 116.93, 112.76, 112.24, 38.99, 32.51, 25.11. \)

6.6. 2-Cyclohexyl-4-thiocyanatophenol (3e)

The title compound was prepared in 61% yield (284 mg, 1.22 mmol) as a yellow oil from 2-cyclohexylphenol (358 mg, 2 mmol) following the general procedure. \(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta = 7.44 (d, J = 2.4 \) Hz, 1H), 7.31 (dd, \( J = 8.4, 2.4 \) Hz, 1H), 6.88 (d, \( J = 8.4 \) Hz, 1H), 6.40 (br s, 1H), 3.05 – 2.87 (m, 1H), 2.10 – 1.74 (m, 5H), 1.68 – 1.08 (m, 5H). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)): \( \delta = 155.41, 136.66, 131.48, 131.06, 117.05, 112.73, 112.59, 37.04, 32.69, 26.68, 26.03. \)

6.7. 2-Ethyl-4-mercaptophenol (4a)

The title compound was prepared as following: a solution of 2-ethyl-4-thiocyanatophenol (3a) (963 mg, 5.38 mmol) in anhydrous THF (100 mL) was added cautiously to a mixture of LiAlH\(_4\) (215 mg, 5.5 mmol) and anhydrous THF (50 mL) at 0 °C. The reaction mixture was stirred at ambient temperature for 4 h under argon. Adding moist THF, water, and 1.0 M HCl destroyed the unreacted LiAlH\(_4\). The mixture was extracted (ethyl acetate, 3x100 mL). The organic layers were combined,
washed with brine, dried (MgSO₄), and concentrated. The residue was purified by column chromatography using hexane/ethyl acetate (4:1) as eluent to give 4a as a light colorless oil in 71% yield (585 mg, 3.8 mmol). ¹H NMR (300 MHz, CDCl₃): δ = 7.28 (d, J = 2.1 Hz, 1H), 7.22 (dd, J = 8.2, 2.3 Hz, 1H), 6.70 (d, J = 8.2 Hz, 1H), 4.86 (s, 1H), 2.61 (q, J = 7.5 Hz, 2H), 1.22 (t, J = 7.5 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): δ = 153.78, 132.46, 130.79, 130.24, 128.62, 115.77, 22.77, 13.64.

6.8. 2-Isopropyl-4-mercaptophenol (4b)

Title compound was prepared in 88% yield (578 mg, 3.44 mmol) as a light yellow oil from 3b (753 mg, 3.9 mmol) following the general procedure. ¹H NMR (300 MHz, CDCl₃): δ = 7.17 (d, J = 2.3 Hz, 1H), 7.03 (dd, J = 8.2, 2.1 Hz, 1H), 6.62 (d, J = 8.2 Hz, 1H), 4.86 (br s, 1H), 3.36 (s, 1H), 3.15 (hept, J = 6.9 Hz, 1H), 1.22 (d, J = 6.9 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ = 151.60, 135.55, 129.62, 129.58, 119.83, 116.07, 26.97, 22.38.

6.9. 2-Methyl-4-[[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl]methylthio]phenol (5a)

The title compound was prepared as following: to a solution of 4-mercapto-2-methyl-phenol (280 mg, 2 mmol) in dry CH₃CN (40 mL) was added Cs₂CO₃ (706 mg, 2 mmol). To this mixture was added dropwise a solution of 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (518 mg, 1.78 mmol) in dry CH₃CN (10 mL). The mixture was stirred for 4 h at ambient temperature under argon, then diluted with water and extracted (ethyl acetate, 3x100 mL). The organic layers were combined, dried (MgSO₄) and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (3:1) to give 5a as a light yellow solid in 96% yield (676 mg, 1.71 mmol). Mp = 126-127 °C. ¹H NMR (300 MHz, CDCl₃): δ = 7.93 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 1.6 Hz, 1H), 6.91 (dd, J = 8.2, 2.1 Hz, 1H), 6.54 (d, J = 8.2 Hz, 1H), 4.03 (s, 2H), 2.17 (s, 3H), 2.03 (s, 3H). ¹³C NMR (75 MHz, CDCl₃): δ = 163.78, 155.25, 151.11, 137.10, 136.22 (distorted q, J = 1.2 Hz), 133.21, 131.49 (q, J = 32.7 Hz), 131.48, 126.45, 125.96 (q, J = 3.8 Hz), 125.54, 123.81 (q, J = 272.3 Hz), 123.16, 115.13, 32.79, 15.76, 14.21.

6.10. 2-Ethyl-4-[[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl]methylthio]phenol (5b)

The title compound was prepared in 66% yield (290 mg, 0.71 mmol) as a yellow solid from 4a (185 mg, 1.2 mmol) and 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (311 mg, 1.07
mmol) following the general procedure. Mp = 132-133 °C. ¹H NMR (300 MHz, CDCl₃): δ = 7.93 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 2.2 Hz, 1H), 6.93 (dd, J = 8.2, 2.3 Hz, 1H), 6.54 (d, J = 8.2 Hz, 1H), 4.03 (s, 2H), 2.57 (q, J = 7.5 Hz, 2H), 2.01 (s, 3H), 1.15 (t, J = 7.5 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): δ = 163.75, 154.82, 151.16, 136.24 (distorted q, J = 0.9 Hz), 135.72, 133.28, 131.61, 131.48 (q, J = 32.7 Hz), 131.46, 126.44, 125.95 (q, J = 3.8 Hz), 123.81 (q, J = 272.2 Hz), 123.24, 115.38, 32.86, 22.91, 14.16, 13.85.

6.11. 2-Iso-propyl-4-[[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl]methylthio]phenol (5c)

The title compound was prepared in 80% yield (576 mg, 1.36 mmol) as a light yellow solid from 4b (321 mg, 1.9 mmol) and 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (495 mg, 1.7 mmol) following the general procedure. Mp = 135-136 °C. ¹H NMR (300 MHz, CDCl₃): δ = 7.93 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.3 Hz, 2H), 7.19 (d, J = 2.1 Hz, 1H), 6.93 (dd, J = 8.2, 2.2 Hz, 1H), 6.53 (d, J = 8.2 Hz, 1H), 4.03 (s, 2H), 3.17 (hept, J = 6.9 Hz, 1H), 1.98 (s, 3H), 1.15 (d, J = 6.9 Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ = 163.75, 154.28, 151.24, 136.28 (distorted q, J = 1.2 Hz), 136.04, 133.28, 133.16, 131.48 (q, J = 32.7 Hz), 131.45, 126.44, 125.95 (q, J = 3.8 Hz), 123.83 (q, J = 272.3 Hz), 123.23, 115.55, 32.91, 26.90, 22.35, 14.10.

