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## Original article

Synthesis, biological evaluation and molecular modeling studies of the PPAR $\beta/\delta$  antagonist CC618

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## ABSTRACT

Herein, we describe the synthesis, biological evaluation and molecular docking of the selective PPAR $\beta/\delta$  antagonist (4-methyl-2-(4-(trifluoromethyl)phenyl)-N-(2-(5-(trifluoromethyl)-pyridin-2-ylsulfonyl)ethyl)thiazole-5-carboxamide), CC618. Results from *in vitro* luciferase reporter gene assays against the three known human PPAR subtypes revealed that CC618 selectively antagonizes agonist-induced PPAR $\beta/\delta$  activity with an IC<sub>50</sub> = 10.0  $\mu$ M. As observed by LC-MS/MS analysis of tryptic digests, the treatment of PPAR $\beta/\delta$  with CC618 leads to a covalent modification of Cys249, located centrally in the PPAR $\beta/\delta$  ligand binding pocket, corresponding to the conversion of its thiol moiety to a 5-trifluoromethyl-2-pyridylthioether. Finally, molecular docking is employed to shed light on the mode of action of the antagonist and its structural consequences for the PPAR $\beta/\delta$  ligand binding pocket.

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## 1. Introduction

The peroxisome proliferator-activated receptors (PPAR $\alpha$ , PPAR $\beta/\delta$  and PPAR $\gamma$ , NR1C1-3) belong to the nuclear hormone receptor superfamily and function as ligand-activated transcription factors [1]. The PPAR receptor subtypes display tissue-specific distribution patterns, with PPAR $\beta/\delta$  being the most abundant in skeletal muscle. The receptors play key roles in the induction of genes involved in carbohydrate and lipid metabolism and storage [2]. PPARs also impart on the processes of wound healing, cell differentiation and cancer [3–5]. Fatty acids and some of their metabolites, as well as synthetic ligands, can modulate transcriptional activity through the PPARs. These observations render support for the notion that the PPAR receptors function as sensors for both endogenously and

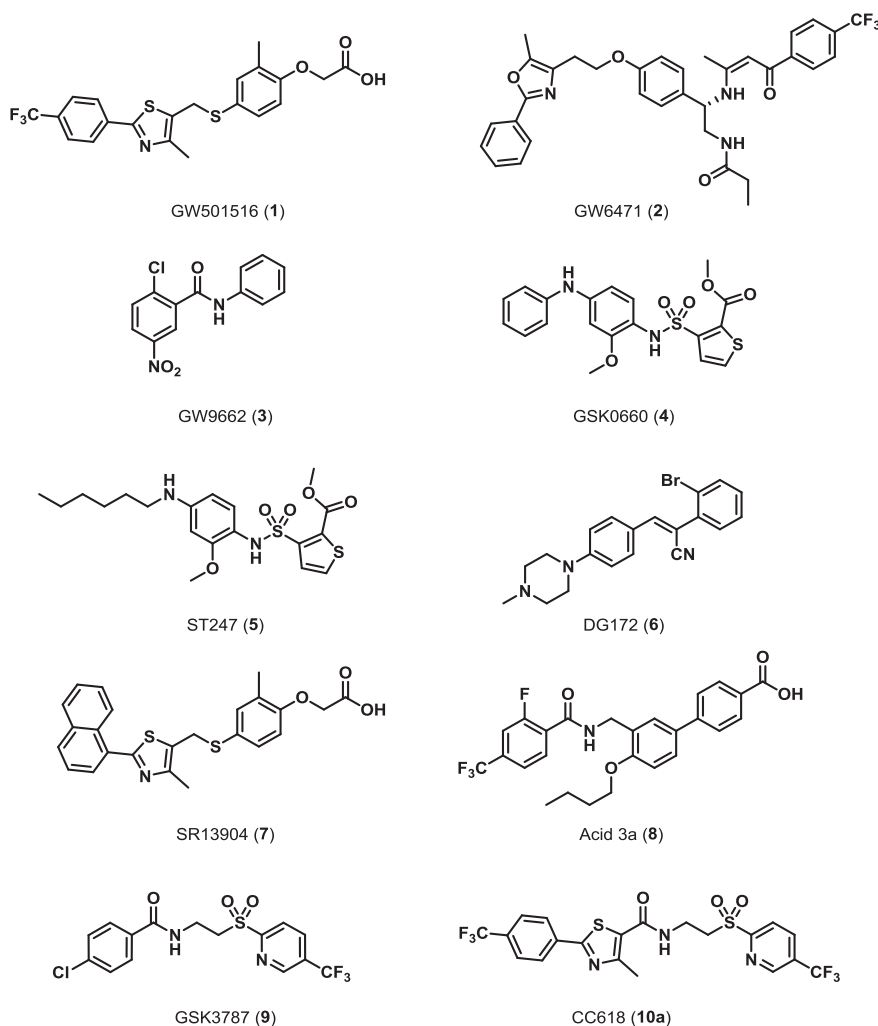
exogenously derived ligands [6]. The synthesis and development of selective ligands have proved instrumental in understanding the biological roles of the PPARs [7]. In the early days of PPAR research, the use of selective agonists against the three known PPAR subtypes was particularly important [8]. In the context of PPAR $\beta/\delta$ , the carboxylic acid GW501516 (1, Fig. 1) [9,10] has seen widespread use as a pharmacological tool. Later, potent and selective PPAR $\alpha$  and PPAR $\gamma$  antagonists, such as GW6471 (2) [11] and GW9662 (3) [12], were introduced (Fig. 1). Recent evidence for the involvement of PPAR $\beta/\delta$  in additional cellular functions [13–16] justifies the need for further development of antagonistic modulators of the  $\beta/\delta$ -subtype [17].

In 2008, Shearer et al. identified GSK0660 (4) via a high-throughput screen against PPARs of a GlaxoSmithKline in-house compound collection. GSK0660 (4) displayed an IC<sub>50</sub> value of 155 nM against PPAR $\beta/\delta$  and nearly ten-fold selectivity over the other PPAR subtypes. The antagonistic properties of GSK0660 (4) were established through the observation of a downregulation of GW501516-induced transcription of *bona fide* PPAR $\beta/\delta$  target genes. However, due to rapid clearance in rodents, GSK0660 (4) was not suited for further *in vivo* studies [18]. Later, Müller and coworkers

Abbreviations: PPAR, peroxisome proliferator-activated receptor; LC-MS, liquid chromatography – mass spectrometry; LBD, ligand-binding domain; LBP, ligand-binding pocket; ABC, ammonium bicarbonate.

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**Fig. 1.** PPAR $\beta/\delta$  modulators: The selective PPAR $\beta/\delta$  agonist GW501516 (**1**), known PPAR antagonists (**2–9**) and CC618 (**10a**).

used GSK0660 (**4**) as a lead-compound for structure-activity studies leading to e.g. the analog ST247 (**5**) [19]. Compound **5** displayed improved bioavailability and binding affinity [20]. The same research group recently developed the acrylonitrile compound DG172 (**6**) which exhibited potent PPAR $\beta/\delta$  antagonism and oral bioavailability [21]. As carboxylic acids are canonical agonists of the PPAR receptors, it is notable that also SR13904 (**7**) [22], reported by Zaveri et al. as well as the acid 3a (**8**) [23], reported by Kasuga et al., exhibited PPAR $\beta/\delta$  antagonism. In 2010, Shearer et al. reported the potent PPAR $\beta/\delta$  antagonist GSK3787 (**9**) [24] which displayed good oral pharmacokinetic properties. This compound was shown to contain a reactive moiety, resulting in the covalent modification of Cys249, a cysteine located centrally in the PPAR $\beta/\delta$  ligand-binding pocket (LBP).

