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Synthesis, molecular modeling studies and biological evaluation of fluorine substituted analogs of GW 501516

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ABSTRACT

(±)-2-Fluoro-2-(2-methyl-4-(((4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)methyl)thio)phenoxy)acetic acid (**2a**) has been prepared and subjected to biological testing against all three subtypes of the PPARs. This compound exhibited agonist effects with EC_{50} values of 560 and 55 nM against PPARα and PPARδ, respectively, in a luciferase assay. Moreover, compound (±)-**2a** also exhibited potent ability to induce oleic acid oxidation in a human myotube cell assay with EC_{50} = 3.7 nM. Compound (±)-**2a** can be classified as a dual PPARα/δ agonist with a 10-fold higher potency against the PPARδ receptor than against the PPARα receptor. Molecular modeling studies revealed that both enantiomers of **2a** bind to the PPARδ receptor with similar binding energies.

1. Introduction

Peroxisome proliferator-activated receptors (PPARα, PPARδ, and PPARγ) are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily.[1] Literature data underline the role of the three subtypes in the expression of genes responsible for the lipid and carbohydrate metabolism by interacting with specific DNA peroxisome proliferator response elements (PPRE).[2]

Molecules activating PPARα have beneficial effects on lipid metabolism by decreasing both serum triglycerides and free fatty acid levels, and increasing high-density lipoprotein level (HDL).[3] PPARγ agonists improve glucose tolerance in type 2 diabetic patients.[4] Several studies have suggested that PPARδ plays an important role in regulating lipid metabolism and energy homeostasis in muscle and adipose tissues.[5–9] Recently, dual agonists became of interest since it has been envisioned they could combine the beneficial effects of two different subtype receptors avoiding their negative actions. Dual activation of PPARs could be a useful tool against several diseases such as metabolic disorders, type 2 diabetes and cardiovascular diseases.[10]

The fluorine atom has found a wide range of applications in medicinal chemistry.[11] For example, the fluorine atom can participate in hydrogen bonding interactions and alter the physical and chemical properties of organic compounds.[12] Therefore, bioisosteric substitution of a hydrogen atom by a fluorine atom is a well known strategy for modification of lead compounds in drug discovery.[13]

We recently published triazole- and thiazole-based analogs of GW 501516 (**1**) which exhibited dual PPARα/δ effects.[14,15] Herein, we report the synthesis and biological evaluation of analogs of **1** where one hydrogen atom at the *alpha*-carbon atom to the carboxylic acid moiety has been replaced with a fluorine atom. We reasoned that such substitutions would afford new agonists with a stronger carboxylic acid with potential stronger binding affinity to the PPARs. These efforts led to the identification of three dual PPARα/δ agonists (Fig. 1). As of today, only a few dual PPARα/δ agonists have been reported.[16]

2. Results and discussion

2.1. Chemistry

Phenols **3a**–**3c**, synthesized as described before,[15,17] were alkylated with (±)-ethyl 2-bromo-2-fluoroacetate to yield esters (±)-**4a**–**4c**. Basic aqueous hydrolysis of (±)-**4a**–**4c** afforded the racemic acids (±)-**2a**–(±)-**2c** in 54–68% yield over the two steps (Scheme 1).

2.2. Biological evaluation

GW 501516 (**1**) and compounds (±)-**2a**–(±)-**2c**, at five different concentrations, were exposed for 96 h to fully differentiated human skeletal muscle cells cultured in 96 well plates. After this

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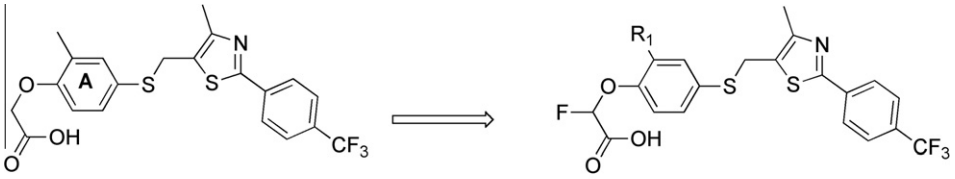
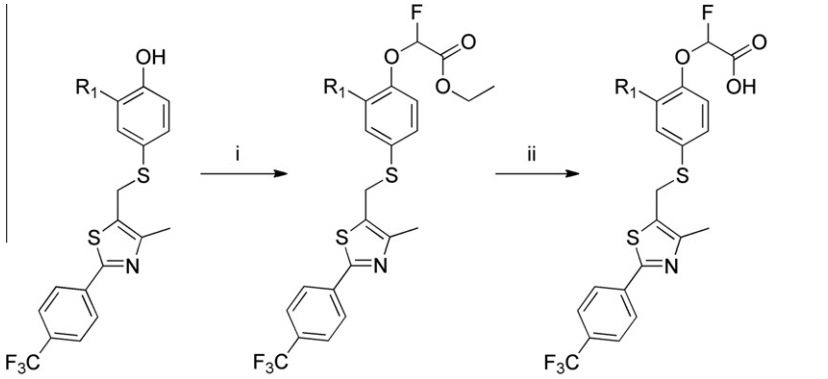


Figure 1. Structures of GW501516 and analogues (±)-2a-(±)-2c.