6.12. Ethyl 2-(2-tert-butyl-4-thiocyanatophenoxy)acetate (6a)

The title compound was prepared as following: to a solution of 2-tert-butyl-4-thiocyanatophenol (3c) (280 mg, 1.35 mmol) in dry CH₃CN (30 mL) was added Cs₂CO₃ (483 mg, 1.48 mmol). To this mixture was added dropwise a solution of ethyl 2-bromoacetate (165 μL, 1.48 mmol) in dry CH₃CN (10 mL). The mixture was stirred for 3 hours at ambient temperature, then diluted with water and extracted (ethyl acetate, 3x100 mL), dried (MgSO₄), and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) to give 6a as a colorless oil in 79% yield (311mg, 1.06 mmol). ¹H NMR (300 MHz, CDCl₃): δ = 7.44 (d, J = 2.5 Hz, 1H), 7.35 (dd, J = 8.5, 2.5 Hz, 1H), 6.72 (d, J = 8.6 Hz, 1H), 4.63 (s, 2H), 4.23 (q, J = 7.1 Hz, 2H), 1.38 (s, 9H), 1.26 (t, J = 7.1 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃): δ = 167.91, 157.98, 141.04, 131.03, 130.93, 114.50, 113.03, 111.51, 65.04, 61.36, 35.18, 29.26, 14.00.
6.13. Ethyl 2-(2-cyclopentyl-4-thiocyanatophenoxy)acetate (6b)

The title compound was prepared in 69% yield (220 mg, 0.72 mmol) as a white solid from 3d (230 mg, 1.05 mmol) and ethyl-2-bromoacetate (129 μL, 1.16 mmol) following the general procedure. Mp = 45-46 °C. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.38 (d, $J$ = 2.4 Hz, 1H), 7.31 (dd, $J$ = 8.6, 2.5 Hz, 1H), 6.70 (d, $J$ = 8.6 Hz, 1H), 4.62 (s, 2H), 4.22 (q, $J$ = 7.1 Hz, 2H), 3.43 – 3.25 (m, 1H), 2.11 – 1.98 (m, 2H), 1.86 – 1.45 (m, 6H), 1.26 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 168.11, 157.18, 137.76, 130.72, 130.42, 114.68, 112.42, 111.44, 65.30, 61.31, 39.26, 32.48, 25.20, 13.99.

6.14. Ethyl 2-(2-cyclohexyl-4-thiocyanatophenoxy)acetate (6c)

The title compound was prepared in 66% yield (257 mg, 0.8 mmol) as a light yellow oil from 3e (285 mg, 1.22 mmol) and ethyl 2-bromoacetate (149 μL, 1.34 mmol) following the general procedure. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.35 (d, $J$ = 2.4 Hz, 1H), 7.30 (dd, $J$ = 8.5, 2.5 Hz, 1H), 6.70 (d, $J$ = 8.6 Hz, 1H), 4.62 (s, 2H), 4.22 (q, $J$ = 7.1 Hz, 2H), 3.07 – 2.91 (m, 1H), 1.93 – 1.66 (m, 5H), 1.52 – 1.15 (m, 8H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 168.13, 156.53, 139.16, 130.58, 130.30, 114.83, 112.45, 111.42, 65.28, 61.30, 37.06, 32.61, 26.71, 26.06, 13.98.

6.15. Ethyl 2-(2-tert-butyl-4-mercaptophenoxy)acetate (7a)

The title compound was prepared as following: to a stirred solution of ethyl 2-(2-tert-butyl-4-thiocyanatophenoxy)acetate (6a) (223 mg, 0.76 mmol) in ethanol (20 mL) at 0 °C 1,4-dithioerythritol (154 mg, 1 mmol) and NaBH$_4$ (38 mg, 1 mmol) were added in portions. The reaction was stirred for 20 min. Adding 1 M HCl destroyed the unreacted NaBH$_4$. The mixture was diluted with water and extracted (diethyl ether, 3x100 mL), dried (MgSO$_4$), and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) to give 7a as a yellow solid in 43% yield (88 mg, 0.33 mmol). Mp = 52-53°C. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.25 (d, $J$ = 2.3 Hz, 1H), 7.11 (dd, $J$ = 8.4, 2.3 Hz, 1H), 6.59 (d, $J$ = 8.4 Hz, 1H), 4.58 (s, 2H), 4.24 (q, $J$ = 7.1 Hz, 2H), 3.35 (s, 1H), 1.38 (s, 9H), 1.28 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 168.60, 155.38, 139.56, 129.99, 129.40, 120.61, 112.46, 65.26, 61.21, 34.89, 29.54, 14.08.

11
6.16. Ethyl 2-(2-cyclopentyl-4-mercaptophenoxy)acetate (7b)

The title compound was prepared in 85% yield (166 mg, 0.59 mmol) as a colorless liquid from 6b (215 mg, 0.70 mmol) following the general procedure. $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 7.18$ (d, $J = 2.3$ Hz, 1H), 7.06 (dd, $J = 8.4$, 2.3 Hz, 1H), 6.58 (d, $J = 8.5$ Hz, 1H), 4.57 (s, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.40 – 3.26 (m, 2H), 2.13 – 1.95 (m, 2H), 1.88 – 1.34 (m, 6H), 1.26 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 168.71, 154.54, 136.21, 129.86, 128.84, 120.99, 112.03, 65.65, 61.10, 39.04, 32.71, 25.31, 14.01.

6.17. Ethyl 2-(2-cyclohexyl-4-mercaptophenoxy)acetate (7c)

The title compound was prepared in 85% yield (200 mg, 0.68 mmol) as a light yellow oil from 6c (257 mg, 0.80 mmol) following the general procedure. $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 7.16$ (d, $J = 2.3$ Hz, 1H), 7.05 (dd, $J = 8.4$, 2.3 Hz, 1H), 6.57 (d, $J = 8.5$ Hz, 1H), 4.57 (s, 2H), 4.22 (q, $J = 7.1$ Hz, 2H), 3.34 (s, 1H), 3.05 – 2.89 (m, 1H), 1.91 – 1.66 (m, 5H), 1.50 – 1.18 (m, 8H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 168.73, 153.87, 137.86, 129.75, 128.77, 121.10, 112.01, 65.64, 61.09, 36.89, 32.84, 26.86, 26.21, 14.02.

6.18. Ethyl 2-{2-methyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoate (8a)

The title compound was prepared as following: to a solution of 2-methyl-4-{[4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl]methylthio}phenol (5a) (120 mg, 0.3 mmol) in dry CH$_3$CN (10 mL) was added Cs$_2$CO$_3$ (147 mg, 0.45 mmol). To this mixture was added dropwise a solution of ethyl 2-bromopropanoate (55 μL, 0.39 mmol) in dry CH$_3$CN (3 mL). The mixture was stirred over night at ambient temperature under argon, then diluted with water and extracted (ethyl acetate, 3x100 mL). The organic layers were combined, dried (MgSO$_4$), and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) to give 8a as a colorless oil in 97% yield (143 mg, 0.29 mmol). $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 7.95$ (d, $J = 8.1$ Hz, 2H), 7.63 (d, $J = 8.2$ Hz, 2H), 7.18 (d, $J = 1.7$ Hz, 1H), 7.07 (dd, $J = 8.4$, 2.1 Hz, 1H), 6.55 (d, $J = 8.5$ Hz, 1H), 4.68 (q, $J = 6.8$ Hz, 1H), 4.15 (q, $J = 7.1$ Hz, 2H), 4.08 (s, 2H), 2.20 (s, 3H), 2.18 (s, 3H), 1.59 (d, $J = 6.8$ Hz, 3H), 1.20 (t, $J = 7.1$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 171.90, 163.04, 156.22, 151.24, 136.72$ (distorted q, $J = 1.2$ Hz), 136.08, 132.06, 131.24 (q, $J = 32.6$ Hz),
130.74, 128.57, 126.37, 125.82 (q, \( J = 3.8 \) Hz), 124.94, 123.91 (q, \( J = 272.2 \) Hz), 112.21, 72.82, 61.22, 32.44, 18.51, 16.15, 14.76, 14.05.

