

Pharmacophore-driven identification of PPAR γ agonists from natural sources

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Received: 3 September 2010 / Accepted: 26 October 2010 / Published online: 11 November 2010
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Abstract In a search for more effective and safe anti-diabetic compounds, we developed a pharmacophore model based on partial agonists of PPAR γ . The model was used for the virtual screening of the Chinese Natural Product Database (CNPD), a library of plant-derived natural products primarily used in folk medicine. From the resulting hits, we selected methyl oleanonate, a compound found, among others, in *Pistacia lentiscus* var. Chia oleoresin (Chios mastic gum). The acid of methyl oleanonate, oleanonic acid, was identified as a PPAR γ agonist through

bioassay-guided chromatographic fractionations of Chios mastic gum fractions, whereas some other sub-fractions exhibited also biological activity towards PPAR γ . The results from the present work are two-fold: on the one hand we demonstrate that the pharmacophore model we developed is able to select novel ligand scaffolds that act as PPAR γ agonists; while at the same time it manifests that natural products are highly relevant for use in virtual screening-based drug discovery.

Keywords Virtual screening · PPAR γ agonist · Natural compounds · Pharmacophore model

Rasmus K. Petersen and Kathrine B. Christensen have equally contributed to this article.

Electronic supplementary material The online version of this article (doi:10.1007/s10822-010-9398-5) contains supplementary material, which is available to authorized users.

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Introduction

PPARs are fatty acid activated transcription factors that belong to the thyroid/retinoid nuclear receptor family. The α , δ , and γ subtypes of PPAR found in mammals, coordinate pathways involved in glucose and lipid homeostasis. PPARs are implicated in the pathology of various disease states including type II diabetes, obesity, dyslipidemia, atherosclerosis, neoplastic diseases and tumors, inflammatory conditions, and neurodegenerative diseases. PPARs are thus targets of numerous drug design and development efforts and the significant role of PPARs in disease treatment is the subject of many studies [1–4].

PPAR γ is predominately expressed in adipose tissue and its significant role in lipid metabolism, adipogenesis, glucose homeostasis, and insulin sensitization is well documented [5–7]. Agonists for this subtype increase adipocyte differentiation and improve the storage of fatty acids. Furthermore, they enhance insulin sensitivity by a not fully understood mechanism that involves PPAR γ activity in adipose tissue, skeletal muscle, macrophages, and liver.

Natural ligands of PPAR γ are fatty acids as well as eicosanoids. Among a large variety of synthetic ligands are thiazolidinediones (TZDs) and some nonsteroidal anti-inflammatory drugs. TZDs have been used in clinical practice to treat type II diabetes for many years and have been shown to lower blood glucose levels and improve insulin sensitivity [8]. While the glucose lowering action of TZDs was well-known as early as 1988, it was not until 1995 that the nuclear receptor PPAR γ was identified as their target and that its activation was shown to be responsible for their therapeutic benefits. However, despite their excellent potencies, administration of TZDs has been associated with severe side effects such as fluid retention, weight gain, cardiac hypertrophy, and hepatotoxicity [9, 10]. Troglitazone, for example, was withdrawn from therapeutic use due to liver toxicity and Farglitazar failed to pass phase III clinical trials due to the emergence of peripheral edema. Since October 2010, Rosiglitazone has been withdrawn from the European market after recommendations by the European Medicines Agency (EMA) and following concerns over excess cardiovascular risks. Though the drug is still available in the United States, the Food and Drug Administration (FDA) has stated that it should be restricted to patients who cannot be successfully treated with other medicine. Pioglitazone is currently in clinical practice despite being also linked to controversial side effects including an increased risk of cardiovascular related death. It is, thus, apparent that the search for PPAR γ ligands with an improved mode of action is an important objective.

The occurrence of undesirable side effects has been linked to the use of TZDs that behave like full PPAR γ agonists [12], where efficacy and side effects have been shown to be intrinsically linked. Higher efficacy compounds are associated with a greater propensity for side effects and increasing doses produce both greater benefits for glucose control as well as greater incidence and higher degrees of side effects. Thus, doses which would produce the maximal clinical benefit of PPAR γ full agonists may not be tolerated by a significant number of patients and the full potential of PPAR γ activation for insulin sensitization and glucose control may not be realized at approved clinical doses.

Partial PPAR γ agonists, on the other hand, are ligands that upon binding to PPAR γ provide diminished conformational stability of the receptor as opposed to full agonists, and thereby also recruit a different set of co-factors than the latter. The alternative co-factor usage is often reflected in suboptimal transcriptional activation of the receptor (defining the low efficacy properties of a partial agonist). It is generally recognized that the selective recruitment of co-factors in response to administration of a partial agonist does not induce the same magnitude of side-

effects as observed for the full agonist TZDs [13]. It has, thus, been suggested that PPAR γ partial agonists fulfill the requirements for beneficial PPAR γ ligands, as they maintain their insulin sensitizing activity without having a strong adipogenic potential. On that account, partial agonists of PPAR γ with decreased side effects on adipose tissue are investigated for their usability in the therapy of type II diabetes [14–16].

