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

Dissertation for the degree of Ph.D.

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Blindern, September 2010 Calin Constantin Ciocoiu

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 EC_{50}-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.

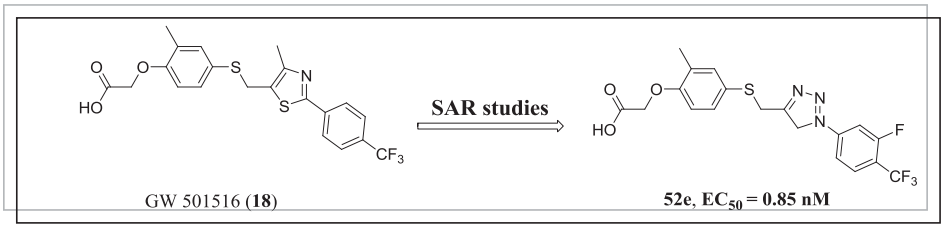
Further SAR studies led to compound **62e** with high potency both in the oleic acid oxidation assay and the luciferase-based transient transfection assay. Moreover, the thiazole **62e** showed a high selectivity towards PPARδ.

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



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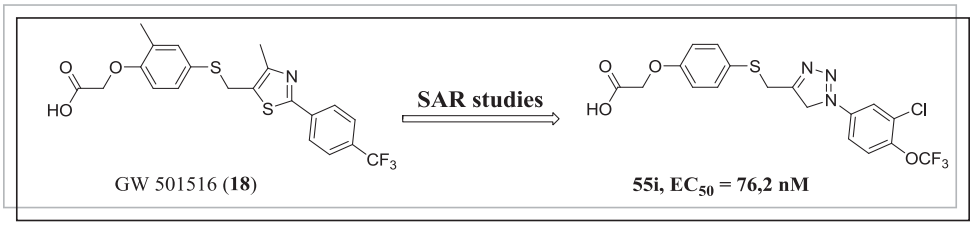

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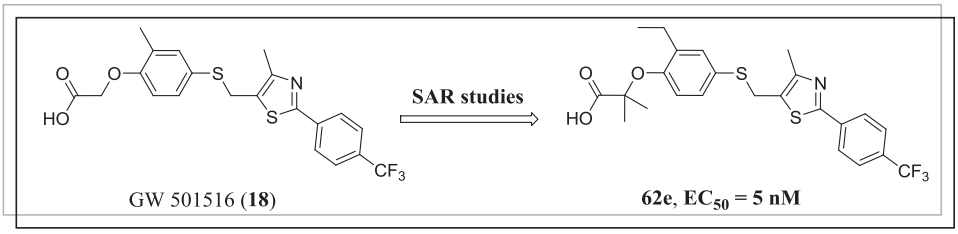
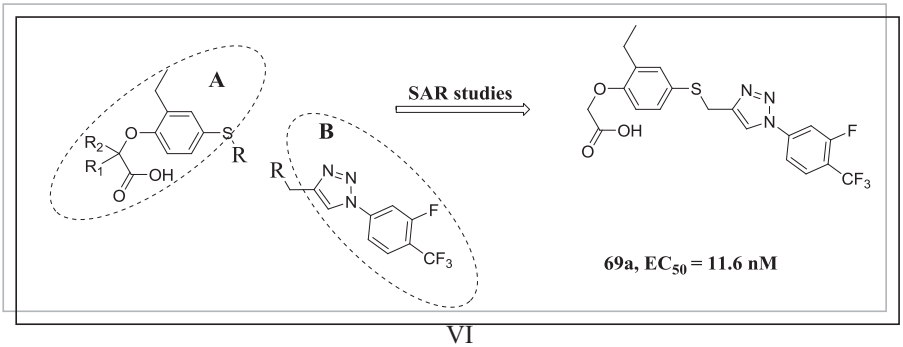
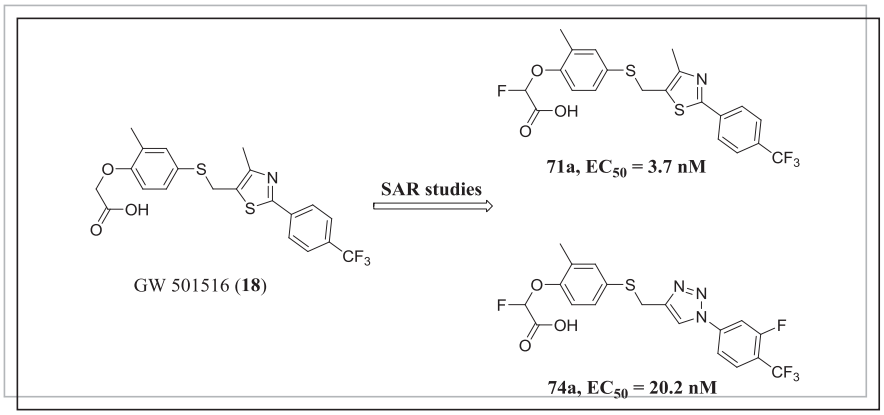


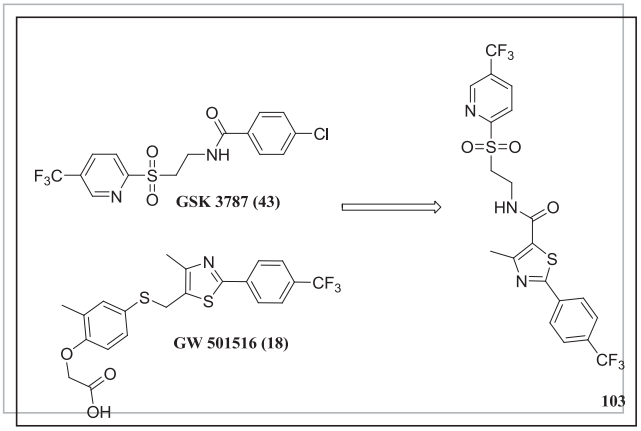
Triazole-based analogues of GW 501516 (**18**). Third series



α-Fluorinated thiazole- and triazole-based analogues of GW 5101516 (**18**)



New potential antagonist of PPARδ



List of abbreviations

BSA	bovine serum albumin
DBD	DNA-binding domain
DMEM	Dulbecco’s modified Eagle’s medium
DPBS	Dulbecco's phosphate buffered saline
EC_{50}	concentration of a compound where 50% of its maximal effect is observed
FCS	fetal calf serum
GSK	GlaxoSmithKline
HDL	high-density lipoprotein
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
IC_{50}	concentration of a compound that inhibits 50% of a given biological process
LBD	ligand-binding domain
LDL	low-density lipoprotein
MS	metabolic syndrome
PBS	phosphate-buffered saline
PDB	protein data bank
PG	prostaglandin
PPAR	peroxisome proliferator-activated receptor
PPRE	peroxisome proliferator response element
PUFA	polyunsaturated fatty acid
SAR	structure activity relationship
VLDL	very low-density lipoprotein

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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.[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.[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.[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.[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.[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.[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-biding 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 $Å^3$ 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 biding 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.[7]

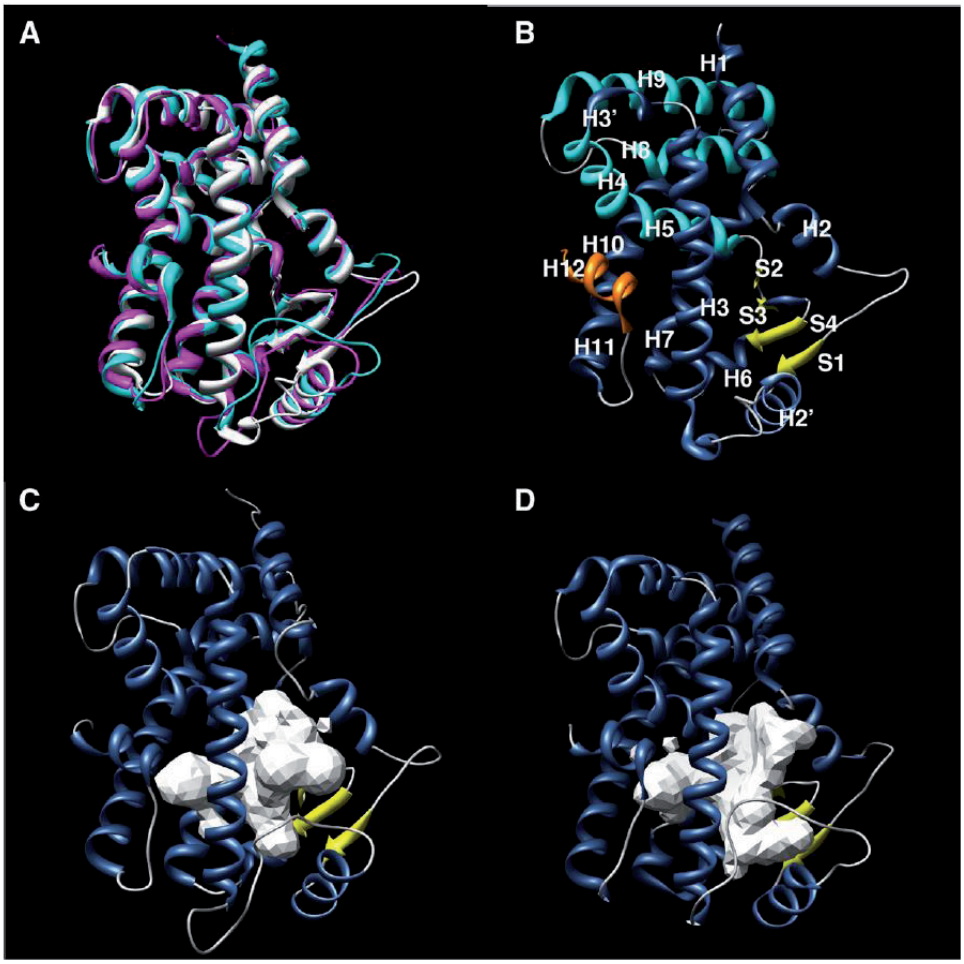


Figure 1.1: (A) Structural superposition of PPARα (white), PPARδ (magenta) and PPARγ (cyan). (B) 3D structure of PPARα. (C) 3D structure of PPARδ. The binding site cavity is shown in white. (D) 3D structure of PPARγ.[6]

PPARγ 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, PPARγ 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]

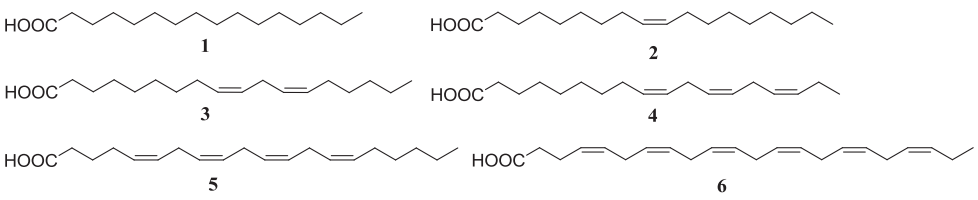


Figure 1.2. Structures of PPARα natural ligands

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 PGA_1(**7**) and PGD_2 (**8**) (**Figure 1.3**).[10,12-14]

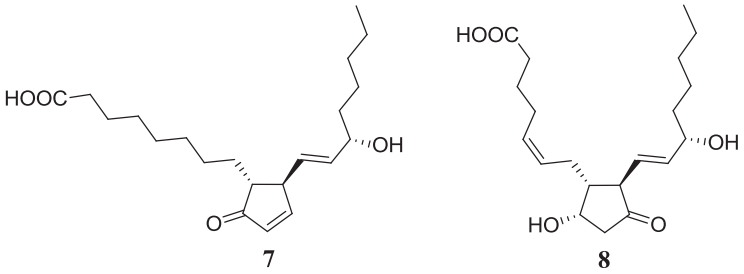


Figure 1.3. Structures of prostaglandins PGA_1 (**7**) and PGD_2 (**8**)

PPARγ was shown to be weakly activated by PUFAs **3**-**6** and prostaglandins **7** and **8** (**Figures 1.2, 1.3**).[10] 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]

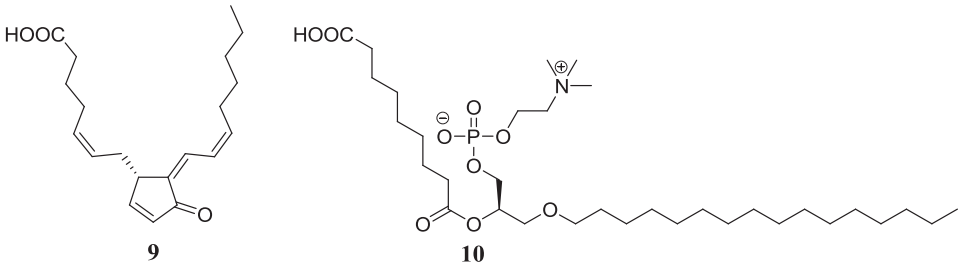


Figure 1.4. Structures of 15d-PGJ_2 (**9**) and hexadecyl azelaoyl phosphatidylcholine (**10**)

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]

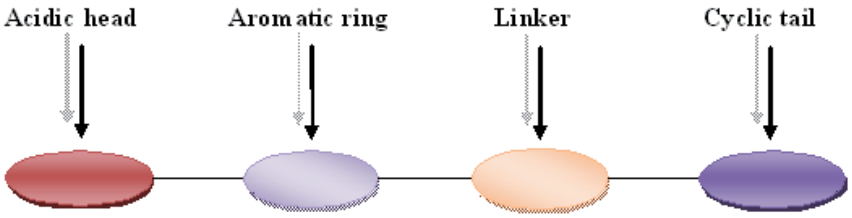


Figure 1.5: General structure of a PPAR agonist

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.[16]

Fibrates marketed as drugs are represented by: benzafibrate (**11**),[17] ciprofibrate (**12**),[18] gemfibrozile (**13**),[16,19] clofibrate (**14**),[18] and fenofibrate (**15**).[17] Structurally, the dimetyhl *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*.[20] Following an extensive activity relationship study (SAR), they discovered that compound **16** possessed high potency against PPARα (EC_{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_{50}-value of 0.06 μM (**Figure 1.6**).[20]

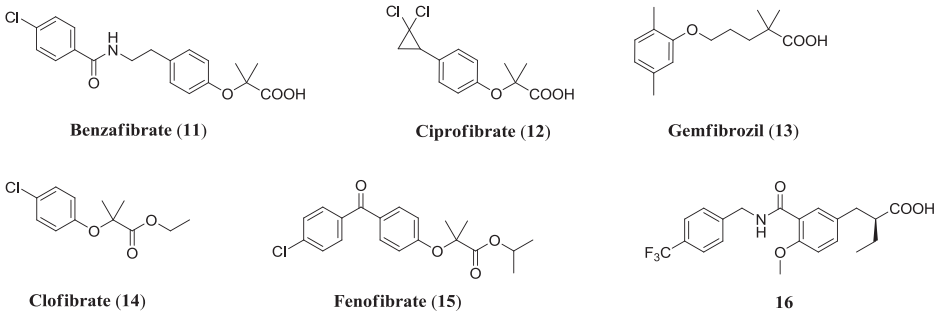


Figure 1.6: Structures of fibrates

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 CF_3 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 EC_{50}-values towards PPARδ of 1 nM, towards PPARα of 1.1 μM, and towards PPARγ of 0.85 μM and 2 μM, respectively.[21]

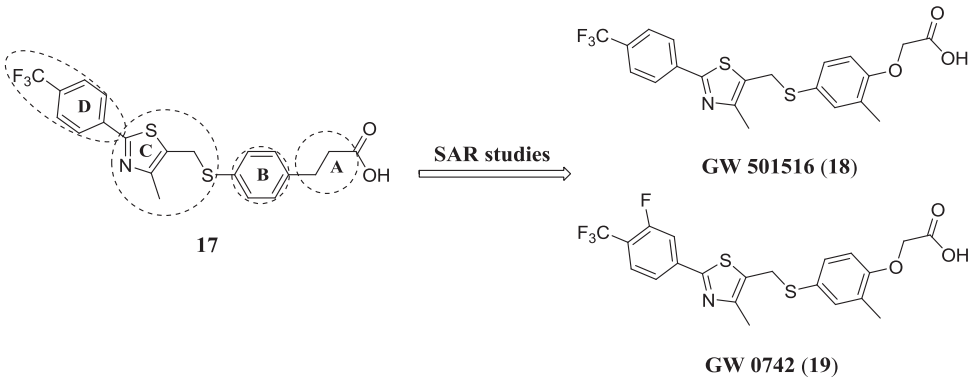
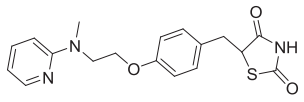


Figure 1.7. Structures of the first selective and potent PPARδ agonists.

