Novel oxazolidinone based PPAR agonists: molecular modeling, synthesis and biological evaluation

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KEYWORDS

oxazolidinone, PPAR agonists, molecular modeling, metabolic syndrome, diabetes

ABSTRACT

A series of new peroxisome proliferator activated receptors (PPAR) chiral ligands have been designed following the accepted three-module structure comprising polar head, linker and hydrophobic tail. The majority of the ligands incorporate the oxazolidinone moiety as a novel polar head, and the nature of the hydrophobic tail has also been varied. Docking studies using the crystal structure of an agonist bound to the ligand binding domain (LBD) of the PPARα receptor have been performed as a tool for their design. Suitable synthetic procedures have been developed and compounds with different stereochemistry have been prepared. Evaluation of basal and ligand induced activity proved that several compounds showed agonist activity at the PPARα receptor, thus validating the oxazolidinone template for PPAR activity. In addition, two compounds **2** and **4** showed dual PPARα / PPARγ agonism, and interesting food intake reduction in rats.

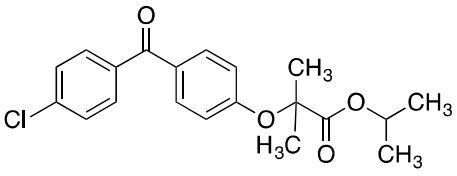
1. INTRODUCTION

The peroxisome proliferator activated receptors PPARs are nuclear receptors activated by the binding of small lipophilic ligands either natural or synthetic. They can induce or repress genes modulating different biochemical processes such as adipogenesis, lipid and glucose metabolism, energy balance, and inflammation among others. Considered an important target in medicinal chemistry, significant efforts have been devoted to search new ligands capable of treating chronic disorders such as high levels of triglycerides, diabetes and metabolic syndrome and chronic inflammatory bowel disease.

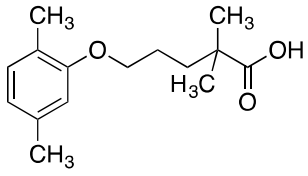
There are three known subtypes of PPAR receptors: alpha (α), gamma (γ) and beta (β)/delta (δ). Whereas PPARα is expressed in tissues with a high rate of fatty acid catabolism and

modulates lipid metabolism and inflammation, PPARγ is predominant in adipose tissue and regulates glucose and lipid metabolism. Finally, the β/δ type is the most ubiquitous and has been less investigated.[1]

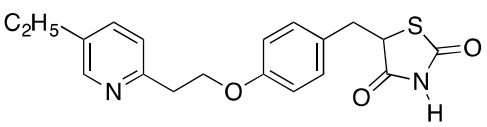
Despite intense research in developing PPAR ligands,[2,3] there are only few marketed drugs targeting PPARα and PPARγ and several promising candidates have failed in clinical trials (Fig.1). The α agonists, represented by fibrates (e.g. Fenofibrate and Gemfibrozil) are clinically used for the treatment of dyslipidemia. The thiazolidindiones (TZDs) or glitazones (e.g. Pioglitazone) are considered as specific ligands for the γ receptor. They are being used for treating hyperglycemia in patients with type 2 diabetes. Due to the considerable structural homology between these PPAR isoforms, there are other kinds of dual ligands that bind to two or more isoforms. The most studied compounds under this description are the glitazars from which the Saroglitazar (*S* enantiomer) has been recently approved and marketed under the trade name Lipaglyn as novel antidiabetic agent.[4] Nowadays, there is a growing interest in developing dual PPARα and PPARγ agonists, since they may have fewer secondary effects compared to selective α and γ ligands.



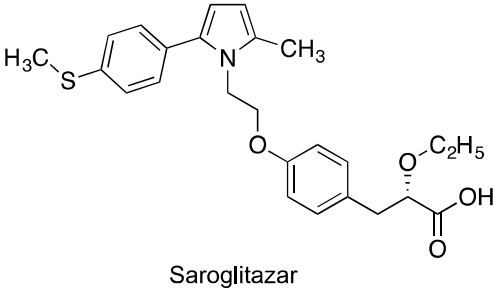
Fenofibrate



Gemfibrozil



Pioglitazone



Saroglitazar

Fig. 1. PPARα and PPARγ marketed drugs.

The LBD (ligand binding domain) is responsible for ligand specificity and PPAR activation. The core of the Y-shaped LBD consists of a set of twelve antiparallel alpha helix (helix 1 to 12) and three-stranded antiparallel beta-sheet. The activation function-2 (AF-2) domain located at the end of C-terminal helix 12 plays a critical role after ligand binding reducing the LBD flexibility and providing an appropriate interface for the cofactors recruitment. A large number of crystal structures of the PPAR-LBD with different ligands have been reported in literature enlightening a well-defined view of the agonists binding mode.[5] In some cases chirality is a crucial point determining the PPAR binding mode and synthetic strategies to deal with these stereoselectivity issues have been recently reviewed.[6] Laghezza *et al.* reported the 2-(aryloxy)-3-phenylpropanoic acids family with PPARα/γ dual agonist activity. The (*S*) and (*R*) enantiomers of the isopropyl derivative were crystallized bound to the LBD of the receptor. The (*S*) stereoisomer behaved as a full and partial agonist of PPARα and γ respectively.[7] In 2002, α-substituted phenylpropanoic acid-type PPAR agonists were reported to bind to the LBD in different conformations indicating multiple binding points inside the binding pocket.[8] New PPARγ scaffolds have also been reported following a virtual screening approach to identify PPAR chemotypes, however further biological studies need to be performed.[9]

The oxazolidinone is a five-membered heterocyclic ring with several applications as organocatalyst and chiral auxiliary (e.g. asymmetric aldol reactions).[10] It is considered a valuable structural motif in medicinal chemistry.[11, 12, 13] Oxazolidinones as chiral auxiliaries have been used by Miyachi and coworkers for the asymmetric synthesis of chiral α-acid derivatives as

PPARγ and α/γ ligands.[14, 15, 16] On the other hand, *in vitro* metabolism studies of a PPARγ ligand structurally similar to the oxazolidinone ring was reported by Agrawal *et al*.[17]

In this paper we describe 14 new PPAR chiral ligands[18] mainly based on an oxazolidinone motif following the three-module structure described by Pirat *et al*.[5] A structure-activity relationship study was conducted by varying the hydrophobic tail and polar head. Moreover, compounds with different stereochemistry were synthesized in order to understand the PPAR binding mode. Docking studies were used as a tool to design these ligands. Some of these ligands showed interesting *in vitro* and *in vivo* pharmacological activities.

2. RESULTS AND DISCUSSION

According to the accepted model, the ligands bind through three chemical features: polar head, linker region and hydrophobic tail (Fig. 2).

In our case, the choice of the hydrophobic tail group was determined by the docking studies going from alkyl chains (alicyclic and cyclic) to aromatic rings trying to occupy the large hydrophobic cavity of the binding site. Besides, the hydrophobic aromatic linker with the appropriate length was introduced. Finally, a polar head such as the oxazolidinone ring or the carboxylic acid should enable the hydrogen bonds in front of the AF-2 helix to stabilize its conformation. The chirality in our ligands has been introduced either on the oxazolidinone ring (substituent in position 4, compounds **1**-**10**) or in the optically active (*S*)-α-acid (compounds **13**, **14**) or in both of them (**11**, **12**).

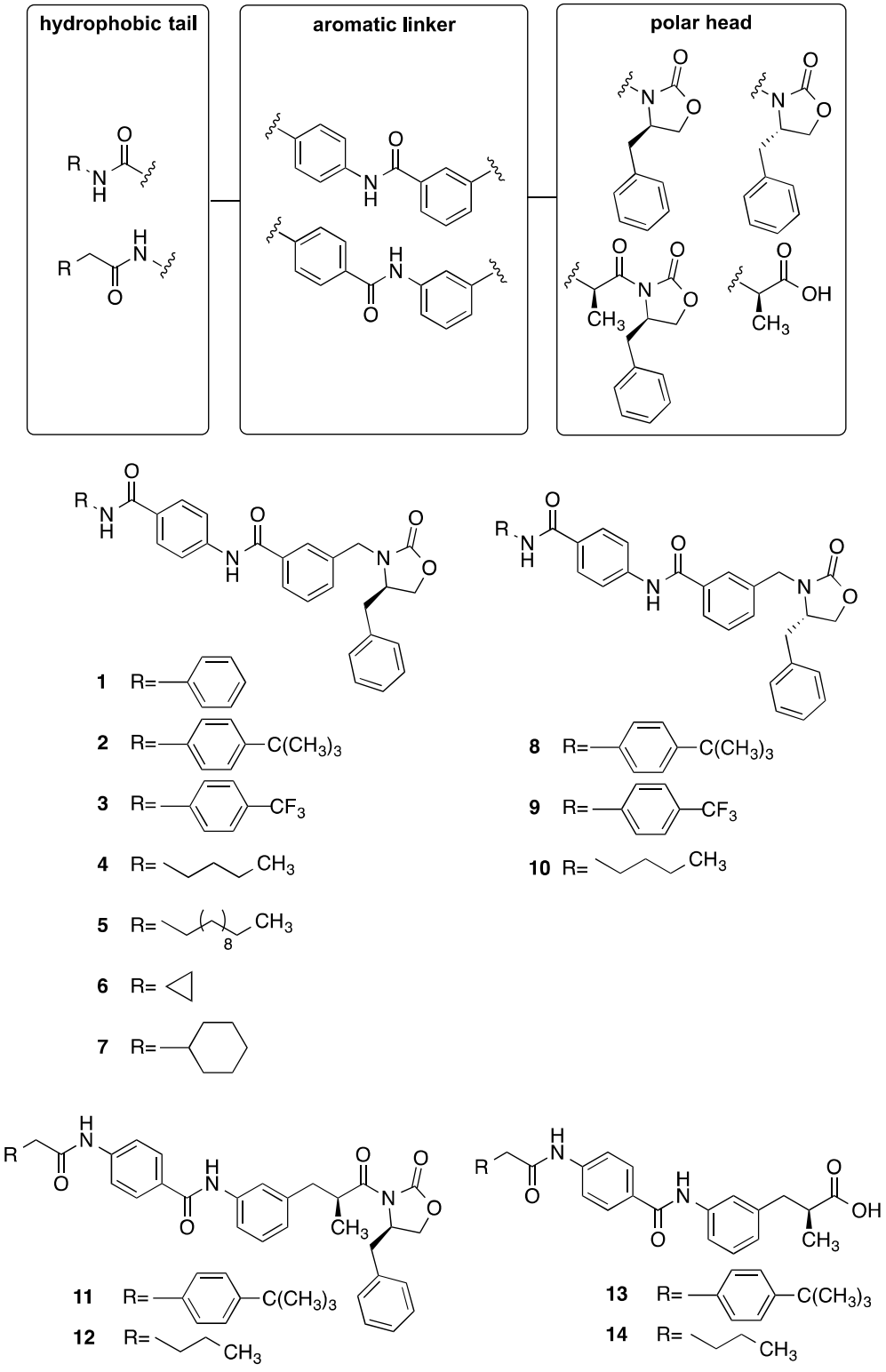


Fig. 2. PPAR chiral ligands general scheme and formulae.

2.1. Docking studies

Docking studies of two known PPAR agonists (GW409544, WY-1463) and compounds **2**, **3**, **4**, **7**, **8**, **9**, **10**, **13**, **14** were carried out by means of AutoDock v4 package. Table 1 summarizes the most stable conformations, the percentage of hydrogen-bonds with the hydroxyl group of Try464 (PPARα) and Tyr473 (PPARγ) of the 100 best docked conformations, the main polar interactions inside the LBD and the docking energy for the selected pose of each compound.

Table 1. Docking of compounds in the LBD of PPARα and γ.

No.	Docking energy (kcal/mol)	Tyr 464 HB (%)	Polar interactions (PPARα)	Docking energy (kcal/mol)	Tyr473 HB (%)	Polar interactions (PPARγ)
GW409544	-18.25	31	Ser280 Tyr314 Tyr464 His440	-14.45	23	Ser289 His323 Tyr473 His449
WY-14643	-8.99	63	Ser280 Tyr314 Tyr464			

			His440			
2	-10.68	4	Tyr314 Tyr464 His-40	-13.55	4	Tyr473 His449
3	-11.93	4	Tyr464 His440			
4	-11.54	12	Tyr464 His440	-11.1	3	Tyr473 His449
7	-12.24	3	Tyr314 Tyr-64 His440			
8	-13.33	13	Ser280 Tyr464 His440	-13.36	7	Ser289 His323 Tyr473
9	-10.85	2	Tyr464			

			His440			
10	-11.24	4	Tyr464 His440	-11.21	4	Tyr473 His449
13	-11.61	15	Ser280 Tyr314 Tyr464 His440	-11.08	7	Ser289 His323 Tyr473 His449
14	-9.99	22	Ser280 Tyr314 Tyr464 His440	-9.76	19	Ser289 His323 Tyr473 His449

The validation of the *in silico* model was performed comparing the X ray data of commercial compound GW409544 bound to the ligand domain of PPARα (PDB:1K71) and to PPARγ (PDB:1K74).[19]

According to the crystal structure, the aromatic rings of GW409544 established several interactions with non-polar amino acids of the PPARα - LBD and PPARγ - LBD. Additionally, GW409544 adopted a conformation within the receptor that allowed the carboxylate group to

form hydrogen bonds with Ser280, Tyr314, His440 and Tyr464 of PPARα and Ser289, His323, His449 and Tyr473 of PPARγ. These polar interactions and specifically the formation of hydrogen bonds with Tyr464 (PPARα) and Tyr473 (PPARγ) typically trigger a conformational change, notably in the activation function-2 (AF-2) helix, which aids in the recruitment of co-regulatory factors to regulate gene transcription.