In this study we have combined *in silico* and *in vitro* approaches for the identification of natural compounds that act as PPAR γ agonists. We have used pharmacophore-based Virtual Screening (VS) for the initial identification of target compounds and subsequently verified one of the hits using bioassay-guided chromatographic fractionation. VS is increasingly used as a cost-effective supplement to high-throughput screening and employs a range of methods for the *in silico* high-throughput assessment of large databases, and rapid evaluation and prioritization of compounds prior to wet-lab testing. Pharmacophore models in particular, involve the identification of the pharmacophoric pattern common to a set of known actives and the use of this pattern in a subsequent search. Pharmacophore-based virtual screening has only recently been used for the identification of novel ligands of PPAR γ of synthetic [17, 18] or natural [19] origin. Experimentally validated PPAR γ agonists from plants are hitherto few and include structurally diverse compounds from various sources such as fruits, vegetables, and medicinal plants [20–23]. Considering that plants have a long history in the traditional treatment of many diseases, including diabetes [24], natural product libraries represent a very promising source of novel PPAR γ ligands.

Materials and computational method

Dataset of natural compounds

Virtual screening for PPAR γ partial agonists was performed on the Chinese Natural Product Database [25] (CNPD v.2004.1). CNPD is a compilation of 57,346 compounds found in plants largely used in TCM (Traditional Chinese Medicine). These compounds originate from 2,611 plant species belonging to 457 different plant genera. After removal of salts, inorganic compounds and duplicates, we extracted 53,180 unique organic compounds in SDF format, which we imported into a Molecular Operating Environment (MOE) v. 2008.10 database [26]. These structures were washed, i.e. for all ionizable groups the correct protonation state at neutral pH conditions was produced, and energy minimized using the MMFF946 force field. Conformations were generated with a stochastic conformational search algorithm.

Partial agonist pharmacophore model

The partial agonist pharmacophore model was developed using the Pharmacophore Elucidator application of the MOE software. The Pharmacophore Elucidator generates a collection of pharmacophore queries from a collection of compounds some or all of which are active against a particular biological target such that all or most of active compounds satisfy the queries. The model was generated using the Unified scheme, based on a compound set of 13 PPAR γ partial agonists, all selective to PPAR γ ; no dual or pan PPAR agonists were included (Table 1). The Unified scheme is the most comprehensive scheme available in MOE and contains 20 different annotation points (such as H-bond donor, h-bond acceptor, hydrophobe, etc.), covering atom, centroid and projected annotations. The pharmacophore was generated on the first aligned solution that was characterized by the highest accuracy and overlap. The model was subsequently used for the virtual screening of the CNPD database. To be considered as a hit, a compound had to fit all the features of the pharmacophore.

Docking of novel PPAR γ partial agonist

Oleanonic acid was docked on the crystal structure 2F4B of PPAR γ using MOE v. 2008.10. Prior to docking, all ligands and water were removed from the protein structure, hydrogens were added, ionization states were assigned, and tethered energy minimization was performed using the MMFF94 force field. The protonation state of oleanonic acid was calculated at physiological pH, with the carboxyl group deprotonated. Docking was performed with a rigid protein and a flexible ligand, using the Alpha PMI placement and the London dG scoring function.

For validation, EHS ([5-(3-{{[6-(phenylcarbonyl)-1-propylnaphthalen-2-yl]oxy}propoxy)-1H-indol-1-yl]acetic acid), the ligand co-crystallized in the PDB complex with PPAR γ , was used. The ligand re-docked in the same position as in the original complex with an RMSD of 1.43 Å. The interaction of the indole acid acetic group of the ligand with His449 is conserved, while the flexibility of the tail of the molecular structure is the one responsible for the deviation on the docking pose from the original in armII.

Fractionation of *P. lentiscus* oleoresin

HPLC-grade acetonitrile (MeCN) was obtained from Fischer Scientific (Leicestershire, UK). Formic acid (FA) 99%, trifluoro acetic acid (TFA) 99 + %, and chloroform-d (CDCl₃) 99.8 atom % D was obtained from Sigma-Aldrich (Steinheim, Germany). Fractions of *P. lentiscus* var. Chia oleoresin were isolated according to [38, 39].