6.19. Ethyl 2-methyl-2-\( \{2\)-methyl-4-\[\( \{(\)methyl-2-\( \)-(4\)-trifluoromethylphenyl\)thiazol-5-y\)l\]methylthio\]phenoxy\]propanoate \( (8b) \)

The title compound was prepared in 83% yield (166 mg, 0.33 mmol) as a yellow oil \( 5a \) (160 mg, 0.4 mmol) and ethyl 2-bromo-2-methylpropanoate (73 \( \mu \)L, 0.52 mmol) following the procedure described for \( 8a \). \( ^1H \) NMR (300 MHz, CDCl\(_3\)): \( \delta = 7.94 \) (d, \( J = 8.1 \) Hz, 2H), 7.63 (d, \( J = 8.2 \) Hz, 2H), 7.16 (d, \( J = 1.8 \) Hz, 1H), 7.03 (dd, \( J = 8.3, 2.2 \) Hz, 1H), 6.53 (d, \( J = 8.5 \) Hz, 1H), 4.19 (q, \( J = 7.1 \) Hz, 2H), 4.08 (s, 2H), 2.18 (s, 3H), 2.15 (s, 3H), 1.56 (s, 6H), 1.20 (t, \( J = 7.1 \) Hz, 3H). \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)): \( \delta = 174.18, 163.01, 154.18, 151.34, 136.80 \) (distorted q, \( J = 1.3 \) Hz), 136.02, 131.45, 130.79 (q, \( J = 32.7 \) Hz), 130.66, 130.45, 126.34, 125.82 (q, \( J = 3.9 \) Hz), 125.44, 123.92 (q, \( J = 272.2 \) Hz), 116.68, 79.22, 61.46, 32.38, 25.36, 16.58, 14.80, 14.03.

6.20. Ethyl 2-\( \{2\)-ethyl-4-\[\( \{(\)methyl-2-\( \)-(4\)-trifluoromethylphenyl\)thiazol-5-y\)l\]methylthio\]phenoxy\]acetate \( (8c) \)

The title compound was prepared in 93% yield (406 mg, 0.82 mmol) as a white solid from \( 5b \) (360 mg, 0.88 mmol) and ethyl 2-bromoacetate (128 \( \mu \)L, 1.15 mmol) following the procedure described for \( 8a \). \( ^1H \) NMR (300 MHz, CDCl\(_3\)): \( \delta = 7.94 \) (d, \( J = 8.1 \) Hz, 2H), 7.63 (d, \( J = 8.2 \) Hz, 2H), 7.16 (d, \( J = 2.1 \) Hz, 1H), 7.13 (dd, \( J = 8.3, 2.3 \) Hz, 1H), 6.58 (d, \( J = 8.3 \) Hz, 1H), 4.59 (s, 2H), 4.21 (q, \( J = 7.1 \) Hz, 2H), 4.08 (s, 2H), 2.62 (q, \( J = 7.5 \) Hz, 2H), 2.14 (s, 3H), 1.25 (t, \( J = 7.1 \) Hz, 3H), 1.14 (t, \( J = 7.5 \) Hz, 3H). \( ^{13}C \) NMR (75 MHz, CDCl\(_3\)): \( \delta = 168.62, 163.01, 156.02, 151.36, 136.76 \) (distorted q, \( J = 1.2 \) Hz), 134.78, 134.20, 132.26, 131.18 (q, \( J = 32.6 \) Hz), 130.68, 126.31, 125.79 (q, \( J = 3.8 \) Hz), 125.20, 123.90 (q, \( J = 272.8 \) Hz), 111.52, 65.40, 61.26, 32.47, 23.07, 14.70, 14.06, 13.85.

6.21. Ethyl 2-\( \{2\)-ethyl-4-\[\( \{(\)methyl-2-\( \)-(4\)-trifluoromethylphenyl\)thiazol-5-y\)l\]methylthio\]phenoxy\]propanoate \( (8d) \)

The title compound was prepared in 93% yield (142 mg, 0.28 mmol) as a yellow oil from \( 5b \) (125 mg, 0.3 mmol) and ethyl 2-bromopropanoate (51 \( \mu \)L, 0.39 mmol) following the procedure described for \( 8a \). \( ^1H \) NMR (300 MHz, CDCl\(_3\)): \( \delta = 7.94 \) (d, \( J = 8.1 \) Hz, 2H), 7.62 (d, \( J = 8.2 \) Hz, 2H), 7.14 (d,


6.22 Ethyl 2-\{(2-ethyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy)-2-methylpropanoate (8e)

The title compound was prepared in 83% yield (131 mg, 0.25 mmol) as a yellow oil from 5b (125 mg, 0.3 mmol) and ethyl 2-bromo-2-methylpropanoate (58 μL, 0.39 mmol) following the procedure described for 8a. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.93 (d, $J = 8.1$ Hz, 2H), 7.62 (d, $J = 8.2$ Hz, 2H), 7.12 (d, $J = 2.3$ Hz, 1H), 7.05 (dd, $J = 8.4$, 2.4 Hz, 1H), 6.52 (d, $J = 8.4$ Hz, 1H), 4.17 (q, $J = 7.1$ Hz, 2H), 4.06 (s, 2H), 2.55 (q, $J = 7.5$ Hz, 2H), 2.12 (s, 3H), 1.57 (s, 6H), 1.17 (t, $J = 7.1$ Hz, 3H), 1.10 (t, $J = 7.5$ Hz, 3H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 171.82, 162.92, 155.76, 151.34, 136.76 (distorted q, $J = 1.3$ Hz), 134.81, 134.25, 132.24, 131.12 (q, $J = 32.6$ Hz), 130.70, 126.28, 125.75 (q, $J = 3.7$ Hz), 123.89 (q, $J = 272.2$ Hz), 124.82, 111.97, 72.48, 61.13, 32.46, 23.17, 18.42, 14.66, 13.99, 13.83.