Scheme 1. Synthesis of compounds (±)-2a-(±)-2c. (i) (±)-Ethyl 2-bromo-2-fluoroacetate, Cs_2CO_3, CH_3CN; (ii) LiOH, H_2O, THF.

period of time, the oleic acid oxidation level was measured by detection of the accumulation of ^{14}C-labeled oxidized oleic acid.[18] The EC_{50}-values for compounds (±)-**2a**–(±)-**2c** obtained using this assay have been compiled in Table 1.

Introduction of a fluorine atom on the *alpha*-carbon to the carboxylic group of GW 501516 (**1**) led to compound (±)-**2a** that exhibited decreased potency compared to GW 501516 (**1**). The EC_{50}-values were determined to be 0.03 nM for **1** and EC_{50} = 3.7 nM for (±)-**2a**, respectively, in the human skeletal muscle cells assay (Table 1).

According to Sternbach and co-authors, the substituent in the *ortho*-position in the A-ring of GW 501516 (**1**) is important for the selectivity of the activation of each of the three PPARs.[19] Hence, we wanted to investigate how the activity and selectivity of the new agonists will be effected by introducing small alkyl groups in this position. Substitution of the methyl group of ring **A** in compound (±)-**2a** with an ethyl group led to compound (±)-**2b** that was almost as potent as (±)-**2a** with EC_{50} = 5.0 nM. When an *iso*-propyl group was introduced instead of the methyl group, the potency decreased significantly. For compound (±)-**2c** the EC_{50}-value was measured as 31.4 nM. Hence, potent agonist effects were observed in this cell-based assay for all three compounds.

The activation towards each subtype of the PPARs were then investigated in a luciferase cell assay.[20] Compounds (±)-**2b** and (±)-**2c** exhibited similar activities towards the PPARα subtype with EC_{50} = 710 and 735 nM, respectively, while (±)-**2a** exhibited slightly higher activity against PPARα (EC_{50} = 560nM, Table 1). Regarding PPARδ activation, compounds (±)-**2b** (EC_{50} = 290) and (±)-**2c** (EC_{50} = 580) were both less potent than (±)-**2a** (EC_{50} = 55 nM). However, compound (±)-**2a** was less potent than

Table 1
Substitution pattern (see Fig. 1) and EC_{50}-values of prepared compounds

Compound	R_1	EC_{50}[a] (nM)	PPARα EC_{50}[b] (nM)	PPARγ EC_{50}[b] (nM)	PPARδ EC_{50}[b] (nM)
(±)-**2a**	Methyl	3.7 ± 0.8	560 ± 25	>1000	55 ± 4
(±)-**2b**	Ethyl	5.0 ± 1.1	710 ± 25	>1000	290 ± 12
(±)-**2c**	*Iso*-propyl	31.4 ± 5.1	735 ± 20	>1000	580 ± 28
GW 501516 (**1**)	—	0.03 ± 0.01	>1000	912 ± 25	2.9 ± 0.4
EHA[c]	—	nd	32 ± 5	>1000	>1000
BRL[d]	—	>1000	>1000	48 ± 8	>1000

[a] EC_{50}-value measured from the oleic acid oxidation human myotube cell assay. Results are of three independent experiments with quadruplicate replicates shown. Values are expressed as means ± SD.
[b] EC_{50}-value measured at different concentration of agonist against each of the three PPARS using the luciferase-based transient transfection system assay. Values are expressed as means ± SD.
[c] (2*E*,4*E*,8*Z*,11*Z*,14*Z*,17*Z*)-Eicosa-2,4,8,11,14,17-hexaenoic acid.
[d] Rosiglitazone.

GW 501516 (**1**) against PPARδ. No activation towards PPARγ was observed for any of the compounds (±)-**2a**–(±)-**2c** (Table 1). Apparently, introducing a larger substituent in the *ortho*-position in the A-ring of **1** afforded a reduction of the potency against the PPARδ receptor for the new fluoro-analogs of lead compound GW 501516 (**1**). These observations are in accord with the structural–activity studies performed on **1** for activation of PPARδ.[19] Introducing a fluoro-atom at the *alpha*-carbon atom to the carboxylic acid moiety had a positive effect on PPARα activation. Hence, the compounds (±)-**2a**–(±)-**2c** can be classified as dual PPARα/δ agonists.