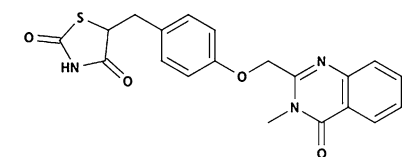
Further sub-fractionation of the most active fractions 4 and 6 was carried out by Semi-preparative HPLC using a Dionex Ulti Mate 3000 pump equipped with a Dionex Ulti Mate Photodiode Array Detector (PDA) and a Develosil ODS-HG-5 RP-18 column (5 μ m; 250 \times 20 mm, Nomura Chemical Co.) as well as a Develosil ODS-HG-5 RP-18 pre-column (5 μ m; 20 \times 50 mm, Nomura Chemical Co.). Flow was 5 mL/min and injection volume was 2 mL. LC-MS data were obtained using a LTQ XL (ESI-2D-iontrap, Thermo Scientific) equipped with an Accela HPLC Pump and PDA Detector as well as an evaporative light scattering detector (ELSD, Sedex 80LT, SEDERE, Alfortville, France). Settings for the mass spectrometer were 36, 26, and 0 (arbitrary units) for sheath, auxiliary, and sweep gas flow rates, respectively. Spray voltage was 5 kV, capillary temperature 275 °C, capillary voltage 3.10 V, tube lens 100 V, and AGC target settings were 3×10^4 and 1×10^4 for full MS and MSⁿ, respectively. Separations were obtained by the solvent gradient A = 1.00% FA in H₂O, B = 1.00% FA in MeCN; 0 min (35% B), 60 min (100% B), 70 min (100% B), 80 min (35% B), 90 min (35% B) on a Luna NH₂-column (5 μ m; 250 \times 4.6 mm, Phenomenex). Flow was 200 μ L/min, temperature 35 °C, and injection volume 10 μ L. 1D and 2D NMR data were acquired on a Bruker AVANCE III 400 MHz system using solvent signals (CDCl₃; δ_H 7.26/ δ_C 77.7) as references.

The neutral fraction (sample 4, 100 mg) was further separated using semi-preparative HPLC by the solvent gradient A = 0.05% TFA in H₂O, B = MeCN; 0 min (50% B), 30 min (100% B), 80 min (100% B), 90 min (50% B), 95 min (50% B) to give four fractions 4-I (41 mg), 4-II (15.7 mg), 4-III (14.5 mg), and 4-IV (9.2 mg). The acidic fraction (sample 6, 100 mg) was likewise separated using semi-preparative HPLC by the solvent gradient A = 0.05% TFA in H₂O, B = MeCN; 0 min (60% B), 10 min (60% B), 40 min (100% B), 90 min (100% B), 100 min (60% B), 105 min (60% B) to give five fractions 6-I (17.3 mg), 6-II (6.6 mg), 6-III (12.3 mg), 6-IV (6.8 mg), and 6-V (41.1 mg).

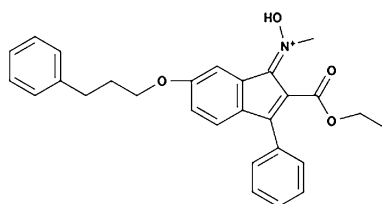
Biological testing of *P. lentiscus* oleoresin fractions

PPAR γ mediated transactivation

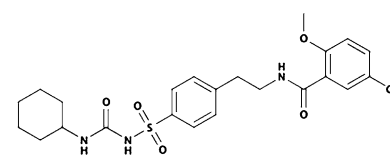
Mouse embryo fibroblasts (MEFs) were propagated in Dulbeccos Modified Eagle's Media (DMEM) supplemented with 10% fetal calf serum and antibiotics. MEFs were transfected in solution by Metafectene (Biontex) lipofection, essentially according to the manufacturer's instructions and seeded in DMEM supplemented with 10% fetal calf serum and antibiotics in 96-well dishes at 24,000 cells/cm². The transfection plasmid mix included the Gal4-responsive luciferase reporter, the expression

Table 1 Compound set of partial PPAR γ agonists and their EC₅₀ values (μ M) utilized for generation of the partial agonist pharmacophore model

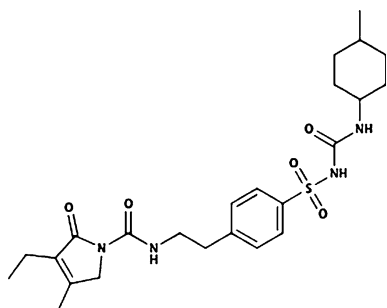
Balaglitazone

EC₅₀ = 1.351 μ M [27]

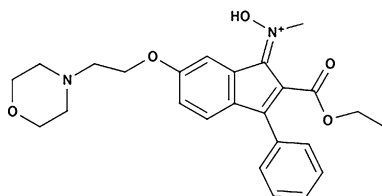
KR-62776

EC₅₀ = 0.05 μ M [28]

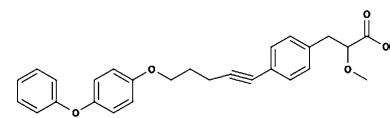
Glibenclamide

EC₅₀ = 7.6 μ M [29]

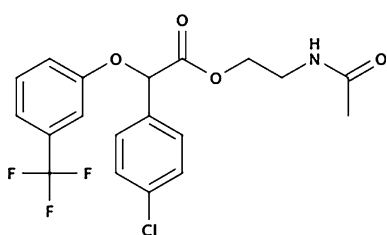
Glimepiride

EC₅₀ = 27 μ M [29]