## 2. Results and discussion

### 2.1. Synthesis of the ligands

The presence of a thiazole- or an oxazole heterocycle connected to an electron-poor phenyl ring appears to be beneficial for the binding of agonists, such as GW501516 (**1**) and other modulators [25–28], to the hydrophobic moieties in the LBP of PPAR $\beta/\delta$  [10,29].

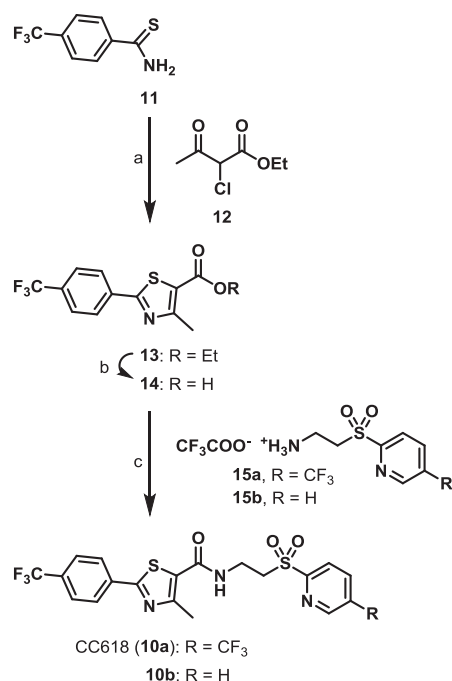
We envisioned that combining the arylthiazole moiety found in the potent and selective PPAR $\beta/\delta$  agonist GW501516 (**1**) with the 5-trifluoromethyl-2-pyridylsulfone moiety of GSK3787 (**9**) would result in antagonism against PPAR $\beta/\delta$  [28].

We thus synthesized CC618 (**10a**), starting with the condensation of thioamide **11** and  $\alpha$ -chloro- $\beta$ -ketoester **12**, as previously described [10], followed by basic hydrolysis, to give acid **14**. Reacting **14** with the ammonium trifluoroacetate salt **15a** [24] under peptide coupling conditions [30], afforded target compound **10a** (Scheme 1). In a similar approach, we prepared an analog of CC618, **10b**, lacking the 5-trifluoromethyl group on the pyridine ring.

### 2.2. In vitro evaluations of the ligands

The antagonistic properties of CC618 (**10a**) against the three known PPARs were first investigated in luciferase reporter gene assays in Cos-1 cells expressing each of the PPAR subtypes.

In the PPAR $\beta/\delta$ -expressing cells, CC618 antagonized the effects of 4 nM of GW501516 with an  $IC_{50}$  = 10.0  $\mu$ M. In this assay, the previously reported antagonist GSK3787 (**9**) displayed an  $IC_{50}$  = 5.0  $\mu$ M (Fig. 2A). The analog **10b**, lacking the 5-trifluoromethyl group, did not display any antagonistic effects (see Supplementary data,



**Scheme 1.** The synthesis of CC618 (**10a**) and analog **10b**: (a) EtOH,  $\Delta$ ; (b) NaOH (aq), EtOH,  $\Delta$ ; (c) **15a** or **15b**, Et<sub>3</sub>N, DCC, HOBT, CH<sub>2</sub>Cl<sub>2</sub>.

Fig. S1). On the other hand, neither CC618 (**10a**) nor GSK3787 (**9**) displayed any agonistic effects in our assay (Fig. 2B). Furthermore, in Cos-1 cells expressing PPAR $\alpha$ , only weak antagonistic effects were observed upon co-treatments of **10a** at various concentrations and 200  $\mu$ M of bezafibrate [31]. An even weaker antagonism was observed in Cos-1 cells expressing PPAR $\gamma$ , in the presence of various concentrations of **10a** and 10  $\mu$ M of rosiglitazone [32] (see Figs. S3 and S4 in the Supplementary data). These results demonstrate that CC618 (**10a**) is a selective PPAR $\beta/\delta$  antagonist.

### 2.3. CC618 covalently modifies Cys249 in PPAR $\beta/\delta$

To further investigate the fate of the PPAR $\beta/\delta$  receptor upon treatment with CC618 (**10a**) or GSK3787 (**9**), we performed LC-MS/MS analyses of the peptides obtained by reductive alkylation and trypsinization of PPAR $\beta/\delta$ , after a 15 min treatment with 10  $\mu$ M of CC618 (**10a**) or GSK3787 (**9**) (see Experimental section for details). In both cases we found that the mass of Cys249 increased by 145 Da, as evidenced by the y-ion series (Fig. 3). This mass

difference corresponds to the addition of a trifluoromethylpyridyl fragment to the cysteine, with concurrent loss of the thiol proton. These results are in accordance with previous mass spectrometrical observations for GSK3787 [24].

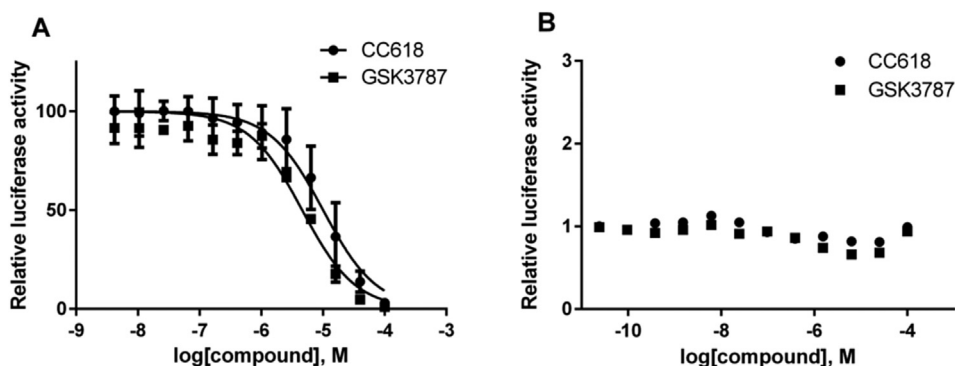
The presence of a reactive cysteine located centrally in the PPAR $\beta/\delta$  ligand binding pocket is a known trait, conserved throughout the PPARs [33]. The reactivity of the 5-trifluoromethyl-2-pyridylsulfone moiety towards a thiol or a thiolate, on the other hand, may be rationalized through a nucleophilic aromatic substitution on an electron-poor 2-pyridylsulfone, also called an *ipso*-substitution. In substitution reactions of alkylsulfonyl aromatics with thiolates or alkoxides, the sulfone moiety acts as the leaving group in the form of an alkylsulfinate (Scheme 2) [34,35].

The reactivity of the compounds **9** and **10a** towards nucleophiles, compared to simple arylsulfones, should thus be enhanced by the electron-withdrawing natures of both the pyridine nitrogen and the 5-trifluoromethyl group [35]. In a chemical context, a 5-*H* pyridylsulfone is reactive towards alkali metal alkoxide nucleophiles [34], but as compound **10b** did not display any antagonistic effects in the reporter assay, the presence of the 5-trifluoromethyl group on the pyridine ring appears to be necessary for the observed reactivity with Cys249. However, the observed effect of the presence of the 5-trifluoromethyl group, in the context of the PPAR $\beta/\delta$  LBP, may be of both a steric and an electronic nature.

