

Virtual docking of 2-phenylthio-2-(methyl)propanoic acid derivatives: insights into new selective PPAR α agonists

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INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor superfamily of ligand-activated transcription factors. There are three known subtypes, namely, PPAR α , PPAR γ , and PPAR δ distributed in a spatial fashion among different tissues. PPARs form heterodimers with retinoid X receptor (RXR) and activate subsets of genes controlling lipids, carbohydrate, and energy homeostasis.¹ Thus, inadequate activation or inactivation of PPARs is directly linked to pathological processes such as type 2 diabetes, cardiovascular diseases, obesity, and dyslipidemia. One of the subtypes, PPAR α , is present at high density mainly in the liver and regulates the expression of genes encoding lipid and lipoprotein metabolism. Fibrates are hypolipidemic agents that are very efficient in lowering elevated triglyceride concentrations.² Their action on lipid metabolism is mediated principally by activation of PPAR α , leading to altered

expression of genes involved in lipid and lipoprotein metabolism. Although fibrates are ligands of PPAR α , their affinity is weak and their subtype-selectivity is poor.³ PPAR α is also involved in cardiac hypertrophy, a pathology related to hypertension, myocardial infarct, valvular pathologies and hypertrophic cardiac myopathy.⁴ Recent studies have demonstrated that cardiac hypertrophy is not only an adaptive state that precedes heart failure, but it is a sufficient risk factor for ischaemic heart disease, arrhythmia and sudden death. During the development of cardiac hypertrophy, myocardial substrates switch from fatty acid to glucose as major source of energy. This metabolic switch involves PPAR α deactivation at transcriptional level as well as at post-translational level. Consequently, the activation of PPAR α receptor could have a beneficial effect on these pathologies. The aim of this work is the enhancement of α -subtype selectivity

for new compounds belonging to a thioisobutyrate series.⁵ So, we docked literature agonists on the X-ray crystal structures of PPAR α and PPAR γ in complex with GW-409544⁶ and GI-262570⁷ respectively (known potent PPAR agonists; pdb accession code 1K7L and 1FM9). We performed a calibration curve using Emodel values (as derived from Glide software⁸) versus transactivation activity values. Then we developed a classification method using EVA molecular description,⁹ to improve and potency versus PPAR α subtype receptor on 13 thioisobutyrate derivatives. A new lead compound, **21** (ST1983 or the corresponding acid ST2622) containing the indole moiety, was identified. Subsequently we enumerated a library of new thioisobutyrate meta- and para-phenoxy derivatives, using commercially available indoles, to explore new structural features.

MATERIALS AND METHODS

Computational methods

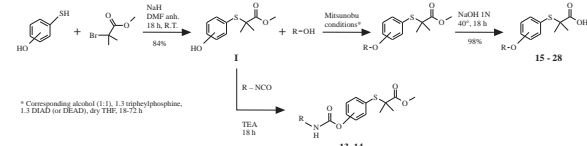
The X-ray crystal structures of both PPAR α and PPAR γ used in this work contain bound ligands: the potent agonists GW-409544 and GI-262570 respectively. Water molecules of crystallization were removed from the complexes, and the proteins prepared for docking using the PPREP utility; docking calculations were performed using the Glide program and the 2001 implementation of the OPLS-AA force field (FirstDiscovery v2.7¹⁰). The binding site, for which the various energy grids were calculated and stored, is defined in terms of two concentric boxes: the bounding box, which must contain the centre of any acceptable ligand pose, and the enclosing box, which must contain all ligand atoms of an acceptable pose. Cubes with an edge length of 14 Å and centred at the midpoint of the longest atom-atom distance in the respective co-crystallized ligands defined the bounding boxes in both proteins. Default input parameters were used in all of the docking calculations. Upon completion of each docking calculation at least three different poses per ligand were stored and examined. Glide's primary scoring function is GlideScore (abbreviated as GScore). Data reported in literature^{6,7} and our evidences, however, suggested that GScore did not satisfactorily correlate with experimental activity values and another scoring function used by Glide, Emodel, was explored. The Emodel function is itself derived from a combination of the GScore, Coulombic, and van der Waals energies and the strain energy of the ligand, and it is reported to give better agreement with experimental data.