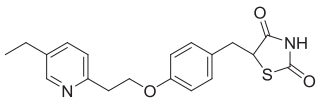
1.3.2.3 Peroxisome proliferator-activated receptors γ agonists

Molecules acting as agonist on PPARγ 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 PPARγ agonist action, on reduction of LDL and elevation of HDL levels.[22,23]

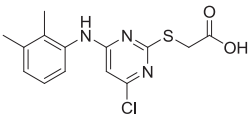
Thieme *et al.*[24] reported in 2010 new PPARγ agonists based on pirinixic acid (**22**) (**Figure 1.8**) which was known for its moderately dual PPARα/γ agonistic effects. The authors stressed the importance of the substitution *alpha* to the carboxylic moiety of **22** for increasing the selectivity towards the γ subtype. *α*-Substitution increased the activation of PPARα as well, but it was the size of the substituent that dictated the preference for PPARγ. According to crystallographic data, the LBD of PPARγ was observed to be larger than the one of PPARα. Hence, bulkier aromatic residues can be better accommodated. Bulky substituents 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 PPARγ activity. These modifications led to compound **23** (**Figure 1.8**) reported as the most potent out of the published compounds.[24]



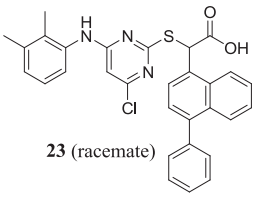
Rosiglitazone (**20,** racemate)



Pioglitazone (**21**, racemate)



Pirinixic acid (**22**)



23 (racemate)

Figure 1.8. Structures of PPARγ 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.[8] Even if some agonists have been synthesized there is no available information on their clinical activities.[25]

Kasuga *et al.*[26,27] and Shen *et al.*[28,29] 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.[20,27] 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, CF_3, OCF_3) 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**: $EC_{50}\alpha$ = 150 nM, $EC_{50}\delta$ = 840 nM, and (*S*)-**24**: $EC_{50}\alpha$ = 12 nM, $EC_{50}\delta$ = 23 nM).[26-29]

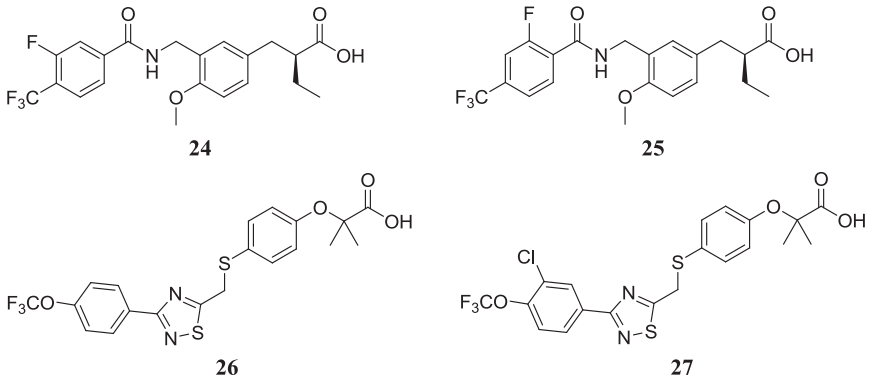


Figure 1.9. Structures of some dual PPARα/δ agonists.

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**),[4] thiazolidinediones (**29**),[32] 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**: $EC_{50}\alpha = 0.19$ μM, $EC_{50}\gamma = 1.5$ 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**: $EC_{50}\alpha = 0.03$ μM, $EC_{50}\gamma = 1.1$ μM, and (*S*)-**31**: $EC_{50}\alpha = 2.2$ μM, $EC_{50}\gamma = 3.4$ μM).[34]

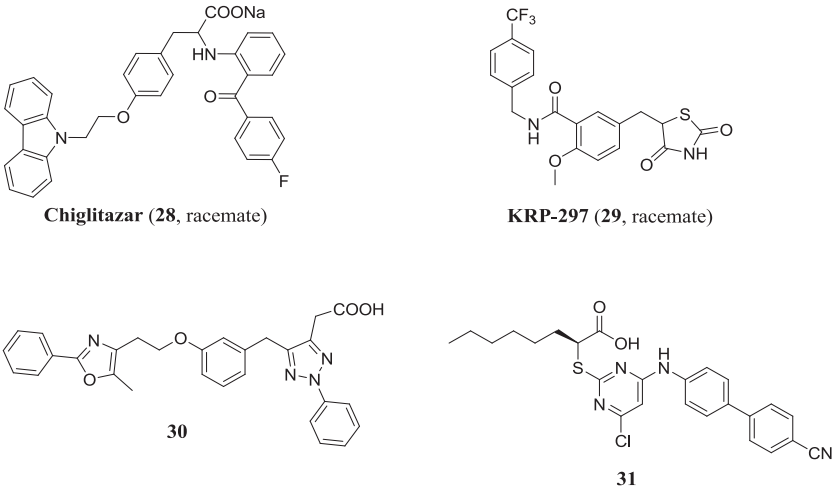


Figure 1.10. Structures of different PPARα/γ dual agonists.

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, CH_3, C_2H_5, CF_3) were desirable for enhancing dual PPARγ/δ activity as observed for compounds **32**-**36** (**Figure 1.11**).[35,36]

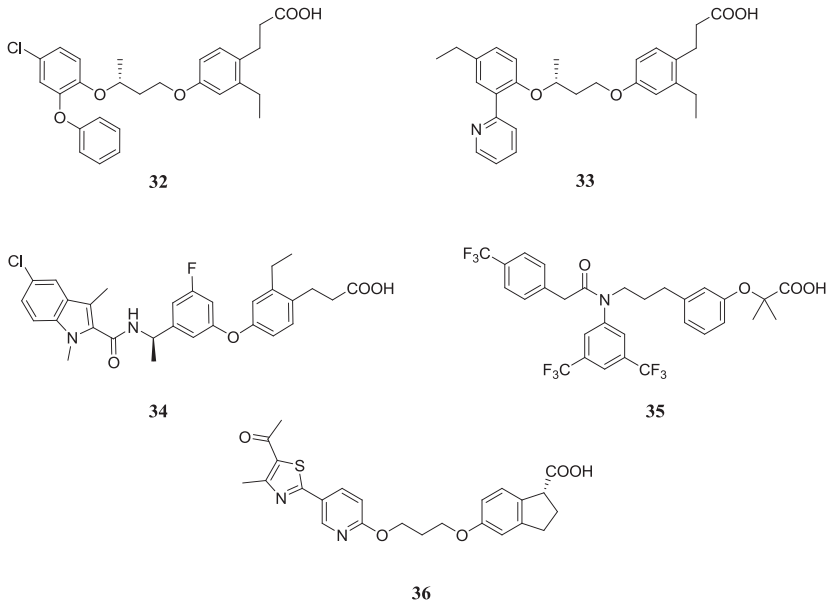


Figure 1.11. Structures of PPAR γ/δ dual agonists

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.[15]

Kasuga *et al.*[40] reported a potent pan agonist, compound **37** (**Figure 1.12**), developed from a substituted *α*-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**).[41,42] Common structural features with compound **37** can be noticed: *α*-substitution to the carboxylic group (compound **38**) and lipophilic substituents on the hydrophobic tail.

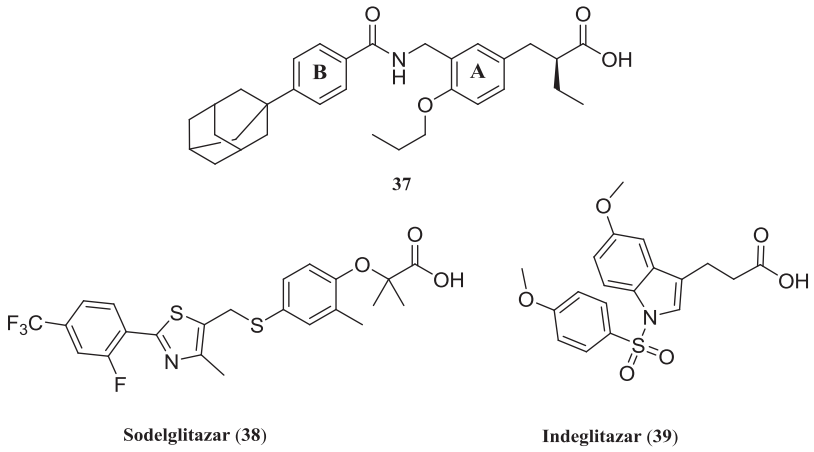


Figure 1.12. Structures of pan agonists.

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 IC_{50}-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 obesity[46] and of breast cancer.[47] GW 9662 (**41**) (**Figure 1.12**) proved to be a selective full antagonist of PPARγ with an IC_{50}-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**)[43] and GSK 3787 (**43**)[49] (**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 a potent antagonist towards the δ-subtype with an IC_{50}-value in an antagonist assay of 300 nM.[43] 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.[49]

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.[50]

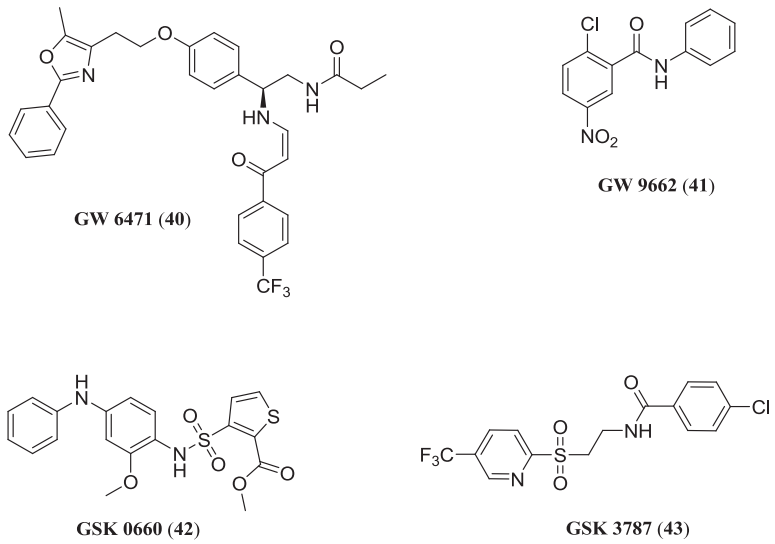


Figure 1.12. Chemical structure of antagonists.

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.[51] 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.

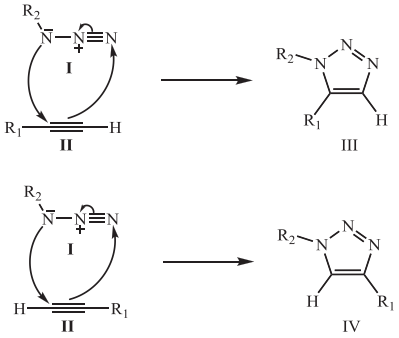
"Click chemistry", a concept conceived by Sharpless in 2001, encompasses simple, efficient, selective, and versatile chemical transformations.[52] A chemical reaction can be classified as a "click reaction" if it fulfils certain criteria: "*A click reaction must be of wide scope, giving consistently 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*" [53]

There have been indentified 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.[53]

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.[54]

The mechanism proposed for the Huisgen cycloaddition involves a concerted mechanism with two σ-bonds formed simultaneously (**Figure 1.1**).[55,56]



Scheme 1.1. Proposed mechanism for the Huisgen cycloaddition[56]

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-dibsustituted regioisomer.[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.[59]

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

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.

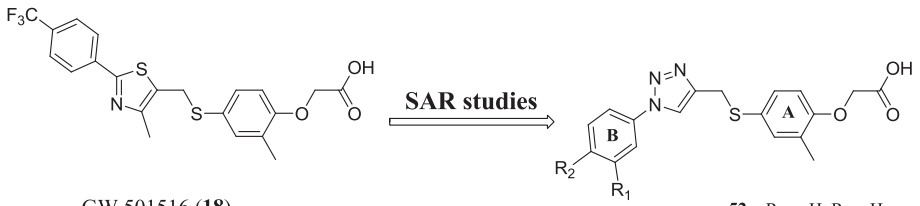
2. RESULTS AND DISCUSSIONS

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 R_1 and R_2 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 rule[64,65] 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]



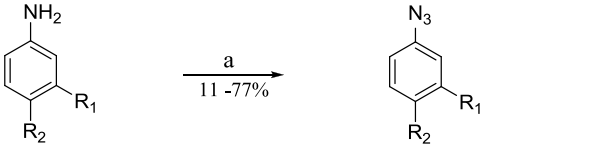
GW 501516 (**18**)

52a: R_1 = H, R_2 = H
52b: R_1 = H, R_2 = CH_3
52c: R_1 = F, R_2 = CH_3
52d: R_1 = H, R_2 = CF_3
52e: R_1 = F, R_2 = CF_3
52f: R_1 = H, R_2 = OCH_3
52g: R_1 = F, R_2 = OCH_3
52h: R_1 = H, R_2 = OCF_3
52i: R_1 = Cl, R_2 = OCF_3
52j: R_1 = F, R_2 = OCF_3

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]

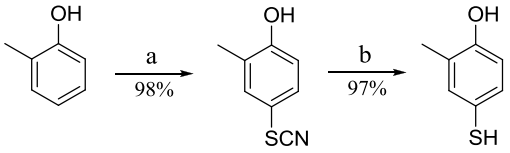


44a-44j

45a: R_1 = H, R_2 = H, (36%)
45b: R_1 = H, R_2 = CH_3, (41%)
45c: R_1 = F, R_2 = CH_3, (70%)
45d: R_1 = H, R_2 = CF_3, (55%)
45e: R_1 = F, R_2 = CF_3, (46%)
45f: R_1 = H, R_2 = OCH_3, (11%)
45g: R_1 = F, R_2 = OCH_3, (49%)
45h: R_1 = H, R_2 = OCF_3, (57%)
45i: R_1 = Cl, R_2 = OCF_3, (77%)
45j: R_1 = F, R_2 = OCF_3, (49%)

Scheme 2.1. (a) $NaNO_2$, NaN_3, H_2O, HCl.

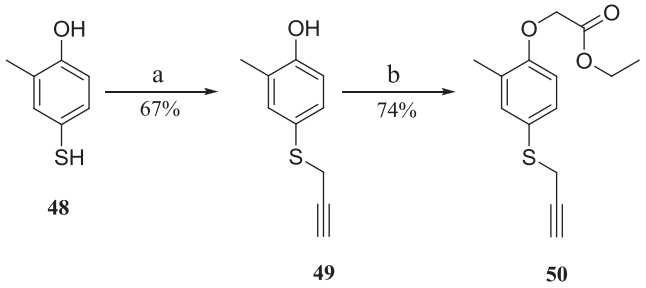
Para-mercaptophenol **48** was prepared according to a literature procedure which consisted in bromination of phenol **46** followed by the reaction with NaSCN to give **47** in 98% yield. Thiocyanate **47** was reduced with $LiAlH_4$ to the thiol **48** in 97% yield. (**Scheme 2.2**).[68]



46 **47** **48**

Scheme 2.2. (a) Br_2, NaBr, NaSCN, MeOH; (b) $LiAlH_4$, THF.

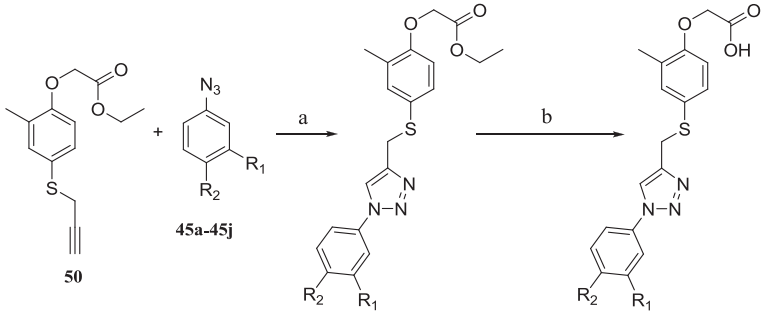
Thiol **48** was alkylated with propargyl bromide affording the phenolic sulphide **49**. Alkylation of phenol **49** with ethyl bromoacetate produced terminal alkyne **50** in 50% yield over the two steps (**Scheme 2.3**).[68]



48 49 50

Scheme 2.3. (a) Propargyl bromide, Cs_2CO_3, CH_3CN; (b) Ethyl bromoacetate, Cs_2CO_3, CH_3CN.

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%.



51a: R_1 = H, R_2 = H, (80%)
51b: R_1 = H, R_2 = CH_3, (92%)
51c: R_1 = F, R_2 = CH_3, (59%)
51d: R_1 = H, R_2 = CF_3, (96%)
51e: R_1 = F, R_2 = CF_3, (77%)
51f: R_1 = H, R_2 = OCH_3, (72%)
51g: R_1 = F, R_2 = OCH_3, (78%)
51h: R_1 = H, R_2 = OCF_3, (61%)
51i: R_1 = Cl, R_2 = OCF_3, (95%)
51j: R_1 = F, R_2 = OCF_3, (53%)

52a: R_1 = H, R_2 = H, (37%)
52b: R_1 = H, R_2 = CH_3, (58%)
52c: R_1 = F, R_2 = CH_3, (48%)
52d: R_1 = H, R_2 = CF_3, (66%)
52e: R_1 = F, R_2 = CF_3, (42%)
52f: R_1 = H, R_2 = OCH_3, (40%)
52g: R_1 = F, R_2 = OCH_3, (44%)
52h: R_1 = H, R_2 = OCF_3, (53%)
52i: R_1 = Cl, R_2 = OCF_3, (47%)
52j: R_1 = F, R_2 = OCF_3, (63%)

Scheme 2.4. (a) Sodium ascorbate, $CuSO_4$, *tert*-BuOH, H_2O; (b) LiOH, THF, H_2O.

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 ((9*Z*)-octadec-9-enoic acid) was measured by detection of the accumulation of ^{14}C-labeled oxidized oleic acid.[69] 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 (PPARα, PPARδ and PPARγ) 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

Compound	R_1	R_2	EC_{50}(nM)[a]
52a	H	H	n.d.[b]
52b	H	CH_3	n.d.
52c	F	CH_3	n.d.
52d	H	CF_3	1.80
52e	F	CF_3	0.85
52f	H	OCH_3	n.d.
52g	F	OCH_3	n.d.
52h	H	OCF_3	10.0
52i	Cl	OCF_3	12.0
52j	F	OCF_3	1.4
GW 501516 (**18**)	-	-	0.07

[a] Results of three replicates
[b] n.d., not determined: EC_{50}-value was not calculate 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_{50}-values of 1.8 nM, 0.85 nM, and 1.4 nM, respectively (**Table 2.1**, **Figures 2.2A-C**, **Figure 2.4A**).