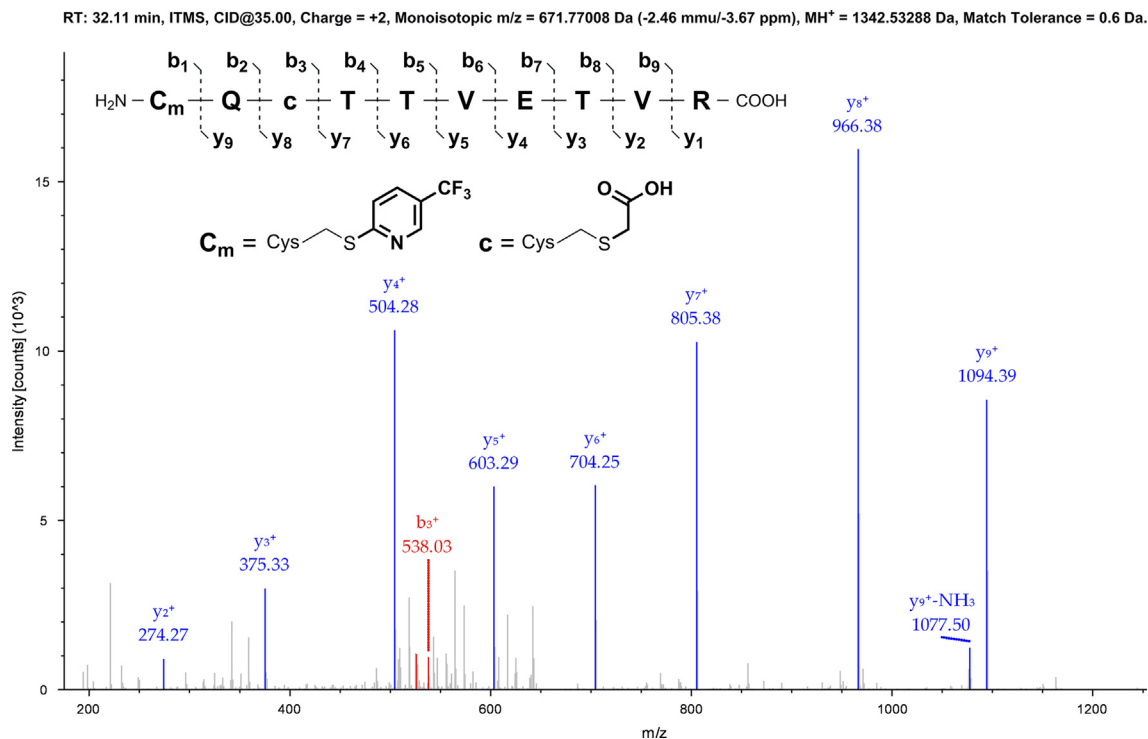
### 2.4. Molecular modeling

In order to visualize and compare the receptor–ligand interactions of CC618 (**10a**) and GSK3787 (**9**) prior to an eventual reaction with Cys249, we docked the intact ligands to the PPAR $\beta/\delta$  LBD (PDB: 3GZ9) using Molsoft ICM [36]. As depicted in Figs. 4 and 5, CC618 (**10a**) and GSK3787 (**9**) are found to bind with similar orientations in relation to the LBP, with C–S distances of 4.3 Å and 3.7 Å, respectively, between their C-2 pyridine ring carbons and the sulfur atom of Cys249. The 5-trifluoromethyl-2-pyridylsulfone moieties of both compounds occupy the arm of the LBP leading to the residues Tyr473, His413 and His287. Canonically, these residues accommodate the fatty acid carboxylate head group and form a hydrogen bonding network that is thought to be responsible for the stabilization of helix 12, through Tyr473 – a central trait in the pathway of classical agonism in nuclear hormone receptors [37]. The perturbation of this stabilization been hypothesized to be a mechanism of nuclear receptor antagonism [38].

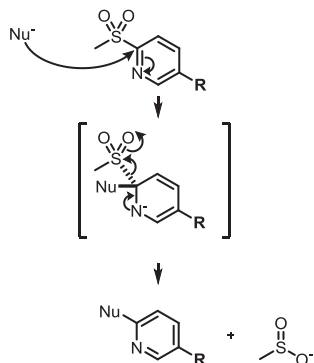
Currently, there are no single crystal x-ray structures of PPAR $\beta/\delta$  in complex with an antagonist available in the RCSB Protein Data Bank. Thus, we chose to employ the covalent docking protocol CovDock [39] implemented in the Schrödinger Suite [40] (see



**Fig. 2.** Effects of CC618 (**10a**) and GSK3787 (**9**) on PPAR $\beta/\delta$  in Cos-1 cells. A: Antagonist measurements (versus transcription induced with 4 nM GW501516). B: Agonist measurements (**10a** or **9** alone). The maximum response of the assay was determined as shown in Fig. S2 in the Supplementary data.



**Fig. 3.** LC-MS/MS analysis of PPAR $\beta/\delta$  treated with CC618 (**10a**): Representative MS/MS spectrum of the peptide C<sub>m</sub>QcTTVETVR (residues 249–258, C<sub>m</sub> = modified Cys249, c = carboxymethylated Cys251), obtained from tryptic digestion of human PPAR $\beta/\delta$  after incubation with 10  $\mu$ M of **10a**. Comparison of the mass of the tryptic peptide MH<sup>+</sup> and ion y9<sup>+</sup>, indicates that Cys249 has an increased mass of 145.13369 Da corresponding, within the match tolerance, to that of the 5-trifluoromethyl-2-pyridyl fragment.

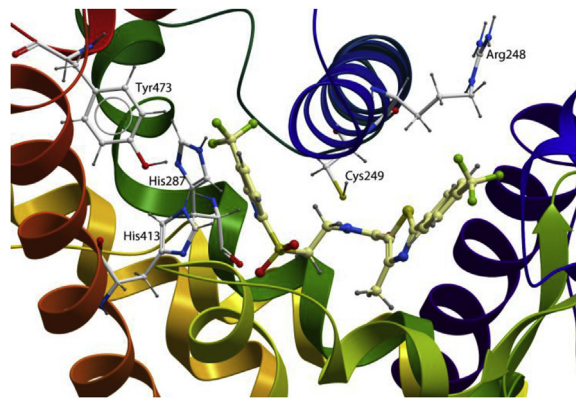


**Scheme 2.** Mechanistic insight: Schematic representation of a nucleophilic aromatic substitution at the 2-position of a 5-R-2-(methylsulfonyl)pyridine, in which R = H, CF<sub>3</sub>.

Supplementary data for details) in order to visualize possible conformational states of the Cys249 thioether resulting from a reaction with compounds **9** or **10a**, assuming that their chemical reactivity (*vide supra*) is relevant in the context of the protein. The three lowest energy poses of the 5-trifluoromethyl-2-pyridyl thioether (based on their Prime Energy property) are shown in Fig. 6. The obtained possible conformations of the 5-trifluoromethyl-2-pyridyl thioether in the PPAR $\beta/\delta$  LBP indicate that the covalently attached fragment may perturb Tyr473, His413 or His287 (and thus destabilize helix 12). It is also possible that the 5-trifluoromethyl-2-pyridyl thioether causes receptor inhibition by impeding the access of agonists to the arm of the LBP leading to the mentioned residues.