For the PPARα ligand binding domain, the AutoDock suite successfully reproduced the binding mode for GW409544, showing a root mean square deviation (RMSD) of one of the most stable binding conformations (pose #55) of 0.7 Å in comparison with the experimental geometry, and the same hydrogen bond patterns (Fig. 3A).[20] An additional reference, the ligand WY-1463, which is the control ligand used in the *in vivo* tests, has also been included in the docking studies (Table 1).

The most stable docking solutions for the different ligands were analyzed, and the results suggest the existence of different potential binding modes of each compound onto the structure of the PPARα-LBD. The hydrophobic tails of each compound were assigned to the same hydrophobic region occupied by the synthetic agonist GW409544 in the crystal structure of the PPARα-LBD (Fig. 3).[17] For these solutions, the oxazolidinone (CO) and carboxylic acid polar groups were close to the hydroxyl group of the lateral chain of Tyr464, which donated a hydrogen bond. The binding modes were stabilized by the hydrogen bond network with: Tyr314, Tyr464 and His440 (**2**, **7**), Tyr 464 and His440 (**3**, **4**, **9** and **10**), Ser280, Tyr464 and His440 (**8**) and with the four residues just compounds **13** and **14**. The chirality induced by the substituent attached to the oxazolidinone ring in position 4 is responsible for the orientation of the CO group towards the polar residues. For instance, enantiomers **2** and **8** showed some differences in the percentage of hydrogen bond with the hydroxyl group of Tyr464 and in the polar residues

interactions. However, at this point, in the absence of crystallization data, these results must be considered as a theoretical approximation.

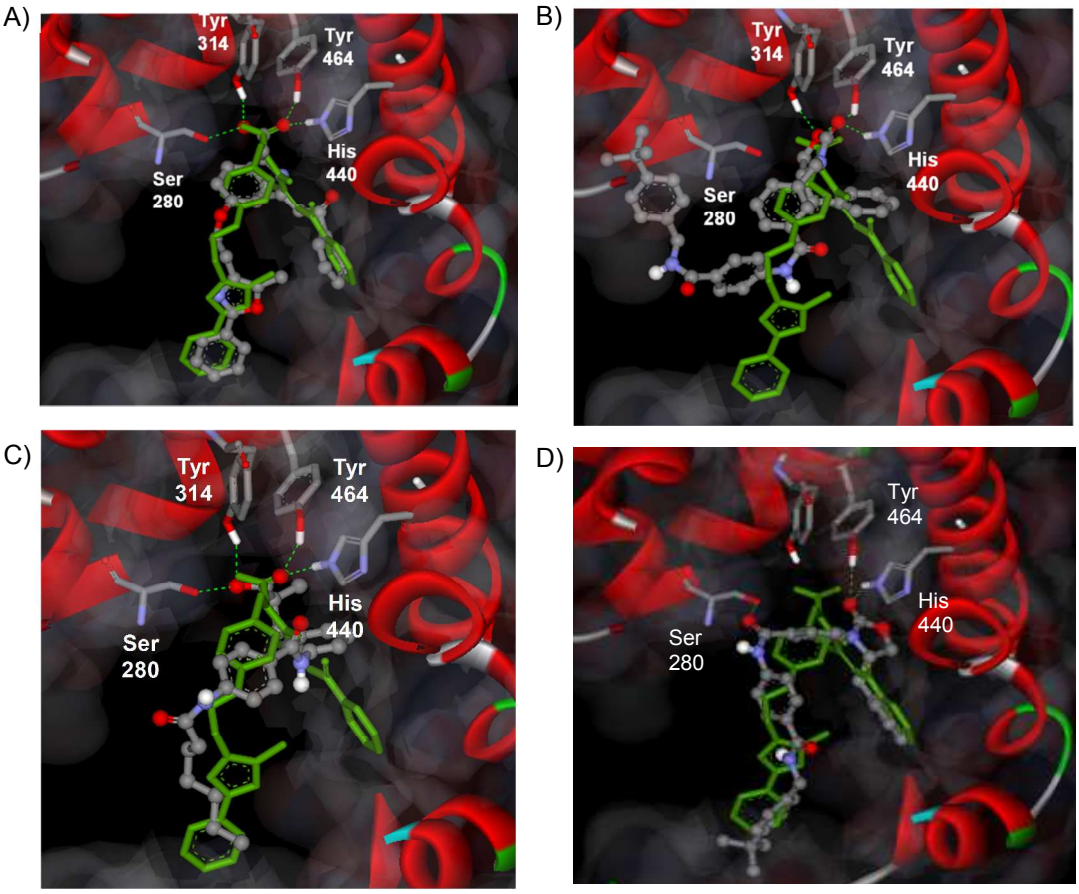


Fig. 3. Docking representation of best location/orientation binding modes. Docking representation of best location/orientation binding modes of GW409544 pose #55 (A), **2** pose #62 (B), **14** pose #3 (C) and **8** pose #4 (D) in balls and sticks colored by atom type. The co-crystallized conformation of GW409544 is shown in green and the protein backbone by ribbons (blue). The most important polar interactions (dashed green lines) and residues involved are shown (Ser280, Tyr314, His440 and Tyr464). All agonist compounds form a hydrogen bond with Tyr464.

In the case of PPARγ-LBD, docking studies were carried out with **2**, **4**, **8**, **10**, **13** and **14**. The hydrophobic tails of these compounds were assigned to the same hydrophobic region occupied by the synthetic agonist GW409544 in the crystal structure of the PPARγ-LBD, whereas the polar heads of the compounds were involved in a hydrogen bond network with the following residues (*i.e.*, Ser289, His323, His449 and Tyr473). Docking representations of the structures of compounds **2** and **4** onto the PPARγ-LBD are represented in Fig. 4.

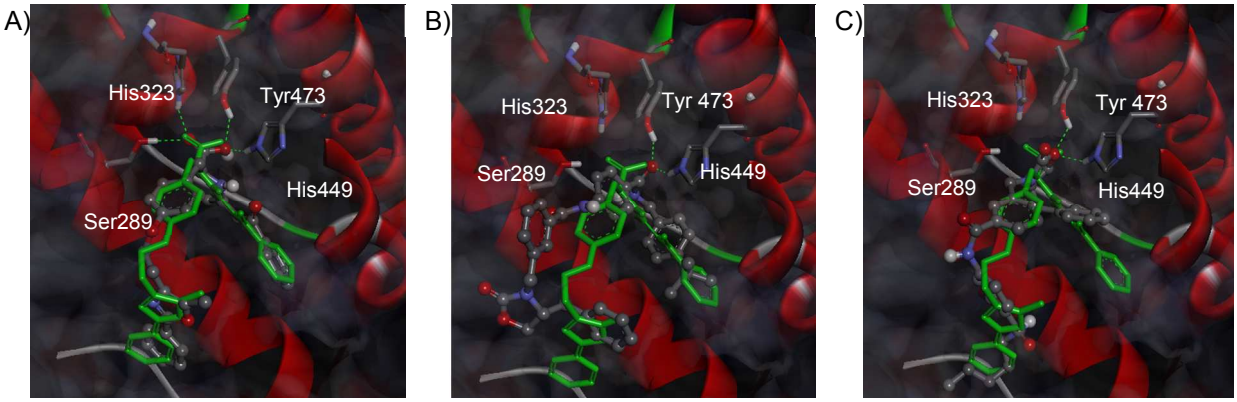
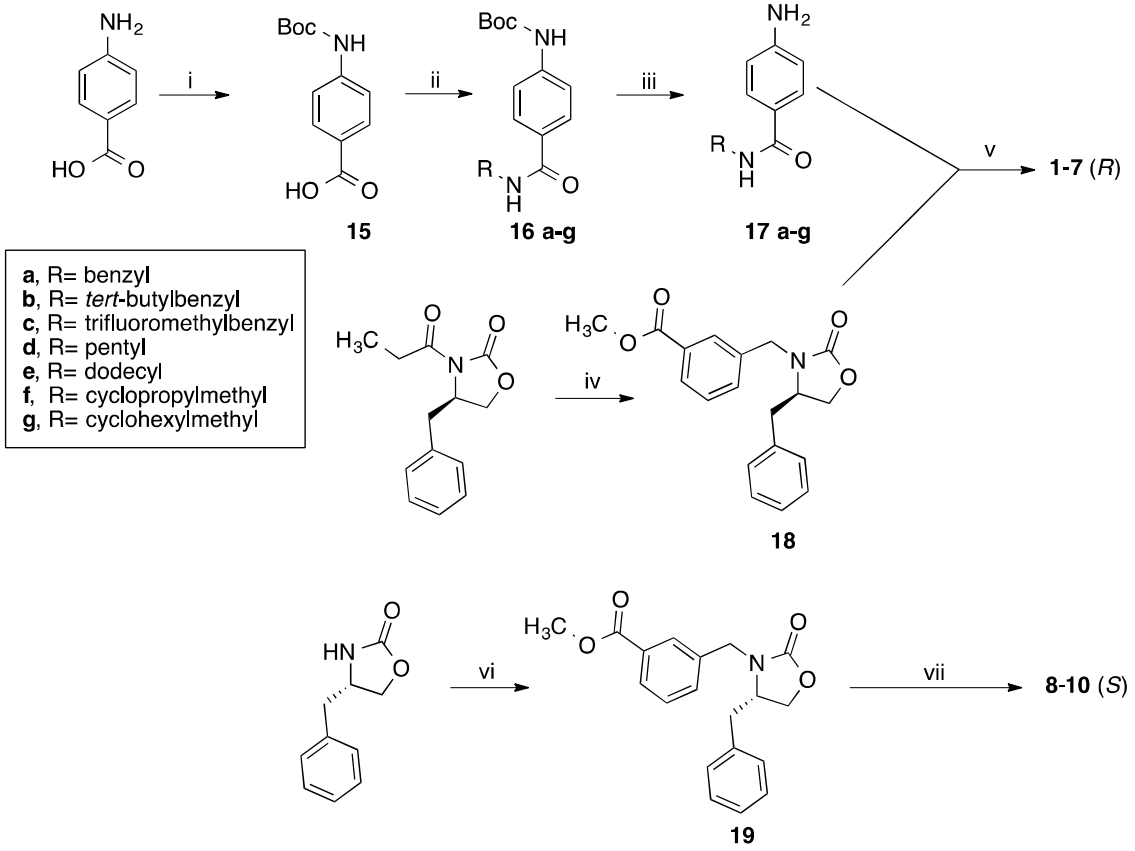


Fig. 4. Docking representation of best location/orientation binding modes to PPARγ-LBD of GW409544 pose #3 (A), **2** pose #4 (B), **4** pose #31 (C) in balls and sticks colored by atom type. The co-crystallized conformation of GW409544 is shown in green and the protein backbone by ribbons (blue). The most important polar interactions (dashed green lines) and residues involved are shown (Ser289, His323, Tyr473, and His449). All agonist compounds form a hydrogen bond with Tyr473.

2.2. Chemistry

For the preparation of compounds **1-10** we followed the synthetic strategy depicted in Scheme 1.



Reagents: i) Boc_2O, Et_3N, dioxane/H_2O (2:1), r.t., 24h (89%); ii) coupling agent, HOBt, Et_3N, R-NH_2, solvent, r.t., 12h (31-84%); iii) CH_2Cl_2/TFA (1:1), r.t., 12h (80-92%); iv) ^tBuOK, Methyl 3-(bromomethyl)benzoate, THF anh., r.t., 7h (62%); v) $Al(CH_3)_3$, THF, mw, 125ºC, 20min (40-50%); vi) Methyl 3-(bromomethyl)benzoate, Cs_2CO_3, THF anh./CH_3CN anh. (1:1), r.t. 6h (66%); vii) **17**, $Al(CH_3)_3$, THF, mw, 125ºC, 20min (80-89%).

Scheme 1. Synthesis of compounds **1**-**10**.