6.23 Ethyl 2-\{(2-iso-propyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy)acetate (8f)

The title compound was prepared in 75% yield (130 mg, 0.26 mmol) as a yellow oil from 5c (144 mg, 0.34 mmol) and ethyl 2-bromoacetate (49 μL, 0.44 mmol) following the procedure described for 8a. $^1$H NMR (300 MHz, CDCl$_3$): δ = 7.93 (d, $J = 8.1$ Hz, 2H), 7.62 (d, $J = 8.2$ Hz, 2H), 7.12 (d, $J = 2.3$ Hz, 1H), 7.13 (d, $J = 8.3$, 2.3 Hz, 1H), 6.58 (d, $J = 8.3$ Hz, 1H), 4.59 (s, 2H), 4.21 (q, $J = 7.1$ Hz, 2H), 4.06 (s, 2H), 3.31 (hept, $J = 6.9$ Hz, 1H), 2.09 (s, 3H), 1.24 (t, $J = 7.1$ Hz, 3H), 1.13 (d, $J = 6.9$ Hz, 6H). $^{13}$C NMR (75 MHz, CDCl$_3$): δ = 168.56, 162.97, 155.45, 151.39, 138.43, 136.72 (distorted q, $J = 1.2$ Hz), 132.42, 132.26, 131.12 (q, $J = 32.6$ Hz), 130.70, 126.26, 125.74 (q, $J = 3.8$ Hz), 125.14, 123.87 (q, $J = 271.9$ Hz), 111.61, 65.38, 61.20, 32.49, 26.73, 22.31, 14.57, 14.01.
6.24. Ethyl 2-{2-iso-propyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoate (8g)

The title compound was prepared in 94% yield (170 mg, 0.32 mmol) as a yellow oil from 5c (144 mg, 0.34 mmol) and ethyl 2-bromopropanoate (57 μL, 0.44 mmol) following the procedure described for 8a. $^1$H NMR (300 MHz, CDCl₃): δ = 7.94 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 2.3 Hz, 1H), 7.10 (dd, J = 8.4, 2.3 Hz, 1H), 6.56 (d, J = 8.4 Hz, 1H), 4.71 (q, J = 6.8 Hz, 1H), 4.13 (q, J = 7.1 Hz, 2H), 4.05 (s, 2H), 3.31 (hept, J = 6.9 Hz, 1H), 2.09 (s, 3H), 1.59 (d, J = 6.8 Hz, 3H), 1.25 − 1.07 (m, 9H). $^{13}$C NMR (75 MHz, CDCl₃): δ = 171.83, 162.97, 155.20, 151.40, 138.43, 136.75 (distorted q, J = 1.3 Hz), 132.45, 132.27, 131.13 (q, J = 32.6 Hz), 130.75, 126.27, 125.75 (q, J = 3.8 Hz), 124.76, 123.88 (q, J = 272.1 Hz), 111.98, 72.44, 61.14, 32.55, 26.78, 22.33, 18.43, 14.59, 14.00.

6.25. Ethyl 2-{2-iso-propyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}-2-methylpropanoate (8h)

The title compound was prepared in 53% yield (96 mg, 0.18 mmol) as a yellow oil from 5c (144 mg, 0.34 mmol) and ethyl 2-bromo-2-methylpropanoate (66 μL, 0.44 mmol) following the procedure described for 8a. $^1$H NMR (300 MHz, CDCl₃): δ = 7.93 (d, J = 8.1 Hz, 2H), 7.62 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 2.3 Hz, 1H), 7.06 (dd, J = 8.4, 2.4 Hz, 1H), 6.51 (d, J = 8.4 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 4.06 (s, 2H), 3.25 (hept, J = 6.9 Hz, 1H), 2.09 (s, 3H), 1.57 (s, 6H), 1.17 (t, J = 7.1 Hz, 3H), 1.09 (d, J = 6.9 Hz, 6H). $^{13}$C NMR (75 MHz, CDCl₃): δ = 174.19, 163.01, 153.26, 151.41, 140.18, 136.75 (distorted q, J = 1.3 Hz), 132.49, 131.70, 131.20 (q, J = 32.6 Hz), 130.76, 126.31, 125.80 (q, J = 3.8 Hz), 125.06, 123.91 (q, J = 272.0 Hz), 116.24, 78.95, 61.40, 32.52, 26.81, 25.33, 22.44, 14.59, 13.95.

6.26. Ethyl 2-{2-tert-butyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}acetate (8i)

The title compound was prepared as following: to a solution of ethyl 2-(2-tert-buty1-4-mercaptophenoxy)acetate (7a) (88 mg, 0.33 mmol) in dry CH₃CN (10 mL) was added Cs₂CO₃ (117 mg, 0.33 mmol). To this mixture was added dropwise a solution of 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (98 mg, 0.34 mmol) in dry CH₃CN (5 mL). The mixture was stirred for 4 h at ambient temperature under argon, then diluted with water and extracted (ethyl acetate,
3x100 mL), dried (MgSO₄), and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (4:1) to give 8i as a colorless oil in 94% yield (163 mg, 0.31 mmol). \(^1\)H NMR (300 MHz, CDCl₃): \(\delta = 7.94 (d, J = 8.1 \text{ Hz}, 2H), 7.63 (d, J = 8.2 \text{ Hz}, 2H), 7.21 (d, J = 2.2 \text{ Hz}, 1H), 7.18 (dd, J = 8.2, 2.3 \text{ Hz}, 1H), 6.60 (d, J = 8.3 \text{ Hz}, 1H), 4.60 (s, 2H), 4.23 (q, J = 7.1 Hz, 2H), 4.06 (s, 2H), 2.07 (s, 3H), 1.31 (s, 9H), 1.26 (t, J = 7.1 Hz, 3H). \(^{13}\)C NMR (75 MHz, CDCl₃): \(\delta = 168.41, 163.08, 156.98, 151.53, 139.44, 136.77\) (distorted q, J = 1.3 Hz), 133.27, 132.80, 130.74, 131.20 (q, J = 32.6 Hz), 126.32, 125.81 (q, J = 3.8 Hz), 124.66, 123.91 (q, J = 272.1 Hz), 112.16, 65.14, 61.28, 34.86, 32.60, 29.42, 14.60, 14.07.

6.27. \textbf{Ethyl 2-\{2-cyclopentyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio\}phenoxy\}acetate (8j)}

The title compound was prepared in 58% yield (114 mg, 0.21 mmol) as a yellow oil from 7b (100 mg, 0.36 mmol) and 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (107 mg, 0.37 mmol) following the procedure described for 8i. \(^1\)H NMR (300 MHz, CDCl₃): \(\delta = 7.94 (d, J = 8.1 \text{ Hz}, 2H), 7.63 (d, J = 8.2 \text{ Hz}, 2H), 7.18 – 7.11 (m, 2H), 6.59 (d, J = 8.2 \text{ Hz}, 1H), 4.59 (s, 2H), 4.22 (q, J = 7.1 Hz, 2H), 4.06 (s, 2H), 3.40 – 3.22 (m, 1H), 2.11 (s, 3H), 2.06 – 1.87 (m, 2H), 1.78 – 1.37 (m, 6H), 1.25 (t, J = 7.1 Hz, 3H). \(^{13}\)C NMR (75 MHz, CDCl₃): \(\delta = 168.62, 163.02, 155.53, 151.50, 137.58, 136.76\) (distorted q, J = 1.2 Hz), 133.15, 132.25, 131.18 (q, J = 32.6 Hz), 130.74, 126.30, 125.78 (q, J = 3.6 Hz), 125.04, 123.90 (q, J = 272.3 Hz), 111.67, 65.53, 61.25, 38.91, 32.73, 32.55 25.31, 14.66, 14.07.

