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Identification of PPAR γ Agonists from Natural Sources Using Different *In Silico* Approaches

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Abstract

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Peroxisome proliferator-activated receptor γ plays an important role in lipid and glucose homeostasis and is the target of many drug discovery investigations because of its role in diseases such as type 2 diabetes. Activation of peroxisome proliferator-activated receptor γ by agonists leads to a conformational change in the ligand-binding domain altering the transcription of several target genes involved in glucose and lipid metabolism, resulting in, for example, facilitation of glucose and lipid uptake and amelioration of insulin resistance, and other effects that are important in the treatment of type 2 diabetes. Peroxisome proliferator-activated receptor γ partial agonists are compounds with diminished agonist efficacy compared to full agonists; however, they maintain the antidiabetic effect of full agonists but do not induce the same magnitude of side effects. This mini-review gives a short introduction to *in silico* screening methods and recent research ad-

vances using computational approaches to identify peroxisome proliferator-activated receptor γ agonists, especially partial agonists, from natural sources and how these ligands bind to the peroxisome proliferator-activated receptor γ in order to better understand their biological effects.

Abbreviations

▼
Cdk5: cyclin-dependent kinase 5
H3: Helix H3
H12: Helix H12
LBD: ligand-binding domain
LBP: ligand-binding pocket
PDB: protein data bank
PPAR: peroxisome proliferator-activated receptor
TZD: thiazolidinedione
T2D: type 2 diabetes
VS: virtual screening

Introduction

▼
Natural products have been and continue to be rich sources for drug discovery. Today over 60% of the drugs that are on the market derive from natural sources [1, 2]. Peroxisome proliferator-activated receptor γ (PPAR γ) plays an essential role in lipid and glucose homeostasis and is the target for many drug discovery efforts because of its role in diseases, such as T2D [3–5]. Obesity-linked phosphorylation of PPAR γ by the protein kinase Cdk5 seems to be involved in the pathogenesis of insulin resistance, and thus the development of T2D. Blocking the phosphorylation by PPAR γ ligands (agonists) can restore a more normal non-diabetic pattern of gene expression [6]. The TZDs, a class of synthetic insulin-sensitizing drugs, target and activate PPAR γ . Activation of PPAR γ by

agonists, such as TZDs, leads to a conformational change in the LBD altering the transcription of several target genes involved in glucose and lipid metabolism, resulting in, for example, the facilitation of glucose and lipid uptake, stimulation of glucose oxidation, a decrease in free fatty acid levels, and the amelioration of insulin resistance [3–5]. Administration of TZDs can cause severe side effects, which have been linked to their behavior as full agonists of PPAR γ [7]. PPAR γ partial agonists are compounds with diminished agonist efficacy that maintain the antidiabetic effect of full agonists but usually do not induce the same magnitude of side effects [8]. The different pharmacology properties of full and partial agonists indicate that changes in the ligand-receptor interaction are responsible for these differences.

A wide variety of promising PPAR γ partial agonists of plant origin have been identified using a bioassay-guided approach. Bioassay-guided fractionation is a very efficient method for the discovery of natural products with interesting bioactivities from natural sources, but it is a tedious process involving time-consuming separation steps combined with biological test models *in vitro* and/ or *in vivo*. In recent years, the field of computational techniques has been evolving towards applying *in silico* VS to support the research in drug discovery, design, development, and optimization [9–11].

In silico screening has been established as one of the most important computational techniques used for prioritizing compounds to be selected for experimental testing. This mini-review gives a short introduction to *in silico* screening methods and provides an assessment of the current state-of-the-art computational approaches to identify PPAR γ agonists, especially partial agonists, from natural sources and discusses how these ligands can bind to PPAR γ in order to better understand their biological effects.

The Complexity of Natural Products with Respect to Molecular Modelling

Natural products have several advantages, but also challenges, when viewed from the perspective of a medicinal chemist. Undoubtedly, they show high diversity, bioavailability, and contain bio-privileged structures [12]. However, to work with natural products in biochemical assays, they have to be isolated from multicomponent mixtures using complex separation procedures requiring expertise in phytochemistry and analytical chemistry. Furthermore, natural products show higher chemical complexity than classical "drug-like" molecules, including higher flexibility, a higher number of ketones and hydroxyl groups, and often a large number of chiral centers [13, 14]. This complexity results in further challenges for computational chemistry and *in silico* VS, especially in terms of handling stereochemistry in the correct way and in covering conformational space when dealing with highly flexible molecules.