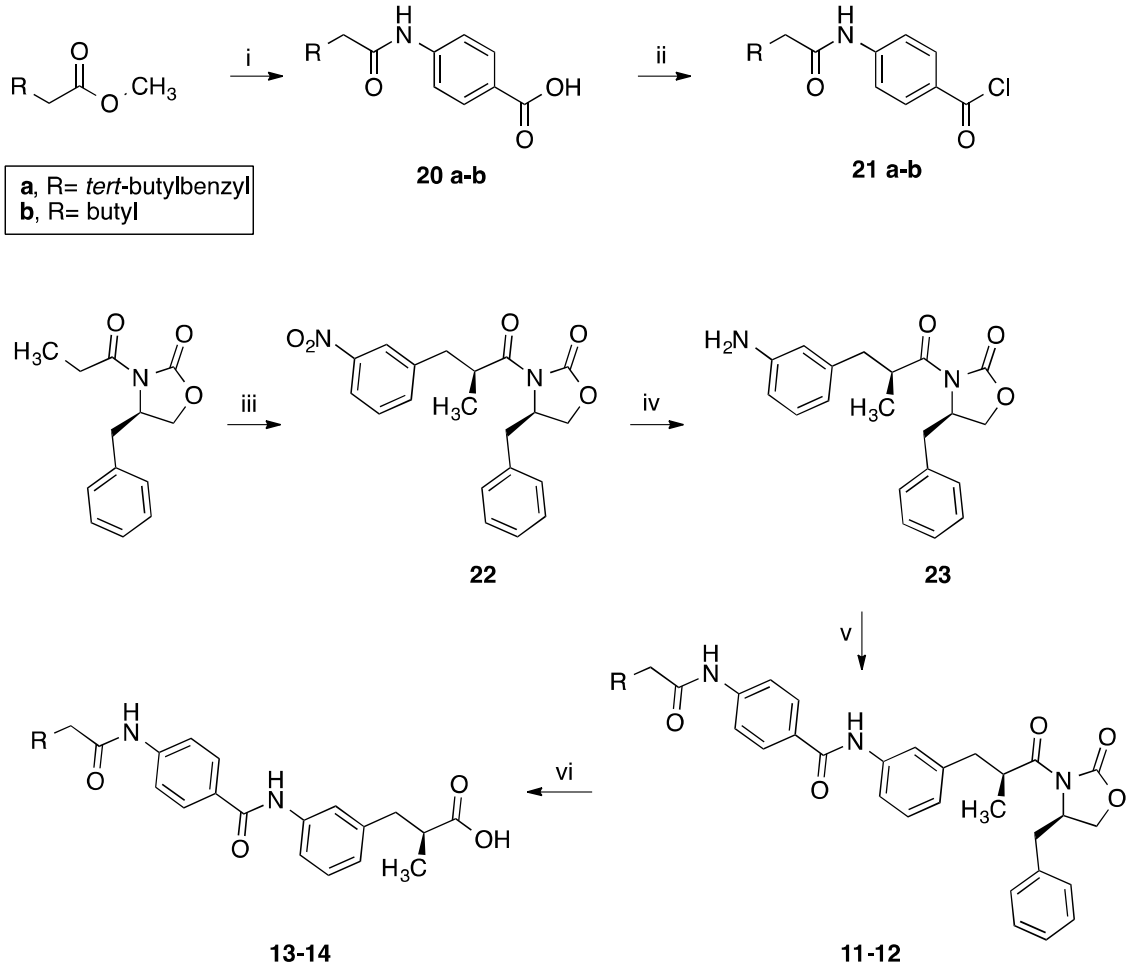
The key intermediate **17** was synthesized in three steps. Starting from commercially available 4-aminobenzoic acid, protection of the amine with Boc anhydride provided **15**. Amide bond formation between the carboxylic group of **15** with several amines (Fig. 1) using EDCI or PyBOP gave intermediate **16** in moderate yields. The *tert*-butyl carbamate **16** was cleaved under anhydrous acidic conditions giving **17** in good yields. The oxazolidinone motif was incorporated after a two-step synthesis starting from the commercially available (*R*)-4-benzyl-3-propionyl-2-oxazolidinone in the case of compounds (**1**-**7**) and from the (*S*)-4-benzyl oxazolidinone for

compounds (**8-10**). In the first case, the use of ^{t}BuOK at room temperature triggered a cross-alkylation reaction in which the oxazolidinone anion attacked the benzyl bromide derivative as we have recently reported, providing the oxazolidinone derivative **18** in moderate yields.[21] In the second case, the alkyl halide underwent a nucleophilic substitution under basic conditions giving the (*S*)-4-benzyl oxazolidinone derivative **19**. The final step to obtain compounds (**1-10**) involved a microwave-assisted amide bond formation between an ester (**18**) or (**19**) and various amine derivatives (**17**) mediated by a trimethyl aluminium complex.

The synthesis of compounds **11-14** is summarized in Scheme 2. For these compounds, two different hydrophobic tails were selected: the *p-tert*-butylbenzyl and the butyl one. Intermediate **21** was synthesized in two steps starting from commercially available methyl *p-tert*-butylphenylacetate or methyl hexanoate, an amide bond formation with the two different amines followed by the acid conversion to acyl chloride gave **21** in moderate yield.

The second fragment of this synthesis (compound **23**) is obtained in two steps. First, an Evans' asymmetric alkylation in which the (*R*)-4-benzyl-3-propionyl-2-oxazolidinone was treated with 3-nitrobenzyl bromide under Evans' protocol (NaHMDS at -78 ºC) provided **22**. Secondly, a Palladium-catalyzed reduction of the aromatic nitro group to amine gave **23** in good yield.

Compounds **11** and **12** resulted from the amide bond formation between the acyl chloride **21** and the amine derivative **23**. Then, a hydrogenolysis of the oxazolidinone ring using $LiOH/H_2O_2$ gave the chiral acid derivatives in alpha position, compounds **13** and **14**.



Reagents: i) 4-aminobenzoic acid, $Al(CH_3)_3$, THF, mw, 125ºC, 20min (65-70%); ii) $SOCl_2$, THF, 80ºC, 6h; iii) NaHMDS, THF anh., 3-nitrobenzyl bromide, -78ºC, 6h (70%); iv) H_2/Pd-C, THF/EtOAc (1:2), 21 psi, r.t., 12h (90%); v) **21**, THF, r.t., 12h, (34-68%); vi) LiOH, H_2O_2, 0ºC-r.t., 6h, (62-70%).

Scheme 2. Synthesis of compounds **11-14**.

2.3. Pharmacology

Luciferase reporter gene assays were performed with extracts from MCF-7 cells that were transiently transfected with a reporter construct containing four copies of the human CPTI DR1-type RE (for PPARs) and the indicated expression vectors for PPARα, RXRα, NCoR (CoR). Cells were treated for 16 h with 10μM of different compounds: DMSO (solvent), the reference PPARα agonist GW7647, the endogenous PPARα agonist OEA, the reference PPARγ

Rosiglitazone, and compounds 1 to 14. MD Simulation of luciferase activity was normalized to the basal activity of PPARα-RXRα−SRC1 in the presence of the solvent (DMSO). Details on the methodology have been published previously.[22,23,24,25,26]

Compounds **2**, **4**, **5**, **6**, **8**, **11**, **12**, **13** and **14** showed agonist activity at the PPARα receptor. Among them, compounds **2** and **4** displayed moderate agonism at the PPARγ receptor so they may be considered as interesting dual PPARα/γ agonists. These compounds also showed key important interactions in the docking studies with both PPAR isoforms. Flexibility in the hydrophobic tail is desired (**4**) over rigid hydrogenated rings (**7**) in accordance with the performed docking studies. The (*R*) stereochemistry at the oxazolidinone ring is preferred over the (*S*) one, and the oxazolidinone ring choice as polar head with a shorter linker resulted to be slightly better than the existing one in compounds **11** and **12**. On the other hand, a difference of one order of magnitude in activity was observed between the carboxylate polar head of **14** and the oxazolidinone ring in **12** with the same hydrophobic tail and linker. This fact correlates perfectly with the docking studies where the (*S*)-α acid derivative showed interactions with all the key residues at the LBD and a high HB percentage with Tyr464. As we can observe from the *in vitro* and docking results, interactions with the polar residues are important but in some cases flexibility and length of the ligand compensate the loss of HB with the polar residues in the LBD. This could explain why smaller ligands like (*R*)-oxazolidinone **2** show similar *in vitro* results to (*S*)-α acid **13**.

Considering the EC_{50} obtained at PPARα receptors, the data suggest an intermediate position in between fibrates and the natural ligand OEA. However, the efficacy at the receptor is not strictly related to *in vivo* efficacy. The most successful PPARα agonists used in human therapy,

the fibrates, (i.e. fenofibrate, gemfibrozil) have reported EC_{50} at the PPARα receptor in the micromolar range, more than 2-fold than those of compounds **2** and **4**.[27]

Based on this assumption, for the *in vivo* studies we selected compounds **2** and **4** since they behaved as dual agonists with interesting PPARα EC_{50} values and moderate affinity for PPARγ isoform.[20,28] *In vivo* pharmacology was tested using the model of feeding behavior in food deprived animals, that provides a good screening test for PPARα agonists with efficacy in animal models of obesity and metabolic disorders.[29,30] Long term-effects such as reductions in plasma lipids will be analyzed in future studies.

Table 2. Pharmacological properties of compounds **1-14**.

No.	**PPARα activation EC_{50} (nM)**	**PPARγ activation EC_{50} (nM)**
OEA	152±31	-
GW7647	6.5±1	-
Rosiglitazone	-	87.0±11
1	$>10^{-4}$ M	$>10^{-4}$ M
2	670±157	1298±261
3	$>10^{-4}$ M	$>10^{-4}$ M
4	821±139	1622±388
5	6623±1232	$>10^{-4}$ M
6	1978±213	-
7	$>10^{-4}$ M	$>10^{-4}$ M
8	756±125	-
9	$>10^{-4}$ M	-
10	$>10^{-4}$ M	-

11	966±233	-
12	1922±408	-
13	866±188	-
14	981±476	-

EC_{50} values calculated in the presence of the different compounds by GraphPad Prism 4. Results are the mean ± SEM. of 3 experiments.

***In vivo* food intake studies**

The acute administration of compound **2** induced a dose-dependant reduction of food intake in adult obese male rats that had been deprived of food for 24 h. Compound **2** at the effective dose of 3.0 mg/kg is able to reduce food intake around 50% being active up to 120 min. This effect disappears after 240 min (Fig. 5).

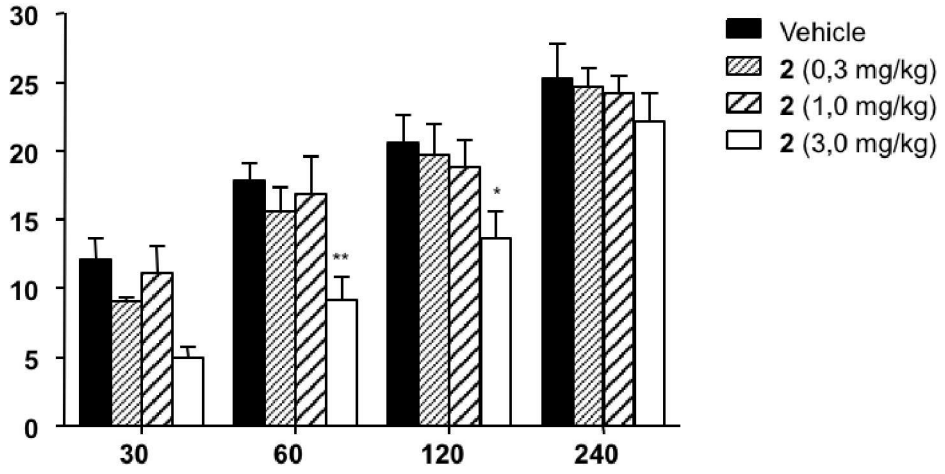


Fig 5. Feeding inhibition induced by acute administration of compound **2**. Time course of the inhibition of food intake induced by **2** at doses of 0.3, 1.0 and 3.0 mg/kg. The compound is active up to 120 min after administration (0'-60', $P<0.05$; 60'-120', $P<0.05$). Abscissa represents time (min) while the ordinate axis represents the cumulative food intake (mg/kg).

The acute administration of compound **4** induced a dose-dependant reduction of food intake at the effective doses of 1.0 and 3.0 mg/kg (Fig. 6). This compound remained active 240 min after dosing and surprisingly it continued exerting its effect 24 h later, an effect only observed before in nanoformulated oral oleoylethanolamide.[31] Therefore, compound **4** is an interesting candidate for chronic administration studies (Fig. 7).

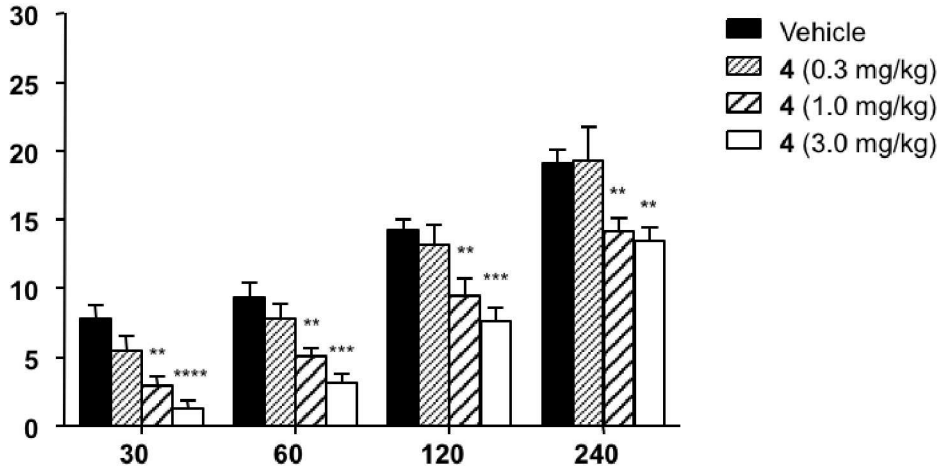


Fig 6. Feeding inhibition induced by acute administration of compound **4**. Time course of the inhibition of food intake induced by **4** at doses of 0.3, 1.0 and 3.0 mg/kg. The compound is active up to 240 min after administration at 1.0 mg/kg (0'-240', $P<0.01$) and at 3.0 mg/kg (0'-30', $P<0.0001$; 30'-120', $P<0.001$; 120'-140', $P<0.01$). Abscissa represents time (min) while the ordinate axis represents the cumulative food intake (mg/kg).