6.28. \textbf{Ethyl 2-\{2-cyclohexyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio\}phenoxy\}acetate (8k)}

The title compound was prepared in 79% yield (151 mg, 0.27 mmol) as a light yellow oil from 7c (100 mg, 0.34 mmol) and 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (101 mg, 0.35 mmol) following the procedure described for 8i. \(^1\)H NMR (300 MHz, CDCl₃): \(\delta = 7.94 (d, J = 8.1 \text{ Hz}, 2H), 7.62 (d, J = 8.2 \text{ Hz}, 2H), 7.14 (dd, J = 8.3, 2.3 \text{ Hz}, 1H), 7.11 (d, J = 2.2 \text{ Hz}, 1H), 6.58 (d, J = 8.3 Hz, 1H), 4.58 (s, 2H), 4.21 (q, J = 7.1 Hz, 2H), 4.05 (s, 2H), 3.01 – 2.85 (m, 1H), 2.08 (s, 3H), 1.87 – 1.58 (m, 5H), 1.45 – 0.99 (m, 8H). \(^{13}\)C NMR (75 MHz, CDCl₃): \(\delta = 168.62, 162.97, 155.53, 151.50, 137.58, 136.76\) (d, J = 1.2 Hz), 133.15, 132.34, 131.14 (q, J = 32.6 Hz), 130.67, 126.28, 125.74 (q, J = 3.7 Hz), 124.99, 123.88 (q, J = 272.3 Hz), 111.65, 65.48, 61.22, 36.79, 32.84, 32.53, 26.83, 26.14, 14.59, 14.05.
2-{2-Methyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoic acid (2a)

The title compound was prepared as following: to a stirred solution of ethyl 2-{2-methyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoate (8a) (170 mg, 0.34 mmol) in THF (10 mL) and H₂O (5 mL) at 0 °C was added slowly 215 μL of 2.0 M LiOH. The reaction mixture was stirred until TLC indicated completion of the reaction. The mixture was diluted with 50 mL H₂O, acidified with 0.1 M HCl, extracted (diethyl ether, 3x50 mL), dried (MgSO₄), and concentrated. The residue was recrystallized from ethyl acetate/hexane to give 2a as a white solid in 50% yield (80 mg, 0.17 mmol). Mp = 78-79 °C. ¹H NMR (300 MHz, DMSO-d₆): δ = 12.97 (br s, 1H), 8.03 (d, J = 8.1 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 7.22 (d, J = 1.6 Hz, 1H), 7.15 (dd, J = 8.4, 2.1 Hz, 1H), 6.72 (d, J = 8.5 Hz, 1H), 4.80 (q, J = 6.7 Hz, 1H), 4.33 (s, 2H), 2.19 (s, 3H), 2.13 (s, 3H), 1.50 (d, J = 6.7 Hz, 3H). ¹³C NMR (75 MHz, DMSO-d₆): δ = 172.92, 161.70, 155.51, 151.08, 136.53 (distorted q, J = 1.0 Hz), 134.36, 131.55, 130.74, 129.61 (q, J = 31.9 Hz), 127.29, 126.34, 126.11 (q, J = 3.8 Hz), 124.38, 124.00 (q, J = 272.3 Hz), 112.46, 71.81, 30.54, 18.28, 15.85, 14.60. MS (ESI) m/z 466.10 [M-H]

6.30. 2-Methyl-2-{2-methyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoic acid (2b)

The title compound was prepared as following: to a stirred solution of ethyl 2-methyl-2-{2-methyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoate (8b) (166 mg, 0.33 mmol) in THF (10 mL) and H₂O (5 mL) was added 1 mL aqueous solution of 2.0 M t-BuOK. The reaction mixture was refluxed for 24 h. After the completion, the mixture was diluted with 50 mL H₂O, acidified with 0.1 M HCl, extracted (diethyl ether, 3x50 mL), dried (MgSO₄), and concentrated. The residue was recrystallized from ethyl acetate/hexane to give 2b as a white solid in 45% yield (72 mg, 0.15 mmol). Mp = 125-126 ºC. ¹H NMR (300 MHz, DMSO-d₆): δ = 8.02 (d, J = 8.2 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 1.8 Hz, 1H), 7.02 (dd, J = 8.7, 2.0 Hz, 1H), 6.79 (d, J = 8.6 Hz, 1H), 4.29 (s, 2H), 2.17 (s, 3H), 2.07 (s, 3H), 1.40 (s, 6H). ¹³C NMR (75 MHz, DMSO-d₆): δ = 173.84, 161.64, 156.36, 150.98, 136.53 (distorted q, J = 1.3 Hz), 134.21, 131.61, 130.81, 129.58 (q, J = 32.0 Hz), 126.84, 126.33, 126.07 (q, J = 3.8 Hz), 124.00 (q, J = 272.2 Hz),
123.18, 112.45, 73.66, 30.77, 18.75, 15.96, 14.62. MS (ESI) m/z 480.0 [M-H], HRMS calcd for C_{23}H_{22}F_{3}NO_{3}S_{2} [M]^{+}: 481.0993; found: 481.0988.

6.31. 2-{2-Ethyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}acetic acid (2c)

The title compound was prepared in 43% yield (86 mg, 0.18 mmol) as a white solid from 8c (206 mg, 0.42 mmol) following the procedure described for 2a. Mp = 141-142 °C. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta = 8.00\) (d, \(J = 8.1\) Hz, 2H), 7.78 (d, \(J = 8.4\) Hz, 2H), 7.15 – 7.04 (m, 2H), 6.69 (d, \(J = 9.0\) Hz, 1H), 4.28 (s, 2H), 4.26 (s, 2H), 2.51 (q, \(J = 7.4\) Hz, 2H), 2.13 (s, 3H), 1.05 (t, \(J = 7.5\) Hz, 3H). \(^1^3\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta = 172.85, 161.65, 156.65, 151.03, 136.53\) (distorted q, \(J = 1.2\) Hz), 132.95, 132.54, 131.74, 131.16, 129.58 (q, \(J = 32.0\) Hz), 126.28, 122.86, 126.09 (q, \(J = 3.8\) Hz), 123.99 (q, \(J = 272.1\) Hz), 112.24, 67.33, 30.95, 22.63, 14.55, 13.90. MS (ESI) m/z 466.1 [M-H], HRMS calcd for C_{22}H_{20}F_{3}NO_{3}S_{2} [M]^{+} : 467.0837; found: 467.0858.