### 3. Conclusion

We have described the synthesis and biological evaluation of the selective PPAR $\beta/\delta$  antagonist CC618 (**10a**). The inhibitory effect of



**Fig. 4.** CC618 (**10a**) docked into PPAR $\beta/\delta$  (PDB: 3GZ9). Color coding of atoms: Red O, blue N, grey H, green F, mustard S and beige C in CC618 (**10a**); white C in PPAR $\beta/\delta$ . The coloring of the ribbons representing the PPAR $\beta/\delta$  backbone is purple, blue, green, yellow, orange and red from N-terminal to C-terminal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

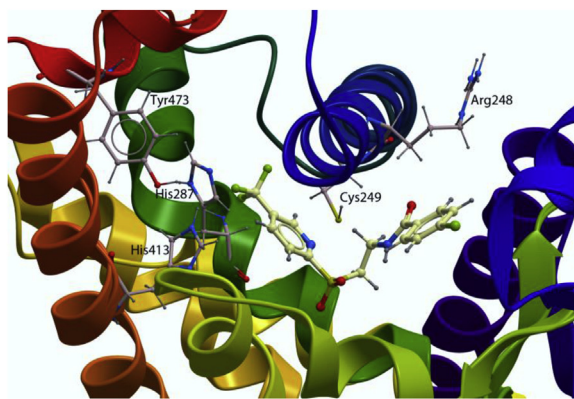
**10a** did not exceed that of GSK3787 (**9**), but our results render support for the covalent mode of action of the 5-trifluoromethyl-2-pyridylsulfone class of PPAR $\beta/\delta$  antagonists [24]. Moreover, non-covalent and covalent docking of CC618 (**10a**) and GSK3787 (**9**) to the PPAR $\beta/\delta$  LBP shed light on their similar mode of receptor inhibition.

## 4. Experimental section

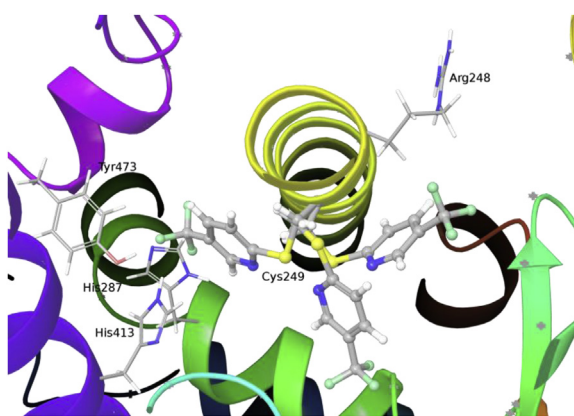
### 4.1. General

All commercially available reagents and solvents were used in the form they were supplied without any further purification. The





**Fig. 5.** GSK3787 (**9**) docked into PPAR $\beta/\delta$  (PDB: 3GZ9). Color coding of atoms: Red O, blue N, grey H, green F, mustard S and beige C in GSK3787 (**9**); white/pink C in PPAR $\beta/\delta$ . The coloring of the ribbons representing the PPAR $\beta/\delta$  backbone is purple, blue, green, yellow, orange and red from N-terminal to C-terminal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



**Fig. 6.** Covalent docking: Three favorable poses (based on their Prime-energy property) of the 5-(trifluoromethyl)-2-pyridyl thioether resulting from the covalent modification of Cys249 of PPAR $\beta/\delta$  (PDB: 3GZ9), generated with CovDock [39] from the Schrödinger Suite [40]. Color coding of atoms: Red O, blue N, white H, cyan F, yellow S and grey C. The coloring of the ribbons representing the PPAR $\beta/\delta$  backbone is brown, orange, yellow, green, blue and purple from N-terminal to C-terminal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stated yields are based on isolated material. The melting points are uncorrected. Thin layer chromatography was performed on silica gel 60 F<sub>254</sub> aluminum-backed plates fabricated by Merck. Flash column chromatography was performed on silica gel 60 (40–63  $\mu$ m) fabricated by Merck. NMR spectra were recorded on a Bruker AVII-400 or a Bruker DPX-300 spectrometer at 400 or 300 MHz, respectively, for  $^1\text{H}$  NMR and at 101 or 75 MHz, respectively, for  $^{13}\text{C}$  NMR. Coupling constants ( $J$ ) are reported in Hz and chemical shifts are reported in parts per million ( $\delta$ ) relative to the central residual solvent resonance in  $^1\text{H}$  NMR ( $\text{CDCl}_3 = \delta$  7.27 and  $\text{DMSO}-d_6 = \delta$  2.50) and the central carbon solvent resonance in  $^{13}\text{C}$  NMR ( $\text{CDCl}_3 = \delta$  77.00 ppm and  $\text{DMSO}-d_6 = \delta$  39.43). Mass spectra were recorded at 70 eV on a Waters Prospec Q spectrometer using EI as the method of ionization. High-resolution mass spectra were recorded on a Waters Prospec Q spectrometer using EI as the method of ionization. Determination of chemical purity of intermediates and final products was performed by HPLC on an Agilent Technologies 1200 Series instrument with a diode array detector set at 254 nm and equipped with a C18 reverse phase

column (Eclipse XDB-C18). The compounds **10a** and **10b** were >98% chemically pure, as judged by HPLC analyses. The TFA-salts **15a** and **15b** were prepared by deprotection of the corresponding *N*-tert butyl carbamates [24] with TFA in MeCN and used without further purification. Compounds **13** [30] and **14** [10] are known compounds. GSK3787 (**9**) [24] and GW501516 (**1**) [10] were prepared as previously described. Ammonium bicarbonate (ABC), 1,4-dithiothreitol (DTT), iodoacetic acid (IAA) all of analytical grade, formic acid for mass spectrometry (FA, ~98%), and sequencing grade L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin from bovine pancreas were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile hypergrade for LC–MS (MeCN) and dimethyl sulfoxide (DMSO, dried p.a.) were purchased from Merck, Darmstadt, Germany. MilliQ™ (mqH<sub>2</sub>O) water was produced in-house using a MilliQ™-system (Merck Millipore, Billerica, MA, USA).

## 4.2. Synthesis

### 4.2.1. Ethyl 4-methyl-2-4-(trifluoromethyl)phenyl-1,3-thiazole-5-carboxylate (**13**) [30]

To a suspension of 4-(trifluoromethyl)-thiobenzamide (3.0 g, 14.7 mmol) in EtOH (150 mL) was added, at room temperature, ethyl 2-chloroacetate (2.4 g, 2.0 mL, 14.7 mmol). The solution was refluxed for 40 h, and the solvent removed under reduced pressure. The solid material was stirred with cold hexane (50 mL) for 60 min, filtered, and washed with hexane ( $2 \times 25$  mL). Drying gave compound **13** (3.5 g, 75%) that was sufficiently pure for use in the next step. The spectroscopic data were in accord with those published.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.05 (d,  $J = 8.1$  Hz, 2H), 7.70 (d,  $J = 8.1$  Hz, 2H), 7.35 (q,  $J = 7.0$  Hz, 2H), 2.77 (s, 3H), 1.40 (t,  $J = 7.0$  Hz, 3H).