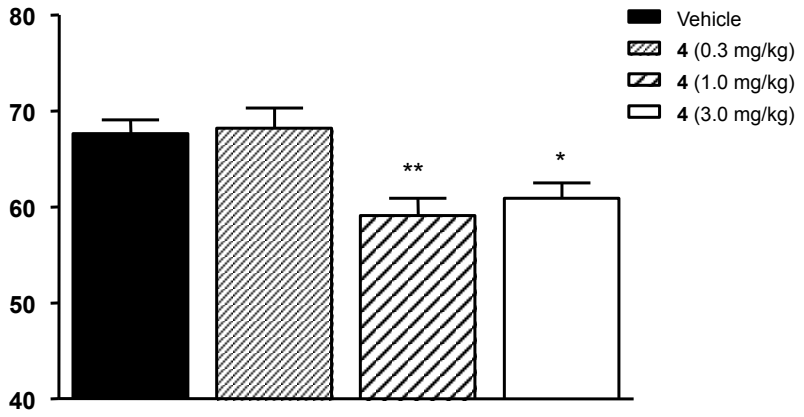


Fig 7. Feeding inhibition results 24 h after compound **4** administration. The compound **4** at 1.0 mg/kg ($P<0,01$) remained active over 24 h after dosing in a dose-dependent manner. Abscissa represents time (24 h) while the ordinate axis represents the cumulative food intake (mg/kg).

In summary, based on molecular modeling and docking studies we have prepared novel PPAR ligands incorporating an oxazolidinone ring. Pharmacological assays, both *in vitro* and *in vivo* of these chiral PPAR ligands have shown that the oxazolidinone motif is an interesting structural feature for PPAR activity. The easy way to synthetically reach these compounds and to perform structural modifications make them interesting leads for further development. Two of them (compounds **2** and **4**) can be considered as dual well-balanced PPARα/γ ligands. Furthermore, they have shown interesting *in vivo* properties that make them attractive candidates for further research in the treatment of metabolic syndrome.

3. EXPERIMENTAL SECTION

Chemistry. General methods

All chemicals were purchased from commercial suppliers and used without further purification. TLC: precoated silica-gel 60 254 plates (Merck), detection by UV light (254 nm). Flash-column Chromatography (FC): Kieselgel 60 (230–400 mesh; Merck). Melting points (mp) were determined in open capillaries with a Gallenkamp capillary melting-points apparatus. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance 300 spectrometer operating at 300.13 MHz and 75.47 MHz respectively, in $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are reported in ppm on the δ scale. In the case of multiplets, the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; and m, multiplet. Coupling constants are expressed in hertz. Hydrogenation reactions were carried out in a Shaker type hydrogenation apparatus (Parr). Elemental analysis was determined with a LECO Elemental Analyzer CHNS-932. LC-MS analyses were performed using an Alliance 2695 (Waters) with a diode array UV/Vis detector Waters 2996 and interfaced to a Micromass ZQ mass spectrometer. Analyses were performed using reversed phase HPLC silica based columns: column Bridge C18 3.5 mm. (2.1 x 100 mm). Using an injection volume of 3 mL, a flow rate of 0.25 mL/min and gradient elution (15 to 95 % over 10 min) of acetonitrile in water. Acetonitrile contains 0.08% v/v formic acid and water contains 0.1% v/v formic acid. Analyses were monitored at 254 nm wavelength. Flash chromatography was performed using a Biotage Isolera™ flash purification system.

General Procedure for the Synthesis of 1-10. To a solution of **18** or **19** (1 Eq) and the corresponding amines (2 Eq) in dry THF, it was added $Al(CH_3)_3$ in heptane 2.0 M (2 Eq). The mixture was heated at 125 ºC for 35 min in a microwave reactor. The reaction mixture was poured into an erlenmeyer, cooled in an ice bath and 1 N HCl aqueous solution was slowly added

until the effervescence ended. The mixture was taken to an extraction funnel and Et_2O was added. The residue was purified by flash chromatography.

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-(benzylcarbamoyl)phenyl]benzamide (1). Compound **18** (0.06 g, 0.18 mmol), compound **17a** (0.08 g, 0.36 mmol), $Al_3(CH_3)_3$ in heptane 2.0 M (0.2 mL, 0.36 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.04 g, 40 %). M.p. 120 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.47 (s, 1H, NH), 8.95 (t, J = 6.0 Hz, 1H, NHCH$_2$), 8.04 – 7.78 (m, 6H, H-Ar), 7.64 – 7.48 (m, 4H, H-Ar), 7.45 – 7.13 (m, 8H, H-Ar), 4.68 (m, 1H), 4.47 (d, J = 6.0 Hz, 2H, NHCH$_2$), 4.41-4.31 (m, 1H), 4.22 (m, 1H), 4.08 – 3.99 (m, 1H), 4.03 – 3.83 (m, 1H), 3.05 (dd, J_{ab}= 13.5, J_{a4}= 4.2 Hz, 1H), 2.75 (dd, J_{ba}= 13.5, J_{b4}= 8.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 165.6 (CO), 165.5 (CO), 158.6 (C), 139.8, 137.3, 136.1, 135.1, 135.5, 131.0, 129.2 (2C), 128.7, 128.5 (2C), 128.2 (3C), 127.9 (2C), 127.2 (3C), 126.8, 126.7 (2C), 119.5 (2C), 66.3 (C), 55.3 (C), 45.1 (C), 42.5 (CH_2), 37.2 (C). HPLC/MS: gradient elution (40 to 95 % over 10 min) of acetonitrile in water, retention time 4.61 min $[M+H]^+$= 520. Elemental analysis calcd. for $C_{32}H_{29}N_3O_4$: C, 73.97; H, 5.63; N, 8.09; found C, 73.88; H, 5.66; N, 8.08.

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-((4-(*tert*-butyl)benzyl)carbamoyl)phenyl]benzamide (2). Compound **18** (0.06 g, 0.18 mmol), compound **17b** (0.08 g, 0.36 mmol), $Al(CH_3)_3$ in heptane 2.0 M (0.2 mL, 0.36 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.05 g, 44 %). M.p. 110 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.91 (s, 1H, NH), 7.83-7.80 (m, 1H, H-Ar), 7.78 – 7.63 (m, 5H, H-Ar), 7.44 – 7.15 (m, 9H, H-Ar), 7.07 – 6.96 (m, 2H, H-Ar), 6.66 (t, J = 5.7 Hz, 1H,

NHCH$_2$), 4.74 - 4.65 (m, 1H), 4.57 (d, J = 5.4 Hz, 2H, NHCH$_2$), 4.21 – 4.07 (m, 2H), 4.00 – 3.90 (m, 1H), 3.87 – 3.76 (m, 1H), 3.04 (dd, J_{ab}= 13.6, J_{a4}= 4.8 Hz, 1H), 2.63 (dd, J_{ba}= 13.6, J_{b4}= 8.5 Hz, 1H), 1.30 (s, 9H, 3CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$) δ (ppm) 166.8 (CO), 165.8 (CO), 158.6, 150.5, 141.2, 139.7, 136.5, 135.2, 131.2, 129.8, 129.2, 128.9 (2C), 128.9 (2C), 127.9 (2C), 127.7 (2C), 127.2, 127.1, 126.6, 125.6 (2C), 119.9 (2C), 67.1 (C), 55.8 (C), 46.0 (C), 43.8 (CH$_2$), 38.6 (C), 34.5 (C(CH$_3$)$_3$), 31.3 (3CH$_3$). HPLC/MS: gradient elution (40 to 95 % over 10.0 min) of acetonitrile in water, retention time 8.83 min [M+H]$^+$= 576. Elemental analysis calcd. for $C_{36}H_{37}N_3O_4$: C, 75.11; H, 6.48; N, 7.30; found C, 75.00; H, 6.75; N, 7.70.

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-((4-(trifluoromethyl)benzyl)carbamoyl)phenyl]benzamide (3). Compound **18** (0.1 g, 0.31 mmol), compound **17c** (0.18 g, 0.61 mmol), Al(CH$_3$)$_3$ in heptane 2.0 M (0.3 mL, 0.61 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.09 g, 50 %). M.p. 114 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.49 (s, 1H, NH), 9.07 (t, J = 6.1 Hz, 1H, CH$_2$NH), 7.98 – 7.77 (m, 6H, H-Ar), 7.69 (m, 2H, H-Ar), 7.53 (m, 5H, H-Ar), 7.35 – 7.10 (m, 4H, H-Ar), 4.68 (d, J = 15.6 Hz, 1H) 4.55 (d, J = 5.8 Hz, 2H, CH$_2$NH), 4.41 (m, 1H), 4.22 (m, 1H), 4.04 (m, 1H), 3.92 (m, 1H), 3.05 (dd, J_{ab} = 13.5, J$_{a4}$= 4.2 Hz, 1H), 2.75 (dd, J_{ba} = 13.6, J_{b4}= 8.1 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ (ppm) 165.8 (CO), 165.7 (CO), 157.7 (C), 144.7, 141.9, 139.2, 137.3, 136.1, 135.1, 131.0, 129.2 (2C), 129.0, 128.7, 128.5 (3C), 128.0 (2C), 127.9 (3C), 127.85, 127.6, 126.8, 126.7, 125.2, 119.5, 66.3 (C), 55.3 (C), 45.1 (C), 42.3 (NHCH$_2$), 37.2 (C). HPLC/MS: gradient elution (40 to 95 % over 10.0 min) of acetonitrile in water, retention time 10.2 min [M+H]$^+$= 588. Elemental analysis calcd. for $C_{33}H_{28}F_3N_3O_4$: C, 67.45; H, 4.80; N, 7.15; found C, 67.69; H, 5.00; N, 7.14.

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-(pentylcarbamoyl)phenyl]benzamide (4). Compound **18** (0.10 g, 0.31 mmol), compound **17d** (0.13 g, 0.61 mmol), $Al(CH_3)_3$ in heptane 2.0 M (0.31 mL, 0.61 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The crude was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.07 g, 45 %). M.p. 140 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 10.46 (s, 1H, NH), 8.35 (t, J = 5.6 Hz, 1H, $CH_2\underline{NH}$), 7.91 (m, 1H, H-Ar), 7.86 (bs, 5H-Ar), 7.58 – 7.51 (m, 2H, H-Ar), 7.32 – 7.17 (m, 5H, H-Ar), 4.69 (m, 1H), 4.42 (m, 1H), 4.23 (m, 1H), 4.06 (m, 1H), 4.00 – 3.89 (m, 1H), 3.25 (m, 2H, $NH\underline{CH_2}$), 3.06 (dd, J_{ab}= 13.6, J_{a4}= 4.2 Hz, 1H), 2.77 (dd, J_{ba}= 13.6, J_{b4}=8.1 Hz, 1H), 1.52 (m, 2H), 1.42 – 1.18 (m, 4H), 0.85 (t, J= 6.8 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 166.0 (CO), 165.9 (CO), 158.1, 141.9, 137.6, 136.5, 135.5, 131.4, 130.1, 129.6 (2C), 129.1, 128.9 (2C), 128.2 (2C), 127.6, 127.2, 127.1, 119.8 (2C), 66.7 (C), 55.7 (C), 45.5 (C), 40.7 (CH_2), 37.6 (C), 29.2 (CH_2), 29.1 (CH_2), 22.3 (CH_2), 14.3 (CH_3). HPLC/MS: gradient elution (40 to 95 % over 10.0 min) of acetonitrile in water, retention time 5.09 min $[M+H]^+$= 500. Elemental analysis calcd. for $C_{30}H_{33}N_3O_4$: C, 72.12; H, 6.66; N, 8.41; found C, 72.40; H, 6.83; N, 8.68.

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-(dodecylcarbamoyl)phenyl]benzamide (5). Compound **18** (0.06 g, 0.18 mmol), compound **17e** (0.11 g, 0.36 mmol), $Al(CH_3)_3$ in heptane 2.0 M (0.18 mL, 0.36 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The organic layers were dried and the solvent was evaporated under reduced pressure and the crude was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.05 g, 45 %). M.p. 115 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.69 – 8.56 (m, 1H, NH), 7.94 – 7.64 (m, 5H, H-Ar), 7.53 – 7.19 (m, 4H, H-Ar), 7.13 – 7.00 (m, 3H, H-Ar), 6.34 – 6.14 (m, 1H, H-Ar), 4.81 (m, 1H), 4.30 –

4.13 (m, 1H), 4.02 (m, 1H), 3.94 – 3.79 (m, 1H), 3.45 - 3.30 (m, 1H), 3.06 (dd, J_{ab}= 13.6, J_{a4}= 4.8 Hz, 1H), 2.66 (dd, J_{ba}= 13.6, J_{b4}= 8.6 Hz, 1H), 1.60 – 1.55 (m, 2H, CH_2), 1.46 – 1.15 (m, 18H, 9CH_2), 0.87 (t, J = 6.7 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.9 (CO), 165.6 (CO), 158.6 (C), 140.7, 139.7, 136.7, 135.4, 135.3, 131.4, 130.5, 129.3, 129.0 (2C), 127.8 (2C), 127.3, 127.0, 126.6, 119.9 (2C), 116.7, 67.2 (C), 55.7 (C), 46.1 (C), 40.2 (CH_2), 38.7 (C), 31.9 (CH_2), 29.6 (4CH_2), 29.3 (3CH_2), 27.0 (CH_2), 22.7 (CH_2), 14.1 (CH_3). HPLC/MS: gradient elution (40 to 95 % over 10.0 min) of acetonitrile in water, retention time 9.93 min $[M+H]^+$= 599. Elemental analysis calcd. for $C_{37}H_{47}N_3O_4$: C, 74.34; H, 7.93; N, 7.03; found C, 74.20; H, 7.50; N, 7.04.