Computer-assisted Drug Design and Virtual Screening

In order to deal with the high experimental effort in isolating and testing natural products, *in silico* VS methods have become a successful technique to enhance experimental success rates by prioritizing those natural products that show a high probability to bind to a specific target. Plant extracts containing several potential bioactive natural products can thus be prioritized for testing according to computational predictions for each contained constituent. Subsequent separation is only performed if the extract shows biological activity and the high efforts of separation [15] can be guided by the hypotheses generated *in silico* [16].

Several computer-aided methods exist for VS, which can roughly be divided into structure- and ligand-based methods. Structure-based modelling relies on the availability of an experimentally determined structure of the macromolecular target under investigation. The Protein Data Bank (PDB) [17, 18] represents the largest public repository of protein and nucleic acid structures determined by X-ray crystallography or NMR and currently comprise more than a hundred thousand macromolecular structures, many of them with co-crystallized ligands.

Table 1 The most important programs and algorithms used for docking.

Program	Algorithm	Ref.
AutoDock	Lamarckian Genetic Algorithm	[64]
DOCK	Volume- or shape-based algorithm	[65]
FlexX	Incremental ligand fragmentation and reconstruction	[66]
Glide	Systematic search	[67]
GOLD	Genetic algorithm	[68]
LigandFit	Monte Carlo approaches	[69]
ParaDOCKs	Particle swarm optimization and other metaheuristics	[70]
Surflex	Surface-based molecular similarity methods	[71]

The most popular structure-based approaches are docking and structure-based 3D pharmacophores. While docking flexibly fits a ligand into a protein-binding site [19–22], structure-based 3D pharmacophores describe protein-ligand interactions by an ensemble of chemical features that can then be used for VS [23]. When employed for the VS of large molecule databases, docking suffers from a high false positive rate, which is mainly caused by problems in scoring [24–26]. Nevertheless, docking remains the method of choice when investigating a binding mode and generating ideas for further lead optimization [27]. The most important docking methods are summarized in **Table 1**. Structure-based 3D pharmacophores can be optimized to contain those chemical features that are known to be important for binding and can thus, if developed carefully, represent predictive VS filters [28].

Despite recent advances in protein crystallization, especially with respect to membrane-bound proteins [29], not all relevant macromolecular targets [30] can be crystallized. In those cases, ligand-based methods are used to overcome this limitation. Based on the hypothesis that different ligands bind at the pocket in a similar manner, a similarity search with respect to known active compounds can be performed. In case of a ligand-based pharmacophore, common chemical features are derived using a 3D overlay [31]. More simple approaches use steric similarity to the most active ligand to perform VS [26, 32].

However, further research into lesser-understood biochemical processes is necessary to improve the reliability of VS as a stand-alone process for identifying bioactive constituents. These processes include protein flexibility and induced-fit adaptations, the role of water in solvation, desolvation, and ligand binding, the involvement of electrostatics, as well as the stereochemistry and conformational space of the ligands [18, 33]. Another factor that can limit VS productivity is the amount of information available when building a compound library. Although a huge amount of information is available when considering natural products for the treatment and prevention of diseases, they do not include all potential bioactive natural products. Finally, VS also has its limitation when considering the concentration needed for a ligand to elicit its therapeutic effect. Docking predicts ligands that may elicit the desired activity, but bioassays are needed to further refine the group of viable candidates to a selected group of hits that at a specific concentration will activate the protein, e.g., PPAR γ . However, as mentioned in the introduction and in the beginning of this section, VS can be useful after the fractionation of extracts and structural elucidation of major components in the fractions to aid in identifying which constituents are potential bioactive compounds, such as PPAR γ partial agonists.

Peroxisome Proliferator-Activated Receptor γ As a Target Protein

PPAR γ exist as two isoforms, PPAR γ 1 and PPAR γ 2, which are expressed in many tissues. In humans, both PPAR γ 1 and γ 2 are abundant in adipose tissue but are, for example, present at low levels in skeletal muscle [34]. In order to understand the binding mode of ligands toward PPAR γ , currently, 124 crystal structures of PPAR γ LBD are available of which 109 contain co-crystallized ligands [17]. The binding mode of partial agonists to PPAR γ has been intensively studied revealing different binding modes, which allowed for a more detailed investigation and subsequent identification of potent PPAR γ partial agonists [35–38].