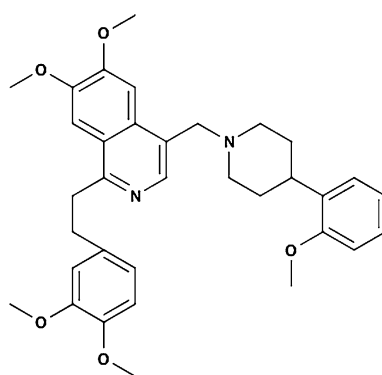
KR-62980

EC₅₀ = 0.015 μ M [30]

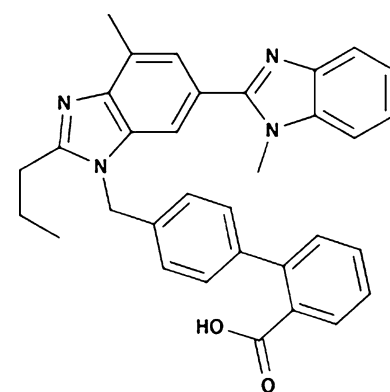
LSN862

EC₅₀ = 0.239 μ M [31]

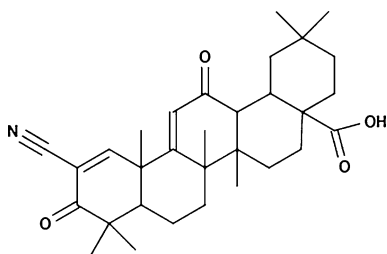
Halofenate

EC₅₀ = 30 μ M [32]

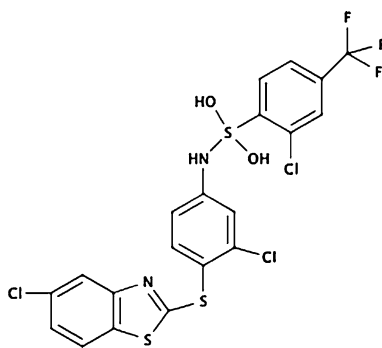
PA-082

EC₅₀ = 0.26 μ M [33]

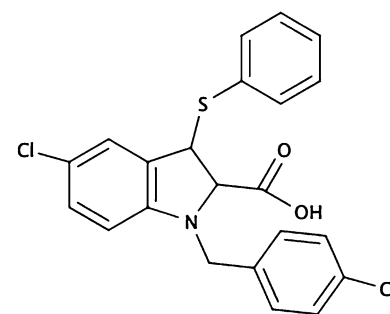
Telmisartan

EC₅₀ = 0.0045 μ M [34]

CDDO

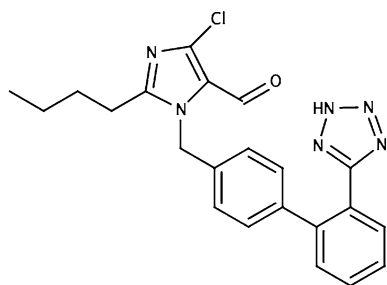
EC₅₀ = 0.030 μ M [35]

T2384

EC₅₀ = 0.56 μ M [36]

nTZDpa

EC₅₀ = 0.057 μ M [13]

Table 1 continued

EXP-3179

EC₅₀ = 17.1 μM [37]

vector for the fusion between the Gal4 DNA-binding domain and the human PPAR γ ligand binding domain, (Gal4(DBD)-hPPAR γ (LBD)), and a CMV Renilla luciferase normalization vector (pRL-CMV, Promega). Six hours after addition of transfection mix to the cells, the media was changed to DMEM supplemented with vehicle (0.2% DMSO), positive control (1 μM Rosiglitazone) or compound (in competition assay cells were incubated with 0.3% DMSO or 0.3 μM Rosiglitazone and increasing concentrations of oleanonic acid as indicated). Approximately 18 h later, cells were harvested and lysates analyzed for Photinus and Renillaluciferase activity by luminometry. All experiments were performed in at least triplicate and each sample measured in duplicate. Luminometer raw data were analyzed in Microsoft Excel spreadsheets and