2.3. Molecular modeling

Molecular modeling studies were performed in order to investigate the binding ability of the two enantiomers of compound (±)-**2a** exhibited towards the PPARδ receptor. The docking indicated that both (*S*)-**2a** and (*R*)-**2a** could be oriented in two putative positions at the binding site. In the following these positions are termed pose A and pose B. The main differences between the two poses were the orientation of the carboxyl acid end of the molecules, and hence also of the fluorine atom. The two docking poses of (*S*)-**2a** and (*R*)-**2a** in PPARδ are shown in Figure 2a–d.

The docking poses were compared with the binding mode of the reported agonist 2,3-dimethyl-4-((2-(prop-2-yn-1-yloxy)-4-((4-(trifluoromethyl)phenoxy)methyl)phenyl)sulfanylphenoxy)acetic acid in the PPARδ X-ray crystal structure.[21] The comparison revealed that docking pose A was more in agreement with the binding mode of the agonist in the PPARδ X-ray crystal structure than pose B. The interaction energies are given in Table 2.

The docking of (*S*)-**2a** and (*R*)-**2a** revealed key interactions with amino acids Arg284, Cys285, His323, His449 and Tyr473 (Fig. 2a–d). In pose A, the fluorine atom of both (*S*)-**2a** and (*R*)-**2a** was oriented towards Met453 (Fig. 2a and c). The carboxylic group of (*S*)-**2a** was rotated towards Thr289 (Fig. 2a), while the carboxylic group of (*R*)-**2a** was oriented towards His323, His449 and Tyr473 (Fig. 2c). The orientation of the carboxylic group of (*R*)-**2a** was al-

Table 2
Interaction energies of (*S*)-**2a** and (*R*)-**2a** oriented in pose A and pose B

Pose	(*S*)-**2a** (kcal/mol)	(*R*)-**2a** (kcal/mol)
A	−15.82	−14.03
B	−14.78	−15.23