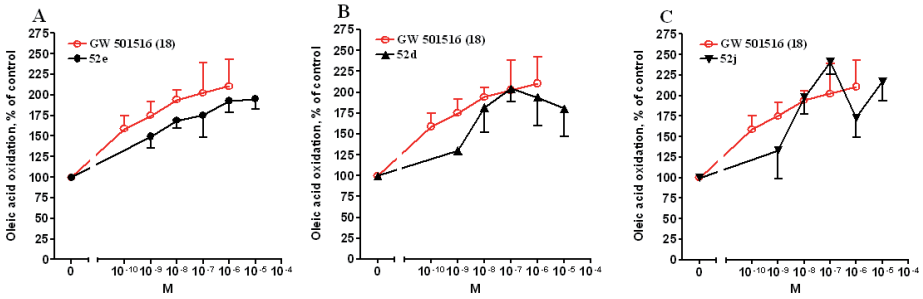


Figure 2.2. Oxidation of oleic acid in human myotubes in the presence of 1,4-disubstituted 1,2,3-triazoles **52d, 52e** and **52j** at five different concentrations. GW 501516 (**18**) was used as positive control.

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

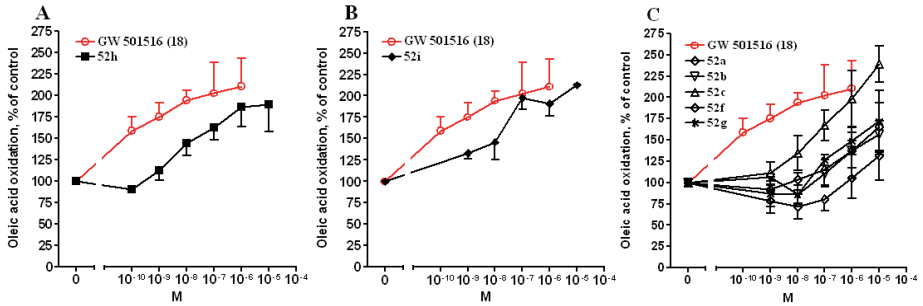


Figure 2.3. Oxidation of oleic acid in human myotubes in the presence of 1,4-disubstituted 1,2,3-triazoles **52a-52c** and **52f-52i** at different concentrations. GW 501516 (**18**) was used as positive control.

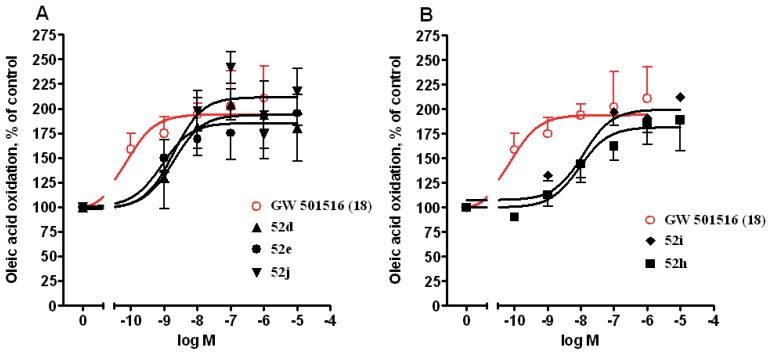


Figure 2.4. Non-linear fit curves of oxidation of oleic acid in human myotubes in the presence of different concentrations of 1,4-disubstituted 1,2,3-triazoles **52d**, **52e**, **52j (A)**; **52i** and **52h** (**B**). GW 501516 (**18**) was used as positive control.

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 (2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-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**).

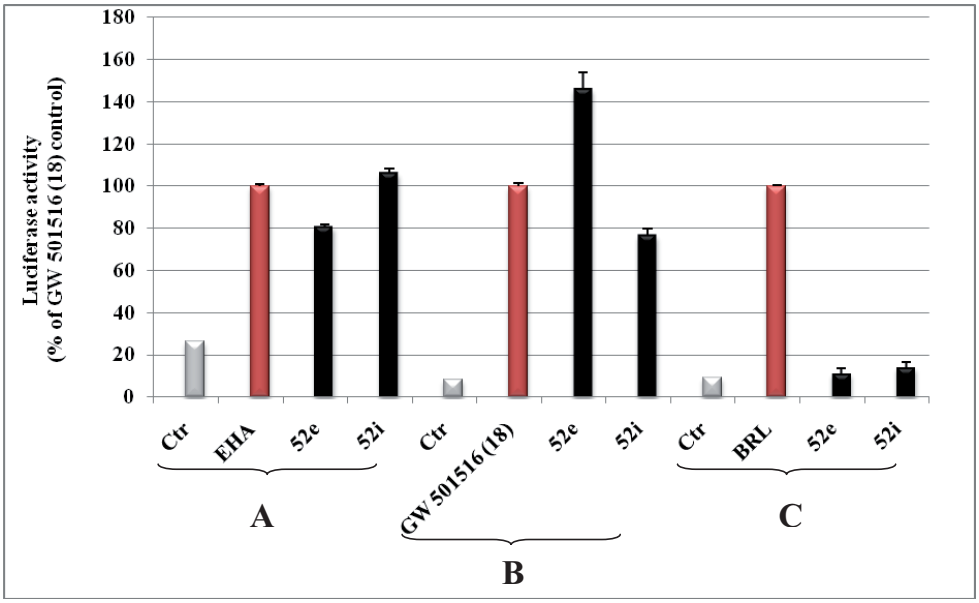


Figure 2.5. Activation of the ligand-binding domain of PPARα (**A**), PPARδ (**B**), PPARγ (**C**) of triazoles **52e** and **52i**. Positive controls: PPARα: **EHA** ((2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-2,4,8,11,14,17-hexaenoic acid); PPARδ: GW 501516 (**18**); PPARγ: **Rosiglitazone** (BRL).

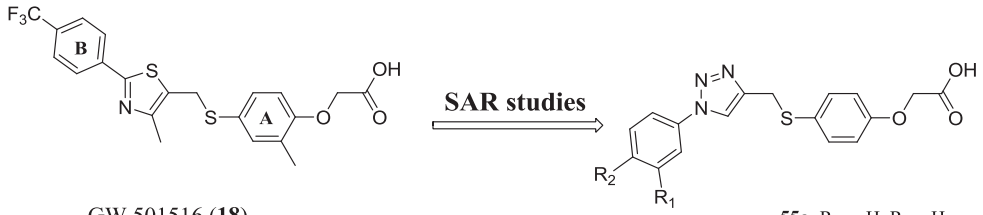
2.1.1.4 Conclusions

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.[27] Dual activation of PPARα and PPARδ could combine the decrease of the levels of triglycerides in blood, due to PPARα,[70] with the enhancement of both the fatty acid metabolism and the HDL cholesterol level, due to PPARδ.[71] 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**).



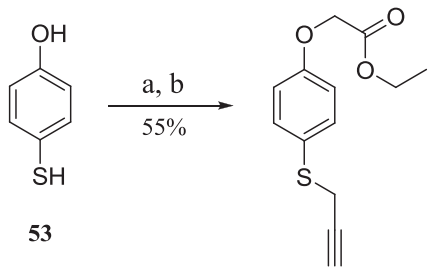
GW 501516 (**18**)

55a: R_1 = H, R_2 = H
55b: R_1 = H, R_2 = CH_3
55c: R_1 = F, R_2 = CH_3
55d: R_1 = H, R_2 = CF_3
55e: R_1 = F, R_2 = CF_3
55f: R_1 = H, R_2 = OCH_3
55g: R_1 = F, R_2 = OCH_3
55h: R_1 = H, R_2 = OCF_3
55i: R_1 = Cl, R_2 = OCF_3
55j: R_1 = F, R_2 = OCF_3

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

2.1.2.1 Synthesis

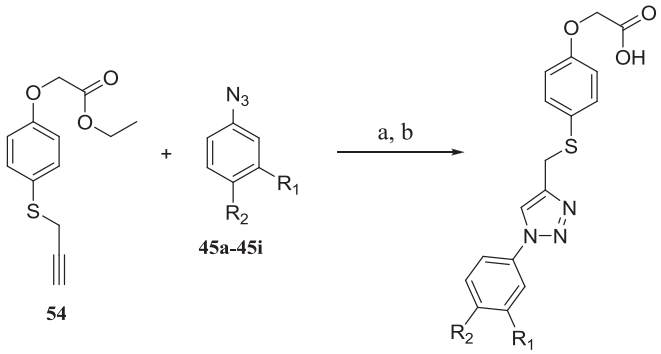
The first step in the 1,4-disubstituted 1,2,3-triazoles **55a-55i** synthesis selective alkylation, using a one pot procedure, of the thiol-, and the phenolic group of compound **53** with propargyl bromide and with ethyl bromoacetate, respectively, to the terminal alkyne **54** in 55% yield (**Scheme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



53 **54**

Scheme 2.5. (a) Propargyl bromide, Cs_2CO_3, CH_3CN; (b) Ethyl bromoacetate, Cs_2CO_3, CH_3CN.

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%).



55a: R_1 = H, R_2 = H, 54%
55b: R_1 = H, R_2 = CH_3, 60%
55c: R_1 = F, R_2 = CH_3, 60%
55d: R_1 = H, R_2 = CF_3, 65%
55e: R_1 = F, R_2 = CF_3, 67%
55f: R_1 = H, R_2 = OCH_3, 26%
55g: R_1 = F, R_2 = OCH_3, 34%
55h: R_1 = H, R_2 = OCF_3, 85%
55i: R_1 = Cl, R_2 = OCF_3, 85%

Scheme 2.6. (a) Sodium ascorbate, $CuSO_4$, *tert*-BuOH, H_2O; (b) LiOH, THF, H_2O.

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 ^{14}C-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** (EC_{50} = 12.0 nM).

Table 2.2. Substitution pattern (see **Figure 2.1**) and EC_{50}-values of prepared compounds

Compound	R_1	R_2	EC_{50}(nM)[a]
55a	H	H	n.d.[b]
55b	H	CH_3	n.d
55c	F	CH_3	n.d.
55d	H	CF_3	n.d.
55e	F	CF_3	n.d.
55f	H	OCH_3	n.d
55g	F	OCH_3	n.d.
55h	H	OCF_3	n.d.
55i	Cl	OCF_3	76.2
GW 501516 (**18**)	-	-	0.12

[a]Results 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.

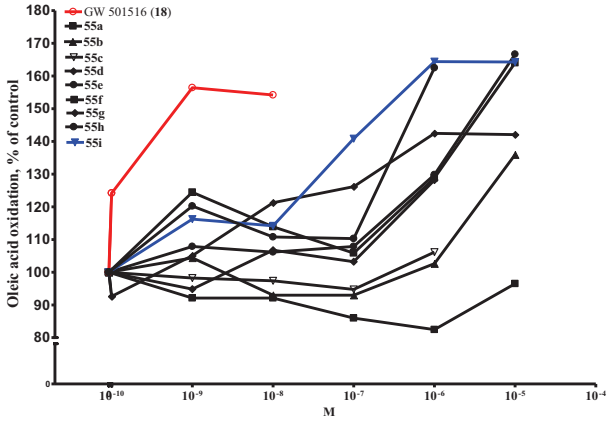


Figure 2.7. Oxidation of oleic acid in human myotubes in the presence of 1,4-disubstituted 1,2,3-triazoles **55a-55i** at different concentrations. GW 501516 (**18**) was used as positive control.

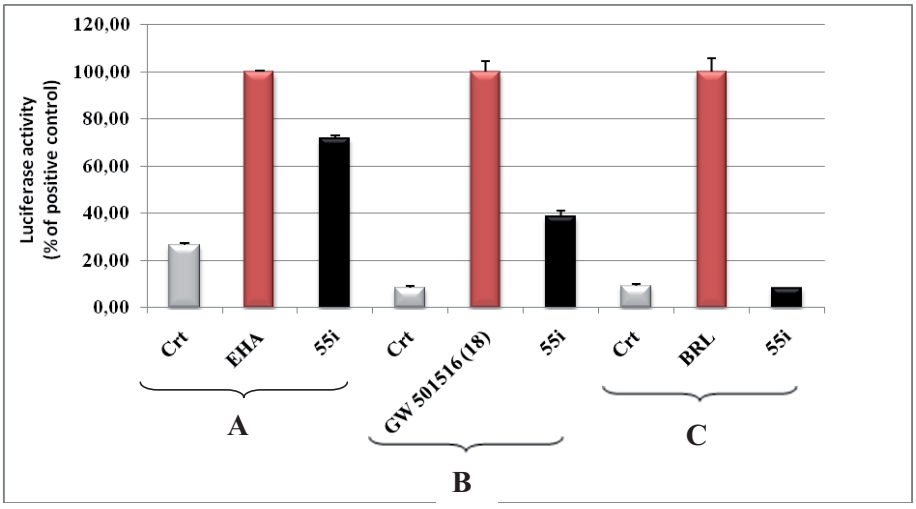


Figure 2.8. Activation of the ligand-binding domain of PPARα (**A**), PPARδ (**B**), PPARγ (**C**) by compound **55i** in a luciferase-based transfection assay. Positive controls: PPARα: **EHA** ((2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-2,4,8,11,14,17-hexaenoic acid); PPARδ: GW 501516 (**18**); PPARγ: **Rosiglitazone** (BRL).

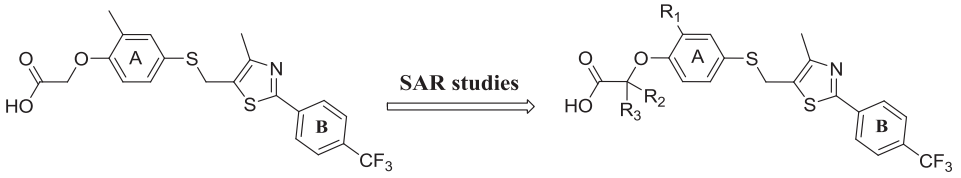
These results revealed the importance of the *ortho*-methyl substituent for the activation of PPARs. Sznaidman *et al.*[21] also reported a reduction of the efficacy for their thiazole-based agonists when an *ortho*-methyl group on a corresponding benzene ring (**A**) was replaced by a hydrogen atom.

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.[28,29]



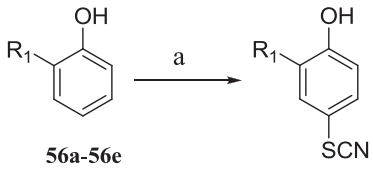
GW 501516 (**18**)

(±)-**62a**: R_1 = Methyl, R_2 = Methyl, R_3 = H
62b: R_1 = Methyl, R_2 = Methyl, R_3 = Methyl
62c: R_1 = Ethyl, R_2 = H, R_3 = H
(±)-**62d**: R_1 = Ethyl, R_2 = Methyl, R_3 = H
62e: R_1 = Ethyl, R_2 =Methyl, R_3 = Methyl
62f: R_1 = Isopropyl, R_2 = H, R_2 = H
(±)-**62g**: R_1 = Isopropyl, R_2 = Methyl, R_3 = H
62h: R_1 = Isopropyl, R_2 = Methyl, R_3 = Methyl
62i: R_1 = *tert*-Butyl, R_2 = H, R_3 = H
62j: R_1 = Cyclopentyl, R_2 = H, R_3 = H
62k: R_1 = Cyclohexyl, R_2 = H, R_3 = H

Figure 2.9. Structures of GW 501516 (**18**) and prepared analogues **62a-62k**.

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]

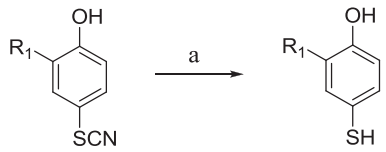


56a-56e

57a: R_1 = Ethyl, (70%)
57b: R_1 = Isopropyl, (81%)
57c: R_1 = *tert*-Butyl, (68%)
57d: R_1 = Cyclopentyl, (83%)
57e: R_1 = Cyclohexyl, (61%)

Scheme 2.7. (a) Br_2, NaBr, NaSCN, MeOH.

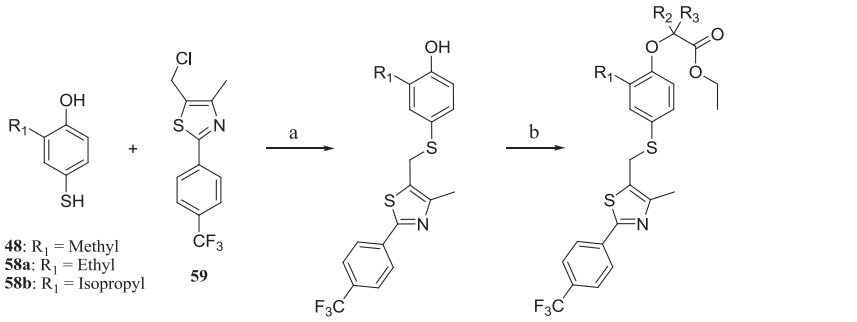
Thiocyanates **57a** and **57b** were reduced with $LiAlH_4$ to mercaptophenols **58a** and **58b** in 71-88% yield (**Scheme 2.8**). 4-Mercapto-2-methylphenol (**48**), **58a** and **58b**, respectively, were reacted with 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole (**59**) in the presence of Cs_2CO_3 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 Cs_2CO_3 to yield esters **61a-61h** (**Scheme 2.9**).