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4 ((cyclopropyl)carbamoyl)phenyl]benzamide (6). Compound **18** (0.18 g, 0.56 mmol), compound **17f** (0.18 g, 0.67 mmol), $Al(CH_3)_3$ in heptane 2.0 M (1.11 mL, 2.24 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane (4:6) yielding a white solid (0.05 g, 2 %). ^{1}H NMR (300 MHz, MeOD) δ (ppm) 7.90 – 7.88 (m, 1H), 7.84 (bs, 2H), 7.79 – 7.77 (m, 2H), 7.55 – 7.51 (m, 4H), 7.30 – 7.26 (m, 2H), 7-18 – 7.16 (m, 2H), 4.81 (d, *J*= 4.8 Hz, 1H), 4.36 (d, *J*= 4.4 Hz 1H), 4.30 – 4.26 (m, 1H), 4.15 – 4.12 (m, 1H), 4.06 – 4.02 (m, 1H), 3.11 (dd, *J*= 13.7, 5.1 Hz, 1H), 2.87 – 2.80 (m, 2H), 0.83 – 0.79 (m, 2H), 0.66 – 0.63 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 169.7 (C), 167.2 (C), 159.5 (C), 141.7 (C), 137 (C), 135.9 (C), 135.3 (C), 131.1 (2CH), 129.5 (C), 128.9 (2CH), 128.8 (2CH), 128.4 (2CH), 126.9 (2CH), 126.7 (CH), 119.8 (2CH), 67.2 (CH_2), 55.9 (CH), 45.5 (CH_2), 37.8 (CH_2), 22.6 (CH), 5.14 (2CH_2).

(*R*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-((cyclohexylmethyl)carbamoyl)phenyl]benzamide (7). Compound **18** (0.06 g, 0.18 mmol),

compound **17g** (0.08 g, 0.36 mmol), $Al(CH_3)_3$ in heptane 2.0 M (0.18 mL, 0.36 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.04 g, 42 %). M.p. 130 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 9.08 (s, 1H, NH), 7.85 (m, 1H, H-Ar), 7.78 – 7.61 (m, 5H, H-Ar), 7.45 – 7.16 (m, 5H, H-Ar), 7.09 – 6.96 (m, 2H, H-Ar), 6.50 (t, J = 6.0 Hz, 1H, CH_2NH), 4.73 (m, 1H), 4.21 – 4.06 (m, 2H), 4.00 (m, 1H), 3.93 – 3.79 (m, 1H), 3.25 (m, 2H, CH_2NH), 3.03 (dd, J_{ab}= 13.6, J_{a4}= 4.9 Hz, 1H), 2.63 (dd, J_{ba} = 13.6, J_{b4}= 8.6 Hz, 1H), 1.82 – 1.52 (m, 6H), 1.32 – 1.10 (m, 4H), 0.93 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.9 (CO), 165.5 (CO), 158.5 (C), 140.8, 136.8, 135.4, 135.3, 131.4, 130.5, 129.3, 129.0, 127.9 (3C), 127.3 (2C), 127.0, 126.6, 119.8, 119.7 (2C), 67.2 (C), 55.7 (C), 46.3 (CH_2NH), 46.1, 38.8 (C), 38.0 (CH), 30.9 ($2CH_2$), 26.4 (CH_2), 25.8 ($2CH_2$). HPLC/MS: gradient elution (40 to 95 % over 10.0 min) of acetonitrile in water, retention time 5.71 min $[M+H]^+$= 527. Elemental analysis calcd. for $C_{32}H_{35}N_3O_4$: C, 73.12; H, 6.71; N, 7.99; found C, 73.44; H, 6.58; N, 7.52.

(*S*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-((4-(*tert*-butyl)benzyl)carbamoyl)phenyl]benzamide (8). Compound **19** (0.20 g, 0.62 mmol), compound **17b** (0.21 g, 0.74 mmol), $Al(CH_3)_3$ in heptane 2.0 M (1.24 mL, 2.48 mmol) in THF (10 mL) were heated at 125 ºC for 20 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane (8:2) yielding a white solid (0.02 g, 4 %). M.p. 171 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.32 (s, 1H, H), 7.89 – 7.71 (m, 6H), 7.48 – 7.22 (m, 9H, H), 7.06 (d, J= 7.53 Hz, 2H), 6.44 (s, 1H), 4.81 (d, J= 15.28 Hz, 1H), 4.61 (s, 2H), 4.23 – 4.15 (m, 2H, H), 4.04 (dd, J= 5.78, 8.91 Hz, 1H), 3.89 (m, 1H), 3.08 (dd, J= 4.69, 13.47 Hz, 1H), 2.68 (dd, J= 8.42, 13.47 Hz, 1H), 2.17 (s, 9H). ^{13}C NMR (MHz, $CDCl_3$) δ 166.7, 165.4, 158.5, 150.7, 140.9, 136.8, 135.3, 135.0, 131.5, 129.4, 129.0, 128.1, 127.8, 127.3, 127.0, 126.6, 125.7, 119.8, 67.2, 55.7,

46.1, 43.9, 38.8, 34.5, 31.3. HPLC/MS: gradient elution 30-95% in 10 min, retention time=7.66, m/z $[M+H]^+$= 576. Elemental analysis calcd. for $C_{36}H_{37}N_3O_4$: C, 75.11; H, 6.48; N, 7.30; found C, 75.10; H, 6.29; N, 7.18.

(*S*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-((4-(trifluoromethyl)benzyl)carbamoyl)phenyl]benzamide (9). Compound **19** (0.20 g, 0.62 mmol), compound **17c** (0.22 g, 0.74 mmol), $Al(CH_3)_3$ in heptane 2.0 M (1.24 mL, 2.48 mmol) in THF (10 mL) were heated at 125 ºC for 35 min in a microwave reactor. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.32 g, 87 %). M.p. 176 ºC. ^{1}H NMR (300 MHz, MeOD) δ (ppm) 7.93-7.78 (m, 4H), 7.65-7.50 (m, 7H), 7.27-7.15 (m, 6H), 4.75 (d, *J*= 15.32 Hz, 1H), 4.61 (d, *J*= 5.84 Hz, 2H), 4.57-4.50 (m, 1H), 4.48-4.32 (m, 1H), 4.26-4.15 (m, 1H), 4.10-4.02 (m, 1H), 3.08 (dd, *J*= 4.88, 13.53 Hz, 1H), 2.81 (dd, *J*= 7.78, 13.35 Hz, 1H). ^{13}C NMR (75 MHz, MeOD) δ (ppm) 169.6 (CO), 168.6 (CO), 160.9, 145.0, 143.3, 138.4, 137.3, 136.6, 133.7, 132.5, 130.8, 130.3, 130.2, 129.8, 129.2, 127.1 (c, *J*= 30.31 Hz), 126.8, 126.4 (c, *J*= 9.17 Hz), 126.1 (d, *J*= 214.1 Hz), 124.7, 121.3, 68.6, 57.3, 46.8, 44.0, 39.2. HPLC/MS: gradient elution (30 to 95 % over 10 min), retention time 6.84 min $[M+H]^+$= 588.

(*S*)-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]-*N*-[4-(pentylcarbamoyl)phenyl]benzamide (10). Compound **19** (0.20 g, 0.62 mmol), compound **17d** (0.15 g, 0.74 mmol), $Al(CH_3)_3$ in heptane 2.0 M (1.24 mL, 2.48 mmol) in THF (10 mL) were heated at 125 ºC for 10 min in a microwave reactor. The crude was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.25 g, 80 %). M.p. 267 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.36 (bs, 1H, NH), 7.92-7.82 (m, 3H), 7.57-7.53 (m, 4H), 7.31-7.19 (m, 6H), 4.69 (d, *J*=15.61 Hz, 1H), 4.42 (d, *J*= 15.54 Hz, 1H), 4.26-4.20 (m, 1H), 4.08-4.03 (m, 1H), 3.98-3.89 (m,

1H), 3.36-3.32 (m, 2H), 3.05 (dd, *J*= 3.78, 13.32 Hz, 1H), 2.75 (dd, *J*= 8.12, 13.43 Hz, 1H), 1.56-1.47 (m, 2H), 1.32-1.26 (m, 4H), 0.87 (t, *J*= 6.46 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 165.6 (CO), 165.5 (CO), 157.7, 141.5, 137.3, 136.1, 135.1, 131.0, 129.7, 129.3, 128.7, 128.5, 127.8, 126.8, 126.7, 119.5, 66.3, 55.3, 45.1, 38.7, 37.2, 28.9, 28.7, 21.9, 13.9. HPLC/MS: gradient elution (30 to 95 % over 10 min), retention time 6.30 min $[M+H]^+$= 500.

***N*-[3-((*S*)-3-((*R*)-4-benzyl-2-oxooxazolidin-3-yl)-2-methyl-3-oxopropyl)phenyl)-4-(2-(4-(*tert*-butyl)phenyl)acetamido]benzamide (11).**

To a solution of **23** (0.16 g, 0.56 mmol) in dry THF was added **21a** in dry THF (10 mL) under nitrogen atmosphere and stirred for 12 h. The reaction mixture was treated with 1 N HCl aqueous solution (10 mL) and extracted with AcOEt (20 mL). The organic layer was washed with saturated aqueous NH_4Cl and dried over $MgSO_4$. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.24 g, 68 %). M.p. 130 ºC. $[\alpha]_D^{20}$= -16.9. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.75 (m, 2H, H-Ar), 7.68 – 7.60 (m, 1H, H-Ar), 7.54 (m, 2H, H-Ar), 7.44 (m, 3H, H-Ar), 7.32 – 7.20 (m, 6H, H-Ar), 7.13 – 7.00 (m, 3H, H-Ar), 4.66 (m, 1H, CH), 4.23 – 4.00 (m, 3H, CH_2/CH), 3.74 (s, 2H, $\underline{CH_2}CO$), 3.25 – 3.06 (m, 2H), 2.63 (m, 2H), 1.34 (s, 9H, $3CH_3$), 1.18 (d, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 176.4 (CO), 176.3 (CO), 169.5 (CO), 153.1 (C-2), 150.9, 140.8, 140.2, 138.0, 135.1, 130.8, 129.4 (2C), 129.2 (2C), 129.1, 128.9 (2C), 128.1 (2C), 127.3, 126.3 (2C), 125.5, 120.8, 119.3 (2C), 118.4, 66.0 (C), 55.2 (C), 44.5 (CH_2), 39.7 (C), 39.6 (C), 37.8 (C), 34.6 ($\underline{C}(CH_3)$), 31.3 ($3CH_3$), 16.5 (CH_3). HPLC/MS: retention time 8.78 min $[M+H]^+$= 632. Elemental analysis calcd. for $C_{39}H_{41}N_3O_5$: C, 74.14; H, 6.54; N, 6.65; found C, 73.89; H, 6.82; N, 6.41.

***N*-[3-((*S*)-3-((*R*)-4-benzyl-2-oxooxazolidin-3-yl)-2-methyl-3-oxopropyl)phenyl]-4-hexanamidobenzamide (12).** To a solution of **23** (0.39 g, 1.65 mmol) in dry THF was added

21b in dry THF (10 mL) under nitrogen atmosphere and stirred for 12 h. The reaction mixture was treated with 1 N HCl aqueous solution (10 mL) and extracted with AcOEt (20 mL). The organic layer was washed with saturated aqueous NH_4Cl and dried over $MgSO_4$. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.21 g, 34 %). M.p. 141 ºC. $[\alpha]_D^{20}$= -32.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.13 – 8.10 (bs, 1H, NH), 7.86 – 7.81 (bs, 1H, NH), 7.76 – 7.70 (m, 2H, H-Ar), 7.67 – 7.54 (m, 3H, H-Ar), 7.47 – 7.37 (m, 2H, H-Ar), 7.24 – 7.15 (m, 4H, H-Ar), 7.11 – 7.00 (m, 2H, H-Ar), 4.67 – 4.57 (m, 1H), 4.22 – 4.01 (m, 3H), 3.14 – 3.06 (m, 2H), 2.62 – 2.50 (m, 2H), 2.36 (t, *J*= 7.6 Hz, 2H, $\underline{CH_2}CO$), 1.70 – 1.59 (m, 2H), 1.37 – 1.23 (m, 4H), 1.16 (d, *J*= 6.7 Hz, 3H, $\underline{CH_3}CH$), 0.97 – 0.79 (t, *J*= 6.6 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 176.3 (CO), 172.1 (CO), 165.3 (CO), 153.2, 141.3, 140.1, 138.1, 135.1, 129.9, 129.3 (2C), 129.0, 128.9 (2C), 128.2 (2C), 127.3 (2C), 125.5, 121.0, 119.3, 118.6, 66.0, 55.2, 39.7, 39.6, 37.8, 37.7 ($\underline{CH_2}CO$), 31.4 (CH_2), 25.2 (CH_2), 22.4 (CH_2), 16.5 ($\underline{CH_3}CH$), 13.9 (CH_3). HPLC/MS: retention time 7.70 min $[M+H]^+$= 556. Elemental analysis calcd. for $C_{33}H_{37}N_3O_5$: C, 71.33; H, 6.71; N, 7.56; found C, 71.18 ; H, 6.42 ; N, 7.31.