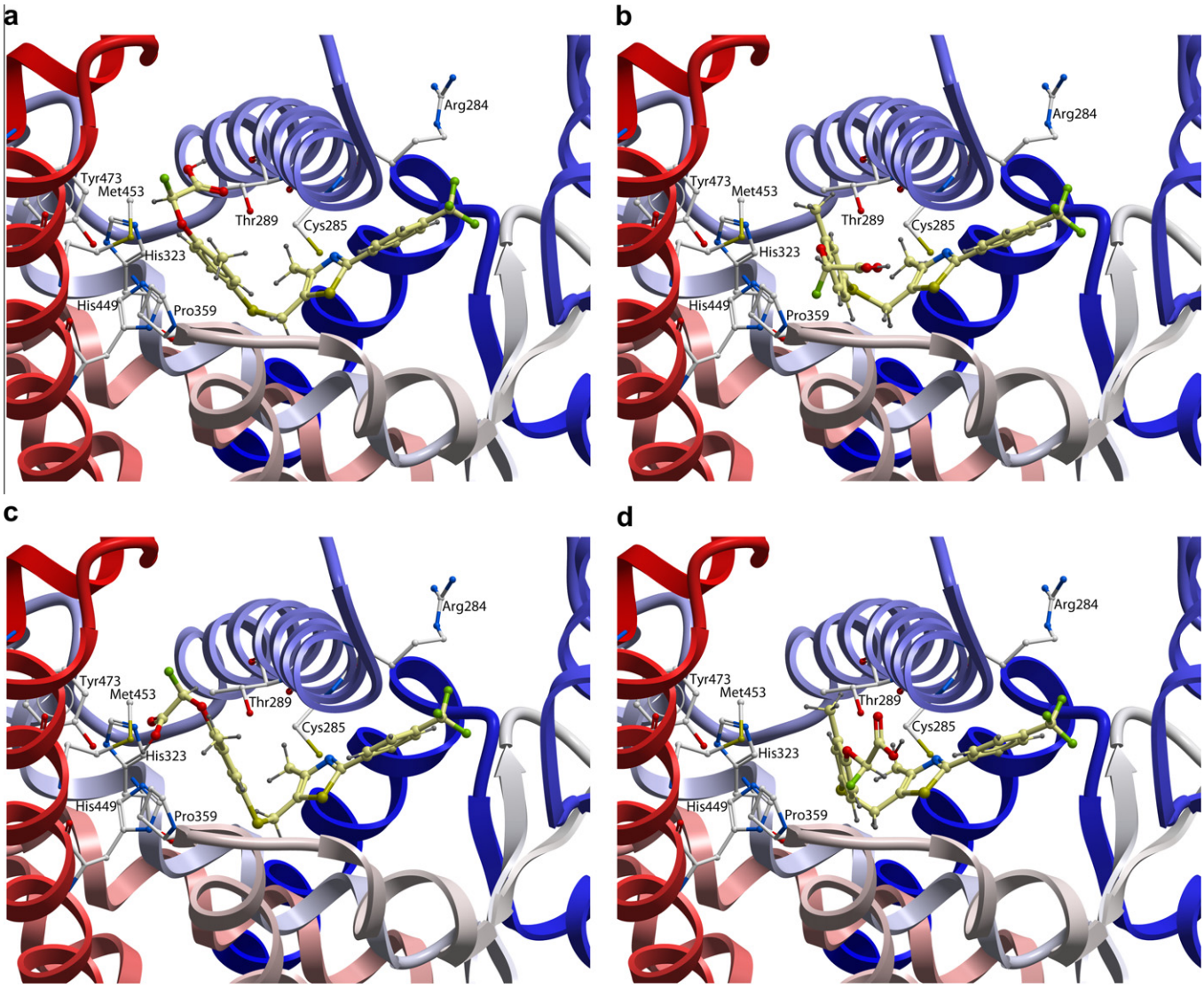


Figure 2 (a–d). The two docking poses of (S)-2a and (R)-2a in PPARδ.

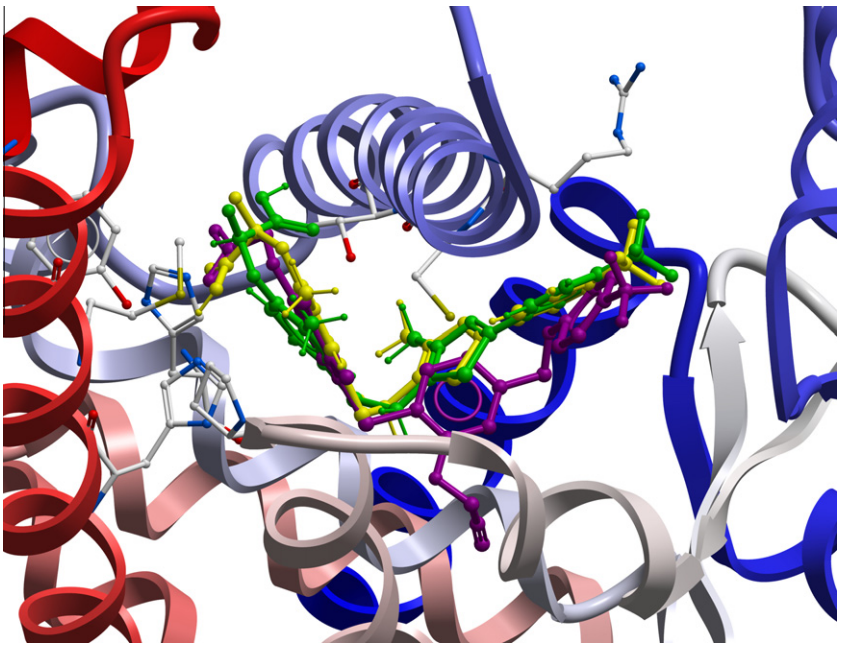


Figure 3. The superposition of (S)-2a and (R)-2a in docking pose A together with a known agonist in the PPARδ X-ray crystal structure.