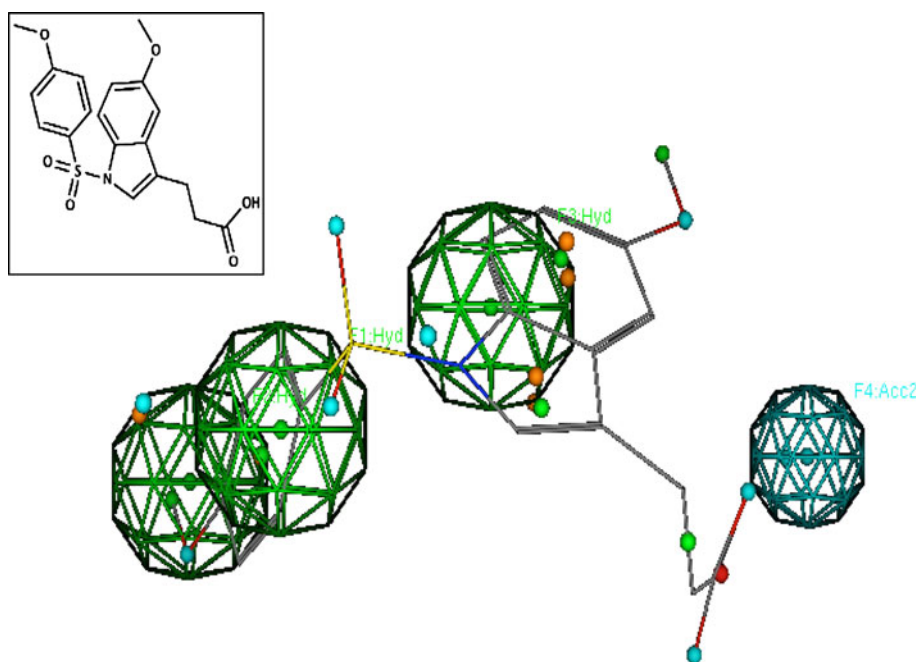
presented as column graphs depicting average values of triplicates and including standard deviations.

Results and discussion

Pharmacophore-based virtual screening

A 4-point pharmacophore model was developed, which consists of one hydrogen bond acceptor, and three hydrophobic spheres (Fig. 1). The model matched all 13 partial agonists of PPAR γ that were employed in the pharmacophore elucidation. The model's performance was validated on a recently discovered partial PPAR γ agonist, Indeglitazar [40], which overlapped with the pharmacophore features

Fig. 1 4-Point pharmacophore model for PPAR γ partial agonists superposed on Indeglitazar (RMSD: 0.50 Å). In MOE, pharmacophoric features are represented by a point encased in a sphere. The spheres depict the location tolerance allowed during virtual screening. Points not encased in spheres are other potential pharmacophore features on the Indeglitazar structure. Blue projected location of hydrogen-bond donor, 1 Å tolerance, green hydrophobic region, 1.4 Å tolerance, orange aromatic center, red CO₂ centroid. On the top left, the 2D structure of Indeglitazar



with a RMSD (Root Mean Square Deviation) = 0.5 Å. The hydrogen-bond acceptor projection (Acc2) feature of the pharmacophore model overlaps well with the propionic acid side chain of Indeglitazar, whereas the hydrophobic features of the model fall on the aromatic rings of the structure. According to Artis et al. [40], the carboxylate group of the propionic acid side chain forms hydrogen-bonds with residues His323, Tyr327, His449, and Tyr473.

In order to prioritize the best hits and reduce the number of false positives from the CNPD database, a volume constrain based on the union of volumes of the 13 partial agonists, as well as the Lipinski drug-like filter were applied. Pharmacophore-based screening of the CNPD database retrieved 939 compounds with $\text{RMSD} \leq 0.5 \text{ \AA}$ (see Supporting Information for list of chemical names and RMSD values).

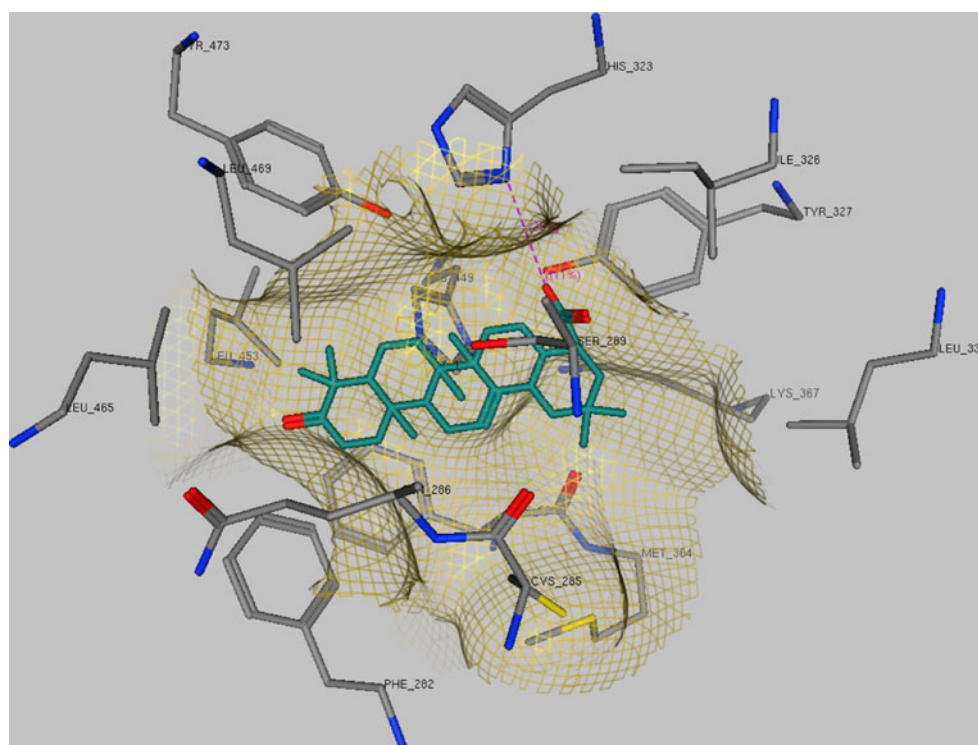
A major bottleneck in virtual screening of natural compound libraries is the unavailability of the natural hits in pure form from commercial suppliers, combined with the costly, time-consuming and sometimes even unfeasible in vitro synthesis of the natural compound, or its isolation from a natural source. Within this framework, we examined carefully the obtained list of hits and selected methyl oleanonate (me-ester-3-oxo-olean-12-en-28-oic acid), ranked 24, for experimental validation of its predicted biological activity on PPAR γ . Methyl oleanonate is a triterpene found in different species of *Pistacia*, some of them endemic to Europe. However, it is not the methyl ester itself that is present in the plant, but the acid,