### 4.2.2. 4-Methyl-2-4-(trifluoromethyl)phenyl-1,3-thiazole-5-carboxylic acid (**14**) [10].

To a cooled solution of **13** (3.5 g, 11.1 mmol) in EtOH (20 mL) was added an aqueous solution of NaOH (20 mL of 6 M, 120.0 mmol) and the mixture was heated at 75–85 °C for 2 h. After evaporation of the ethanol under reduced pressure, the aqueous solution was diluted with water (20 mL) and acidified to pH 1 with concentrated aqueous HCl. The precipitated solid material was filtered and washed with water ( $2 \times 10$  mL) and  $\text{CH}_2\text{Cl}_2$  (10 mL). After drying on a vacuum pump, acid **14** (2.9 g, 92%) was obtained as a colorless powder that was sufficiently pure for use in the next step. The data was in accord with that published.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  13.20–13.45 (bs, 1H), 8.14 (d,  $J = 8.3$  Hz, 2H), 7.84 (d,  $J = 8.3$  Hz, 2H), 2.66 (s, 3H).

### 4.2.3. 4-Methyl-2-(4-(trifluoromethyl)phenyl)-N-(2-(5-(trifluoromethyl)pyridin-2-ylsulfonyl)ethyl)thiazole-5-carboxamide (**10a**)

To a stirred solution of 2-(5-(trifluoromethyl)pyridin-2-ylsulfonyl)ethanamine TFA salt (**15a**, 115 mg, 0.4 mmol) [24] and 4-methyl-2-(4-(trifluoromethyl)phenyl)thiazole-5-carboxylic acid (**14**) (150 mg, 0.4 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL), triethylamine (360  $\mu$ L, 2.6 mmol) was added. To this mixture *N,N'*-dicyclohexylcarbodiimide (124 mg, 0.6 mmol) and 1-hydroxybenzotriazole (81 mg, 0.6 mmol) were then added. The reaction mixture was stirred overnight at ambient temperature. The mixture was washed with  $\text{H}_2\text{O}$  ( $3 \times 50$  mL) and the remaining organic phase was dried ( $\text{MgSO}_4$ ) and concentrated. The residue was purified by column chromatography on silica gel with hexane/ethyl acetate (1:1) to obtain the title compound as a colorless solid (147 mg, 72%). Mp 181–182 °C. The purity (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/ $\text{H}_2\text{O}$ , 7:3, 1.0 mL/min):

$t_r(\text{major}) = 7.61 \text{ min}$   $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ ):  $\delta = 9.18$  (s, 1H), 8.56 (dd,  $J = 8.2, 1.5 \text{ Hz}$ , 1H), 8.28–8.20 (m, 2H), 8.10 (d,  $J = 8.2 \text{ Hz}$ , 2H), 7.87 (d,  $J = 8.3 \text{ Hz}$ , 2H), 3.88 (t,  $J = 6.1 \text{ Hz}$ , 2H), 3.68–3.60 (m, 2H), 2.57 (s, 3H).  $^{13}\text{C NMR}$  (100 MHz, DMSO- $d_6$ ):  $\delta = 164.0, 160.5, 160.0$  (q,  $J = 1.4 \text{ Hz}$ ), 156.1, 147.3 (q,  $J = 3.5 \text{ Hz}$ ), 137.0 (q,  $J = 3.2 \text{ Hz}$ ), 135.8 (q,  $J = 1.2 \text{ Hz}$ ), 130.5 (q,  $J = 32.3 \text{ Hz}$ ), 128.2 (q,  $J = 33.1 \text{ Hz}$ ), 126.9, 126.3 (q,  $J = 3.9 \text{ Hz}$ ), 126.2, 124.0 (q,  $J = 272.4 \text{ Hz}$ ), 122.2 (q,  $J = 273.4 \text{ Hz}$ ), 121.3, 50.3, 33.8, 17.0. HRMS calcd. for  $\text{C}_{20}\text{H}_{15}\text{F}_6\text{N}_3\text{O}_3\text{S}_2 [\text{M}]^+$ : 523.0459; found: 523.0446.

#### 4.2.4. 4-Methyl-2-(4-(trifluoromethyl)phenyl)-N-(2-(pyridin-2-ylsulfonyl)ethyl)thiazole-5-carboxamide (**10b**)

A solution of *tert*-butyl (2-(pyridin-2-ylsulfonyl)ethyl)carbamate [24] (214 mg, 0.75 mmol) in MeCN (3 mL) was cooled on an ice/water bath for a period of 5 min, followed by the dropwise addition of trifluoroacetic acid (3 mL). The solution was stirred for 48 h at ambient temperature, by which time the starting material had been consumed, as observed by TLC. The volatiles were removed at 60 °C under reduced pressure, using a water aspirator. Toluene was added to the crude material and the solvent was removed under the same conditions as above. The resulting slightly brown oil and 4-methyl-2-(4-(trifluoromethyl)phenyl)thiazole-5-carboxylic acid (**14**) (216 mg, 0.75 mmol) were dissolved in  $\text{CH}_2\text{Cl}_2$  (10 mL) and triethylamine (0.7 mL, 5.03 mmol) was added. To this mixture *N,N'*-dicyclohexylcarbodiimide (239 mg, 1.16 mmol) and 1-hydroxybenzotriazole (189 mg, 1.12 mmol) were then added. The reaction mixture was stirred at ambient temperature for 72 h, diluted with  $\text{CH}_2\text{Cl}_2$  (20 mL), and the organic phase washed with  $\text{H}_2\text{O}$  ( $4 \times 15 \text{ mL}$ ), dried ( $\text{MgSO}_4$ ) and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with heptane/ethyl acetate (1:1) to obtain the title compound as a colorless solid (121 mg, 35%). The purity (>98%) was determined by HPLC analysis (Eclipse XDB-C18, MeOH/ $\text{H}_2\text{O}$ , 6:4, 1.0 mL/min):  $t_r(\text{major}) = 11.88 \text{ min}$   $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.71 (d,  $J = 4.6 \text{ Hz}$ , 1H), 8.11 (d,  $J = 7.8 \text{ Hz}$ , 1H), 8.01 (d,  $J = 8.4 \text{ Hz}$ , 2H), 7.98 (partial t,  $J = 7.8 \text{ Hz}$ , 1H), 7.67 (d,  $J = 8.2 \text{ Hz}$ , 1H), 7.56 (dd,  $J = 7.7, 4.8 \text{ Hz}$ , 1H), 7.05 (bt,  $J = 5.8 \text{ Hz}$ , 1H), 3.99 (q,  $J = 5.7 \text{ Hz}$ , 2H), 3.71 (dd,  $J = 7.0, 4.3 \text{ Hz}$ , 2H), 2.74 (s, 3H).  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ )  $\delta$  165.5, 161.5, 157.0, 156.5, 150.2, 138.4, 135.8 (q,  $J = 1.3 \text{ Hz}$ ), 132.2 (q,  $J = 32.8 \text{ Hz}$ ), 127.7, 126.8 (s, 2C), 126.7, 125.9 (q,  $J = 3.8 \text{ Hz}$ , 2C), 123.7 (q,  $J = 272.4 \text{ Hz}$ ), 122.0, 51.8, 33.8, 17.4.  $^1\text{H NMR}$  (400 MHz, DMSO- $d_6$ )  $\delta$  8.78 (d,  $J = 4.7 \text{ Hz}$ , 1H), 8.36 (bt,  $J = 5.5 \text{ Hz}$ , 1H), 8.16 (partial t,  $J = 7.9 \text{ Hz}$ , 1H), 8.13 (d,  $J = 7.7 \text{ Hz}$ , 2H), 8.08 (d,  $J = 7.8 \text{ Hz}$ , 1H), 7.88 (d,  $J = 8.1 \text{ Hz}$ , 2H), 7.72 (dd,  $J = 7.6, 4.8 \text{ Hz}$ , 1H), 3.77 (t,  $J = 6.6 \text{ Hz}$ , 2H), 3.61 (q,  $J = 6.3 \text{ Hz}$ , 2H), 2.59 (s, 3H).  $^{13}\text{C NMR}$  (101 MHz, DMSO- $d_6$ )  $\delta$  163.9, 160.6, 156.5, 155.6, 150.2, 139.0, 135.7 (q,  $J = 1.3 \text{ Hz}$ ), 130.4 (prq,  $J = 32.0 \text{ Hz}$ ), 128.0, 126.9 (s, 2C), 126.8, 126.2 (q,  $J = 3.8 \text{ Hz}$ , 2C), 123.8 (prq,  $J = 272.4 \text{ Hz}$ ), 121.7, 50.1, 33.6, 16.9. HRMS calcd. for  $\text{C}_{19}\text{H}_{16}\text{F}_3\text{N}_3\text{O}_3\text{S}_2 [\text{M}]^+$ : 455.0585; found: 455.0578.