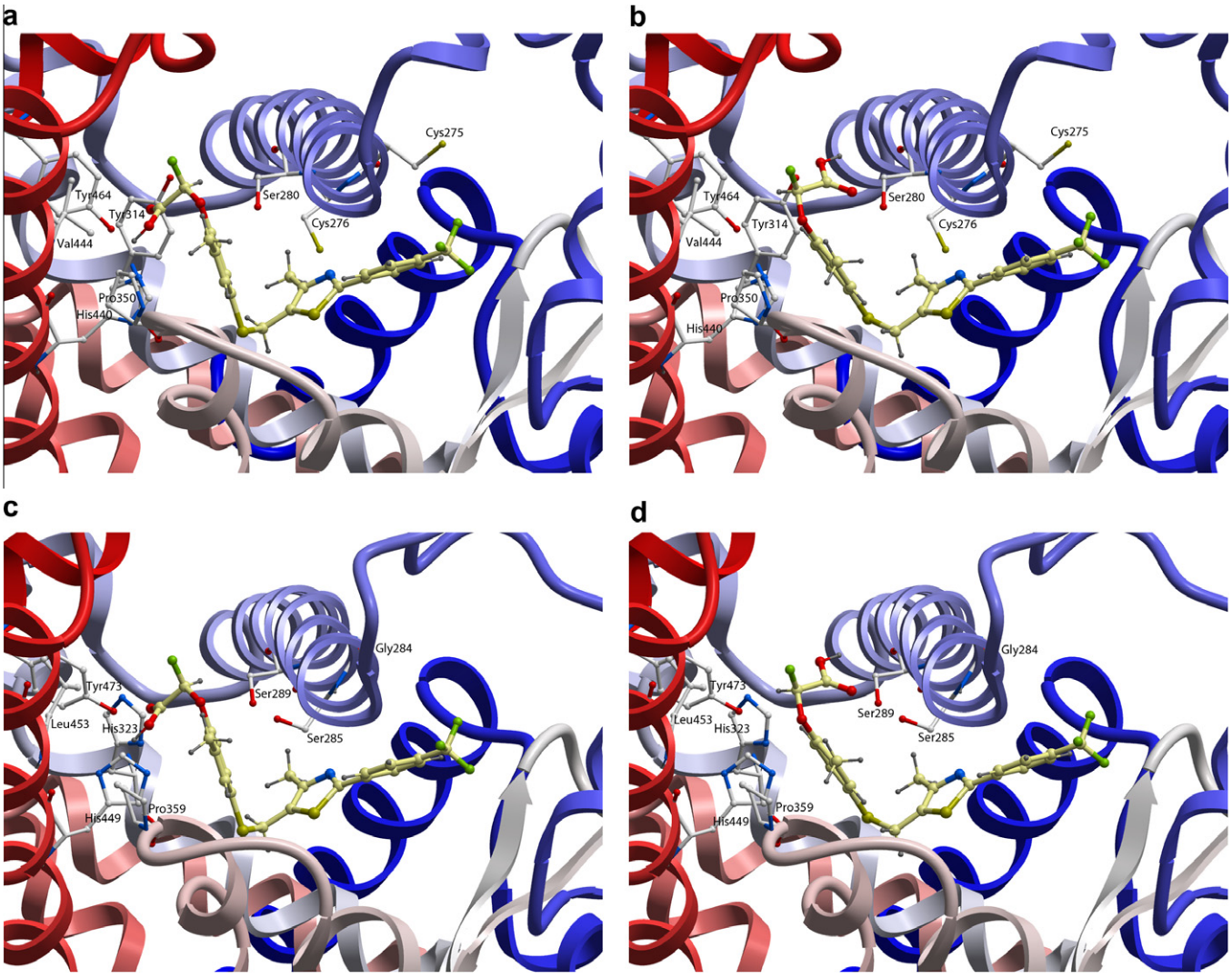


Figure 4 (a–d). (S)-2a and (R)-2a docked into PPARα (4a-4b) and PPARγ (4c-4d), respectively.

most identical to carboxylic group of the agonist in the PPARδ X-ray crystal structure. In pose B, the fluorine atom of both (*S*)-**2a** (Fig. 2b) and (*R*)-**2a** (Fig. 2d) was oriented towards Pro359. The carboxylic group of (*R*)-**2a** was oriented towards Phe282, while the carboxylic group of (*S*)-**2a** was oriented towards Cys285. The superposition of (*S*)-**2a** and (*R*)-**2a** in docking pose A with the known agonist 2,3-dimethyl-4-((2-(prop-2-yn-1-yloxy)-4-((4-(trifluoromethyl)-phenoxy)methyl)phenyl)sulfanylphenoxy) acetic acid in the PPARδ X-ray crystal structure is depicted in Figure 3.

According to calculated binding energies, the affinity of both (*S*)-**2a** and (*R*)-**2a** for the PPARδ receptor is similar, with the affinity of (*S*)-**2a** slightly higher than that of (*R*)-**2a**. When comparing (*S*)-**2a** and (*R*)-**2a** in pose A with the agonist binding mode in the PPARδ X-ray crystal structure, the binding mode of (*R*)-**2a** was more in agreement with the agonist binding model in the PPARδ X-ray crystal structure (Fig. 3). Since our molecular modeling studies revealed that (*S*)-**2a** and (*R*)-**2a** have similar binding affinity, we did not investigate the asymmetric synthesis or racemate resolution. In order to investigate differences in intermolecular interactions between (*S*)-**2a** and (*R*)-**2a** with PPARα, PPARγ and PPARδ, both enantiomers were also docked into PPARα and PPARγ. These studies are shown in Figure 4a–d.

When (*S*)-**2a** and (*R*)-**2a** were docked into PPARα and PPARγ, it was observed that the sulfur atoms of both of the ligands interacted with Cys285 (PPARδ) and Cys276 (PPARα), respectively. The corresponding amino acid in PPARγ is Ser285. These observations may, at least in part, explain the dual agonist effects towards PPARα, and PPARδ.