oleanonic acid. This inconsistency in the CNPD is most likely due to the fact that GC–MS is primarily used for identification of these triterpenes and methylation is a necessary step in this technique for identification of acids. Oleanonic acid has primarily been identified as its methyl ester in the oleoresin from *Pistacia lentiscus* var. Chia [38, 39, 41] that is uniquely cultivated in southern Chios, a Greek island in the Aegean. Interestingly, in local traditional medicinal praxis, Chios mastic gum dispersed in water is used as antidiabetic medication [42].

Docking of oleanonic acid to PPAR γ

In order to determine the putative binding mode and the potential ligand-target interactions involved in the binding of oleanonic acid to PPAR γ , the former was docked on the Ligand Binding Domain (LBD) of the PDB (Protein Data Bank) entry 2F4B [43]. Figure 2 shows the best docking pose, where the carboxylic moiety of the compound forms hydrogen bonds with His323 and Tyr327 on helix 4/5 of armI, which is akin to the binding mode of Indeglitazar [40]. In addition, the remainder of the ligand is stabilized with several interactions in the hydrophobic pocket consisting of residues Gln286, Met364, Leu453 and Leu469. The fact that there are no predicted hydrogen bonding interactions between the carboxyl group and residues His449 and Tyr473 of the AF2 helix hints towards oleanonic acid being a partial PPAR γ agonist, which was indeed our initial aim during the development of the pharmacophore model.

Fig. 2 Docking pose of oleanonic acid in the LBD of PPAR γ , which shows that the carboxylic moiety of the compound forms hydrogen bonds with His323 and Tyr327 on helix 4/5 of armI. In addition, the remainder of the ligand is stabilized with several interactions in the hydrophobic pocket consisting of residues Gln286, Met364, Leu453 and Leu469



Pistacia lentiscus oleoresin; fractionation and biological testing

Mastic gum has been known from the time of Pedanius Dioscurides, (70ac) [11] and has been used in Greek folklore medicine for various gastrointestinal disorders such as gastralgia, dyspepsia, and peptic ulcer for more than 2,500 years. Furthermore, folklore medicine indicates that mastic gum components may be active against diabetes. Mastic gum has been reported to possess anti-inflammatory [44], antimicrobial [45], anticancer [44, 46, 47] as well as gastric and duodenal antiulcer activity [48, 49]. Consumption of mastic gum has also recently been associated with cardiovascular protection, where in vivo decrease in the cholesterol levels in the serum [50] and in vitro inhibition of oxidation of human low-density lipoproteins (LDL) [51] are reported. In previous studies, the oleoresin of *P. lentiscus* var. Chia was shown to exhibit antioxidant [52] and radical scavenging activities (Assimopoulou and Papageorgiou, unpublished data). The metabolites responsible for these reported bioactivities of *P. lentiscus* oleoresin have not been fully clarified. However, some studies on the biological activities of one of the major components, oleanonic acid do exist and include cytotoxic, uterotonic, and anti-*Helicobacter* activities [53–59]. In a recent report, Giner-Larza et al. [60] demonstrated the inflammatory activity of both oleanonic and oleanolic acid and suggested that the presence of the keto group in the former augments its anti-inflammatory activity in models related with the activation of 5-lipoxygenase. Investigations into the composition of *Pistacia lentiscus* oleoresin are generally based on examinations of its acidic and neutral fractions [38, 39, 41]. In the acidic fractions of *P. lentiscus* oleoresin, the triterpenes oleanonic acid, isomasticadienonic acid and masticadienonic acid are the major metabolites. In the neutral fraction, a much larger number of compounds are present but the dominating ones are 28-norolean-17-en-3-one and oleanonic aldehyde [38].