57a, **57b**

58a: R_1 = Ethyl, 71%
58b: R_1 = Isopropyl, 88%

Scheme 2.8. (a) $LiAlH_4$, THF;



48: R_1 = Methyl
58a: R_1 = Ethyl
58b: R_1 = Isopropyl

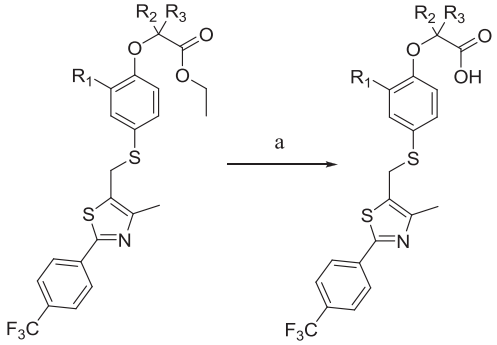
59

60a: R_1 = Methyl, (96%)
60b: R_1 = Ethyl, (66%)
60c: R_1 = Isopropyl, (80%)

(±)-61a: R_1 = Methyl, R_2 = Methyl, R_3 = H, (97%)
61b: R_1 = Methyl, R_2 = Methyl, R_3 = Methyl, (83%)
61c: R_1 = Ethyl, R_2 = H, R_3 = H, (93%)
(±)-61d: R_1 = Ethyl, R_2 = Methyl, R_3 = H, (93%)
61e: R_1 = Ethyl, R_2 =Methyl, R_3 = Methyl, (83%)
61f: R_1 = Isopropyl, R_2 = H, R_2 = H, (75%)
(±)-61g: R_1 = Isopropyl, R_2 = Methyl, R_3 = H, (94%)
61h: R_1 = Isopropyl, R_2 = Methyl, R_3 = Methyl, (53%)

Scheme 2.9. (a) Cs_2CO_3, CH_3CN; (b) Ethyl 2-bromoesters, Cs_2CO_3, CH_3CN.

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 $(CH_3)_3COK$. Only the racemates of **62a**, **62d** and **62g** were prepared.



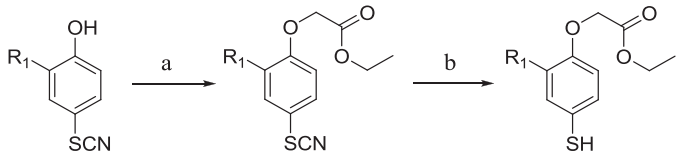
61a-61h

(±)-62a: R_1 = Methyl, R_2 = Methyl, R_3 = H, (50%)
62b: R_1 = Methyl, R_2 = Methyl, R_3 = Methyl, (45%)
62c: R_1 = Ethyl, R_2 = H, R_3 = H, (43%)
(±)-62d: R_1 = Ethyl, R_2 = Methyl, R_3 = H, (50%)
62e: R_1 = Ethyl, R_2 =Methyl, R_3 = Methyl, (60%)
62f: R_1 = Isopropyl, R_2 = H, R_2 = H, (35%)
(±)-62g: R_1 = Isopropyl, R_2 = Methyl, R_3 = H, (66%)
62h: R_1 = Isopropyl, R_2 = Methyl, R_3 = Methyl, (48%)

Scheme 2.10. (a) (i) LiOH, THF, H_2O; (ii) $(CH_3)_3COK$, THF, H_2O, reflux.

Since the treatment of thiocyanates **57c-57e** with $LiAlH_4$ afforded large quantities of the corresponding disulphide dimers,[72] a different approach was used in order to synthesize compounds **62i-62k**.

First, an alkylation reaction with ethyl 2-bromoacetate in the presence of Cs_2CO_3 of compounds **57c-57e** was performed, followed by the reduction with $NaBH_4$ and 1,4-dithioerythritol[72] to afford **64a-64c** in 61-77% yield (**Scheme 2.11**).



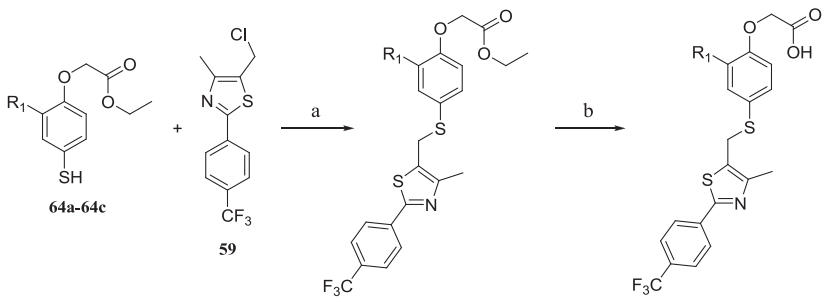
57c: R_1 = *tert*-Butyl
57d: R_1 = Cyclopentyl
57e: R_1 = Cyclohexyl

63a: R_1 = *tert*-Butyl, (79%)
63b: R_1 = Cyclopentyl, (69%)
63c: R_1 = Cyclohexyl, (66%)

64a: R_1 = *tert*-Butyl, (43%)
64b: R_1 = Cyclopentyl, (85%)
64c: R_1 = Cyclohexyl, (85%)

Scheme 2.11. (a) Ethyl 2-bromoacetate, Cs_2CO_3, CH_3CN; (b) $NaBH_4$, 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**).



61i: R_1 = Isopropyl, (94%)
61j: R_1 = Cyclopentyl, (58%)
61k: R_1 = Cylcohexyl, (79%)

62i: R_1 = Isopropyl, (44%)
62j: R_1 = Cyclopentyl, (39%)
62k: R_1 = Cyclohexyl, (48%)

Scheme 2.12. (a) Cs_2CO_3, CH_3CN; (b) LiOH, THF, H_2O.

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 EC_{50}-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**: EC_{50} = 4.15 nM, **62f**: EC_{50} = 4.15 nM, **62i**: EC_{50} = 5.51 nM, **62j**: EC_{50} = 16.60 nM, **62k:** EC_{50} = 17.30 nM) (**Table 2.3**). A similar pattern was noticed for compounds (±)-**62d** and (±)-**62g**, and **62b**, **62e** and **62h**, respectively ((±)-**62d**: EC_{50} = 0.36 nM and (±)-**62g**: EC_{50} = 5.79 nM; **62b**: EC_{50} = 0.24 nM, **62e**: EC_{50} = 0.54 nM and **62h**: EC_{50} = 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**: EC_{50} = 0.65 nM, (±)-**62d**: EC_{50} = 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**: EC_{50} = 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**: EC_{50} = 0.31 nM, (±)-**62d**: EC_{50} = 0.36 nM). Methyl substitution of the second hydrogen atom in (±)-**62d** afforded compound **62e** that showed a significant decline of the potency ((±)-**62d**: EC_{50} = 0.36 nM, **62e**: EC_{50} = 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** (EC_{50} = 4.15 nM) were replaced by one methyl group, affording compound (±)-**62g** (EC_{50} = 5.79 nM), or two methyl groups, affording **62h** (EC_{50} = 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

Compound	R_1	R_2	R_3	EC_{50} (nM)[a]
(±)-**62a**	Methyl	Methyl	H	0.65
62b	Methyl	Methyl	Methyl	0.24
62c	Ethyl	H	H	0.31
(±)-**62d**	Ethyl	Methyl	H	0.36
62e	Ethyl	Methyl	Methyl	0.54
62f	Isopropyl	H	H	4.15
(±)-**62g**	Isopropyl	Methyl	H	5.79
62h	Isopropyl	Methyl	Methyl	9.11
62i	*tert*-Butyl	H	H	5.51
62j	Cyclopentyl	H	H	16.60
62k	Cyclohexyl	H	H	17.30
GW 501516 (**18**)	Methyl	H	H	0.10

[a]Results of three replicates

The effects compounds **62a-62k** exhibited on all of the peroxisome proliferator-activated receptors (PPARα, PPARδ and PPARγ) in a luciferase-based transient transfection system was also investigated. Compounds **62a**-**62e**, **62g** and **62h** showed a higher activation of both PPARα as well as PPARδ at 10 μM concentrations than the positive controls (**Figures 2.10**-**2.11**). Compounds **62i** and **62j** activated only the PPARδ 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 PPARγ (**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**).[21] 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.

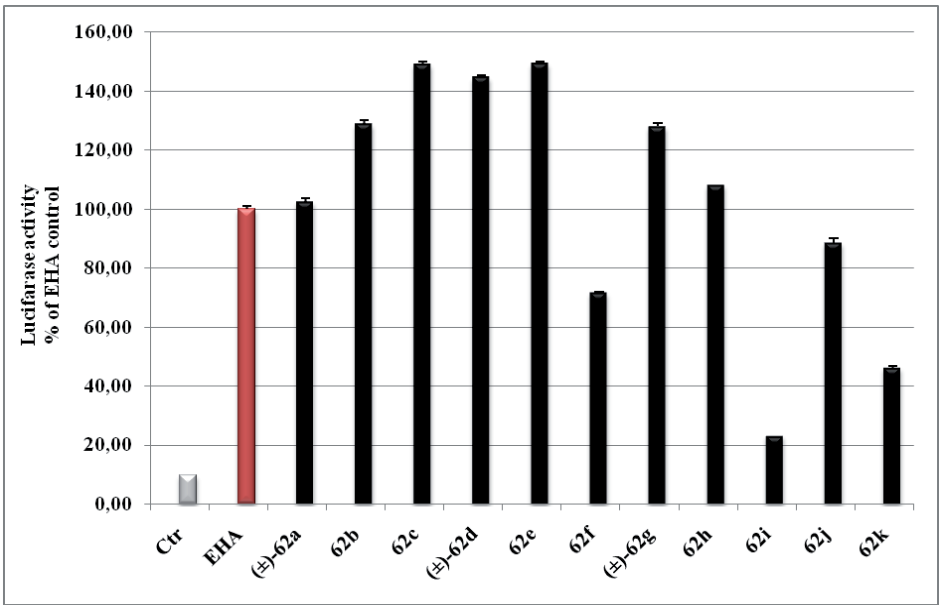


Figure 2.10. Activation of the ligand-binding domain of PPARα by compounds **62a-62k**. Positive control: **EHA** ((2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-2,4,8,11,14,17-hexaenoic acid.

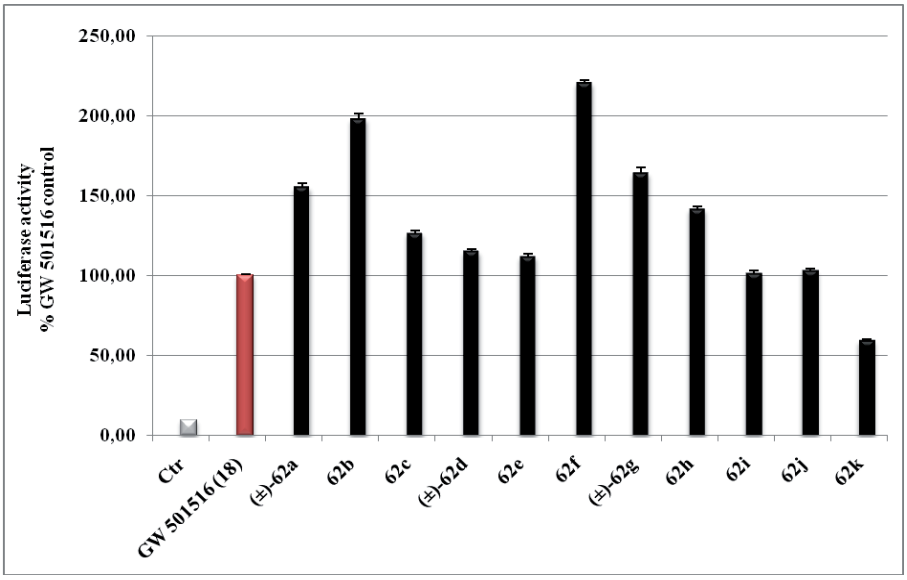


Figure 2.11. Activation of the ligand-binding domain of PPARδ by compounds **60a-60k**. Positive control: GW 501516 (**18**).

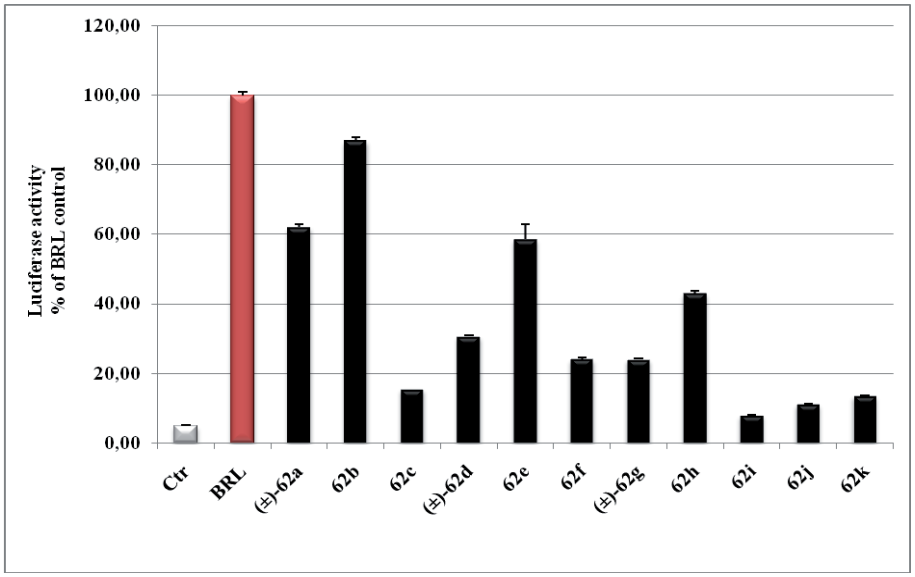


Figure 2.12. Activation of the ligand-binding domain of PPARγ by compounds **62a-62k**. Positive control: **Rosiglitazone** (BRL).

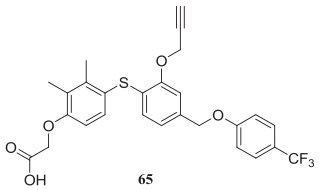
2.1.3.4 Molecular modelling

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[74]. In the activated receptor conformation of PPARδ, the amino acids His323, His449 and Tyr473 are essential for agonist interactions.[6,75]

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)phenyl-thio]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 R_1-position. In the docked complex of **62e** the ethyl group in R_1 points in the direction of Thr289, Ile326 and Phe327. A larger substituent R_1 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.



65

Figure 2.13. Structure of the agonist 2-{2,3-dimethyl-4-[2-propargyloxy-4-((4-trifluoromethylphenoxy)methyl)phenylthio]phenoxy}acetic acid.

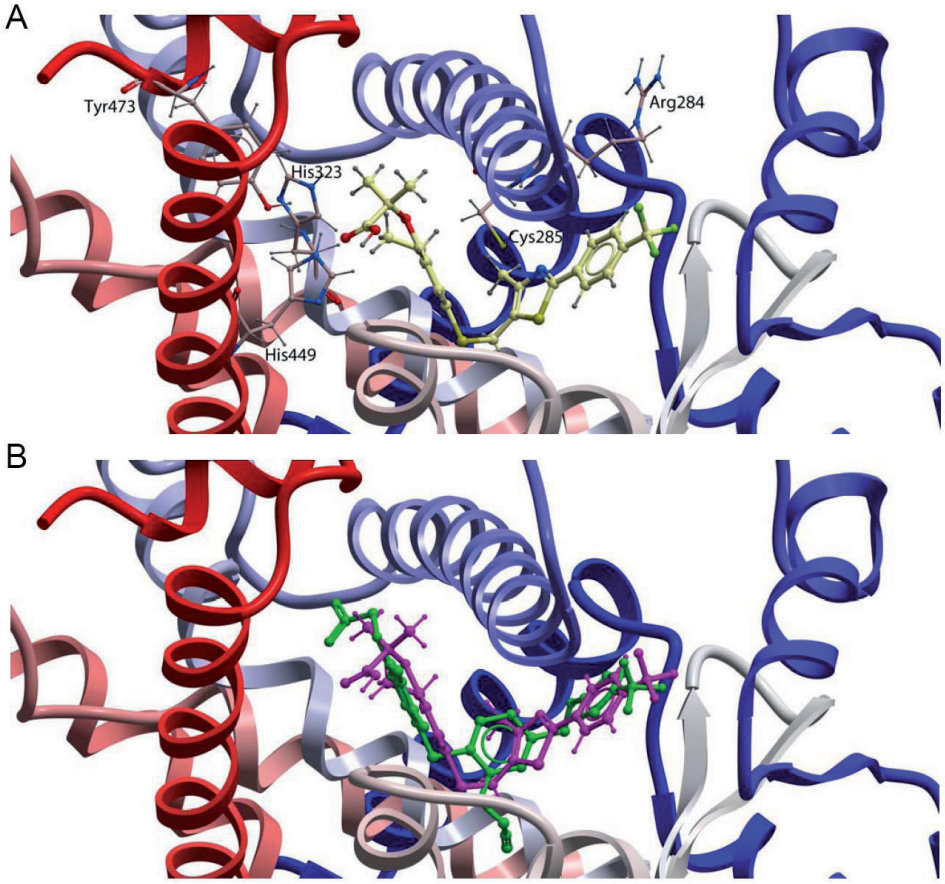


Figure 2.14.**A**. **62e** docked into PPARδ. Colour coding: red O, blue N, grey H, yellow C in **62e**, white C in PPARδ. Colouring of the Cα traces of PPARδ is blue *via* white to red from N-terminal to C-terminal. **B**. The docked complex of **62e** (purple) superimposed on the X-ray structure complex of the agonist 2-{2,3-dimethyl-4-[2-prop-2-ynyloxy-4-((4-trifluoromethylphenoxy)methyl)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**).