6.32. 2-{2-Ethyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}propanoic acid (2d)

The title compound was prepared in 50% yield (69 mg, 0.14 mmol) as a white solid from 8d (142 mg, 0.28 mmol) following the procedure described for 2a. Mp = 127-128 °C. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta = 12.95\) (br s, 1H), 8.02 (d, \(J = 8.1\) Hz, 2H), 7.79 (d, \(J = 8.3\) Hz, 2H), 7.17 (dd, \(J = 8.4, 2.3\) Hz, 1H), 7.12 (d, \(J = 2.3\) Hz, 1H), 6.73 (d, \(J = 8.5\) Hz, 1H), 4.81 (q, \(J = 6.7\) Hz, 1H), 4.30 (s, 2H), 2.52 (q, \(J = 7.3\) Hz, 2H), 2.13 (s, 3H), 1.49 (d, \(J = 6.7\) Hz, 3H), 1.06 (t, \(J = 7.5\) Hz, 3H). \(^1^3\)C NMR (75 MHz, DMSO-\(d_6\)): \(\delta = 172.97, 161.73, 155.23, 151.18, 136.55\) (distorted, \(J = 1.2\) Hz), 133.37, 133.05, 131.52, 131.22, 129.62 (q, \(J = 32.0\) Hz), 126.30, 126.08 (q, \(J = 3.7\) Hz), 124.22, 124.00 (q, \(J = 272.0\) Hz), 112.42, 71.67, 30.77, 22.70, 18.26, 14.50, 13.85. MS (ESI) m/z 480.1 [M-H], HRMS calcd for C_{23}H_{22}F_{3}NO_{3}S_{2} [M]^{+} : 481.0993; found: 481.0980.

6.33. 2-{2-Ethyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}2-methylpropanoic acid (2e)

The title compound was prepared in 60% yield (74 mg, 0.15 mmol) as a white solid from 8e (131 mg, 0.25 mmol) following the procedure described for 2b. Mp = 132-133 °C. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta = 13.07\) (br s, 1H), 8.02 (d, \(J = 8.1\) Hz, 2H), 7.80 (d, \(J = 8.3\) Hz, 2H), 7.15 (dd, \(J = 8.4,
2.4 Hz, 1H), 7.13 (d, J = 2.2 Hz, 1H), 6.62 (d, J = 8.3 Hz, 1H), 4.31 (s, 2H), 2.49 (q, 7.5 Hz, 2H), 2.13 (s, 3H), 1.50 (s, 6H), 1.04 (t, J = 7.5 Hz, 3H). 13C NMR (75 MHz, DMSO-d6): δ = 175.04, 161.73, 153.12, 151.21, 136.54 (distorted q, J = 1.2 Hz), 134.96, 133.54, 131.39, 130.71, 129.62 (q, J = 32.0 Hz), 126.29, 126.10 (q, J = 3.7 Hz), 124.76, 124.00 (q, J = 272.1 Hz), 116.22, 78.32, 30.65, 24.98, 22.92, 14.49, 13.97. MS (ESI) m/z 494.0 [M-H]−, HRMS calcd for C24H24F3NO3S2 [M]+: 495.1150; found: 495.1173.

6.34. 2-[(2-Iso-propyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy)acetic acid (2f)

The title compound was prepared in 35% yield (43 mg, 0.09 mmol) as a white solid from 8f (130 mg, 0.26 mmol) following the procedure described for 2a. Mp = 146-147 °C. 1H NMR (300 MHz, DMSO-d6): δ = 12.95 (br s, 1H), 8.02 (d, J = 8.0 Hz, 2H), 7.80 (d, J = 8.2 Hz, 2H), 7.20 (dd, J = 8.4, 1.7 Hz, 1H), 7.07 (d, J = 1.5 Hz, 1H), 6.80 (d, J = 8.5 Hz, 1H), 4.69 (s, 2H), 4.29 (s, 2H), 3.22 (hept, J = 6.9 Hz, 1H), 2.07 (s, 3H), 1.06 (d, J = 6.9 Hz, 6H). 13C NMR (75 MHz, DMSO-d6): δ = 170.01, 161.77, 154.98, 151.26, 137.09, 136.57 (distorted q, J = 1.2 Hz), 131.59, 131.48, 130.92, 129.61 (q, J = 32.1 Hz), 126.29, 126.10 (q, J = 3.8 Hz), 124.26, 124.00 (q, J = 272.1 Hz), 112.20, 64.73, 30.91, 26.15, 22.16, 14.43. MS (ESI) m/z 480.1 [M-H]−, HRMS calcd for C23H22F3NO3S2 [M]+: 481.0993; found: 481.0973.

6.35. 2-[(2-Iso-propyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy)propanoic acid (2g)

The title compound was prepared in 66% yield (113 mg, 0.23 mmol) as a white solid from 8g (183 mg, 0.35 mmol) following the procedure described for 2a. Mp = 170-171 °C. 1H NMR (300 MHz, DMSO-d6): δ = 12.97 (br s, 1H), 8.03 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H), 7.19 (dd, J = 8.5, 2.3 Hz, 1H), 7.06 (d, J = 2.2 Hz, 1H), 6.74 (d, J = 8.6 Hz, 1H), 4.83 (q, J = 6.7 Hz, 1H), 4.29 (s, 2H), 3.21 (hept, J = 6.9 Hz, 1H), 2.06 (s, 3H), 1.50 (d, J = 6.7 Hz, 3H), 1.06 (d, J = 6.9 Hz, 6H). 13C NMR (75 MHz, DMSO-d6): δ = 172.90, 161.77, 154.67, 151.29, 137.21, 136.58 (distorted q, J = 1.4 Hz), 131.58, 131.48, 131.04, 129.61 (q, J = 32.0 Hz), 126.30, 126.11 (q, J = 3.7 Hz), 124.13, 124.00 (q, J = 272.2 Hz), 112.51, 71.59, 30.91, 26.23, 22.09, 18.22, 14.40. MS (ESI) m/z 494.1 [M-H]−, HRMS calcd for C24H24F3NO3S2 [M]+: 495.1150; found: 495.1147.
6.36. 2-{2-Iso-propyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}-2-methylpropanoic acid (2h)

The title compound was prepared in 48% yield (44 mg, 0.09 mmol) as a white solid from 8h (96 mg, 0.18 mmol) following the procedure described for 2b. Mp = 122-123 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 8.03$ (d, $J = 8.1$ Hz, 2H), 7.82 (d, $J = 8.3$ Hz, 2H), 7.17 (dd, $J = 8.5$, 2.2 Hz, 1H), 7.06 (d, $J = 2.2$ Hz, 1H), 6.62 (d, $J = 8.5$ Hz, 1H), 4.30 (s, 2H), 3.16 (hept, $J = 6.9$ Hz, 1H), 2.07 (s, 3H), 1.49 (s, 6H), 1.04 (d, $J = 6.9$ Hz, 6H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 175.15, 161.79, 152.64, 151.31, 139.06, 136.58$ (distorted q, $J = 1.3$ Hz), 131.49, 131.23, 130.98, 129.61 (q, $J = 32.0$ Hz), 126.30, 126.15 (q, $J = 3.6$ Hz), 124.57, 124.02 (q, $J = 272.2$ Hz), 116.36, 78.41, 30.83, 26.34, 25.02, 22.21, 14.40. MS (ESI) $m/z$ 508.0 [M-H], HRMS calcd for C$_{25}$H$_{26}$F$_3$NO$_3$S$_2$ [M]+: 509.1306; found: 509.1295.