### 4.3. In vitro luciferase assays

#### 4.3.1. Antagonist measurements in luciferase-based transient reporter system [25,41].

Cos-1 cells (ATCC no. CRL-1650) were maintained in DMEM (Invitrogen, Carlsbad, CA) containing gentamicin (10  $\mu\text{g/mL}$ ) and fetus bovine serum (10%), at 37 °C in a humidified atmosphere of 5%  $\text{CO}_2$ . Cell confluence never exceeded 80% before sub-culturing or transfection. The pSG5-Gal4-hPPAR-LBD ( $\alpha$ ,  $\gamma$  and  $\beta/\delta$  subtypes) expression constructs were generous gifts from Dr. Hilde Nebb, University of Oslo, and the pGL3-5XUAS-SV40 luciferase reporter construct was purchased from Promega Corporation, Madison, WI. Cos-1 cells were transiently transfected with 1.7  $\mu\text{g}$  of the expression plasmids and 8.5  $\mu\text{g}$  of the reporter construct per  $1 \times 10^7$  cells. Transient transfection was achieved using the Neon electroporation

system (Invitrogen). Cells were seeded ( $2 \times 10^4/\text{well}$ ) in 96-plates (white F96 microwell, Nalge Nunc Int., Rochester, NY) and allowed to attach (5 h) before the test compounds were added. Transfected cells were treated for 19 h with either CC618 (**10a**) or **10b** in dilution series, as indicated on the x-axis in Fig. 2A and in Fig. S1, using vehicle supplemented with 4 nM of the PPAR $\beta/\delta$  agonist GW501516 (**1**). All values are given relative to the luciferase activity obtained by activating the reporter gene system with 4 nM GW501516 (**1**). For comparison, the antagonist activity of GSK3787 (**9**) is demonstrated in the same assay system. All values are relative to the luciferase activity obtained by activating the reporter gene system with 207  $\mu\text{M}$  of GW501516 (**1**), as indicated on the x-axis in Fig. S2. The  $\text{EC}_{50}$ -value of GW501516 (**1**) was found to be 0.5 nM. Luc protein expression was developed (One-Glo Luc assay system, Promega Biosciences, CA USA) and quantified on a luminometer (Envision, PerkinElmer, MA, US). The figures were generated using GraphPad Prism version 6.0, GraphPad Software, La Jolla, California, USA.

### 4.4. Liquid chromatography – mass spectrometry

#### 4.4.1. Ligand treatment and in-solution digestion of N-His<sub>6</sub>-PPAR $\beta/\delta$

Human recombinant N-His<sub>6</sub>-PPAR $\beta/\delta$  (10  $\mu\text{g}$ , 1 mg/mL, Cayman Chemical), stored at –80 °C ( $\text{CO}_2$  (s)), was thawed on an ice/water bath, diluted to a concentration of 50  $\mu\text{g/mL}$  using 190  $\mu\text{L}$  of freshly made 50 mM ABC buffer, fractionated to 10 stock solutions of 20  $\mu\text{L}$  and subsequently refrozen at –30 °C. Prior to ligand treatment and in-solution digestion, 10  $\mu\text{L}$  of the above described 50  $\mu\text{g/mL}$  stock solution of human recombinant N-His<sub>6</sub>-PPAR $\beta/\delta$  was thawed on ice in a Protein LoBind Eppendorf tube (Eppendorf AG, Hamburg, Germany) and subsequently diluted with 10  $\mu\text{L}$  of a freshly made solution of 375  $\mu\text{M}$  of DTT in 50 mM ABC buffer. The solution was left on ice for 5 min and then added 10  $\mu\text{L}$  of a 30  $\mu\text{M}$  solution of compound **9** or **10a** in 50 mM ABC buffer (w/3.9% of MeCN), resulting in a final concentration of 125  $\mu\text{M}$  of DTT [42] and 10  $\mu\text{M}$  of ligand. The solution was incubated at ambient temperature for 15 min, diluted with 55  $\mu\text{L}$  of 50 mM ABC buffer and added 5  $\mu\text{L}$  of a solution containing 50 mM DTT in 50 mM ABC buffer. After incubation for 15 min at 95 °C and subsequent cooling to ambient temperature, 5  $\mu\text{L}$  of a freshly prepared solution of 250 mM iodoacetic acid (IAA) in 50 mM ABC buffer was added and the solution was incubated in the dark at ambient temperature for 20 min. Following alkylation, 5  $\mu\text{L}$  of a freshly prepared solution of containing 2.5  $\mu\text{g/mL}$  bovine trypsin in 50 mM ABC buffer (trypsin:protein ratio, 1:40 (w/w)) was added and the sample was incubated at 37 °C overnight using a Thermomixer Comfort (Thermo Scientific) at 800 rpm. The final protein concentration was 5  $\mu\text{g/mL}$  in all experiments.

#### 4.4.2. LC-MS/MS analysis

Samples of 20  $\mu\text{L}$  of the proteolytic peptide mixtures were injected into the Chromeleon Xpress-controlled Dionex HPLC system (Thermo Scientific, Bremen, Germany) and trapped on a C18, 5 mm  $\times$  300  $\mu\text{m}$  i.d. Acclaim PepMap 100 (5  $\mu\text{m}$ ) enrichment column (Thermo Scientific). The loading mobile phase consisting of 3% MeCN, 0.1% FA and 97%  $\text{mqH}_2\text{O}$ , was delivered at 10  $\mu\text{L/min}$  for 4 min. The analytes were transferred to a 150  $\times$  0.075 mm i.d. Acclaim PepMap 100 (pore size 100 Å, particle diameter 3  $\mu\text{m}$ ; Thermo Scientific) at 300 nL/min. The mobile phases consisted of A: 5% MeCN, 3% DMSO, 0.1% FA and 92%  $\text{mqH}_2\text{O}$ , and B: 95% MeCN, 3% DMSO, 0.1% FA and 2%  $\text{mqH}_2\text{O}$ . A linear gradient was run from 0% to 50% B in 60 min. Subsequently, the elution strength was increased to 100% B, before the column was regenerated for at least 10 column volumes. Total analysis time per run was 87 min. The LC setup was connected to an Xcalibur 2.0.7-controlled LTQ Discovery Orbitrap