The molecular modeling studies reported herein will be used for the future development of more potent dual PPARα/δ agonists.

3. Conclusions

Bioisosteric substitution of one of the two hydrogen atoms on the *alpha*-carbon atom to the carboxylic group with a fluorine atom led to three new dual PPARα/δ agonists. Compared to GW 501516 (**1**), these substitutions had a positive effect on PPARα activation, while the activation of PPARδ was reduced. No significant activity was observed against PPARγ. The binding pocket in the PPARδ receptor has been characterized as large.[22] Based on the results reported herein, the introduction of a small substituent, such as a fluorine atom, at the *alpha*-carbon atom to the carboxylic acid moiety, affords potent dual PPARα/δ agonists. Molecular modeling studies and calculated binding energies showed that the affinity of both (*S*)-**2a** and (*R*)-**2a** for the PPARδ receptor is similar. Based on these results, it is likely that the enantiomers of the agonists reported herein will exhibit similar potency as agonists against PPARδ and PPARα, respectively.

4. Experimental

4.1. 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 (ppm, δ) relative to $CDCl_3$ (7.24 ppm for ^1H and 77.00 ppm for ^{13}C) or DMSO-d_6 (2.50 ppm for ^1H and 39.51 ppm for ^{13}C). Melting points were measured using a Barnstead Electrothermal apparatus and are uncorrected. Flash chromatography was performed on Silica Gel 60 (40–63 μm, Fluka). Mass spectra were recorded at 70 eV with Fission's VG Pro spectrometer. High resolution mass spectra were performed with a VG Prospec mass spectrometer. LC/MS analyses were performed on an Agilent Technologies 1200 Series (Eclipse XDB-C18, 5 μm 4.6 × 150 mm), coupled with an Agilent 6310 ion trap. According to LC/MS spectra, all final compounds submitted to the biological testing had a purity >99%.

4.2. Ethyl 2-fluoro-2-(2-methyl-4-((4-methyl-2-(4-trifluoro methylphenyl)thiazol-5-yl)methylthio)phenoxy)-acetate ((±)-4a)

Cs_2CO_3 (143 mg, 0.44 mmol) was added to a solution of 2-methyl-4-((4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio)phenol (**3a**) (160 mg, 0.4 mmol)in dry CH_3CN (10 mL). To this mixture was added dropwise a solution of ethyl 2-bromo-2-fluoroacetate (60 μL, 0.48 mmol) in dry CH_3CN (3 mL). The mixture was stirred over night at rt under argon, then diluted with water and extracted with ethyl acetate (3 × 100 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 (3:1) as eluent to give (±)-**4a** as a yellow oil in 88% yield (177 mg, 0.35 mmol). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.93 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 2.1, 1H), 7.16 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.99 (dd, *J* = 8.3, 0.7 Hz, 1H), 5.84 (d, *J* = 59.4 Hz, 1H), 4.32 (qd, *J* = 7.1, 0.7 Hz, 2H), 4.13 (s, 2H), 2.22 (s, 3H), 2.21 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.77 (d, *J* = 31.7 Hz), 163.11, 154.11 (d, *J* = 2.8 Hz), 151.32, 136.64 (distorted q, *J* = 1.3 Hz), 135.22, 131.22, 131.20 (q, *J* = 32.6 Hz), 130.26, 129.73 (d, *J* = 1.6 Hz), 129.38, 126.29, 125.77 (q, *J* = 3.8 Hz), 123.86 (q, *J* = 272.2 Hz), 116.68 (d, *J* = 1.0 Hz), 102.76 (d, *J* = 232.6 Hz), 62.49, 31.70, 15.88, 14.81, 13.88; MS (EI) *m*/*z*500.1 $[M+H]^+$; HRMS calcd for $C_{23}H_{21}F_4NO_3S_2$ $[M]^+$: 499.0899; found: 499.0900.