(*S*)-3-[3-(4-(2-(4-(*tert*-butyl)phenyl)acetamido)benzamido)phenyl]-2-methylpropanoic acid (13).

To a solution of **11** (0.14 g, 0.22 mmol) in THF/H_2O (3:1) (30 mL) at 0 ºC were added 30 % H_2O_2 aqueous solution (0.1 mL, 0.88 mmol) and LiOH (0.01 g, 0.44 mmol) in H_2O (2 mL). The solution was stirred for 2 h at r.t. The reaction mixture was treated with 1 N HCl aqueous solution (1 mL) and extracted with AcOEt (2x 20 mL). The organic layers were washed with brine and dried over $MgSO_4$. The residue was purified: Biotage KP-Sil 25g, Ethanol/CH_2Cl_2 (0-6 %) yielding a white solid (0.07 g, 70 %). M.p 180.9 ºC. $[\alpha]_D^{20}$= 11.1. ^{1}H NMR (300 MHz,

DMSO-d_6) δ (ppm) 12.16 (s, 1H, COOH), 10.40 (s, 1H, NH), 10.04 (s, 1H, NH), 7.92 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.64 – 7.57 (m, 2H, H-Ar), 7.34 (m, 2H, H-Ar), 7.26 (m, 3H, H-Ar), 6.91 (m, 1H, H-Ar), 3.62 (s, 2H, $\underline{CH_2}CO$), 2.89 (m, 1H,), 2.66 – 2.54 (m, 2H), 1.26 (s, 9H, $3CH_3$), 1.05 (d, J = 6.5 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 176.7 (CO), 169.7 (CO), 164.7 (CO), 148.9 , 142.1, 139.9, 139.1, 132.6, 130.3, 129.1, 128.8 (2C), 128.5 (2C), 128.3, 125.1 (2C), 123.9, 120.8, 118.2 (2C) , 42.9 ($\underline{CH_2}CO$), 40.6, 38.7, 34.1 ($\underline{C}(CH_3)_3$), 31.1 ($3CH_3$), 16.7 (CH_3). HPLC/MS: gradient elution (40 to 95 % over 10.0 min) of acetonitrile in water, retention time 6.72 min $[M+H]^+$= 473. Elemental analysis calcd for $C_{29}H_{32}N_2O_4$: C, 73.7; H, 6.83; N, 5.93; found C, 74.00; H, 7.00; N, 5.70.

(*S*)-3-[3-(4-hexanamidobenzamido)phenyl]-2-methylpropanoic acid (14). To a solution of **12** (0.10 g, 0.18 mmol) in THF/H_2O (3:1) (30 mL) at 0 ºC were added 30 % H_2O_2 aqueous solution (0.1 mL, 0.72 mmol) and LiOH (0.008 g, 0.36 mmol) in H_2O (2 mL). The solution was stirred for 2 h at r.t. The reaction mixture was treated with 1 N HCl aqueous solution (1 mL) and was extracted with AcOEt (2x 20 mL). The organic layers were washed with brine and dried over $MgSO_4$. The residue was purified: Biotage KP-Sil 25g, CH_3OH/CH_2Cl_2 (0-6 %) yielding a white solid (0.09 g, 62 %). M.p 200 ºC. $[\alpha]_D^{20}$= 12.9. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.18 (s, 1H, COOH), 10.13 (s, 1H, NHCO), 10.03 (s, 1H, NHCO), 7.91 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H), 7.68 – 7.53 (m, 2H), 7.23 (m, 1H), 6.91 (m, 1H), 2.90 (m, 1H), 2.61 (m, 3H), 2.33 (t, J = 7.4 Hz, 2H, $\underline{CH_2}CO$), 1.58 (m, 2H, CH_2), 1.28 (m, 4H, $2CH_2$), 1.05 (d, J = 6.2 Hz, 3H, $\underline{CH_3}CH$), 0.87 (t, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 176.7 (CO), 171.7 (CO), 164.7 (CO), 142.2, 139.9, 139.1, 128.7, 128.5 (2C), 128.2, 124.0, 120.7, 118.2, 118.1 (2C), 40.6 (CH), 38.6, 36.4 ($\underline{CH_2}CO$), 30.9 (CH_2), 24.6 (CH_2), 21.9 (CH_2), 16.7

(<u>CH_3</u>CH), 13.8 (CH_3). HPLC/MS: retention time 7.93 min $[M+H]^+$= 236. Elemental analysis calcd. for $C_{23}H_{28}N_2O_4$: C, 69.68; H, 7.12; N, 7.07; found C, 70.21; H, 7.08; N, 6.95.

4-[(*tert*-butoxycarbonyl)amino)]benzoic acid (15). To a solution of 4-aminobenzoic acid (0.2 g, 1.5 mmol) in water/dioxane (1:2, 6 mL) were added Et_3N (0.4 mL, 3.0 mmol) and di-*tert*-butyl dicarbonate (0.6 g, 3.0 mmol). It was stirred at room temperature for 24 h. The solvent was evaporated and the residue was acidified with 1 N HCl aqueous solution. The obtained precipitate washed with water yielding **15** as white solid (0.47 g, 89 %). M.p. 181 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.21 (s, 1H, COOH), 9.71 (s, 1H, NH), 7.82 (d, J = 8.7 Hz, 2H), 7.54 (d, J= 8.7 Hz, 2H), 1.44 (s, 9H, $3CH_3$).^{13}C NMR (75 MHz, DMSO-d_6) δ 167.4 (CO_2H), 152.9 (CONH), 144.1, 130.3 (C), 124.4, 117.6 (C), 80.0 (<u>C</u>$(CH_3)_3$), 28.4 ($3CH_3$). HPLC-MS: retention time 4.25 min $[M+H]^+$= 237. Elemental analysis calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90; found C, 60.84; H, 6.09; N, 5.95.

General Procedure for the Synthesis of 16a-g. To a cooled mixture of **15** (1 Eq) in THF or DMF was added Et_3N (1.2 Eq), HOBt (1.2 Eq) and corresponding coupling reagents (1.2 Eq). Then, corresponding amines were added and mixture was stirred for 12 h at r.t. The reaction mixture was treated with 1 N HCl aqueous solution and extracted with AcOEt. The organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography.

4-[*N*-(*tert*-butoxycarbamoyl)amino)]benzylamine (16a). To solution of **15** (0.20 g, 0.84 mmol) in THF at 0 ºC were added PyBOP (0.53 g, 1.01 mmol), HOBt (0.14 g, 1.01 mmol), Et_3N (0.14 mL, 1.01 mmol) and benzylamine (0.11 mL, 1.01 mmol). The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.19 g, 80 %). M.p. 150 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H, NH), 8.86 (t, J = 6.0 Hz, 1H,

NHCH$_2$), 7.80 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 8.7 Hz, 2H), 7.38 – 7.18 (m, 5H), 4.45 (d, *J* = 5.9 Hz, 2H, NHCH$_2$), 1.47 (s, 9H, 3CH$_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.7 (CO), 152.5 (CO), 142.3, 139.8, 128.2 (C), 128.0 (C), 127.6, 127.1, 126.1, 117.1, 79.4 (C(CH$_3$)$_3$), 42.5 (NHCH$_2$), 28.0 (3CH$_3$). HPLC-MS: retention time 9.05 min [M+H]$^+$= 327. Elemental analysis calcd. for $C_{19}H_{22}N_2O_3$: C, 69.92; H, 6.79; N, 8.58; found C, 70.16; H, 6.49; N, 8.58.

4-[*N*-(*tert*-butoxycarbamoyl)amino)]-*N*-(*tert*-butyl)benzylamine (16b). To solution of **15** (0.30 g, 1.26 mmol) in THF (20 mL) at 0 ºC were added PyBOP (0.79 g, 1.52 mmol), HOBt (0.21 g, 1.52 mmol), Et$_3$N (0.21 mL, 1.52 mmol) and 4-(*tert*-butyl)-benzylamine (0.08 g, 1.52 mmol). The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.3 g, 60 %). M.p. 112 ºC. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 7.72 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J*= 8.4 Hz, 2H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J*= 8.6 Hz, 2H) 6.77 (s, 1H, NH), 6.37 (t, *J* = 5.7 Hz, 1H, NHCH$_2$), 4.59 (d, *J* = 5.4 Hz, 2H, NHCH$_2$), 1.51 (s, 9H, 3CH$_3$), 1.31 (s, 9H, 3CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$) δ (ppm) 166.7 (CO), 152.3 (CO), 150.6, 141.5, 135.2, 128.5, 128.0 (C), 127.8 (C), 125.7 (C), 117.7 (C), 81.0 (OC(CH$_3$)$_3$), 43.8 (CH$_2$), 34.5 (C(CH$_3$)$_3$), 31.3 (3CH$_3$), 28.3 (3CH$_3$). HPLC-MS: gradient elution (15 to 95 % over 5 min) of acetonitrile in water, retention time 5.71 min [M+H]$^+$= 383. Elemental analysis calcd. for $C_{23}H_{30}N_2O_3$; C, 72.22; H, 7.91; N, 7.32; found C, 72.51; H, 7.95; N, 7.20.

4-[*N*-(*tert*-butoxycarbamoyl)amino)]-*N*-(4-trifluoromethyl)benzylamine (16c). To solution of **15** (0.10 g, 0.42 mmol) in THF (20 mL) at 0 ºC were added PyBOP (0.26 g, 0.51 mmol), HOBt (0.07 g, 0.51 mmol), Et$_3$N (0.07 mL, 0.51 mmol) and 4-(trifluoromethyl)-benzylamine (0.07 mL, 0.51 mmol). The residue was purified: Biotage KP-Sil 25g, AcOEt/Hexane from (5:95) up to (10:90) gave **16c** as white solid (0.14 g, 84 %). M.p. 141 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.61 (s, 1H, NH), 8.96 (t, *J* = 6.0 Hz, 1H, NHCH$_2$), 7.81 (d, *J* = 8.8 Hz, 2H,

CH-Ar), 7.68 (d, *J* = 8.8 Hz, 2H, CH-Ar), 7.56 – 7.47 (m, 4H, CH-Ar), 4.52 (d, *J* = 5.8 Hz, 2H, NHCH$_2$), 1.47 (s, 9H, 3CH$_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 165.8 (CO), 152.5 (CO), 144.7, 142.4, 128.0, 127.8 (C), 127.3 (C), 125.9 (C), 125.1 (c, *J*= 268 Hz, CF$_3$), 117.1 (C), 79.4 (OC(CH$_3$)$_3$), 42.2 (CH$_2$), 28.0 (3CH$_3$). HPLC-MS: retention time 5.24 min $[M+H]^+$= 395. Elemental analysis calcd. for $C_{20}H_{21}F_3N_2O_3$: C, 60.91; H,5.37; N, 7.10; found C, 61.10; H, 5.25; N, 6.88.

***tert*-butyl (4-(pentylcarbamoyl)phenyl)carbamate (16d).** To solution of **15** (0.3 g, 1.26 mmol) in THF (20 mL) at 0 ºC were added PyBOP (0.78 g, 1.52 mmol), HOBt (0.21 g, 1.52 mmol), Et$_3$N (0.21 mL, 1.52 mmol) and 1-pentylamine (0.17 mL, 1.52 mmol). The residue was purified: Biotage KP-Sil 25g, 2 % CH$_3$OH in CH$_2$Cl$_2$ yielding an orange solid (0.12 g, 33 %). M.p. 99 ºC. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 7.70 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.6 Hz, 2H), 6.70 (bs, 1H, NHCH$_2$), 6.08 (s, 1H, NH), 3.41 – 3.38 (m, 2H, NHCH$_2$CH$_2$), 1.52 (s, 9H, 3CH$_3$), 1.40 – 1.29 (m, 6H, 3CH$_2$), 0.91 (t, *J*= 6.8 Hz, 3H, CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$) δ (ppm) 166.8 (CO), 152.3 (CO), 141.2, 129.0, 127.9 (C), 117.7 (C), 81.0 (OC(CH$_3$)$_3$), 40.0 (CH$_2$), 29.4 (CH$_2$), 29.1 (CH$_2$), 28.2 (3CH$_3$), 22.4 (CH$_2$), 13.9 (CH$_3$). HPLC-MS: retention time 5.03 min $[M+H]^+$= 307. Elemental analysis calcd. for $C_{17}H_{26}N_2O_3$: C, 66.64; H, 8.55; N, 9.14; found C, 66.20; H, 8.30; N, 9.11.

***tert*-butyl (4-(dodecylcarbamoyl)phenyl)carbamate (16e).** To solution of **15** (0.20 g, 0.84 mmol) in THF (20 mL) at 0 ºC were added PyBOP (0.19 g, 1.01 mmol), HOBt (0.19 g, 1.01 mmol), Et$_3$N (0.14 mL, 1.01 mmol) and 1-dodecylamine (0.23 mL, 1.01 mmol). The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.22 g, 65 %). M.p. 110 ºC. ^{1}H NMR (300 MHz, CDCl$_3$) δ (ppm) 7.69 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.6 Hz, 2H), 6.70 (s, 1H, NHCO-O), 6.12 (bs, 1H, NHCH$_2$), 3.42 (m, 2H, CH$_2$NH), 1.61

(m, 2H, CH_2), 1.52 (s, 9H, $3CH_3$), 1.42 – 1.17 (m, 18H, $9CH_2$), 0.86 (t, J =7.1, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.8 (CO), 152.3 (CO), 141.3, 128.9, 127.9, 117.7, 81.0 ($O\underline{C}(CH_3)_3$), 40.1 (CH_2N), 31.9 (CH_2), 29.7 (CH_2), 29.6 (CH_2), 29.54 (CH_2), 29.50 (CH_2), 29.3 ($3CH_2$), 28.3 ($3CH_3$), 27.0 (CH_2), 22.6 (CH_2), 14.1 (CH_3). HPLC-MS: retention time 6.0 min $[M+H]^+$= 405. Elemental analysis calcd. for $C_{24}H_{40}N_2O_3$: C, 71.25; H, 9.97; N, 6.92; found C, 71.08; H, 10.12; N, 6.63.