6.37. 2-{2-Tert-butyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}acetic acid (2i)

The title compound was prepared in 44% yield (42 mg, 0.08 mmol) as a white solid from 8i (94 mg, 0.18 mmol) following the procedure described for 2a. Mp = 105-106 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 12.97$ (br s, 1H), 8.04 (d, $J = 8.1$ Hz, 2H), 7.81 (d, $J = 8.3$ Hz, 2H), 7.26 (dd, $J = 8.4$, 2.2 Hz, 1H), 7.00 (d, $J = 2.3$ Hz, 1H), 6.83 (d, $J = 8.5$ Hz, 1H), 4.69 (s, 2H), 4.26 (s, 2H), 2.01 (s, 3H), 1.23 (s, 9H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 169.82, 161.86, 156.67, 151.45, 138.02, 136.58$ (distorted q, $J = 1.2$ Hz), 132.34, 131.98, 131.55, 129.62 (q, $J = 31.9$ Hz), 126.30, 126.13 (q, $J = 3.8$ Hz), 124.01 (q, $J = 272.1$ Hz), 123.64, 113.01, 64.70, 34.38, 31.12, 29.18, 14.32. MS (ESI) $m/z$ 494.0 [M-H], HRMS calcd for C$_{24}$H$_{24}$F$_3$NO$_3$S$_2$ [M]$^+$: 495.1150; found: 495.1138.

6.38. 2-{2-Cyclopentyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}acetic acid (2j)

The title compound was prepared in 39% yield (37 mg, 0.07 mmol) as a white solid from 8j (94 mg, 0.18 mmol) following the procedure described for 2a. Mp = 88-89 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 8.02$ (d, $J = 8.1$ Hz, 2H), 7.80 (d, $J = 8.3$ Hz, 2H), 7.14 (dd, $J = 8.4$, 2.2 Hz, 1H), 7.01 (d, $J = 2.1$ Hz, 1H), 6.70 (d, $J = 8.5$ Hz, 1H), 4.31 (s, 2H), 4.25 (s, 2H), 3.30 – 3.14 (m, 1H), 2.07 (s, 3H), 1.93 – 1.77 (m, 2H), 1.63 – 1.28 (m, 6H). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 173.09, 161.70, 156.70, 151.18, 136.55$ (distorted q, $J = 1.2$ Hz), 134.44, 131.84, 131.46, 131.34, 129.58 (q, $J =$
31.7 Hz), 126.26, 126.09 (q, \( J = 3.9 \) Hz), 124.00 (q, \( J = 272.2 \) Hz), 122.72, 112.27, 67.27, 38.25, 32.34, 31.07, 24.85, 14.50. MS (ESI) \( m/z \) 506.2 [M-H]^-, HRMS calcd for \( \text{C}_{25}\text{H}_{24}\text{F}_3\text{NO}_3\text{S}_2 \) [M]^+: 507.1150; found 507.1161.

6.39. 2-{2-Cyclohexyl-4-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio]phenoxy}acetic acid (2k)

The title compound was prepared in 48% yield (64 mg, 0.12 mmol) as a white solid from 8k (135 mg, 0.25 mmol) following the procedure described for 2a. Mp = 144-145°C. \(^1\)H NMR (300 MHz, DMSO-\( d_6 \)): \( \delta = 12.94 \) (br s, 1H), 8.04 (d, \( J = 8.1 \) Hz, 2H), 7.81 (d, \( J = 8.3 \) Hz, 2H), 7.21 (dd, \( J = 8.5, 2.2 \) Hz, 1H), 6.76 (d, \( J = 2.2 \) Hz, 1H), 6.76 (d, \( J = 8.6 \) Hz, 1H), 4.88 (s, 2H), 4.27 (s, 2H), 2.91 – 2.76 (m, 1H), 2.04 (s, 3H), 1.70 – 1.51 (m, 5H), 1.36 – 1.04 (m, 5H). \(^{13}\)C NMR (75 MHz, DMSO-\( d_6 \)): \( \delta = 170.06, 161.80, 155.09, 151.42, 136.60 \) (distorted q, \( J = 1.0 \) Hz), 136.13, 131.82, 131.77, 131.51, 129.61 (q, \( J = 32.1 \) Hz), 126.30, 126.08 (q, \( J = 3.6 \) Hz), 124.02 (d, \( J = 272.1 \) Hz), 123.94, 112.14, 64.73, 36.07, 32.26, 30.98, 26.42, 25.58, 14.40. MS (ESI) \( m/z \) 520.0 [M-H]^-, HRMS calcd for \( \text{C}_{26}\text{H}_{26}\text{F}_3\text{NO}_3\text{S}_2 \) [M]^+: 521.1306; found: 521.1310.

6.40. Measurement of oleic acid oxidation

Satellite cells were isolated from the Musculus obliquus internus abdominis of healthy donors. The biopsies were obtained with informed consent and approval by the Regional Committee for Research Ethics, Oslo, Norway. The cells were cultured in DMEM (5.5 mM glucose) with 2% FCS, 2% Ultroser G, penicillin/streptomycin (P/S) and amphotericin B until 70-80% confluent. Myoblast differentiation to myotubes was then induced by changing medium to DMEM (5.5 mM glucose) with 2% FCS, 25 pM insulin, P/S and amphotericin B. Experiments were performed after 7 days of differentiation, and preincubation with agonists was started after 3 days. The substrate, [1-\(^{14}\)C]oleic acid (1 \( \mu \)Ci/mL, 100 \( \mu \)M), was given in DPBS with 10 mM HEPES and 1 mM L-carnitine. A 96-well UNIFILTER® microplate was mounted on top of the CellBIND® plate as described before [19], and the cells were incubated at 37 °C for 4 hours. The CO\(_2\) trapped in the filter was counted by liquid scintillation (MicroBeta®, PerkinElmer) and normalized against protein content. EC\(_{50}\)-values were calculated with GraphPad Prism, version 4.
6.41. Luciferase-based transient transfection system