4.3. Ethyl 2-fluoro-2-(2-ethyl-4-((4-methyl-2-(4-trifluorometh ylphenyl)thiazol-5-yl)methylthio)phenoxy)-acetate ((±)-4b)

The title compound was prepared in 80% yield (122 mg, 0.24 mmol) as a yellow oil from 2-ethyl-4-((4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio)phenol (**3b**)(123 mg, 0.3 mmol) according to the general procedure described for (±)-**4a**. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.94 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.22–7.14 (m, 2H), 7.00 (dd, *J* = 8.3, 1.1 Hz, 1H), 5.86 (d, *J* = 59.4 Hz, 1H), 4.33 (qd, *J* = 7.1, 1.5 Hz, 2H), 4.13 (s, 2H), 2.60 (q, *J* = 7.6 Hz, 2H), 2.19 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.12 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.78 (d, *J* = 31.8 Hz), 163.15, 153.91 (d, *J* = 2.9 Hz), 151.40, 136.66 (distorted q, *J* = 1.3 Hz), 135.49 (d, *J* = 1.6 Hz), 134.18, 131.57, 131.24 (q, *J* = 32.6 Hz), 130.31, 129.30, 126.31, 125.80 (q, *J* = 3.8 Hz), 123.87 (q, *J* = 272.2 Hz), 116.53 (d, *J* = 1.0 Hz), 102.70 (d, *J* = 232.4 Hz), 62.49, 31.85, 22.98, 14.77, 14.04, 13.89; MS (EI) *m*/*z* 514.1 $[M+H]^+$; HRMS calcd for $C_{24}H_{23}F_4NO_3S_2$ $[M]^+$: 513.1055; found: 513.1062.

4.4. Ethyl 2-fluoro-2-(2-isopropyl-4-((4-methyl-2-(4-trifluoro methylphenyl)thiazol-5-yl)methylthio)phenoxy)-acetate ((±)-4c)

The title compound was prepared in 89% yield (155 mg, 0.29 mmol) as a yellow oil from 2-isopropyl-4-((4-methyl-2-(4-trifluoromethylphenyl)thiazol-5-yl)methylthio)phenol (**3c**) (140 mg, 0.33 mmol) according to the general procedure described for (±)-**4a**. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.93 (d, *J* = 8.1 Hz, 2H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.23–7.16 (m, 2H), 7.00 (dd, *J* = 9.1, 1.0 Hz, 1H), 5.86 (d, *J* = 59.3 Hz, 1H), 4.33 (qd, *J* = 7.1, 1.6 Hz, 2H), 3.26 (hept, *J* = 6.9 Hz, 1H), 2.15 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H), 1.11 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.75 (d, *J* = 31.9 Hz), 163.14, 151.44, 153.42 (d, *J* = 3.0 Hz), 139.73 (d, *J* = 1.6 Hz), 136.64 (distorted q, *J* = 1.2 Hz), 131.94, 131.70, 131.22 (q, *J* = 32.6 Hz), 130.35, 129.23, 126.28, 125.78 (q, *J* = 3.8 Hz),

123.86 (q, J = 272.1 Hz), 116.52 (d, J = 1.1 Hz), 102.73 (d, J = 232.4 Hz), 62.48, 31.97, 26.81, 22.47, 14.70, 13.88; MS (EI) m/z 528.1 $[M+H]^+$; HRMS calcd for $C_{25}H_{25}F_4NO_3S_2$ $[M]^+$: 527.1212; found: 527.1219.

4.5. 2-Fluoro-2-(2-methyl-4-((4-methyl-2-(4-trifluoro methylphenyl)thiazol-5-yl)methylthio)phenoxy)acetic acid ((±)-2a)

To a stirred solution of (±)-**4a** (177 mg, 0.35 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 complete hydrolysis. The mixture was diluted with 50 mL H_2O, acidified with 0.1 M HCl, extracted with diethyl ether, (3 × 50 mL), dried over $MgSO_4$, and concentrated. The residue was recrystallized from ethyl acetate/hexane to give (±)-**2a** as a colorless solid in 65% yield (108 mg, 0.23 mmol). Mp 161–162 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.05 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 1.9 Hz, 1H), 7.25 (dd, J = 8.5, 1.9 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 6.27 (d, J = 58.9 Hz, 1H), 4.43 (s, 2H), 2.26 (s, 3H), 2.16 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.37 (d, J = 30.2 Hz), 161.85, 153.12 (d, J = 2.4 Hz), 151.17, 136.48 (distorted q, J = 1.3 Hz), 133.66, 131.27, 129.90, 129.69 (q, J = 32.1 Hz), 128.79, 128.72 (d, J = 1.2 Hz), 126.34, 126.08 (q, J = 3.7 Hz), 124.00 (q, J = 272.1 Hz), 116.25, 102.31 (d, J = 228.8 Hz), 29.79, 15.57, 14.70; MS (EI) m/z 470.10 $[M-H]^-$; HRMS calcd for $C_{21}H_{17}F_4NO_3S_2$ $[M]^+$: 471.0586; found: 471.0570.