***tert*-butyl (4-((cyclopropyl)carbamoyl)phenyl)carbamate (16f).** To a solution of **15** (0.50 g, 2.11 mmol) in DMF (25 mL) at 0 ºC were added EDCI (0.61g, 3.17 mmol), HOBt (0.43 g, 3.17 mmol), Et_3N (0.88 mL, 6.33 mmol) and cyclopropylamine (0.22 mL, 3.17 mmol). The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.28 g, 48 %). M.p. 184 ºC. ^{1}H NMR (300 MHz, MeOD) δ (ppm) 7.72 (d, J = 8.8 Hz, 2 H), 7.48 (d, J = 8.8 Hz, 2H), 2.82 (dq, J = 7.4, 3.9 Hz, 1H), 1.52 (s, 9H), 0.93 – 0.73 (m, 2H), 0.69 – 0.50 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 164.6 (CO), 155.2 (CO), 144.5 (C), 139.15 (C), 129.6 (2CH), 119.1 (2CH), 81.6 (C), 29.0 ($3CH_3$), 24.3 (CH), 6.95 ($2CH_2$). HPLC-MS: retention time 7.84 min $[M+H]^+$= 277. Elemental analysis calcd. for $C_{15}H_{20}N_2O_3$: C, 65.2; H,7.3; N, 10.14; found C, 68.18; H, 6.94; N, 10.08.

***tert*-butyl (4-((cyclohexylmethyl)carbamoyl)phenyl)carbamate (16g).** To a solution of **15** (0.14 g, 0.57 mmol) in THF (20 mL) at 0 ºC were added EDCI (0.17 g, 0.88 mmol), HOBt (0.12 g, 0.88 mmol), Et_3N (0.12 mL, 0.88 mmol) and cyclohexylamine (0.11 mL, 0.88 mmol). The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.06 g, 31 %). M.p. 114 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 9.56 (s, 1H, NH), 8.24 (t, J = 5.8 Hz, 1H, $\underline{NH}CH_2$), 7.74 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 3.05 (m, 2H, $NH\underline{CH_2}$), 1.67 (m, 5H), 1.47 (s, 9H, $3CH_3$), 1.24 – 1.01 (m, 4H), 0.90 (m, 2H). ^{13}C NMR

(75 MHz, DMSO-d_6) δ (ppm) 165.7 (CO), 152.6 (CO), 142.0, 128.06, 127.9 (C), 117.0 (C), 79.4 (O<u>C</u>$(CH_3)_3$), 45.3 ($NHCH_2$), 37.5 (C), 30.5 (C), 28.0 ($3CH_3$), 26.1 (C), 25.4 (C). HPLC-MS: retention time 6.02 min $[M+H]^+$= 333. Elemental analysis calcd. for $C_{19}H_{28}N_2O_3$: C, 68.65; H,8.49; N, 8.43; found C, 68.40; H, 8.70; N, 8.20.

General Procedure for the Synthesis of 17a-g. The corresponding *N*-Boc amino acid (**16a-g**) was treated with a mixture of TFA and dichloromethane (1:1, 40 mL) at room temperature for 12 h. The mixture reaction was quenched with aqueous 1 N NaOH (15 mL). The organic layer separated, washed with water and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure obtaining the desired compounds.

4-amino-*N*-benzylbenzamide (17a). General method was used with compound **16a** (0.35 g, 1.07 mmol). White solid (0.22 g, 92 %). M.p. 150 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 8.55 (t, *J* = 6.1 Hz, 1H, <u>NH</u>CH_2), 7.62 (d, *J* = 8.6 Hz, 2H), 7.40 – 7.17 (m, 5H, H-Ar), 6.54 (d, *J* = 8.6 Hz, 2H), 5.60 (bs, 2H, NH_2), 4.42 (d, *J* = 5.9 Hz, 2H, NH<u>CH_2</u>). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 166.2 (CO), 151.6, 140.3, 128.7 (C), 128.1, 127.1, 126.5, 125.3, 124.2, 121.0, 112.5 (C), 42.3 (CH_2). HPLC-MS: retention time 8.5 min $[M+H]^+$= 227. Elemental analysis calcd. for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38. found C, 74.39; H, 6.43; N, 12.55.

4-amino-*N*-(4-(*tert*-butyl)benzyl)benzamide (17b). General method was used with compound **16b** (0.20 g, 0.52 mol). White solid (0.13 g, 90 %). M.p. 113 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.62 (d, *J* = 8.3 Hz, 2H), 7.37 (d, *J* = 8.2 Hz, 2H), 7.29 (d, *J* = 8.2 Hz, 2H), 6.64 (d, *J* = 8.3 Hz, 2H), 4.59 (d, *J* = 5.4 Hz, 2H, NH<u>CH_2</u>), 1.32 (s, 9H, $3CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.0 (CO), 150.5, 149.5, 135.5, 128.7 (C), 127.7 (C), 125.6 (C), 124.0, 114.1 (C), 43.7 (CH_2), 34.5 (<u>C</u>$(CH_3)_3$), 31.3 ($3CH_3$). HPLC-MS: retention time 4.67 min $[M+H]^+$= 283.

Elemental analysis calcd. for $C_{18}H_{22}N_2O$: C, 76.56; H, 7.85; N, 9.92. found C, 76.71; H, 8.10; N, 9.94.

4-amino-*N*-(4-(trifluoromethyl)benzyl)benzamide (17c). General method was used with compound **16c** (0.20 g, 0.51 mol). White solid (0.14 g, 92 %). M.p. 113 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.63 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.44 (d, *J* = 7.8 Hz, 2H), 6.65 (d, *J* = 8.0 Hz, 2H), 6.42 (bs, 1H, NHCH$_2$), 4.67 (d, *J* = 5.8 Hz, 2H, NHCH$_2$), 4.34 – 3.49 (bs, 2H, NH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.2 (CO), 149.8, 142.8, 131.1, 128.8 (C), 127.9 (C), 125.7 (C), 125.6, 124.9 (c, *J*= 268 Hz, CF_3), 114.1 (C), 43.4 (CH_2). HPLC-MS: retention time 4.21 min $[M+H]^+$= 295. Elemental analysis calcd. for $C_{15}H_{13}F_3N_2O$: C, 61.22; H, 4.45; N, 9.52. found C, 61.23; H, 4.60; N, 9.60.

4-amino-*N*-pentylbenzamide (17d). General method was used with compound **16d** (0.76 g, 3.68 mmol). White solid (0.44 g, 85 %). M.p. 100 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.58 (d, *J* = 8.6 Hz, 2H), 6.64 (d, *J* = 8.6 Hz, 2H), 6.01 (bs, 1H, $NHCH_2$), 3.40 – 3.37 (m, 2H, NHCH$_2$CH$_2$), 1.57 – 1.51 (m, 2H, NHCH$_2$CH$_2$), 1.43 – 1.24 (m, 4H, 2CH_2), 0.99 – 0.79 (t, *J*= 6.8 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.2 (CO), 149.4, 128.5 (C), 124.4, 114.1 (C), 40.0 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 22.4 (CH_2), 14.0 (CH_3). HPLC-MS: retention time 3.7 min $[M+H]^+$= 207. Elemental analysis calcd. for $C_{12}H_{18}N_2O$: C, 69.87; H, 8.80; N, 13.58. found C, 69.23; H, 8.60; N, 13.60.

4-amino-*N*-dodecylbenzamide (17e). General method was used with compound **16e** (0.20 g, 0.49 mmol). White solid (0.14 g, 92 %). M.p. 112 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.59 (d, *J*= 8.6 Hz, 2H), 6.65 (d, *J*= 8.6 Hz, 2H), 6.05 – 5.91 (bs, 1H, NH, $NHCH_2$), 3.40 (m, 2H, NHCH$_2$), 1.65 – 1.50 (m, 2H, NHCH$_2$CH$_2$), 1.45 – 1.16 (m, 18H, 9CH_2), 0.87 (t, *J*= 7.1, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 167.2 (CO), 149.3, 128.9, 127.8, 114.1, 39.9

(NHCH$_2$), 31.9 (CH$_2$), 29.8 (CH$_2$), 29.6 (3CH$_2$), 29.3 (3CH$_2$), 27.0 (CH$_2$), 22.7 (CH$_2$), 14.1 (CH$_3$). HPLC-MS: retention time 11.0 min $[M+H]^+$= 305. Elemental analysis calcd. for $C_{19}H_{32}N_2O$: C, 74.95; H, 10.59; N, 9.20; found C, 74.60; H, 10.55; N, 9.16.

4-amino-*N*-cyclopropylbenzamide (17f). General method was used with compound **16f** (0.55 g, 1.99 mmol). White solid (0.22 g, 63 %). M.p. 142 ºC. ^{1}H NMR (300 MHz, MeOD) δ 7.57 (d, *J*= 8.7 Hz, 2H), 6.64 (d, *J*= 8.7 Hz, 2H), 4.86 (s, 4H), 2.85 – 2.71 (m, 1H), 0.83 – 0.70 (m, 2H), 0.65 – 0.53 (m, 2H). ^{13}C NMR (75 MHz, MeOD) δ 172.1 (CO), 153.2 (C), 129.9 (C), 123.1 (2CH), 114.6 (2CH), 23.8 (CH), 6.6 (2CH$_2$). HPLC-MS: retention time 1.2 min $[M+H]^+$= 177. Elemental analysis calcd. for $C_{10}H_{12}N_2O$: C, 68.16; H, 6.86; N, 15.90; found C, 67.97; H, 6.80; N, 15.75.

4-amino-*N*-(cyclohexylmethyl)benzamide (17g). General method was used with compound **16g** (0.20 g, 0.60 mmol). White solid (0.11 g, 80 %). M.p. 150 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (t, *J* = 5.8 Hz, 1H, CH$_2$NH), 7.54 (d, *J* = 8.6 Hz, 2H), 6.50 (d, *J* = 8.6 Hz, 2H), 5.60 – 5.48 (m, 2H, NH$_2$), 3.08 – 2.92 (m, 2H, CH$_2$NH), 1.66 – 1.62 (m, 5H), 1.47 – 1.41 (m, 1H), 1.13 – 1.11 (m, 3H), 0.95 – 0.77 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 165.7 (CO), 142.0, 128.1, 127.6 (C), 116.0 (C), 45.3 (NHCH$_2$), 37.5 (C), 30.5 (C), 26.1 (C), 25.4 (C). HPLC-MS: retention time 5.34 min $[M+H]^+$= 333. Elemental analysis calcd. for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06; found C, 72.60; H, 8.55; N, 12.16.

(*R*)-methyl-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]benzoate (18). To a solution of (*R*)- 4-benzyl-3-propionyl-2-oxazolidinone (1.2 g, 5.24 mmol) in anhydrous THF (13 mL) was added potassium *tert*-butoxide (0.6 g, 5.24 mmol) and stirred for 45 min under nitrogen atmosphere at room temperature. Then methyl 3-bromomethyl benzoate (1.0 g, 4.36 mmol) was added to the stirred solution. The reaction mixture was stirred for 6 h. A saturated solution of NH$_4$Cl (15 mL)

was added and the reaction extracted with ethyl acetate (30 mL). The combined organic extracts were washed successively with H_2O (30 mL), brine (30 mL) and dried over Na_2SO_4. The solvent was evaporated and the crude purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.43 g, 62 %). M.p. 62 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.06 – 7.95 (m, 1H, H-Ar), 7.90 – 7.88 (m, 1H, H-Ar), 7.54 – 7.40 (m, 2H, H-Ar), 7.36 – 7.22 (m, 3H, H-Ar), 7.12 – 7.02 (m, 2H, H-Ar), 4.86 (d, J = 15.4 Hz, 1H), 4.18 (m, 2H), 4.10 – 4.02 (m, 1H), 3.93 (s, 3H, CH_3), 3.89 – 3.77 (m, 1H), 3.09 (dd, J_{ab}= 13.6, J_{a4}= 4.7 Hz, 1H), 2.66 (dd, J_{ba}= 13.6, J_{b4}= 8.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 166.5 (CO), 157.9, 136.4, 135.4, 132.4, 130.8, 129.3, 129.1, 129.0, 128.9, 127.1, 67.0, 55.5, 51.9 (OCH_3), 45.9, 38.3. HPLC-MS: retention time 4.8 min $[M+H]^+$= 326. Elemental analysis calcd. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31; found C, 70.31; H, 5.67; N, 4.60.