In the present study, six samples were initially isolated and analyzed for PPAR γ activity, as listed in Table 2,

Table 2 Samples of oleoresin of *P. lentiscus* var. Chia, its subfractions and oleoresin of *P. terebinthus* var. Chia that were tested for their ability to activate PPAR γ

Sample #	Sample
1	<i>P. lentiscus</i> oleoresin
2	<i>P. terebinthus</i> oleoresin*
3	Mastic oil
4	Neutral fraction
5	Acidic fraction I
6	Acidic fraction II

* Species similar to *P. lentiscus*

including the oleoresin of *P. lentiscus* var. Chia (sample 1) and its subfractions (samples 3–6), as well as the oleoresin of *P. terebinthus* var. Chia, a species very similar to *P. lentiscus* (sample 2). Although not all of these fractions contained oleanonic acid they were included in the experimental testing as many compounds structurally similar to oleanonic acid are present in these samples.

As seen from Fig. 3, fractions 4 and 6 possessed the ability to stimulate PPAR γ -mediated transactivation and consequently they were selected for further fractionations. Fraction 6 was also the one with the highest content of oleanonic acid as observed from LC–MS (data not shown).

Separation of the major components present in the active sub-fractions was achieved using semi-preparative reversed phase HPLC. The neutral fraction (sample 4) of *P. lentiscus* oleoresin was separated into four fractions and the acidic fraction (sample 6) into five fractions. Testing of these fractions revealed the presence of PPAR γ modulating activity in the sub-fractions 4-I, 6-I, and 6-II (Fig. 4).

LC–MS analysis showed that fraction 6-II almost exclusively contains oleanonic acid, supporting the pharmacophore model prediction for this molecule. LC–MS analysis of fractions 4-I and 6-I revealed that these fractions contain several compounds. However, as we in the present study focused on oleanonic acid, we did not examine which of these compounds were responsible for the observed activation of PPAR γ . The acquired LC–MS data were compared to data from literature both from GC–

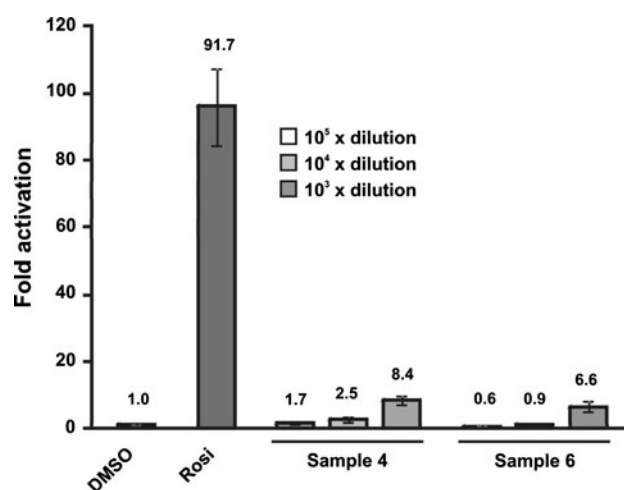


Fig. 3 PPAR γ activating properties of *P. lentiscus* oleoresin neutral fraction (sample 4) and acidic fraction II (sample 6). In transient transfection experiments fractions of *P. lentiscus* oleoresin were able to induce transcriptional activation of a luciferase reporter driven by a fusion between the Gal4 DNA-binding domain and the human PPAR γ ligand-binding domain (Gal4(DBD)-hPPAR γ (LBD)). Shown is the relative activation compared to the DMSO vehicle, with each column representing the average \pm standard deviation ($n \geq 3$) of a representative experiment. The PPAR γ full agonist Rosiglitazone (Rosi) is included as a positive control for PPAR γ agonist activation

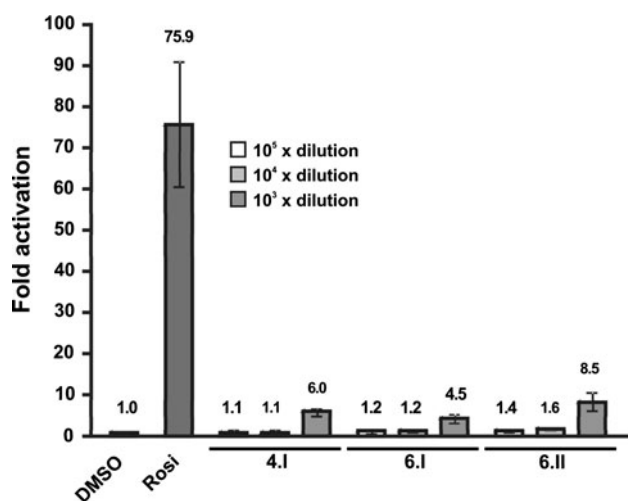


Fig. 4 PPAR γ activating properties of *P. lentiscus* oleoresin sub-fractions 4.I, 6.I and 6.II. In transient transfection experiments fractions of *P. lentiscus* oleoresin were able to induce transcriptional activation of a luciferase reporter driven by Gal4 (DBD)-hPPAR γ (LBD). Shown is the relative activation compared to the DMSO vehicle, with each column representing the average \pm standard deviation ($n \geq 3$) of a representative experiment. The PPAR γ full agonist Rosiglitazone (Rosi) is included as a positive control for PPAR γ agonist activation

MS and LC–MS (1–3) and further verified by 1D and 2D NMR.