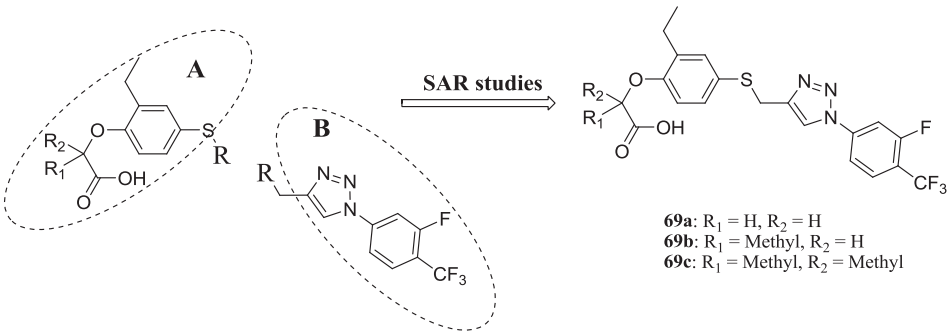
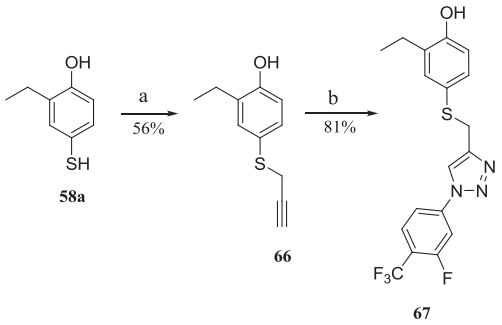


Figure 2.15. Structures of prepared agonists **69a-69c**.

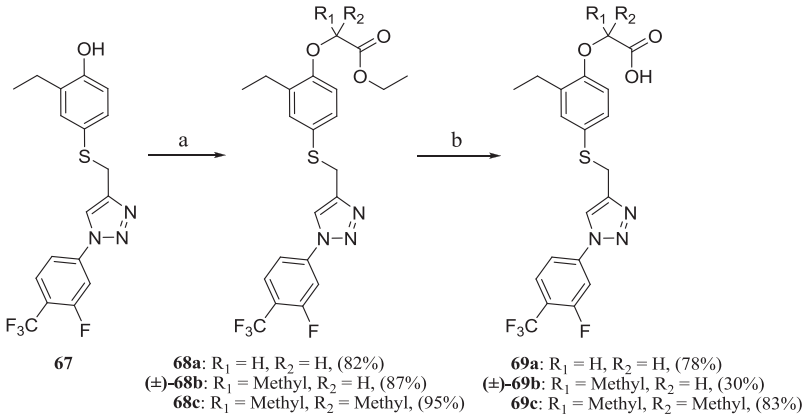
2.1.4.1 Synthesis

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 Cs_2CO_3 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' conditions[57] to afford compound **67** in 45% yield over two steps (**Scheme 2.13**).



Scheme 2.13. (a) Propargyl bromide, Cs_2CO_3, CH_3CN; (b) 4-Azido-2-fluoro-1-trifluoromethylbenzene (**45e**), sodium ascorbate, $CuSO_4$, *tert*-BuOH, H_2O.

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 $(CH_3)_3COK$. Only the racemate of **69b** was prepared.



Scheme 2.14. (a) Ethyl 2-bromoesters, Cs_2CO_3, CH_3CN; (b) (i) LiOH, THF, H_2O; (ii) $(CH_3)_3COK$, THF, H_2O, 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 **subchapter 2.1.1.2**. The EC_{50}-values for triazoles **69a-69c** were obtained from **Figure 2.17** and are presented in **Table 2.4**.

Table 2.4. Substitution pattern (see **Figure 2.15**) and EC_{50}-values of prepared compounds

Compound	R_1	R_2	EC_{50}(nM)[a]
69a	H	H	11.6
(±)-**69b**	Methyl	H	34.8
69c	Methyl	Methyl	n.d.[b]
GW 501516 (**18**)	-	-	0.04

[a] 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 1/300th 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**: EC_{50} = 0.85 nM, **69a**: EC_{50} = 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 EC_{50} 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**.

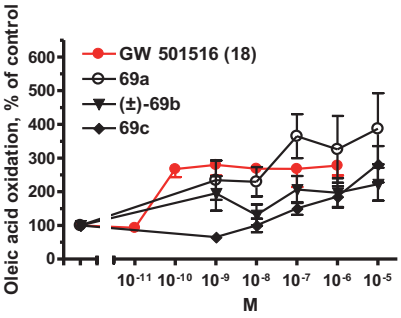


Figure 2.16. Oxidation of oleic acid in human myotubes in the presence of compounds **69a-69c** at different concentrations. GW 501516 (**18**) was used as positive control.

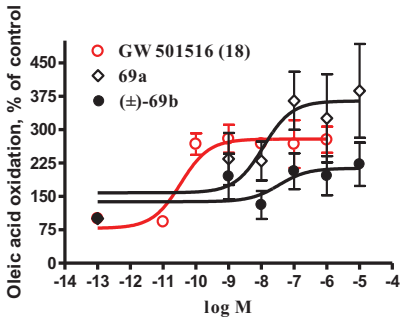


Figure 2.17. Non-linear fit curves of oxidation of oleic acid in human myotubes in the presence of different concentrations of compounds **69a** and (±)-**69b**. GW 501516 (**18**) was used as positive control.

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**).

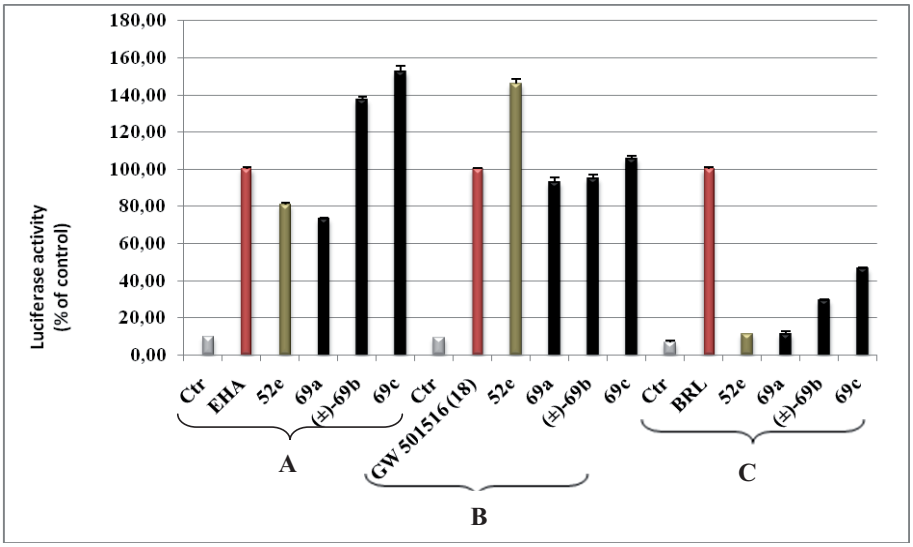


Figure 2.18. Activation of the ligand-binding domain of PPARα (**A**), PPARδ (**B**), PPARγ (**C**) of triazoles **52e** and **69a-69c**. Positive controls for PPARα, PPARδ and PPARδ: **EHA** ((2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-2,4,8,11,14,17-hexaenoic acid), GW 501516 (**18**), and Rosiglitazone (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]

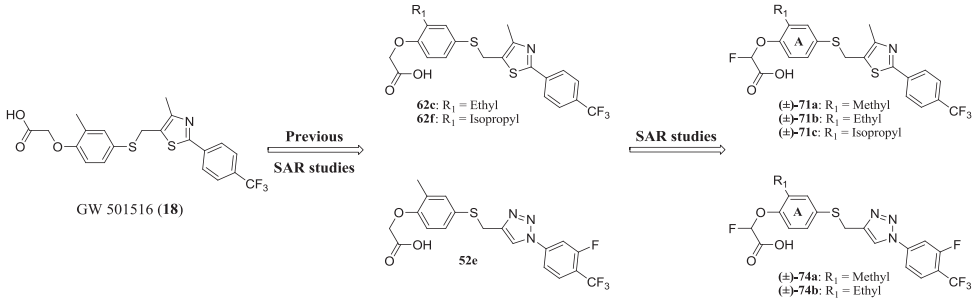
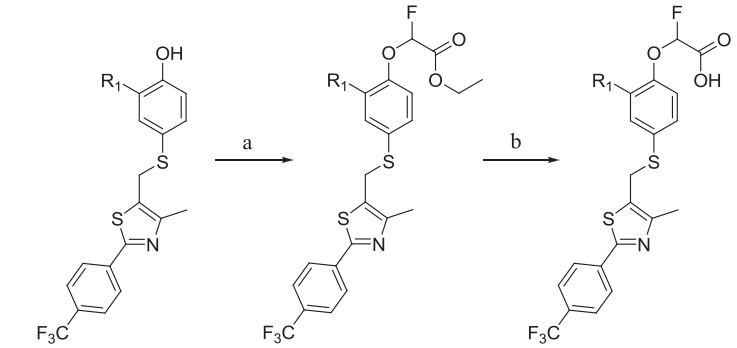


Figure 2.19. Structures of GW 501516 (**18**) and prepared analogues.

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 p*K*a-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.

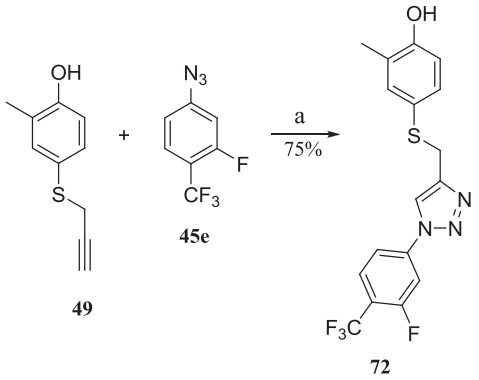
2.1.5.1 Synthesis

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**).



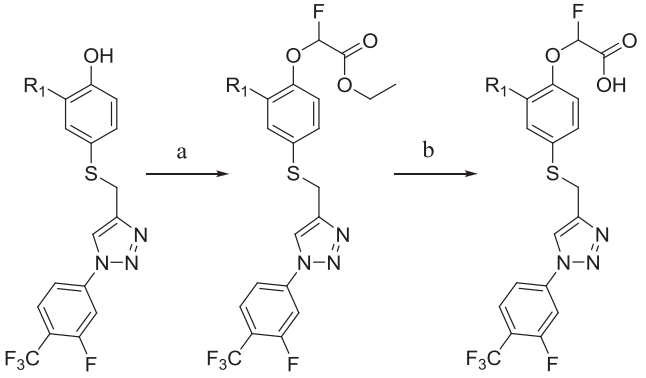
Scheme 2.15. (a) Ethyl 2-bromo-2-fluoroacetate , Cs_2CO_3, CH_3CN; (b) LiOH, THF, H_2O.

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)[57] to yield the intermediate triazole **72** in 75% yield (**Scheme 2.16**).



Scheme 2.16. (a) Sodium ascorbate, $CuSO_4$, *tert*-BuOH, H_2O.

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**).



67: R_1 = Ethyl
72: R_1 = Methyl

(±)-73a: R_1 = Methyl, (93%)
(±)-73b: R_1 = Ethyl, (90%)

(±)-74a: R_1 = Methyl, (78%)
(±)-74b: R_1 = Ethyl, (67%)

Scheme 2.17. (a) Ethyl 2-bromo-2-fluoroacetate, Cs_2CO_3, CH_3CN. (b) LiOH, THF, H_2O.

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

Compound	R_1	EC_{50}(nM)[a]
(±)-**71a**	CH_3	3.7
(±)-**71b**	Ethyl	5.04
(±)-**71c**	Isopropyl	31.4
GW 501516 (**18**)	-	0.03

[a] Results of three replicates

Table 2.6. Substitution pattern (see **Figure 2.19**) and EC_{50}-values of prepared compounds

Compound	R_1	EC_{50}(nM)[a]
(±)-**74a**	CH_3	20.2
(±)-**74b**	Ethyl	n.d.[b]
GW 501516 (**18**)	-	0.03

[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).

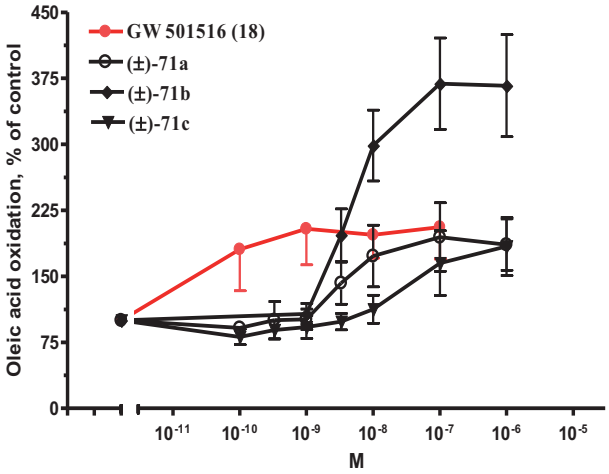


Figure 2.20. Oxidation of oleic acid in human myotubes in the presence of racemates **71a-71c** at different concentrations. GW 501516 (**18**) was used as positive control.

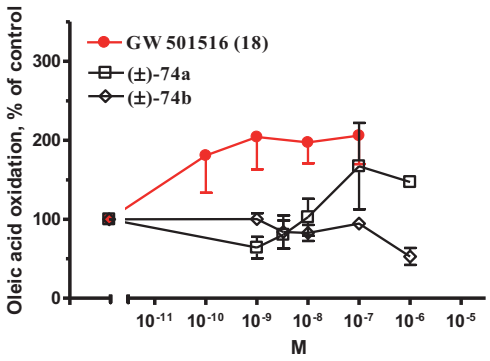


Figure 2.21. Oxidation of oleic acid in human myotubes in the presence of racemates **74a** and **74b** at different concentrations. GW 501516 (**18**) was used as positive control.

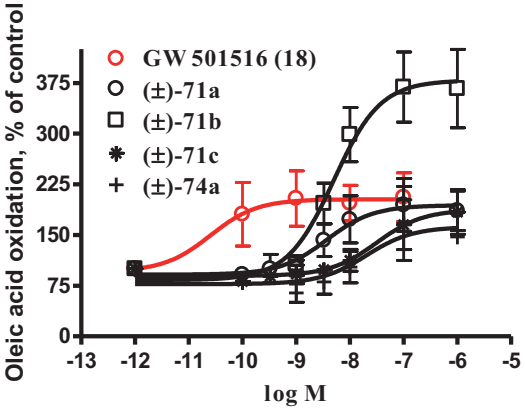


Figure 2.22. Non-linear fit curves of oxidation of oleic acid in human myotubes in the presence of different concentrations of racemates **71a-71c** and **74a**. GW 501516 (**18**) was used as positive control.

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**).

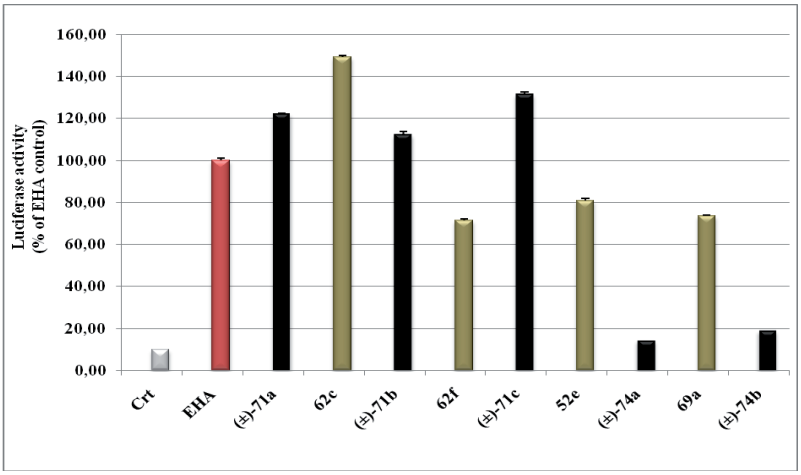


Figure 2.23. Activation of the ligand-binding domain of PPARα by the compounds (±)-**71a**, **62c**, (±)-**71b**, **62f**, (±)-**71c**, **52s**, (±)-**74a**, **69a** and (±)-**74b**. Positive control: **EHA** ((2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-2,4,8,11,14,17-hexaenoic acid.

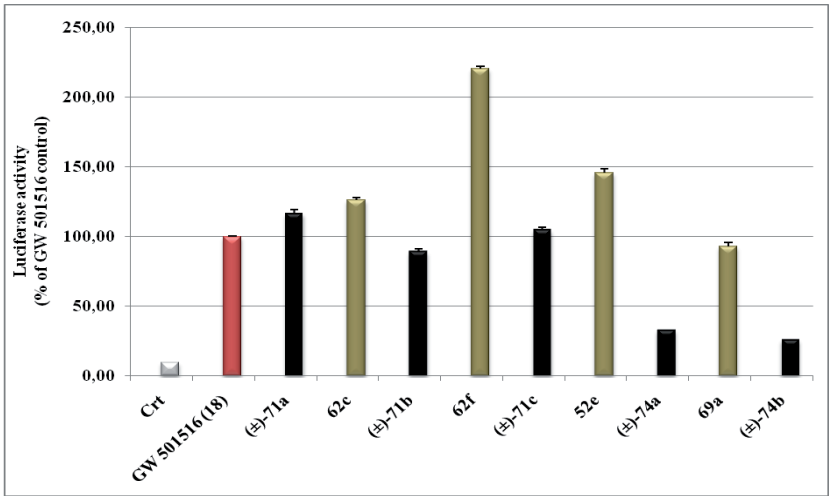


Figure 2.24. Activation of the ligand-binding domain of PPARδ by the compounds (±)-**71a**, **62c**, (±)-**71b**, **62f**, (±)-**71c**, **52s**, (±)-**74a**, **69a** and (±)-**74b**. Positive control: GW 501516 (**18**).