(*S*)-methyl-3-[(4-benzyl-2-oxooxazolidin-3-yl)methyl]benzoate (19). To a solution of (*S*)- 4-benzyl-3-propionyl-2-oxazolidinone (0.5 g, 2.82 mmol) in anhydrous THF/CH_3CN (20 mL, 1:1) was added Cs_2CO_3 (2.76 g, 8.46 mmol) and stirred for 45 min under nitrogen atmosphere at room temperature. Then methyl 3-bromomethyl benzoate (1.3 g, 5.64 mmol) was added to the stirred solution. The reaction mixture was stirred for 6 h. A saturated solution of NH_4Cl (25 mL) was added and the reaction extracted with ethyl acetate (2x30 mL). The combined organic extracts were washed successively with water (30 mL), brine (30 mL) and dried over Na_2SO_4. The solvent was evaporated and the crude was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding a white solid (0.60 g, 66 %). M.p. 61.1 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.00 (d, J= 6.5 Hz, 1H), 7.90 (s, 1H), 7.52 - 7.40 (m, 2H), 7.35 – 7.17 (m, 3H), 7.10 - 6.99 (m, 2H), 4.86 (d, J= 15.4 Hz, 1H), 4.24 – 4.10 (m, 2H), 4.08 – 3.98 (m, 1H), 3.93 (s, 3 H), 3.88 – 3.73 (m, 1H), 3.09 (dd, J= 13.6, 4.8 Hz, 1H), 2.66 (dd, J= 13.6, 8.9 Hz,

1H). ^{13}C NMR (MHz, $CDCl_3$) δ (ppm) 166.6, 158.3, 136.3, 135.3, 132.6, 130.7, 129.3, 129.1, 129.0, 128.9, 127.2, 67.0, 55.5, 52.2, 46.1, 38.6. HPLC-MS: retention time 9.06 min $[M+H]^+$= 326. Elemental analysis calcd. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31; found C, 70.42; H, 5.62; N, 4.13.

4-[2-(4-(*tert*-butyl)phenyl)acetamido]benzoic acid (20a). 4-aminobenzoic acid (0.20 g, 1.46 mmol), methyl *p*-*tert*-butylphenylacetate (0.45 mL, 2.19 mmol), $Al(CH_3)_3$ in heptane 2.0 M (1.10 mL, 2.19 mmol) in THF (10 mL) were heated at 125 ºC for 40 min in a microwave reactor. The reaction mixture was poured into an erlenmeyer, cooled in an ice bath and 1 N HCl aqueous solution was slowly added until the effervescence ended. The mixture was extracted with AcOEt (2x20 mL) and the organic layer was washed with saturated aqueous NH_4Cl and dried over $MgSO_4$. The residue was purified: Biotage KP-Sil 25g, EtOH/CH_2Cl_2 0-6 % yielding a white solid (0.30 g, 65 %). M.p. 155 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.66 (s, 1H, CO_2H), 10.43 (s, 1H, NH), 7.86 (d, *J*= 8.8 Hz, 2H), 7.69 (d, *J*= 8.8 Hz, 2H), 7.33 (d, *J*= 8.4 Hz, 2H), 7.24 (d, *J*= 8.4 Hz, 2H), 3.61 (s, 2H, $\underline{CH_2}CO$), 1.34 (s, 9H, $3CH_3$). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 169.8(CO), 166.8 (CO), 148.9, 143.2, 132.6, 130.3, 128.7, 125.1, 118.3, 49.2 ($\underline{CH_2}CO$), 34.1 ($\underline{C}(CH_3)_3$), 31.1 ($3CH_3$). HPLC/MS: retention time 5.25 min $[M+H]^+$= 312. Elemental Analysis calcd. for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.80; N, 4.50; found C, 73.10; H, 6.78; N, 4.52.

4-hexanamidobenzoic acid (20b). 4-aminobenzoic acid (0.25 g, 1.81 mmol), ethyl hexanoate (0.6 g, 3.64 mmol), $Al(CH_3)_3$ in heptane 2.0 M (1.82 mL, 3.64 mmol) in THF (20 mL) were heated at 125 ºC for 20 min in a microwave reactor. The reaction mixture was poured into an erlenmeyer, cooled in an ice bath and 1 N HCl aqueous solution was slowly added until the effervescence ended. The precipitate obtained was washed with H_2O (2x10 mL) yielding a white solid (0.30 g, 70 %). M.p. 245 ºC. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 12.65 (s, 1H, CO_2H),

10.15 (s, 1H, NH), 7.86 (d, *J*= 8.7 Hz, 2H), 7.69 (d, *J*= 8.7 Hz, 2H), 2.32 (t, *J*= 7.4 Hz, 2H, $\underline{CH_2}CO$), 1.65 – 1.46 (m, 2H, CH_2), 1.40 – 1.18 (m, 4H, $2CH_2$), 0.95 – 0.75 (t, *J*= 6.7 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm) 171.8 (CO), 166.9 (CO), 143.3, 130.3, 124.7, 118.2, 36.4 ($\underline{CH_2}CO$), 30.8 (CH_2), 24.6 (CH_2), 21.9 (CH_2), 13.8 (CH_3). HPLC/MS: retention time 7.93 min $[M+H]^+$= 236. Elemental Analysis calcd. for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95; found C, 66.50; H, 7.50; N, 5.84.

4-[2-(4-(*tert*-butyl)phenyl)acetamido]benzoyl chloride (21a). To a solution of **20a** (0.10 g, 0.32 mmol) in dry THF was added thionyl clhoride (2 mL). The solution was stirred under reflux for 2 h. the solvent was removed under reduced pressure yielding (0.11 g).

4-hexanamidobenzoyl chloride (21b). To a solution of **20b** (0.10 g, 0.43 mmol) in dry THF was added thionyl clhoride (2 mL). The solution was stirred under reflux for 2 h. the solvent was removed under reduced pressure yielding (0.11 g).

(*R*)-4-benzyl-3-[(*S*)-2-methyl-3-(3-nitrophenyl)propanoyl]oxazolidin-2-one (22). A solution of (*R*)-(-)-4-benzyl-3-propionyl-2-oxazolidinone (0.60 g, 2.58 mmol) in anhydrous THF (11 mL) was stirred for 15 min under nitrogen atmosphere at -78 ºC. Then sodium bis(-trimethylsilyl)amide 1.0 M in THF (2.84 mL, 2.84 mmol) was added dropwise with a syringe to the stirred solution. The reaction mixture was allowed to stir for 1 h at -78 ºC. 1-bromomethyl-3-nitrobenzene (0.61 g, 2.84 mmol) was added and the reaction stirred at this temperature for 6 h. After reaching room temperature a saturated solution of NH_4Cl (15 mL) was added and the reaction extracted with ethyl acetate (30 mL). The combined organic extracts were washed successively with water (30 mL), brine (30 mL), and dried over Na_2SO_4. The residue was purified: Biotage KP-Sil 25g, EtOAc/Hexane from (5:95) up to (10:90) yielding an oil (0.67 g, 70 %). $[\alpha]_D^{20}$= -56.0. ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.22 – 8.04 (m, 2H, H-Ar), 7.66 (dt, *J*

= 7.7, 1.3 Hz, 1H, H-Ar), 7.48 (t, J = 7.9 Hz, 1H, H-Ar), 7.34 – 7.20 (m, 3H, H-Ar), 7.16 – 6.99 (m, 2H, H-Ar), 4.68 (m, 1H, CH), 4.15 – 3.90 (m, 3H), 3.28 (dd, J_{ab}= 13.5, J_{a14}= 7.0 Hz, 1H), 3.14 (dd, J_{ab} = 13.4, J_{a4}= 3.4 Hz, 1H), 2.76 (dd, J_{ba}= 13.5, J_{b14}= 7.6 Hz, 1H), 2.62 (dd, J_{ba} = 13.4, J_{b4}= 9.4 Hz, 1H), 1.21 (d, J= 6.7 Hz, 2H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 175.6 (CO), 153.0, 148.3, 141.3, 139.6, 135.5, 134.9, 129.3 (2C), 128.9 (2C), 127.4, 124.1, 121.6, 66.1, 55.2, 39.5, 39.2, 37.8, 16.6 (CH_3). HPLC/MS: retention time 10.04 min $[M+H]^+$= 368. Elemental analysis calcd. for $C_{20}H_{20}N_2O_5$: C, 65.21; H, 5.47; N, 7.60; found C, 65.50; H, 5.71; N, 7.83.

(*R*)-3-[(*S*)-3-(3-aminophenyl)-2-methylpropanoyl]-4-benzyloxazolidin-2-one (23). The catalytic hydrogenation of **22** (0.25 g, 0.68 mmol) in THF/AcOEt (1:2, 20 mL) adding Pd-C 10% cat. was carried out in mild conditions (21 psi., r.t.) in a PAR reactor over 12 h. Finally, the reaction mixture was filtered through a hydrophilic PVDF filter (30 mm, 0.45 μ) and the solvent removed under reduced pressure yielding **23** as an oil (0.21 g, 90%). $[\alpha]_D^{20}$= -14.8. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.30 – 7.24 (m, 2H, H-Ar), 7.20 (m, 3H, H-Ar), 7.15 – 7.06 (m, 2H, H-Ar), 7.00 (m, 2H, H-Ar), 4.65 (m, 1H), 4.20 – 4.01 (m, 3H), 3.17 – 2.98 (m, 2H), 2.62 (m, 2H), 1.16 (d, J= 6.8 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 176.4 (CO), 153.1 (CO), 140.8, 135.2, 135.2 (2C), 129.2 (2C), 128.9, 127.1, 120.5 (2C), 117.8, 66.0, 55.2, 39.6, 39.2, 37.5, 16.5 (CH_3). HPLC/MS: retention time 6.94 min $[M+H]^+$= 338. Elemental analysis calcd. for $C_{20}H_{22}N_2O_5$: C, 70.99; H, 6.55; N, 8.28; found C, 71.04; H, 6.78; N, 8.19.

Pharmacological Evaluation.

In vivo food intake studies

In order to facilitate the rapid screening of PPARα-related activity we selected the inhibition of food intake in food-deprived animals. Although the gold standard in PPARα activity has been

considered the reduction of triglicerides after chronic administration, the reduction of forced food intake has been identified a reliable action of PPARα agonists. The acute effects of drugs on feeding behavior were analyzed in 24-h food-deprived male Wistar rats weighing 300-350 g from Charles Rivers Laboratories España, S.A. (Barcelona, Spain). Animals were housed individually in cages in a temperature- and humidity-controlled room (22 ºC and 55 % relative humidity) with a 12:12-h yellow light/dark cycle.

Water and food were available *ad libitum*. Animals were handled daily for a week before starting the experiments. The drugs were administered intraperitoneally (i.p.) at doses of 0.3, 1, and 3 mg/kg suspended in Tween 80 and saline as vehicle. After a period of 15 min after drug administration the animals were returned to their home cage and food intake was monitored 30, 60, 120 and 240 min after starting the test. In some cases the food intake was monitored after 24h. At the end of the test, the amount of water consumed was also measured. All animal procedures met the National Institutes of Health guidelines for the care and use of laboratory animals, and the European Communities directive 86/609/EEC regulating animal research.

Computational details

Theoretical calculations. All calculations were performed on an Intel® Core™2 Quad Workstation using Linux Ubuntu kernel 3.4.0.

Preparation of ligands and target macromolecule. ChemAxon Marvin 5.4.1 software package (2013) (http://www.chemaxon.com), accessed 09/10/2012) was used to build the structure of the ligands. Energy minization and partial charges were calculated using PM6 method available in MOPAC 2012.[32]

The three-dimensional structure of PPARα-LBD (1K7L entry, chain A) and PPARγ-LBD (1K74 entry, chain A) were retrieved from the RCSB Protein Data Bank.[33] Ligands, salts, and water molecules were removed, and the tautomeric forms were checked. In order to optimize hydrogen bond networks, the MolProbity server was used to add hydrogen atoms.[34] Finally, Kollman charges were computed through ADT.

To prepare the appropriate file needed for the docking study, non-polar hydrogen atoms were merged and rotatable bonds within the ligands were defined through AutoDockTools v1.5 (ADT) program (The Scripps Research Institute: http://mgltools.scripps.edu/. accessed 22/06/2012) (La Jolla, CA, USA).

Protocol for docking study. Protocol for docking study. Docking experiments of compounds were carried out by means of AutoDock v4 package.[35] For the calculations, a grid box with the dimensions 60x60x60 points was constructed around the binding site based on the location of the co-crystallized ligand GW409544 (1K7L entry, chain A) (1K74 entry, chain B), dimensions of axis were 22.5 Å and spacing of the grid points was 0.375 Å. The Lamarckian genetic algorithm (LGA) procedure was employed and the docking runs were set to 100, the maximum number of generations to 27,000 and the maximum number of energy evaluations to 25,000,000. The rest of the parameters were taken as default.

Analysis of the binding mode. To select the binding mode of each compound, we applied a qualitative analysis based on the location/orientation of the best 100 docked conformations given by AutoDock in relation to the co-crystallized ligand GW409544.[36] Hydrogen bonds and properties in ligand-receptor interaction of the binding mode of each compound were evaluated by using Accelrys Discovery Studio® v2.0 (Accelrys, Inc., San Diego, CA, USA). Measurements of docked conformations RMSD were carried out through ADT.

ASSOCIATED CONTENT

Supporting Information.

Molecular formula strings and the associated biological data is available free of charge on the ACS publications website.

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ABBREVIATIONS

PPAR, peroxisome proliferator activated receptors; LBD, ligand binding domain; AF, activation function; HB, hydrogen bond, NCoR, nuclear receptor co-repressor; RXR, retinoid X receptor ; CPT1-DR1, Carnitine palmitoyltransferase I response element direct repeat 1.

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