4.6. 2-Fluoro-2-(2-ethyl-4-((4-methyl-2-(4-trifluoromethyl phenyl)thiazol-5-yl)methylthio)phenoxy)acetic acid ((±)-2b)

The title compound was prepared in 67% yield (78 mg, 0.16 mmol) as a colorless solid from (±)-**4b** (122 mg, 0.24 mmol) according to the general procedure described for (±)-**2a**. Mp 159–160 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.01 (d, J = 7.8 Hz, 2H), 7.78 (d, J = 7.9 Hz, 2H), 7.32–7.20 (m, 2H), 7.11 (d, J = 8.1 Hz, 1H), 6.28 (d, J = 58.9 Hz, 1H), 4.39 (s, 2H), 2.54 (q, J = 7.4 Hz, 2H), 2.20 (s, 3H), 1.06 (t, J = 7.3 Hz, 3H);^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.39 (d, J = 30.2 Hz), 161.86, 152.90 (d, J = 2.6 Hz), 151.24, 136.50 (distorted q, J = 1.0 Hz), 134.40, 132.75, 131.30 (d, J = 0.9 Hz), 130.43, 129.68 (q, J = 32.0 Hz), 128.61, 126.32, 126.10 (q, J = 3.8 Hz), 123.99 (q, J = 272.4 Hz), 116.07, 102.25 (d, J = 228.9 Hz), 29.97, 22.51, 14.62, 14.09. MS (EI) m/z 484.21 $[M-H]^-$, HRMS calcd for $C_{22}H_{19}F_4NO_3S_2$ $[M]^+$: 485.0742; found: 485.0725.

4.7. 2-Fluoro-2-(2-isopropyl-4-((4-methyl-2-(4-trifluoromethyl phenyl)thiazol-5-yl)methylthio)phenoxy)acetic acid ((±)-2c)

The title compound was prepared in 76% yield (109 mg, 0.22 mmol) as a colorless solid from (±)-**4c** (155 mg, 0.29 mmol) according to the general procedure described for (±)-**2a**. Mp 120–121 °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), 7.28 (dd, J = 8.5, 2.3 Hz, 1H), 7.19 (d, J = 2.2 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 6.28 (d, J = 58.9 Hz, 1H), 4.39 (s, 2H), 3.19 (hept, J = 6.9 Hz, 1H), 2.15 (s, 3H), 1.08 (d, J = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 165.37 (d, J = 30.2 Hz), 161.88, 152.41 (d, J = 2.5 Hz), 151.29, 138.56 (d, J = 1.4 Hz), 136.51 (distorted q, J = 1.3 Hz), 131.38, 130.76, 130.45, 129.66 (q, J = 32.0 Hz), 128.50, 126.31, 126.11 (q, J = 3.8 Hz), 123.99 (q, J = 271.8 Hz), 116.13, 102.28 (d, J = 228.9 Hz), 30.17, 26.38, 22.23 (d, J = 4.4 Hz), 14.56.MS (ESI) m/z 498.34 $[M-H]^-$, HRMS calcd for $C_{23}H_{21}F_4NO_3S_2$ $[M]^+$: 499.0899; found: 499.0881.

4.8. Measurement of oleic acid oxidation[18]

Satellite cells were isolated from the *Musculus obliquusinternus 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 at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air in DMEM (5.5 mM glucose) with 2% FCS, 2% Ultroser G, penicillin/streptomycin (P/S, 100 U/mL/ 100 μg/mL) and amphotericin B (1.25 μg/mL) 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 agonists were added at the following five concentrations: 100, 10, 1, 0.1, 0.01 nM. 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® micro plate was mounted on top of the CellBIND® plate as described before,[18] and the cells were incubated at 37 °C for 4 h. 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.Student's t-test was employed for determination of statistical significance.

4.9. Luciferase-based transient transfection system[20]

COS-1 cells (ATCC no. CRL 1650) were cultured in DMEM supplemented with L-glutamine (2 mM), 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. The agonists were added at the following five concentrations: 1000, 100, 10, 1 and 0.1 nM to the media 5 h after transfection. The positive controls and DMSO (negative control) were added to the media 5 h after transfection. Transfected cells were maintained for 24 h 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. Student's t-test was employed for determination of statistical significance.

4.10. Molecular modeling

The ICM ('Internal Coordinate Mechanics') program[23] (version 3.6-1h) was used for docking and calculation of receptor–ligand interaction energies. The X-ray crystal structure of PPARδ in complex with the agonist (2,3-dimethyl-4-((2-(prop-2-yn-1-yloxy)-4-((4-(trifluoromethyl)phenoxy)methyl)phenyl)sulfanyl)-phenoxy) acetic acid have been solved (PDB code: 3GZ9).[21] The structure of PPARδ from this complex was converted to an ICM object and the receptor maps were calculated based on the agonist position in the X-ray crystal structure complex. (*S*)-**2a** and (*R*)-**2a** were modeled 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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