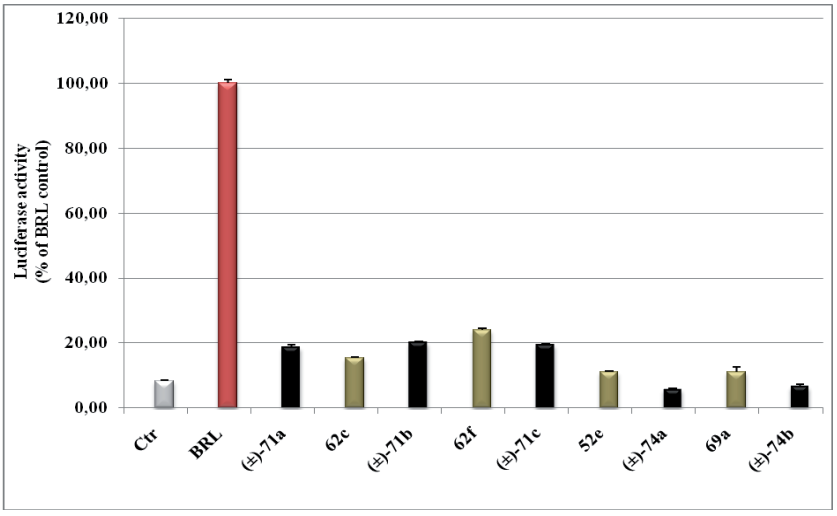


Figure 2.25. Activation of the ligand-binding domain of PPARγ by the compounds (±)-**71a**, **62c**, (±)-**71b**, **62f**, (±)-**71c**, **52s**, (±)-**74a**, **69a** and (±)-**74b**. Positive control: **Rosiglitazone** (BRL).

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.

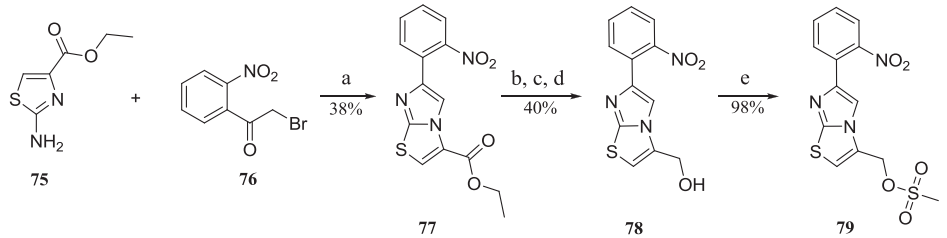
2.2 Preparation and biological evaluation of SRT 1720 (85)

Sirtuins (SIRT1, SIRT2, SIRT3) constitute a class of proteins with important metabolic roles, such as protein deacetylation.[79] 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.[80]

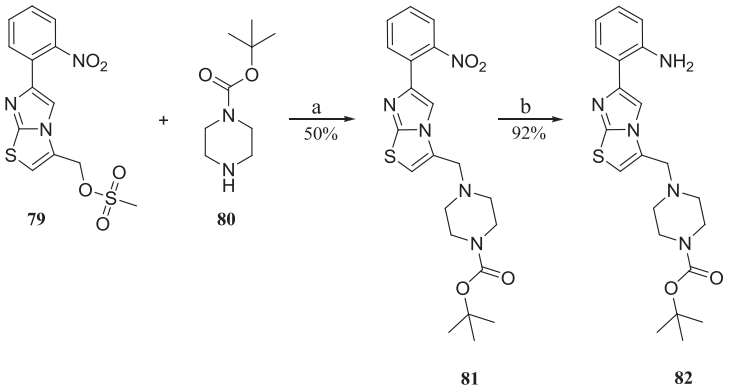
SIRT1 activator SRT 1720 (**85**) was reported in 2007 by a group from Sirtris Pharmaceuticals Inc. as a result of a high-throughput assay. It showed a high selectivity and strong activation toward SIRT1.[80] Given the biological response of SIRT1 activation by **SRT 1720** (**85**), we decided to investigate this molecule using the oleic acid oxidation essay.[69]

2.2.1 Synthesis

The synthesis of SRT 1720 was achieved according to a literature procedure[80] 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**).



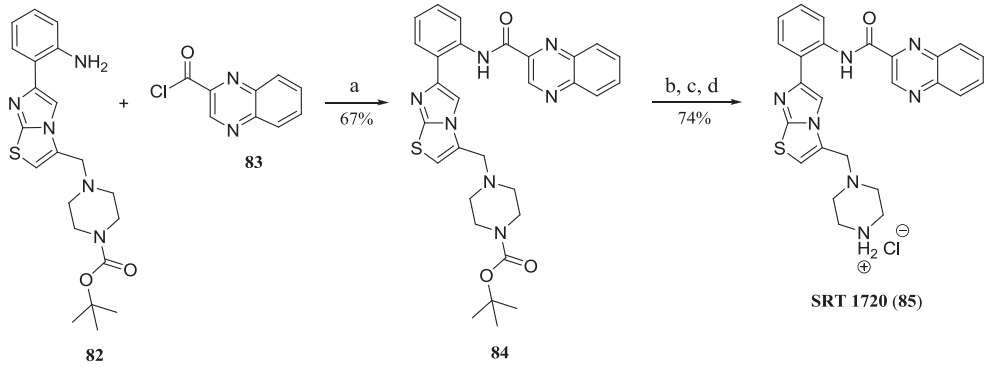
Scheme 2.18. (a) Methyl ethyl ketone, reflux; (b) NaOH, H_2O, THF; (c) Isobutyl chloroformate, (d) $NaBH_4$, *N*-methylmorpholine, THF; (e) Methanesulfonyl chloride , Et_3N, CH_2Cl_2.



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**).

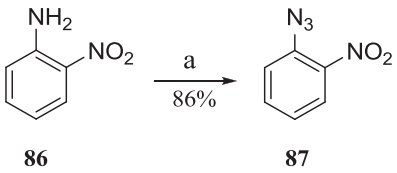
The next step was the amide formation by the nucleophilic attack of amine **82** on 2-quinoxaloyl chloride (**83**) under basic conditions affording compound **84**. The treatment with TFA of the compound **84** produced its TFA salt which was neutralized with $NaHCO_3$, extracted, and treated with HCl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



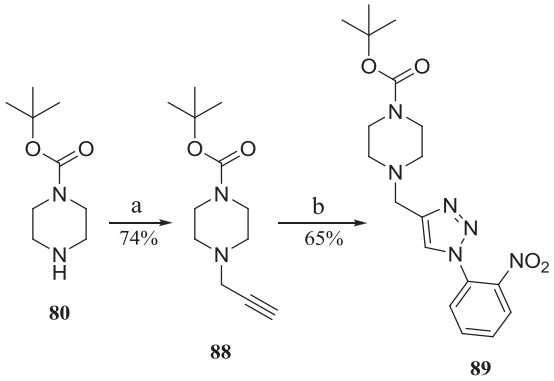
Scheme 2.20. (a) DMAP, DMF; (b) TFA, CH_2Cl_2; (c) $NaHCO_3$, H_2O, extraction; (d) HCl, H_2O, CH_3CN.

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**),[67] 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[57] to the triazole **89** in 48% yield over the two steps (**Scheme 2.22**).

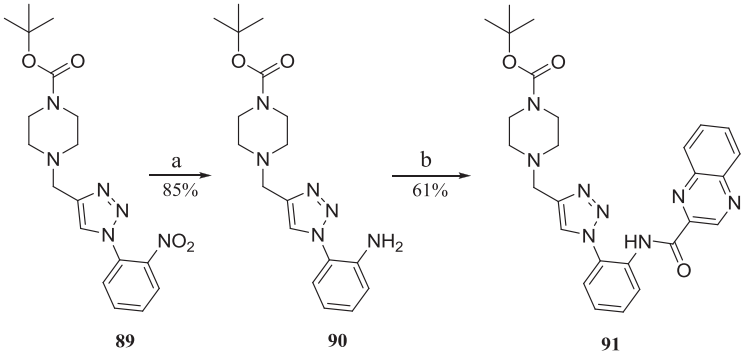


Scheme 2.21. (a) $NaNO_2$, NaN_3, H_2O, HCl



Scheme 2.22. (a) Propargyl bromide, Et_3N, CH_3CN; (b) 1-Azido-2-nitrobenzene (**87**), sodium ascorbate, $CuSO_4$, *tert*-BuOH, H_2O.

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) H_2, Pd/C, MeOH; (b) 2-Quinoxaloyl chloride (**83**), DMAP, DMF.

2.2.3 Biological evaluation of SRT 1720 (85) and the analogue 91

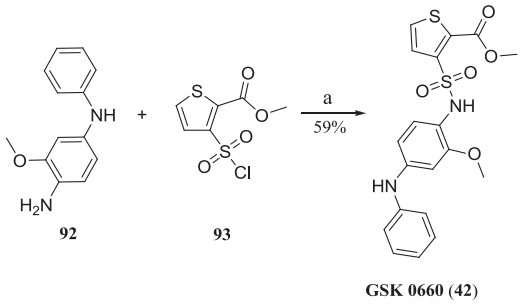
The results of the biological testing of SRT 1720 (**85**) and compound **91** are not yet available.

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.[69] 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.[69]

2.3.1 Preparation of GSK 0660 (42)

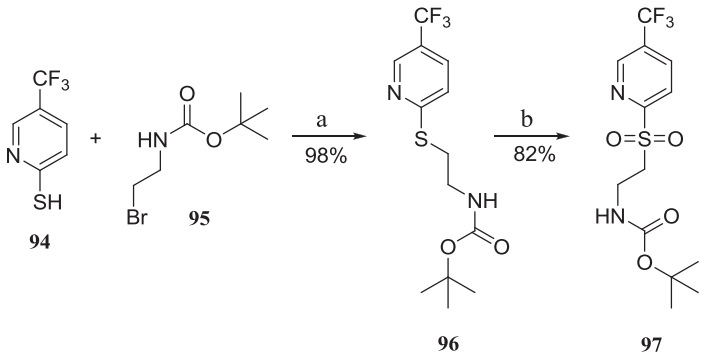
The synthesis of **GSK 0660** (**42**) was performed by a nucleophilic attack of disubstituted amine **92** on sulfone **93** (**Scheme 2.24**).



Scheme 2.24. (a) Pyridine, diethyl ether.

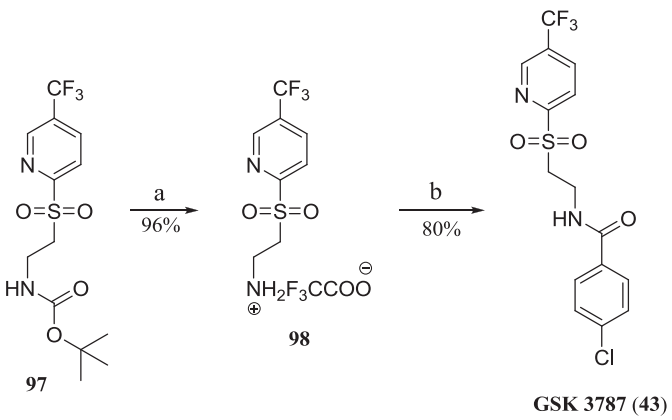
2.3.2 Preparation of GSK 3787 (43).

The synthesis of GSK 3787 (**43**) was conducted according to a literature procedure.[49] 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**).



Scheme 2.25. (a) Et_3N, DMF; (b) Oxone™, acetone, H_2O.

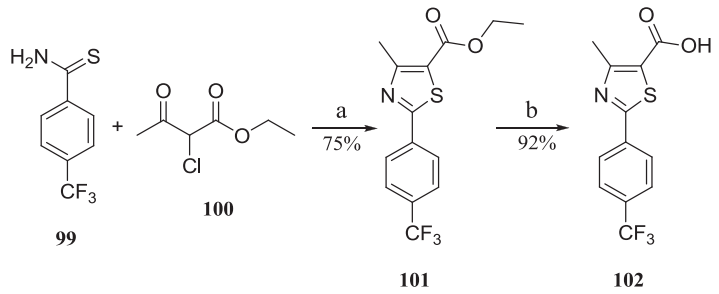
Sulphone **97** was deprotected to compound **98** which reacted with 4-chlorobenzoyl chloride through a nucleophilic substitution to produce GSK 3787 (**43**) in 77% yield over the two steps (**Scheme 2.26**).



Scheme 2.26. (a) TFA, CH_3CN; (b) 4-Chlorobenzoyl chloride, Et_3N, CH_2Cl_2.

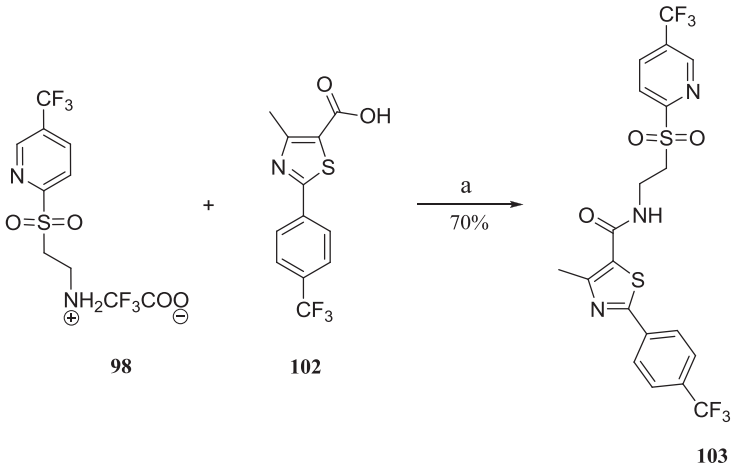
2.3.3 Synthesis of a potential new antagonist of PPARδ (103) (Paper IV)

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**).[81]



Scheme 2.27. (a) EtOH, reflux; (b) LiOH, H_2O, 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, Et_3N, CH_2Cl_2.

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**.

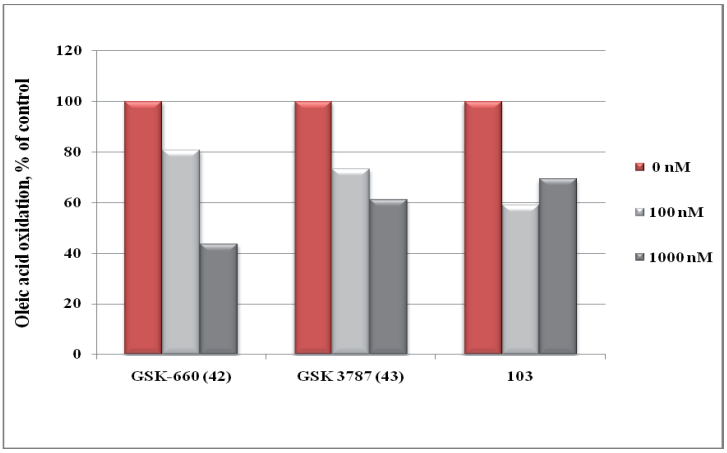


Figure 2.26. Inhibition of the oleic acid oxidation by the prepared antagonists; one replicate.

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.*[49] 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**.

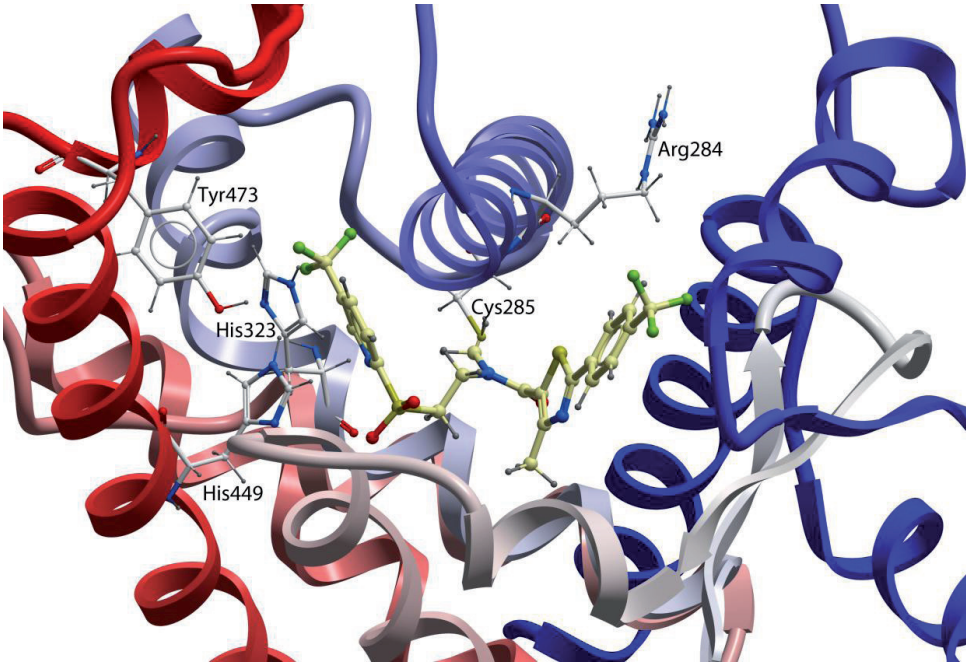


Figure 2.27. Compound **103** docked into PPARδ. Colour coding: red - oxygen, blue - nitrogen, grey - hydrogen, green - fluorine, yellow - carbon in **103**, white - carbon in PPARδ. Colouring of the Cα traces of PPARα is blue via white to red from N-terminal to C-terminal.

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 PPARδ. Activation of PPARα generally increased. No changes in PPARγ activation were observed.

In the case of 1,4-disubstiuted 1,2,3-triazole-based agonists, introduction of a fluorine atom led to a significant reduction of the activation of PPARα and PPARδ. No changes in PPARγ activation were observed.