COS-1 cells (ATCC no. CRL 1650) were cultured in DMEM supplemented with L-glutamine (2MM), penicillin (50 U/mL), streptomycin (50 μg/mL), fungizone (2.5 μg/mL), and 10% inactivated FBS. The cells were incubated at 37 °C in a humidified atmosphere of 5%CO₂ and 95% air and used for transient transfections. Cells were plated in six-well plates 1 day before transfection. Transient transfection by lipofectamin 2000 (Invitrogen, Carlsbad, CA) was performed as described. Each well received 990 ng plasmid: 320 ng reporter ((UAS)5-tk-LUC) (UAS = upstream activating sequence and LUC = luciferase), 640 ng pGL3 basic (empty vector) and 30 ng expression plasmid of either pSG5-GAL4-hPPARα, pSG5-GAL4-hPPARδ and pSG5-GAL4-hPPARγ. 10 μM of the compounds and controls and DMSO (negative control) was added to the media 5 hours after transfection. Transfected cells were maintained for 24 hours before lysis by reporter lysis buffer. Binding of the ligands to the LBD of PPARs activates GAL4 binding to UAS, which in turn stimulates the tk promoter to drive luciferase expression. Luciferase activity was measured using a luminometer (TD-20/20 luminometer Turner Designs, Sunnyvale, CA) and normalized against protein content. The following compounds were used as positive controls: (2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid (EHA), GW 501516 (1) and rosiglitazone (BRL) for PPARα, PPARδ, and PPARγ, respectively. EC₅₀ is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC₅₀-value is the average of three separate tests.

6.42. Docking of 2e into PPARδ

The ICM ('Internal Coordinate Mechanics') program (version 3.6-1h) [24] was used for docking and calculation of the interaction energy. The X-ray crystal structure PPARδ (PDB entry: 3GZ9) [22], with an agonist at the binding site, was converted to and ICM object, and receptor maps where calculated based on the agonist position in the crystal structures. 2e was modelled using the ICM molecule editor and docked into PPARδ using interactive docking, and the interaction energy was calculated using the calcBindingEnergy macro of ICM.

7. Acknowledgment: Professors Arild Chr. Rustan and G. Hege Thoresen, as well as Nataša Nikolić, are gratefully acknowledged for fruitful discussions and assistance with biological testing.
8. References and notes


Figure Legends

**Figure 1.** Structure of GW 501516 (1) and derivatives 2a-2k

**Figure 2.** Activation of the ligand-binding domain of PPARα by compounds 2a-2k. Positive control: EHA ((2E,4E,8Z,11Z,14Z,17Z)-eicosa-2,4,8,11,14,17-hexaenoic acid).

**Figure 3.** Activation of the ligand-binding domain of PPARδ by compounds 2a-2k. Positive control: GW 501516 (1).

**Figure 4.** Activation of the ligand-binding domain of PPARγ by compounds 2a-2k. Positive control: BRL (rosiglitazone).

**Figure 5:** A. 2e docked into PPARδ. Colour coding: red O, blue N, grey H, yellow C in 2e, white C in PPARδ. Colouring of the Ca traces of PPARδ is blue via white to red from N-terminal to C-terminal. B. The docked complex of 2e (purple) superimposed onto the X-ray structure complex of the agonist 2-{2,3-dimethyl-4-[2-prop-2-ynyloxy-4-((4-trifluoromethylphenoxy)methyl)phenylthio]phenoxy}acetic acid (green) (PDB entry: 3GZ9).

**Scheme 1.** Reagents and conditions: (a) LiAlH₄, THF; (b) 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole, Cs₂CO₃, CH₃CN; (c) i) ethyl 2-bromoacetate, Cs₂CO₃, CH₃CN or ii) ethyl 2-bromopropionate, Cs₂CO₃, CH₃CN; (d) LiOH, THF, H₂O, 0 °C (2a, 2c, 2d, 2f, 2g); (CH₃)₃COK, THF, H₂O, reflux (2b, 2e, 2h).

**Scheme 2.** Reagents and conditions: (a) ethyl 2-bromoacetate, Cs₂CO₃, CH₃CN; (b) NaBH₄, 1,4-dithioerythritol, EtOH; (c) 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole, Cs₂CO₃, CH₃CN; (d) LiOH, THF, H₂O, 0 °C.

**Table 1.** Substitution pattern (see Fig. 1) and EC₅₀-values of tested compounds in the oleic acid oxidation assay

*aResults of three experiments*
Figure 1

Calin C. Ciocoiu

GW 501516 (I)

2a: R₁ = CH₃, R₂ = H, R₃ = CH₃
2b: R₁ = CH₃, R₂ = CH₃, R₃ = CH₃
2c: R₁ = Ethyl, R₂ = H, R₃ = H
2d: R₁ = Ethyl, R₂ = H, R₃ = CH₂
2e: R₁ = Ethyl, R₂ = CH₃, R₃ = CH₃
2f: R₁ = iso-Propyl, R₂ = H, R₃ = H
2g: R₁ = iso-Propyl, R₂ = H, R₃ = CH₃
2h: R₁ = iso-Propyl, R₂ = CH₃, R₃ = CH₃
2i: R₁ = Norisobutyryl, R₂ = H, R₃ = H
2j: R₁ = Cyclopentyl, R₂ = H, R₃ = H
2k: R₁ = Cyclobexyl, R₂ = H, R₃ = H
Figure 2
Calin C. Ciocoiu
Figure 3
Calin C. Ciocoiu
Figure 4
Calin C. Ciocoiu
Figure 5
Calin C. Ciocoiu
Scheme 1
Calin C. Ciocoiu
Scheme 2
Calin C. Ciocoiu
### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$R_3$</th>
<th>EC$_{50}$ (nM)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>CH$_3$</td>
<td>H</td>
<td>CH$_3$</td>
<td>0.65</td>
</tr>
<tr>
<td>2b</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>0.24</td>
</tr>
<tr>
<td>2c</td>
<td>Ethyl</td>
<td>H</td>
<td>H</td>
<td>0.31</td>
</tr>
<tr>
<td>2d</td>
<td>Ethyl</td>
<td>H</td>
<td>CH$_3$</td>
<td>0.36</td>
</tr>
<tr>
<td>2e</td>
<td>Ethyl</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>0.54</td>
</tr>
<tr>
<td>2f</td>
<td><em>iso</em>-Propyl</td>
<td>H</td>
<td>H</td>
<td>4.15</td>
</tr>
<tr>
<td>2g</td>
<td><em>iso</em>-Propyl</td>
<td>H</td>
<td>CH$_3$</td>
<td>5.79</td>
</tr>
<tr>
<td>2h</td>
<td><em>iso</em>-Propyl</td>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>9.11</td>
</tr>
<tr>
<td>2i</td>
<td><em>tert</em>-Butyl</td>
<td>H</td>
<td>H</td>
<td>5.51</td>
</tr>
<tr>
<td>2j</td>
<td>Cyclopentyl</td>
<td>H</td>
<td>H</td>
<td>16.60</td>
</tr>
<tr>
<td>2k</td>
<td>Cyclohexyl</td>
<td>H</td>
<td>H</td>
<td>17.30</td>
</tr>
<tr>
<td><strong>GW 501516 (1)</strong></td>
<td>CH$_3$</td>
<td>H</td>
<td>H</td>
<td>0.10</td>
</tr>
</tbody>
</table>

$^a$ Values are in nM.