MS equipped with a Nano-ESI ion source (Thermo Fischer). The nanospray ionization source was operated in the positive ionization mode (360  $\mu\text{m}$  o.d.  $\times$  20  $\mu\text{m}$  i.d. distal coated fused silica emitter, 10  $\mu\text{m}$  i.d. tip (New Objective, Woburn, MA, USA)). The spray voltage was set at 2.2 kV. The heated capillary was kept at 275  $^{\circ}\text{C}$ . The capillary voltage was set at 45 V and the tube lens offset at 120 V. The mass spectrometer was operated in data-dependent positive ion mode. Survey MS scans were performed in the orbitrap analyzer at a resolution of 30,000, over a mass range between  $m/z$  250–2000 Da, with charge state disabled. The up to 6 most intense ions per scan were fragmented by collision induced dissociation (CID) at 35% relative collision energy, activation time of 30 ms, minimum signal required of 500 and analyzed in the linear ion trap. The wide band activation option was enabled and dynamic exclusion of a time window of 15 s was used to minimize the extent of repeat sequencing of the peptides.

#### 4.4.3. Data interpretation

The MS raw files were processed with Proteome Discoverer 1.3 (Thermo Scientific), using the Sequest algorithm, searching against the FASTA file ipi.HUMANpr. May 22, 2014. Enzyme specificity was set to trypsin. The initial parent and fragment ion maximum mass deviations were set to 10 ppm and 0.6 Da, respectively. The search included cysteine carboxymethylation and 5-CF<sub>3</sub>-2-pyridyl modification of cysteine, histidine, arginine or lysine as variable modifications. Peptides had to be fully tryptic, and up to two missed cleavages were allowed. A decoy database search was performed by searching against a database containing the reversed protein sequences with a strict target false discovery rate (FDR) of 0.01 and a relaxed FDR of 0.05.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2015.03.006>.