Further testing of oleanonic acid (fraction 6-II) data in dose response studies revealed that this compound acts as a low potency (mid μ M range) PPAR γ activator with an efficacy reaching app. 20% the activity of the full agonist Rosiglitazone at saturating concentration (Fig. 5).

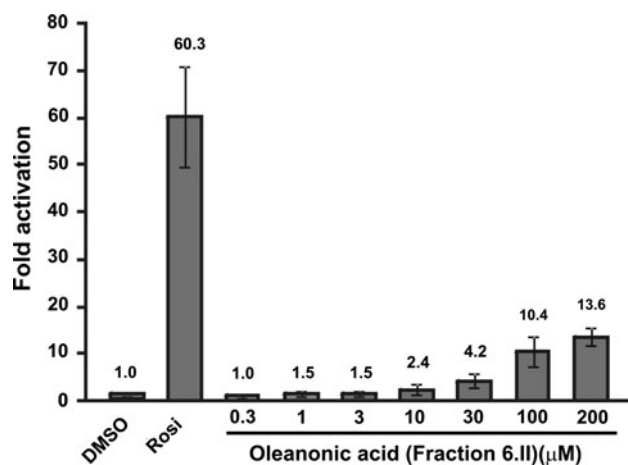


Fig. 5 Oleanonic acid induces transcriptional activation of PPAR γ . In transient transfection experiments oleanonic acid dose dependently activates a luciferase reporter driven by the Gal4 (DBD)-hPPAR γ (LBD). Shown is the relative activation compared to the DMSO vehicle, with each column representing the average \pm standard deviation ($n \geq 6$) of a representative experiment. The PPAR γ full agonist Rosiglitazone (Rosi) is included as a positive control for PPAR γ agonist activation

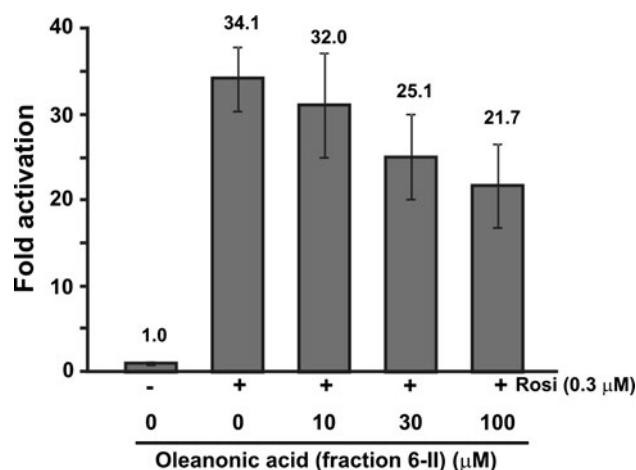


Fig. 6 Oleanonic acid antagonizes Rosiglitazone induced transcriptional activation of PPAR γ . In transient transfection experiments oleanonic acid dose dependently attenuates Rosiglitazone (Rosi) induced PPAR γ -mediated activation of a luciferase reporter. Shown is the relative activation compared to the DMSO vehicle, with each column representing the average \pm standard deviation ($n \geq 6$) in a representative experiment

A competition assay demonstrated that oleanonic acid was able to dose dependently antagonize Rosiglitazone-mediated PPAR γ transcriptional activation (Fig. 6), suggesting that oleanonic acid as predicted by the in silico screening may function as a partial agonist for PPAR γ . Further analyses including determination of the ability of oleanonic acid to recruit co-factors will be required to corroborate whether oleanonic acid is a bona fide partial PPAR γ agonist.

Conclusions

In summary, pharmacophore-based virtual screening of a library of natural compounds, in combination with bioassay-guided chromatographic fractionation, led to the successful identification of oleanonic acid as a new partial agonist for PPAR γ . Oleanonic acid is found in the oleoresin of *Pistacia lentiscus* var. Chia (Chios mastic gum), which, according to local folk medicine, has antidiabetic properties. An often-observed situation among plant remedies, however, is pharmacokinetic synergy, where the pharmacological effect of the plant is the outcome of multiple active substances acting in combination towards the same or multiple biological targets [61]. In this work, we did observe that other compounds able to activate PPAR γ were present in the tested fractions. Furthermore, activities towards other biological targets relevant to disease development should not be ruled out either. It is our intention to further investigate in a subsequent study the full activity profile of oleanonic acid and the other constituents of

P. lentiscus oleoresin towards a complete panel of biological targets related to type-2 diabetes.

Acknowledgments IK acknowledges financial support from the Danish Research Council for Technology and Production Sciences (Grant 274-06-0301). This work was further supported by the Danish Council for Strategic Research (Grant No. 2101-01-0065).

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