The ligands used in this study, along with their transactivation activity values are shown in Table 1 and Table 2. Calculations were performed for all the molecules in their acidic form and dissociated state, even if the in house compound activities were evaluated also for ester forms. EVA description¹¹ for compounds listed in Table 2, was derived from calculated infrared (IR-range) vibrational frequencies. The normal modes of vibration for each input molecule were calculated using Spartan '02¹² where conformations deriving from the selected poses of docking were used. The normal modes of vibration are used to build up a vibrational profile between 0 and 4000 cm⁻¹ which provide a description consisting of ~800 variables. On the basis of PPAR α transactivation values (as shown in Table 2), the compounds having acidic form were grouped in equal active (<100% at 20 μ M; light red), and more active (>200% at 20 μ M; light blue) than WY-14,643 (known potent PPAR α agonist, used as reference compound at 2 μ M). EVA descriptors were used as independent variables, and the two classes values, were used as dependent variables in a PLS-DA method to derive a QSAR model (SIMCA-P+ 10.0¹³). The same model was used to predict the classification of a virtual library (created with TSAR3D v.3.3¹⁴) of indole derivatives mono- or di-substituted on the position 2, 3, 4, 5, 6 and 7. In Table 3 are reported the predicted active compounds and their calculated ADME properties (ACD v.7.0¹⁵).

Chemistry

The starting material for thioisobutyrate synthesis was 3- or 4-hydroxythiophenol, which by reaction with bromo-isobutyrate in the presence of NaH gave intermediates **1** in 84% yield. **1** was reacted with the appropriate substituted alcohol, under Mitsunobu conditions, to give the corresponding esters **15-28** (Scheme 1), whose yields are reported in Table 2. Compounds **13** and **14**, were prepared by reaction of **1** with the appropriate substituted isocyanate. The acidic forms were obtained from the corresponding esters through basic hydrolysis.

Scheme 1



* Corresponding alcohol (1:1, 1:1, 1:3 trihydroxyphenols, 1:1 OHAD (or OHAD), dry THF, 18-72 h)

Biology

Compounds reported in Table 2, on cell-based transactivation assays to evaluate their putative *in vitro* agonist activity on mouse PPAR α and PPAR γ respectively. Compounds were tested on COS-7 cells for the PPAR α specific assay, and their activity was compared with that of 2 μ M WY-14,643, a known selective PPAR α agonist. PPAR γ agonist activity was tested on NIH-3T3 cells, but using 2 μ M rosiglitazone, a selective PPAR γ activator, as reference compound.⁵ Data shown in Table 2 represent the percentages of activity, at 20 μ M, relative to that seen in control cells treated with the reference compound (2 μ M WY-14,643 or rosiglitazone), set to 100%. Binding values for human PPAR γ receptor (pK_i) were also measured, using the method reported in ref. 17 with minor modifications.

Figure 2 (a) **26** (ST2740, coloured by atom) docked in PPAR α receptor (dark orange). (b) **13** (ST2031, coloured by atom) docked in PPAR γ receptor (dark orange). The isoform- and species-specific residue Thr 279 is coloured in light yellow. White dashed lines represent H bonds between agonist and receptor. Hydrogen atoms are not shown.

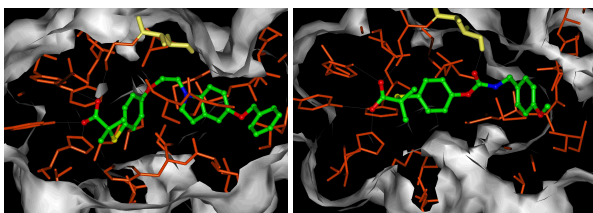


Figure 3. Scatter plot of PLS-DA scores of the molecules used for build up the model (in black) the compounds defined equal active and in red those defined more active than WY-14,643 and of virtual library, predicted by the model (in light grey).

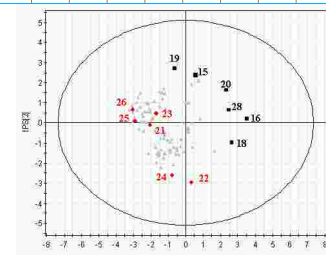


Table 3. Selected compounds with some physico-chemical properties.

N°	NAME	pKa	LogD pH=7.4	Log(1/S) pH=7.4	PPAR α Emodel
29	ST1983_4Cl	3.78	2.4	2.57	-80.9
30	ST1983_5,6MeO	3.78	1.54	2.03	-91.7
31	ST1983_5Cl	3.78	2.48	2.62	-82.9
32	ST1983_5Me	3.78	2.26	2.24	-83.3
33	ST1983_6BrO	3.78	3.37	3.31	-88.2
34	ST1983_6PrO	3.78	2.60	2.54	-93.7
35	ST1983_6MeO	3.78	1.72	2.03	-86.0
36	ST1983_6BuO	3.78	2.95	2.71	-92.3
37	ST1983_7EtO	3.78	2.25	2.35	-84.6
38	ST2394_5BrO	3.63	2.91	2.99	-96.1
39	ST2394_6PhO	3.63	3.33	3.21	-105.8