#### References

- [1] D.J. Mangelsdorf, C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, R.M. Evans, *Cell* 83 (1995) 835–839. [http://dx.doi.org/10.1016/0092-8674\(95\)90199-X](http://dx.doi.org/10.1016/0092-8674(95)90199-X).
- [2] V. Zoete, A. Grosdidier, O. Michielin, *Biochim. Biophys. Acta* 1771 (2007) 915–925. <http://dx.doi.org/10.1016/j.bbap.2007.01.007>.
- [3] L. Michalik, W. Wahli, *Biochim. Biophys. Acta* 1771 (2007) 991–998. <http://dx.doi.org/10.1016/j.bbap.2007.02.004>.
- [4] J.M. Peters, F.J. Gonzalez, *Biochim. Biophys. Acta* 1796 (2009) 230–241. <http://dx.doi.org/10.1016/j.bbcan.2009.06.002>.
- [5] B.P. Kota, T.H.-W. Huang, B.D. Roufogalis, *Pharm. Res.* 51 (2005) 85–94. <http://dx.doi.org/10.1016/j.phrs.2004.07.012>.
- [6] B. Desvergne, L. Michalik, W. Wahli, *Physiol. Rev.* 86 (2006) 465–514. <http://dx.doi.org/10.1152/physrev.00025.2005>.
- [7] T.M. Willson, P.J. Brown, D.D. Sternbach, B.R. Henke, *J. Med. Chem.* 43 (2000) 527–550. <http://dx.doi.org/10.1021/jm990554g>.
- [8] B. Pourcet, J.-C. Fruchart, B. Staels, C. Glineur, *Expert Opin. Emerg. Drugs* 11 (2006) 379–401. <http://dx.doi.org/10.1517/14728214.11.3.379>.
- [9] W.R. Oliver, J.L. Shenk, M.R. Snaith, C.S. Russell, K.D. Plunket, N.L. Bodkin, M.C. Lewis, D.A. Winegar, M.L. Sznaidman, M.H. Lambert, et al., *Proc. Nat. Acad. Sci.* 98 (2001) 5306–5311. <http://dx.doi.org/10.1073/pnas.091021198>.
- [10] M.L. Sznaidman, C.D. Haffner, P.R. Maloney, A. Fivush, E. Chao, D. Goreham, M.L. Sierra, C. LeGrumelec, H.E. Xu, V.G. Montana, M.H. Lambert, T.M. Willson, W.R. Oliver Jr., D.D. Sternbach, *Bioorg. Med. Chem. Lett.* 13 (2003) 1517–1521. [http://dx.doi.org/10.1016/S0960-894X\(03\)00207-5](http://dx.doi.org/10.1016/S0960-894X(03)00207-5).
- [11] H.E. Xu, T.B. Stanley, V.G. Montana, M.H. Lambert, B.G. Shearer, J.E. Cobb, D.D. McKee, C.M. Galaridi, K.D. Plunket, R.T. Nolte, D.J. Parks, J.T. Moore, S.A. Kliewer, T.M. Willson, J.B. Stimmel, *Nature* 415 (2002) 813–817. <http://dx.doi.org/10.1038/415813a>.
- [12] L.M. Leesnitzer, D.J. Parks, R.K. Bledsoe, J.E. Cobb, J.L. Collins, T.G. Consler, R.G. Davis, E.A. Hull-Ryde, J.M. Lenhard, L. Patel, K.D. Plunket, J.L. Shenk, J.B. Stimmel, C. Theraponts, T.M. Willson, S.G. Blanchard, *Biochemistry* 41 (2002) 6640–6650. <http://dx.doi.org/10.1021/bi0159581>.
- [13] G.S. Harmon, M.T. Lam, C.K. Glass, *Chem. Rev.* 111 (2011) 6321–6340. <http://dx.doi.org/10.1021/cr2001355>.
- [14] K.D. Wagner, N. Wagner, *Pharmacol. Ther.* 125 (2010) 423–435. <http://dx.doi.org/10.1016/j.pharmthera.2009.12.001>.
- [15] T. Adhikary, K. Kaddatz, F. Finkernagel, A. Schönbauer, W. Meissner, M. Scharfe, M. Jarek, H. Blöcker, S. Müller-Brüsselbach, R. Müller, *PLoS One* 6 (2011) e16344. <http://dx.doi.org/10.1371/journal.pone.0016344>.
- [16] T. Adhikary, D.T. Brandt, K. Kaddatz, J. Stockert, F. Finkernagel, S. Naruhn, W. Meissner, J. Obert, S. Lieber, M. Scharfe, M. Jarek, P.M. Toth, F. Scheer, W.E. Diederich, S. Reinartz, R. Grosse, S. Müller-Brüsselbach, R. Müller, *Oncogene* 32 (2013) 5241–5252. <http://dx.doi.org/10.1038/ncr.2012.549>.
- [17] A. Amazzalorso, B. De Filippis, L. Giampietro, R. Amoroso, *ChemMedChem* 8 (2013) 1609–1616. <http://dx.doi.org/10.1002/cmdc.201300250>.
- [18] B.G. Shearer, D.J. Steger, J.M. Way, T.B. Stanley, D.C. Lobe, D.A. Grillot, M.A. Iannone, M.A. Lazar, T.M. Willson, A.N. Billin, *Mol. Endocrinol.* 22 (2008) 523–529. <http://dx.doi.org/10.1210/me.2007-0190>.
- [19] S. Naruhn, P.M. Toth, T. Adhikary, K. Kaddatz, V. Pape, S. Dorr, G. Klebe, S. Müller-Brüsselbach, W.E. Diederich, R. Müller, *Mol. Pharmacol.* 80 (2011) 828–838. <http://dx.doi.org/10.1124/mol.111.074039>.
- [20] P.M. Toth, S. Naruhn, V.F. Pape, S.M. Dorr, G. Klebe, R. Müller, W.E. Diederich, *ChemMedChem* 7 (2012) 159–170. <http://dx.doi.org/10.1002/cmdc.201100408>.
- [21] S. Lieber, F. Scheer, W. Meissner, S. Naruhn, T. Adhikary, S. Müller-Brüsselbach, W.E. Diederich, R. Müller, *J. Med. Chem.* 55 (2012) 2858–2868. <http://dx.doi.org/10.1021/jm2017122>.
- [22] N.T. Zaveri, B.G. Sato, F. Jiang, J. Calaoagan, K.R. Laderoute, B.J. Murphy, *Cancer Biol. Ther.* 8 (2009) 1252–1261. <http://dx.doi.org/10.4161/cbt.8.13.8691>.
- [23] J.-i. Kasuga, S. Ishida, D. Yamasaki, M. Makishima, D. Doi, Y. Hashimoto, H. Miyachi, *Bioorg. Med. Chem. Lett.* 19 (2009) 6595–6599. <http://dx.doi.org/10.1016/j.bmcl.2009.10.021>.
- [24] B.G. Shearer, R.W. Wiethe, A. Ashe, A.N. Billin, J.M. Way, T.B. Stanley, C.D. Wagner, R.X. Xu, L.M. Leesnitzer, R.V. Merrihew, T.W. Shearer, M.R. Jeune, J.C. Ulrich, T.M. Willson, *J. Med. Chem.* 53 (2010) 1857–1861. <http://dx.doi.org/10.1021/jm900464j>.
- [25] C.C. Ciocoiu, N. Nikolic, H.H. Nguyen, G.H. Thoresen, A.J. Aasen, T.V. Hansen, *Eur. J. Med. Chem.* 45 (2010) 3047–3055. <http://dx.doi.org/10.1016/j.ejmech.2010.03.035>.
- [26] C.C. Ciocoiu, A.W. Ravna, I. Sylte, T.V. Hansen, *Arch. Pharm. Chem. Life Sci.* 343 (2010) 612–624. <http://dx.doi.org/10.1002/ardp.201000189>.
- [27] C.C. Ciocoiu, A.W. Ravna, I. Sylte, A.C. Rustan, T.V. Hansen, *Bioorg. Med. Chem.* 19 (2011) 6982–6988. <http://dx.doi.org/10.1016/j.bmc.2011.10.020>.
- [28] C. C. Ciocoiu, Ph. D. Thesis, University of Oslo (NO), 2010. <http://urn.nb.no/URN:NBN:no-26907>.
- [29] H.E. Xu, M.H. Lambert, V.G. Montana, D.J. Parks, S.G. Blanchard, P.J. Brown, D.D. Sternbach, J.M. Lehmann, G.B. Wisely, T.M. Willson, S.A. Kliewer, M.V. Milburn, *Mol. Cell.* 3 (1999) 397–403. [http://dx.doi.org/10.1016/S1097-2765\(00\)80467-0](http://dx.doi.org/10.1016/S1097-2765(00)80467-0).
- [30] M.L. Sierra, V. Beneton, A.-B. Boullay, T. Boyer, A.G. Brewster, F. Donche, M.-C. Forest, M.-H. Fouchet, F.J. Gellibert, D.A. Grillot, M.H. Lambert, A. Laroze, C. Le Grumelec, J.M. Linget, V.G. Montana, V.-L. Nguyen, E. Nicodème, V. Patel, A. Penfornis, O. Pineau, D. Pohin, F. Potvain, G. Poulain, C.B. Ruault, M. Saunders, J. Toum, H.E. Xu, R.X. Xu, P.M. Pianetti, *J. Med. Chem.* 50 (2007) 685–695. <http://dx.doi.org/10.1021/jm058056x>.
- [31] A. Tenenbaum, M. Motro, E. Fisman, *Cardiovasc. Diabetol.* 4 (2005) 14. <http://dx.doi.org/10.1186/1475-2840-4-14>.
- [32] T.M. Willson, J.E. Cobb, D.J. Cowan, R.W. Wiethe, I.D. Corea, S.R. Prakash, K.D. Beck, L.B. Moore, S.A. Kliewer, J.M. Lehman, *J. Med. Chem.* 39 (1996) 665–668. <http://dx.doi.org/10.1021/jm950395a>.
- [33] F. Grün, *Biochemical and biological effects of Organotin*, Chapter 3, in: A. Pagliarini, F. Trombetti, V. Ventrella (Eds.), Bentham Science, 2012, pp. 53–69. <http://dx.doi.org/10.2174/978160805265311201010001>.
- [34] N. Furukawa, S. Ogawa, T. Kawai, S. Oae, *Tetrahedron Lett.* 24 (1983) 3243–3246. [http://dx.doi.org/10.1016/S0040-4039\(00\)88146-0](http://dx.doi.org/10.1016/S0040-4039(00)88146-0).
- [35] S. Oae, N. Furukawa, in: Alan R. Katritzky (Ed.), *Advances in Heterocyclic Chemistry*, vol. 48, Academic Press Inc., 1990, pp. 1–63. [http://dx.doi.org/10.1016/S0065-2725\(08\)60337-60342](http://dx.doi.org/10.1016/S0065-2725(08)60337-60342).
- [36] R. Abagyan, M. Totrov, D. Kuznetsov, *J. Comp. Chem.* 15 (1994) 488–506. <http://dx.doi.org/10.1002/jcc.540150503>.
- [37] H.E. Xu, M.H. Lambert, V.G. Montana, K.D. Plunket, L.B. Moore, J.L. Collins, J.A. Oplinger, S.A. Kliewer, R.T. Gampe, D.D. McKee, J.T. Moore, T.M. Willson, *Proc. Nat. Acad. Sci.* 98 (2001) 13919–13924. <http://dx.doi.org/10.1073/pnas.241410198>.



- [38] Y. Hashimoto, H. Miyachi, *Bioorg. Med. Chem.* 13 (2005) 5080–5093. <http://dx.doi.org/10.1016/j.bmc.2005.03.027>.
- [39] K. Zhu, K.W. Borrelli, J.R. Greenwood, T. Day, R. Abel, R.S. Farid, E. Harder, *J. Chem. Inf. Model* 54 (2014) 1932–1940. <http://dx.doi.org/10.1021/ci500118s>.
- [40] Schrödinger Release 2014-2: *Maestro, Version 9.8*, Schrödinger, LLC, New York, NY, 2014.
- [41] I. Tzamelis, H. Fang, M. Ollero, S. Shi, J.K. Hamm, P. Kievit, A.N. Hollenberg, J.S. Flier, *J. Biol. Chem.* 279 (2004) 36093–36102. <http://dx.doi.org/10.1074/jbc.M405346200>.
- [42] F.J. Schopfer, M.P. Cole, A.L. Groeger, C.-S. Chen, N.K.H. Khoo, S.R. Woodcock, F. Golin-Bisello, U.N. Motanya, Y. Li, J. Zhang, et al., *J. Biol. Chem.* 285 (2010) 12321–12333. <http://dx.doi.org/10.1074/jbc.M109.091512>.