5) Selective PPARδ antagonists

An analogue (**105**) of the recently reported selective PPARδ 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™. 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_3CN (20 mL) was added Cs_2CO_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_3CN (25 mL). The mixture was stirred for 2 h at r.t., and then an additional quantity of Cs_2CO_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_3CN (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-1*H*-1,2,3-triazol-4-yl)methylthio]phenoxy}acetic acid (55a)

Sodium ascorbate (120 mg, 0.6 mmol, 20 mol %) and $CuSO_4$ (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/H_2O (1:1).The mixture was stirred at r.t. over night. The formed precipitate was filtered off, washed with aqueous NH_3 (3.5%, 2x50 mL), brine, dissolved in a mixture of THF (30 mL) and H_2O (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 H_2O. 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 $MgSO_4$, and concentrated. The residue was recrystallized from CH_2Cl_2 giving **55a** as a colourless solid in 54% yield (553 mg, 1.6 mmol). Mp 182-183°C. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{17}H_{15}N_3O_3S$ $[M]^{\ddagger}$: 341.0834; found 341.0830.

2-{4-[(1-p-tolyl-1*H*-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, 1mmol) (**45b**) according to the general procedure described for **55a**. Mp 148-149°C. ^{1}H NMR (300 MHz, DMSO-d_6): 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).^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{18}H_{17}N_3O_3S$ $[M]^{\ddagger}$: 355.0991; found 355.0983.

2-{4-[(1-(3-Fluoro-4-methylphenyl)-1*H*-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): δ = 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): δ = 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 $C_{18}H_{16}FN_3O_3S$ $[M]^{\ddagger}$: 373.0896; found 373.0889.

2-{4-[(1-(4-trifluoromethylphenyl)-1*H*-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): δ = 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): δ = 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 $C_{18}H_{14}F_3N_3O_3S$ $[M]^{\ddagger}$: 409.0708; found 409.0701.

2-{4-[(1-(3-Fluoro-4-trifluoromethyphenyl)-1*H*-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, 1mmol) (**45e**) according to the general procedure described for **55a**. Mp 152-153°C. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{18}H_{13}F_4N_3O_3S$ $[M]^{\ddagger}$: 427.0614; found 427.0605.

2-{4-[(1-(4-Methoxyphenyl)-1*H*-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. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.04, 159.20, 157.05, 144.72, 132.19, 130.01, 126.12, 121.62, 121.29, 115.28, 114.87, 64.53, 55.56, 29.24. MS (ESI) *m/z* 370.1 $[M-H]^-$; HRMS calcd for $C_{18}H_{17}N_3O_4S$ $[M]^{\ddagger}$: 371.0940; found 371.0932.

2-{4-[(1-(3-Fluoro-4-methoxyphenyl)-1*H*-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. ^{1}H NMR (300

MHz, DMSO-d_6): δ = 13.03 (bs, 1H), 8.55 (b, 1H), 7.81 (dd, *J* = 12.1, 2.6 Hz, 1H), 7.70 – 7.62 (m, 1H), 7.41 – 7.31 (m, 3H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.66 (s, 2H), 4.23 (s, 2H), 3.90 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.03, 157.06, 151.26 (d, *J* = 245.7 Hz), 147.18 (d, *J* = 10.3 Hz), 144.97, 132.18, 129.57 (d, *J* = 9.0 Hz), 126.05, 121.40, 116.29 (d, *J* = 3.6 Hz), 115.28, 114.48 (d, *J* = 2.5 Hz), 108.71 (d, *J* = 22.9 Hz), 64.53, 56.36, 29.22. MS (ESI) *m/z* 388.1 [M-H]$^-$; HRMS calcd for $C_{18}H_{16}FN_3O_4S$ [M]‡: 389.0846; found 389.0856.

2-{4-[(1-(4-trifluoromethoxyphenyl)-1*H*-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. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 12.99 (bs, 1H), 8.63 (s, 1H), 7.99 (d, *J* = 9.1 Hz, 2H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.66 (s, 2H), 4.25 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.04, 157.11, 147.78 (distorted q, *J* = 1.9 Hz), 145.27, 135.42, 132.32, 125.94, 122.59, 121.93, 121.60, 120.00 (q, *J* = 257.0 Hz), 115.28, 64.52, 29.22. MS (ESI) *m/z* 424.0 [M-H]$^-$; HRMS calcd for $C_{18}H_{14}F_3N_3O_4S$ [M]‡: 425.0657; found 425.0674.

2-{4-[(1-(3-Chloro-4-trifluoromethoxyphenyl)-1*H*-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. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 12.83 (bs, 1H), 8.75 (s, 1H), 8.29 (d, *J* = 2.6 Hz, 1H), 8.02 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.79 (dd, *J* = 9.0, 1.3 Hz, 1H), 7.36 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.66 (s, 2H), 4.25 (s, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.02, 157.12, 145.52, 143.47 (q, *J* = 1.8 Hz), 136.04, 132.29, 127.44, 125.91, 124.42, 122.24, 121.79, 120.28, 120.00 (q, *J* = 259.1 Hz).115.29, 64.52, 29.20. MS (ESI) *m/z* 458.0 [M-H]$^-$; HRMS calcd for $C_{18}H_{13}ClF_3N_3O_4S$ [M]‡: 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_3CN (20 mL), Cs_2CO_3 (847 mg, 2.6 mmol) was added. To this mixture, a solution of propargyl bromide (216 μL, 2.3 mmol) in dry CH_3CN (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)-1*H*-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_2O (1:1), sodium ascorbate (70 mg, 20 mol %) and copper sulphate (28 mg, 10 mol %) were added. The mixture was stirred at r.t. over night. 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), 7.60 (dd, *J* = 10.7, 1.3 Hz, 1H), 7.54 (dd, *J* = 8.5, 0.7 Hz, 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, 3H). ^{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,

121.91 (qd, *J* = 272.3, 1.1 Hz), 120.18, 116.04, 118.44 (qd, *J* = 33.8, 12.6 Hz), 115.08 (d, *J* = 3.9 Hz), 109.05 (d, *J* = 25.6 Hz), 30.62, 22.81, 13.75.

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

To a solution of **67** (150 mg, 0.38 mmol) in dry CH_3CN (20 mL) was added Cs_2CO_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_3CN (3 mL). 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 $MgSO_4$, 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)-1*H*-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,

140.67 (dd, *J* = 10.1, 0.2 Hz), 134.19, 133.36, 130.64, 128.63 (qd, *J* = 4.6, 2.5 Hz), 125.45, 121.95 (qd, *J* = 272.3, 1.2 Hz), 119.80, 117.91 (qd, *J* = 33.7, 12.6 Hz), 114.89 (d, *J* = 3.9 Hz), 111.97, 108.75 (d, *J* = 25.6 Hz), 72.31, 61.10, 30.42, 23.12, 18.38, 13.92, 13.78.

Ethyl 2-{4-[(1-(3-fluoro-4-trifluoromethylphenyl)-1*H*-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* = 9.8 Hz), 136.05, 133.21, 129.92, 128.69 (qd, *J* = 4.5, 2.6 Hz), 125.87, 121.97 (qd, *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-trifluoromethyphenyl)-1*H*-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_2O (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_2O, acidified with 0.1 M HCl, extracted with diethyl ether (3x50 mL), dried over $MgSO_4$, and concentrated. The residue was recrystallized from CH_2Cl_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 $C_{20}H_{17}F_4N_3O_3S$ $[M]^{\ddagger}$: 455.0927; found 455.0917.

2-{4-[(1-(3-Fluoro-4-trifluoromethyphenyl)-1*H*-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. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{21}H_{19}F_4N_3O_3S$ $[M]^{\ddagger}$: 469.1083; found 469.1077.

2-{4-[(1-(3-Fluoro-4-trifluoromethyphenyl)-1*H*-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 H_2O (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 H_2O, acidified with 0.1 M HCl, extracted with diethyl ether (3x50 mL), dried over $MgSO_4$, and concentrated. The residue was recrystallized from CH_2Cl_2 to give **69c** as a colourless solid in 83% yield (146 mg, 0.30 mmol). Mp 155-156°C. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{22}H_{21}F_4N_3O_3S$ $[M]^+$: 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_2O (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, $CDCl_3$): δ = 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, $CDCl_3$): δ =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_3CN (20 mL) was added triethylamine (167 μL, 1.2 mmol). To this mixture a solution of ethyl 3-bromopropyne (90 μL, 1.2 mmol) in dry CH_3CN (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 $MgSO_4$, 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.38, 79.39, 78.17, 73.31, 51.38, 46.75, 43.18, 28.20.

tert-Butyl 4-[1-(2-nitrophenyl)-1*H*-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/H_2O (1:1). The mixture was stirred at r.t over night and then it was diluted with 50 mL water and extracted with CH_2Cl_2 (3x50 mL). The organic phases were washed with aqueous NH_3 (3.5%, 2x500 mL), brine, dried over $MgSO_4$, 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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)-1*H*-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 CH_2Cl_2/MeOH (16:1) as eluent to give **90** as a colourless solid in 85% yield (100 mg, 0.28 mmol). Mp 143-144°C. ^{1}H NMR (300 MHz, $CDCl_3$): 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),

3.42 (t, *J* = 5.9 Hz, 4H), 2.48 (d, *J* = 5.9 Hz, 4H), 1.42 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): 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 over night 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 $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (16:1) as eluent to give **91** as a colourless solid in 61% yield (88 mg, 0.17 mmol). Mp 143-144°C. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 $C_{27}H_{30}F_4N_8O_3$ $[M]^{\ddagger}$: 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 over night 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 $MgSO_4$ and concentrated. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (16:1) as eluent to give **942** as a light green solid in 59% yield (370 mg, 0.88 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): 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, $CDCl_3$): δ = 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 myotubes 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.

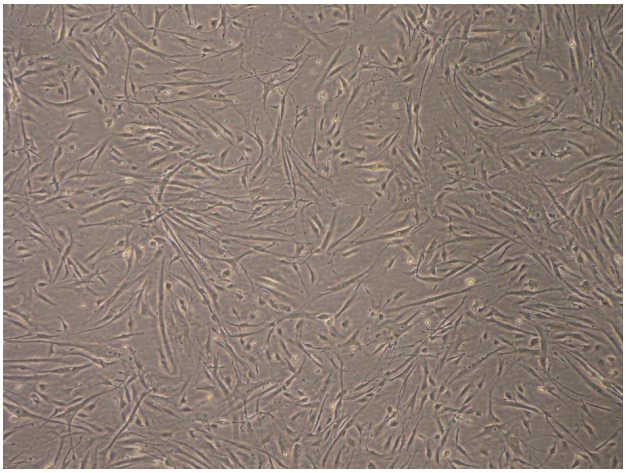


Figure 5.1. Muscle cell culture: 4 days after seeding

The cells were cultured in humidified 5% CO_2 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.

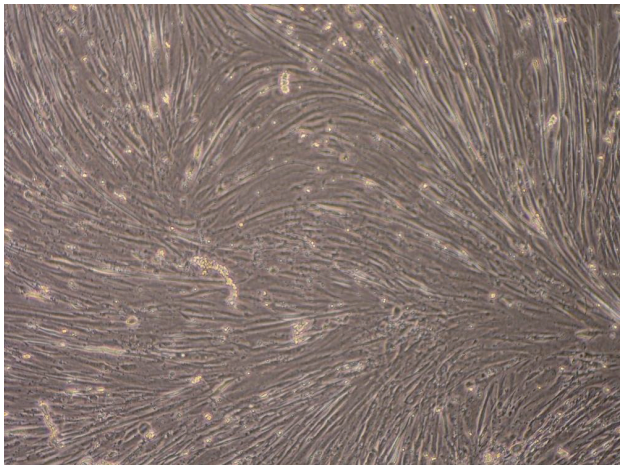


Figure 5.2. Muscle cell culture: after 3 days of differentiation

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-^{14}C]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 CO_2 was trapped as described before.[69] 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®A), and the CO_2 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 ^{14}C-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.[83] 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

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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 7.02 (t, J = 9.0 Hz, 1H) it should be 6.97-7.06 (m, 1H).

Instead of $[M]^{+}$ it should be $[M]^{\ddagger}$.

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 $[M]^{\ddagger}$.

List of papers

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

Calin C. Ciocoiu, Nataša Nikolić, Huyen Hoa Nguyen, G. Hege Thoresen, Arne J. Aasen, Trond Vidar Hansen, *Eur. J. Med. Chem*. **2010**, 45, 3047-3055.

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

Calin C. Ciocoiu, Aina W. Ravna, Ingebrigt Sylte, Trond Vidar Hansen, *Arch. Pharm. Chem. Life Sci.*, Accepted: August 20, 2010.

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

Calin C. Ciocoiu, Aina W. Ravna, Ingebrigt Sylte, Arild C. Rustan, Trond Vidar Hansen, *Bioorg. Med. Chem.*, *manuscript*

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*

I

II

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

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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.

[INSERT FIGURE 1]

2. Chemistry

Compounds **3a**-**3e** were synthesized according to a literature procedure [17]. Thiocyanates **3a** and **3b** were reduced with $LiAlH_4$ 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 Cs_2CO_3 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 Cs_2CO_3 to yield esters **8a**-**8h** which after basic aqueous hydrolysis afforded acids **2a**-**2h** in 51-80% yield (Scheme 1).

[INSERT SCHEME 1]

Treatment of thiocyanates **3c**-**3e** with $LiAlH_4$ afforded large quantities of the corresponding disulfide dimers. Hence, compounds **3c**-**3e** were first reacted with ethyl 2-bromoacetate and then reduced with $NaBH_4$ 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).

[INSERT 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 ^{14}C-labeled oxidized oleic acid [19]. The EC_{50}-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).

[INSERT TABLE 1]

[INSERT FIGURE 2]

[INSERT FIGURE 3]

[INSERT FIGURE 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**: EC_{50} = 0.65 nM) compared to **1**. Introduction of two methyl groups afforded agonist **2b** that was slightly more potent than **2a** (**2b**: EC_{50} = 0.24 nM), but exhibited slightly lower potency than the lead compound **1**.

When the methyl group from the R_1-position of GW 501516 (**1**) was substituted to an ethyl group, a decrease of the potency was noticed. (**2c**: EC_{50} = 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** (EC_{50} = 0.36 nM). Increasing the size of the *ortho*-substituent in the benzene ring **A** (R_1), by substitution with an *iso*-propyl, *tert*-butyl, cyclopentyl or cyclohexyl group, led to a reduction in potency (**2f**: EC_{50} = 4.15 nM; **2i**: EC_{50} = 5.51 nM; **2j**: EC_{50} = 16.60 nM; **2k:** EC_{50} = 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** (R_1) to an ethyl group led to agonist **2e** that exhibited an EC_{50}-value of 0.54 nM. Replacing the ethyl group in **2e** with an *iso*-propyl group afforded agonist **2h** (EC_{50} = 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 R_1, 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** (EC_{50} = 5.79 nM) and **2h** (EC_{50} = 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 EC_{50}-values were determined against all three PPARs using the aforementioned transfection assay. The EC_{50}-value for **2e** against PPARδ was determined to be 5.0 nM, which is slightly lower than the EC_{50}-value of 1.0 nM reported for GW 501516 (**1**) [13]. Interestingly, the EC_{50}-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 (EC_{50} > 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 R_1-position. In the docked complex of **2e** the ethyl group in R_1 points in the direction of Thr289, Ile326 and Phe327. A larger substituent R_1 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 EC_{50} = 5 nM. Moderate potency was observed against PPARα for compound **2e** (EC_{50} = 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 DPX300 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_2Cl_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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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 ($MgSO_4$), 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). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.78, 132.46, 130.79, 130.24, 128.62, 115.77, 22.77, 13.64.

*6.8. 2-Iso-propyl-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. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 CH_3CN (40 mL) was added Cs_2CO_3 (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 CH_3CN (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 ($MgSO_4$) 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. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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. ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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 CH_3CN (30 mL) was added Cs_2CO_3 (483 mg, 1.48 mmol). To this mixture was added dropwise a solution of ethyl 2-bromoacetate (165 µL, 1.48 mmol) in dry CH_3CN (10 mL). The mixture was stirred for 3 hours at ambient temperature, then diluted with water and extracted (ethyl acetate, 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 **6a** as a colorless oil in 79% yield (311mg, 1.06 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): 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). ^{13}C NMR (75 MHz, $CDCl_3$): 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.

*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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 168.73, 153.87, 137.68, 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_3CN (10 mL) was added Cs_2CO_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_3CN (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, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)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 µL, 0.52 mmol) following the procedure described for **8a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)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 µL, 1.15 mmol) following the procedure described for **8a**. Mp = 80-81 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 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, $CDCl_3$): δ = 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-[(4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)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 µL, 0.39 mmol) following the procedure described for **8a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.14 (d,

J = 2.3 Hz, 1H), 7.09 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.55 (d, *J* = 8.4 Hz, 1H), 4.70 (q, *J* = 6.8 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.06 (s, 2H), 2.61 (q, *J* = 7.5 Hz, 2H), 2.13 (s, 3H), 1.59 (d, *J* = 6.8 Hz, 3H), 1.18 (t, *J* = 7.2 Hz, 3H), 1.13 (t, *J* = 7.6 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.

*6.22 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 = 174.12, 162.90, 153.79, 151.35, 136.76 (distorted q, *J* = 1.3 Hz), 135.96, 134.81, 131.65, 131.12 (q, *J* = 32.6 Hz), 130.68, 126.26, 125.74 (q, *J* = 3.8 Hz), 125.11, 123.88 (q, *J* = 272.3 Hz), 116.20, 78.91, 61.36, 32.39, 25.27, 23.40, 14.64, 13.92.