RESULTS and DISCUSSION

Most representative literature agonists are selected based on similarity with in house thioisobutyrate series, with different selectivity for PPAR α or PPAR γ isoform. In Table 1 the structures, the pEC₅₀ transactivation values and the Glide scoring values (Emodel) are reported. Selected crystal structures (1K7L and 1FM9) of human PPARs contain the most active and bulky agonists to be sure that the grid dimensions are sufficient to accommodate a wide set of molecules. Linear relationships between Emodel and PPAR α transactivation values are shown in Figure 1a and b. About PPAR α correlation (Figure 1a), we have obtained R² = 0.94 with n = 9 compounds and an Emodel range of ~100 kJ/mol vs 4.3 order of magnitude of activity; we are confident to predict the activities of external compounds in not very active (pEC₅₀ ≤ 6), active (6 < pEC₅₀ < 8) and very active (pEC₅₀ ≥ 8) with respect to reference compound rosiglitazone, when Emodel ≥ -100, -100 > Emodel > -150 and Emodel ≤ -150 kJ/mol respectively. About PPAR γ correlation (Figure 1b), we have obtained R² = 0.76 with n = 8 compounds and an Emodel range of ~70 kJ/mol vs 4.3 order of magnitude of activity; we are less confident in this case to predict the activities of external compounds in not very active (pEC₅₀ ≤ 6), active (6 < pEC₅₀ < 8) and very active (pEC₅₀ ≥ 8) with respect to reference compound WY-14,643, when Emodel ≥ -100, -100 > Emodel > -130 and Emodel ≤

-130 kJ/mol respectively. Then we have docked the acidic form of thioisobutyrate series on both receptors; in Table 2 we have reported the structures, transactivation values and Emodel values for both PPARs and pK_i values for PPAR γ receptor. About PPAR γ docking, all the compounds have shown an Emodel values > -100 kJ/mol, so they can be classified as not very active with respect to rosiglitazone; the binding data (pK_i) are generally in agreement with this prediction with the exception of compounds **18** (ST2618) and **24** (ST2653) (pK_i > 6). A possible explanation can be found in the presence of bulk lipophilic substituents in meta position for these compounds. We are going to improve this prediction using docking with induced fit procedure. About PPAR α , we have selected the compounds more active than WY-14,643 (in light red in Table 2) and we have pinpointed that a significant increase of Emodel values (~20 kJ/mol) exists in this series, where the **26** (ST2740) is identified as the most active molecule (Emodel = -93.4). Transactivation value of this molecule is already high at lowest concentration (150% at 2 μ M, data not shown). In Figure 2a **26** (ST2740), docked in human PPAR α receptor, is shown. In this series we have identified a possible new lead compound, **21** (ST1983 or its acid form ST2622) showing a potency that can be modulated if the indole ring is appropriately substituted. We also have synthesized two carbanate derivatives (**13** and **14**, ST2031 and ST2208

respectively), to explore new functional groups as linker between the two aromatic moieties. The first one has shown a comparable orientation and an acceptable Emodel value; on the contrary the transactivation experiment didn't evidence activity. In Figure 2b acid form of **13** (ST2031), docked in human PPAR α receptor, accepts a hydrogen bond from Thr 279; this amino acid is not conserved between human and murine PPAR α . This is a possible explanation to the lack of activity on the murine receptor. Another possibility concerns the chemical or enzymatic lability of carbanate group. The amino acid Thr 279 is Arg 288 in PPAR γ and could be used also to enhance the selectivity vs PPAR α . These evidences will be the basis for future works. To evaluate the potency of thioisobutyrate series, shown in light blue and red in Table 2, we have performed a PLS-DA analysis; we have described the structures using EVA descriptors and a two component model is obtained (R² = 0.93 and Q² = 0.82), having a good predictive ability. Figure 3 shows scatter plot of the score values where the two classes of the training set are well separated. Next we have built a virtual library based on commercially available indole derivatives of **21** (ST1983) and of its meta isomer **22** (ST2394), and we have used the PLS-DA model to select good candidates to be synthesized. Grey triangles in Figure 3 represent the prediction set and Table 3 shows the selected compounds as a combination of docking results on PPAR α (Emodel values < -80 kJ/mol) and PLS-DA analysis.

CONCLUSIONS

In this work we have used a set of literature PPARs agonists and a virtual docking software to assess a method to define a scale of selectivity between the two isoforms (PPAR α and PPAR γ) roughly indicating three classes of relative activity: not very active (pEC₅₀ ≤ 6), active (6 < pEC₅₀ < 8), very active (pEC₅₀ ≥ 8) with respect to reference compounds. A thioisobutyrate series was then evaluated and classified in terms of activity for both receptors. To evaluate the potency of this series on PPAR α receptor, then we have set up a mathematical model (PLS/DA), able to build the training set molecules in two classes: equal or more active and less active than the reference compound WY-14,643. From a virtual library enumerated using indole derivatives, we selected a subset of molecules with high probability to be more efficient than its lead compound. New syntheses of indole derivatives on the model of compound **21** (ST1983 or of its corresponding acid ST2622) are in progress to obtain more potent PPAR α and selective compounds.

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