*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), 7.16 (d, *J* = 2.2 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, $CDCl_3$): 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, $CDCl_3$): 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, $CDCl_3$): 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), 7.06 (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, $CDCl_3$): 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*-butyl-4-mercaptophenoxy)acetate (**7a**) (88 mg, 0.33 mmol) in dry CH_3CN (10 mL) was added Cs_2CO_3 (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 CH_3CN (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 ($MgSO_4$), 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, $CDCl_3$): δ = 7.94 (d, *J* = 8.1 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.21 (d, *J* = 2.2 Hz, 1H), 7.18 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.60 (d, *J* = 8.3 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, $CDCl_3$): δ = 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. 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, $CDCl_3$): δ = 7.94 (d, *J* = 8.1 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.18 – 7.11 (m, 2H), 6.59 (d, *J* = 8.2 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, $CDCl_3$): δ = 168.62, 163.02, 156.15, 151.45, 136.77 (distorted q, *J* = 1.3 Hz), 136.23, 133.03, 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. 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, $CDCl_3$): δ = 7.94 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.14 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.11 (d, *J* = 2.2 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, $CDCl_3$): δ = 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.

*6.29. 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 H_2O (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_2O, acidified with 0.1 M HCl, extracted (diethyl ether, 3x50 mL), dried ($MgSO_4$), 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. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $[M-H]^-$, HRMS calcd for $C_{22}H_{20}F_3NO_3S_2$ $[M]^+$: 467.0837; found: 467.0830.

*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 H_2O (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 H_2O, acidified with 0.1 M HCl, extracted (diethyl ether, 3x50 mL), dried ($MgSO_4$), 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 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{23}H_{22}F_3NO_3S_2$ $[M]^+$: 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): δ = 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). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 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 $C_{22}H_{20}F_3NO_3S_2$ $[M]^+$: 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): δ = 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). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 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 $C_{23}H_{22}F_3NO_3S_2$ $[M]^+$: 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) δ = 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{24}H_{24}F_3NO_3S_2$ $[M]^+$: 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. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{23}H_{22}F_3NO_3S_2$ $[M]^+$: 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. ^{1}H NMR (300 MHz, DMSO-d_6): 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). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{24}H_{24}F_3NO_3S_2$ $[M]^+$: 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): δ = 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): δ = 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 $C_{25}H_{26}F_3NO_3S_2$ [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): δ = 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): δ = 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 $C_{24}H_{24}F_3NO_3S_2$ [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): δ = 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): δ = 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 $C_{25}H_{24}F_3NO_3S_2$ $[M]^+$: 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): δ = 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), 6.97 (d, *J* = 2.2 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 4.68 (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): δ = 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 $C_{26}H_{26}F_3NO_3S_2$ $[M]^+$: 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 μCi/mL, 100 μM), 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%CO_2 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: (2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosa-2,4,8,11,14,17-hexaenoic acid (EHA), GW 501516 (**1**) and rosiglitazone (BRL) for PPARα, PPARδ, and PPARγ, respectively. EC_{50} is the concentration of test compounds needed to induce 50% of the maximum luciferase activity. The EC_{50}-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.

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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 ((2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-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 Cα 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) $LiAlH_4$, THF; (b) 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole, Cs_2CO_3, CH_3CN; (c) i) ethyl 2-bromoacetate, Cs_2CO_3, CH_3CN or ii) ethyl 2-bromopropionate, Cs_2CO_3, CH_3CN; (d) LiOH, THF, H_2O, 0 °C (**2a**, **2c**, **2d**, **2f**, **2g**); $(CH_3)_3COK$, THF, H_2O, reflux (**2b**, **2e**, **2h**).

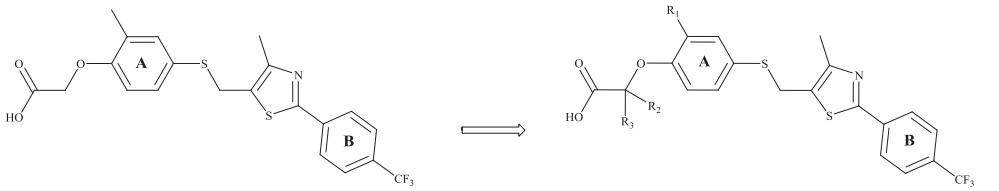
Scheme 2. Reagents and conditions: (a) ethyl 2-bromoacetate, Cs_2CO_3, CH_3CN; (b) $NaBH_4$, 1,4-dithioerythritol, EtOH; (c) 5-chloromethyl-4-methyl-2-(4-trifluoromethylphenyl)thiazole, Cs_2CO_3, CH_3CN; (d) LiOH, THF, H_2O, 0 °C.

Table 1. Substitution pattern (see Fig. 1) and EC_{50}-values of tested compounds in the oleic acid oxidation assay

[a]Results of three experiments

Figure 1

Calin C. Ciocoiu



GW 501516 (**1**)

2a: $R_1 = CH_3$, $R_2 =$ H, $R_3 = CH_3$
2b: $R_1 = CH_3$, $R_2 = CH_3$, $R_3 = CH_3$
2c: R_1 = Ethyl, R_2 = H, R_3 =H
2d: R_1 = Ethyl, R_2 = H, $R_3 = CH_3$
2e: R_1 = Ethyl, $R_2 = CH_3$, $R_3 = CH_3$
2f: R_1 = *iso*-Propyl, R_2 = H, R_3 = H
2g: R_1 = *iso*-Propyl, R_2 = H, $R_3 = CH_3$
2h: R_1 = *iso*-Propyl, $R_2 = CH_3$, $R_3 = CH_3$
2i: R_1 = *tert*-Butyl, R_2 = H, R_3 = H
2j: R_1 = Cyclopentyl, R_2 = H, R_3 = H
2k: R_1 = Cyclohexyl, R_2 = H, R_3 = H

Figure 2

Calin C. Ciocoiu

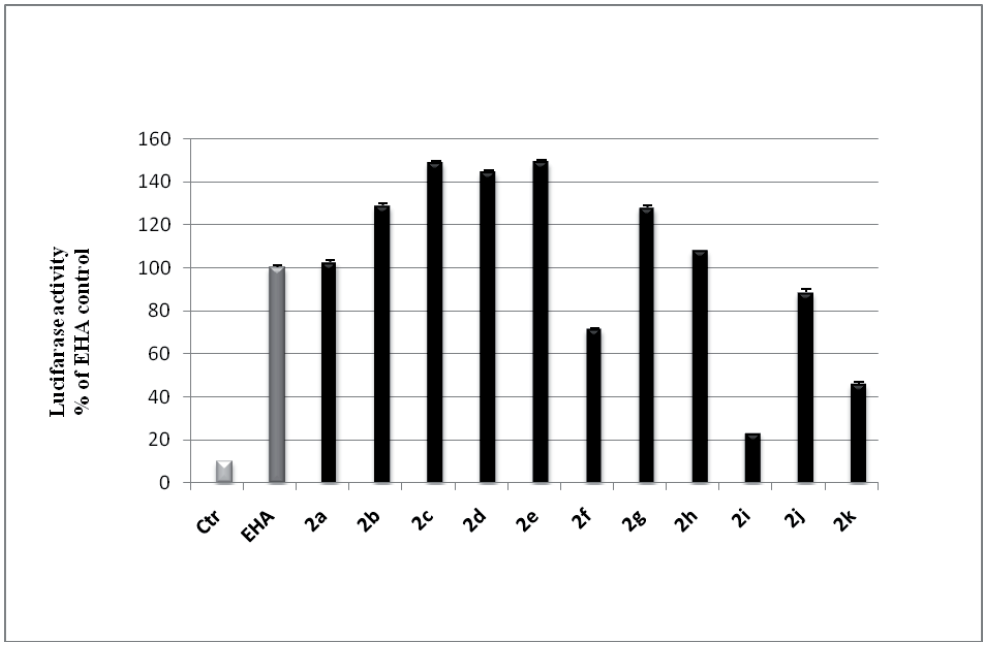


Figure 3

Calin C. Ciocoiu

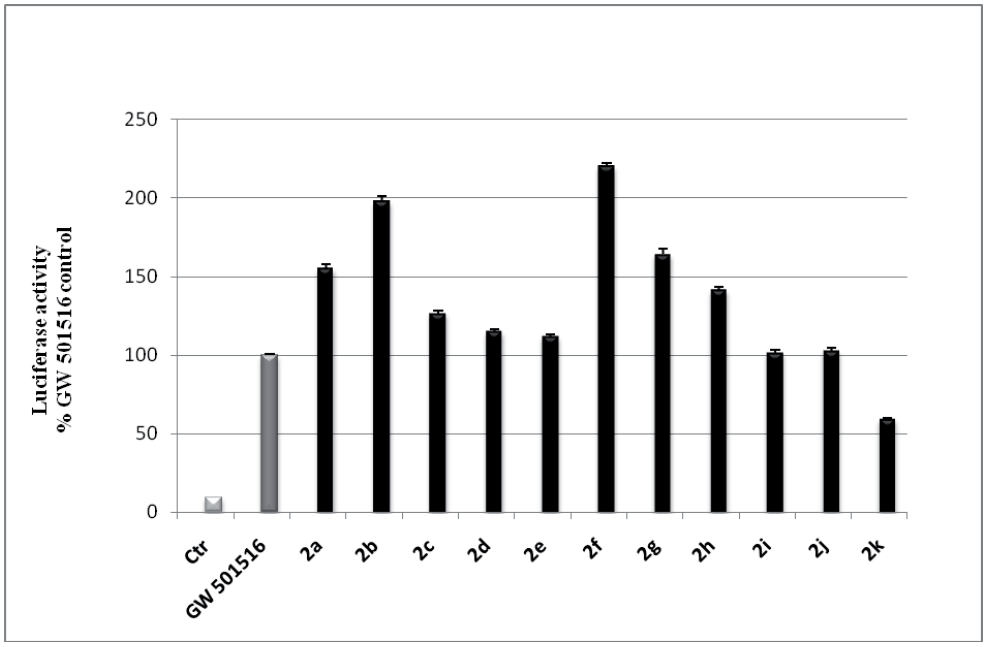


Figure 4

Calin C. Ciocoiu

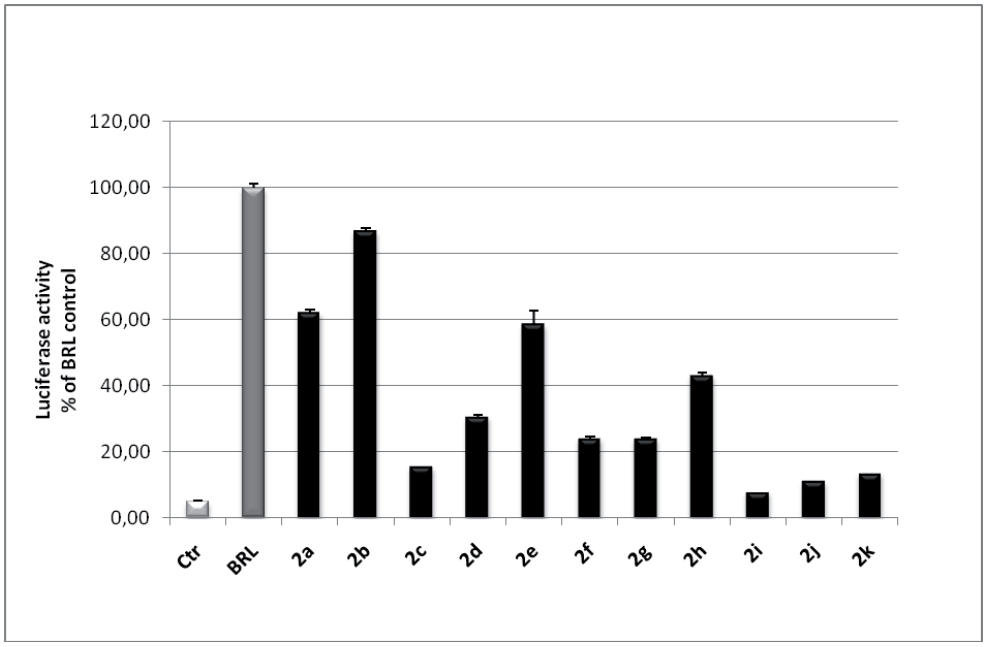
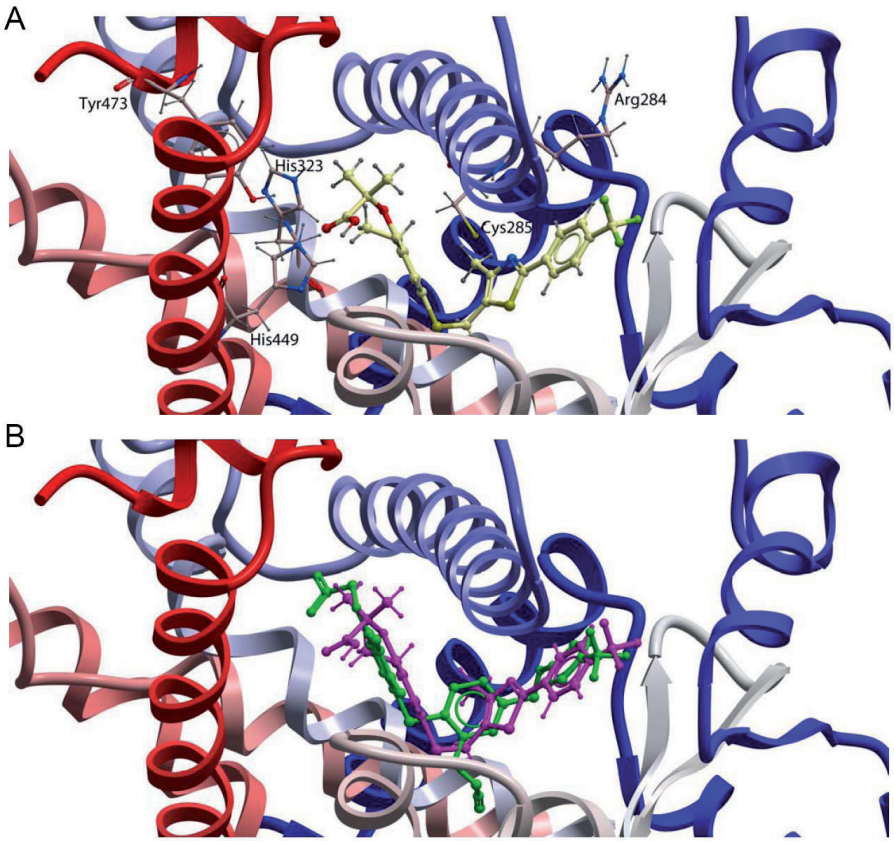


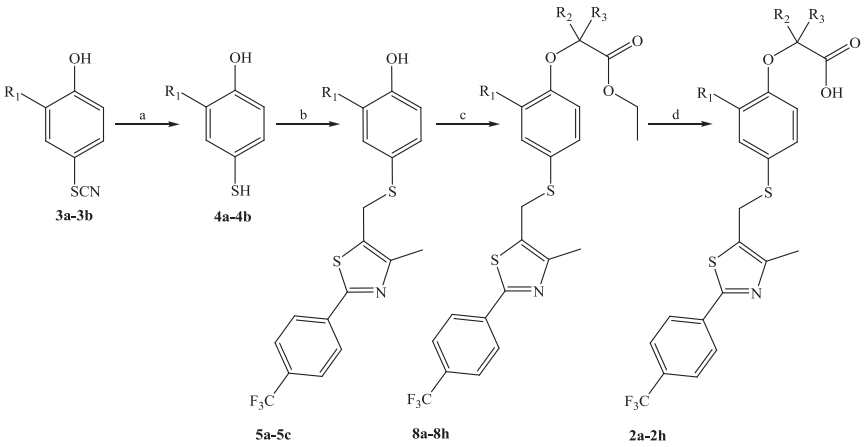
Figure 5

Calin C. Ciocoiu



Scheme 1

Calin C. Ciocoiu



Scheme 2

Calin C. Ciocoiu

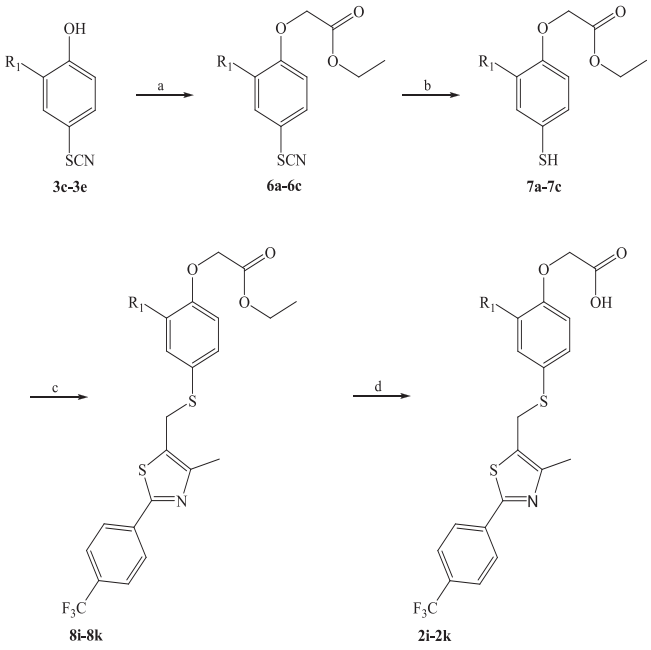


Table 1

Calin C. Ciocoiu

Compound	R_1	R_2	R_3	EC_{50} (nM)[a]
2a	CH_3	H	CH_3	0.65
2b	CH_3	CH_3	CH_3	0.24
2c	Ethyl	H	H	0.31
2d	Ethyl	H	CH_3	0.36
2e	Ethyl	CH_3	CH_3	0.54
2f	*iso*-Propyl	H	H	4.15
2g	*iso*-Propyl	H	CH_3	5.79
2h	*iso*-Propyl	CH_3	CH_3	9.11
2i	*tert*-Butyl	H	H	5.51
2j	Cyclopentyl	H	H	16.60
2k	Cyclohexyl	H	H	17.30
GW 501516 (1)	CH_3	H	H	0.10

III

IV