Investigation of several known crystallographic structures of the PPAR γ LBD bound to an agonist revealed two binding modes in the same LBD, which correspond to full and partial agonists [39]. The LBP of the PPAR γ LBD is a large Y-shaped ligand-binding cavity, consisting of an entrance (arm III) that branches off into two binding pockets (arm I and arm II). Arm I is extended toward helix H12 (H12) and arm II is situated between helix H3 (H3) and the β -sheet (Fig. 1). Arm I is the only substantially polar cavity of the PPAR γ LBD, whereas arm II and the interior of arm III are mainly hydrophobic [40]. Full agonists occupy arm I forming a network of hydrogen bonds with the side chains of amino acids Ser289, His323, His449, and Tyr473. These interactions stabilize H12 and are mainly responsible for the transactivation activity of PPAR γ . In addition, full agonists also occupy arm II through a hydrophobic tail [41,42]. On the other hand, the partial agonists interact mainly with amino residues on regions other than H12 through a hydrogen bond with Ser342 of arm III, but also with arm II through several hydrophobic contacts [35,36]. This binding mode causes a lower degree of H12 stabilization and an increase in the stabilization of H3 that affects the recruitment of co-activators and decreases the transactivation activity of PPAR γ [43,44].

Although co-crystal structures of the PPAR γ LBD bound to ligands give information on atomic differences in the LBD, it provides little insight into the graded activity of the ligands and therefore does not explain why agonists with different PPAR γ transactivation activities show similar insulin-sensitizing potencies. However, recent studies have demonstrated that the antidiabetic efficacy of different ligands correlates with the ligand-binding affinity as well as their ability to inhibit phosphorylation of PPAR γ by Cdk5 at Ser273 in PPAR γ 2 and Ser245 in PPAR γ 1, thereby preventing the unregulated expression of some target genes involved in lipid and glucose homeostasis [6,45,46]. Furthermore, the potency of blockage of Ser273/Ser245 phosphorylation by ligands appears to be associated with the strength of interaction with the backbone amide at Ser342 (graded PPAR γ agonism) and seems not to depend on the degree of classical agonist action of full agonists by stabilizing H12 [45,46]. A substantial part of the antidiabetic effect of full and partial agonists of PPAR γ may therefore be explained by the inhibition of PPAR γ phosphorylation. The side effects of full agonists are therefore likely to occur through the classical agonist action. Thus, an effective partial agonist of PPAR γ with reduced side effects should then have a weak transactivation activity, but a high phosphorylation inhibitory activity on PPAR γ at Ser273/Ser245 [45,46]. Finally, Hughes et al. [47] have recently demonstrated that synthetic ligands designed to mimic the activity of endogenous ligands (fatty acid derivatives) via binding to the canonical hydrophobic LBP of the PPAR γ LBD are able to bind to an alternate site on the LBD. Alternate site

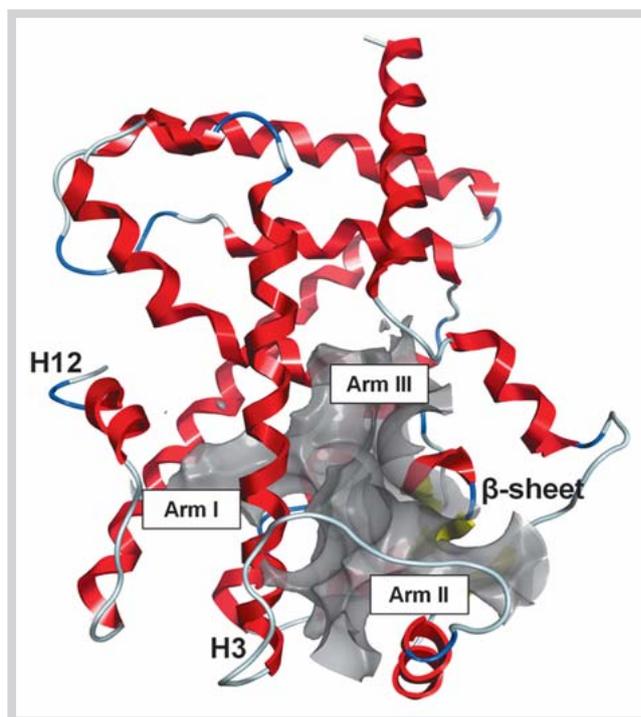


Fig. 1 3D structure of PPAR γ . The crystal structure of PPAR γ (PDB code: 2F4B [72]) is shown with α -helices colored in red, β -sheets in yellow, and the ligand-binding site in grey. (Color figure available online only.)

binding affects the structure and function of PPAR γ , and hence may contribute to the pharmacological response of PPAR γ ligands. Alternate site binding can occur via the following three mechanisms of potential pharmacological relevance [47]: (1) binding of two molecules of the same ligand to PPAR γ , one to the canonical LBP and a second to the alternate binding site; (2) the canonical LBP is "blocked" by a covalently bound irreversible antagonist; and (3) the canonical LBP is covalently bound to an endogenous ligand. If, for example, the PPAR γ LBD is occupied by a covalently bound endogenous ligand, such as a prostaglandin (e.g., 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂) or an oxidized fatty acid (e.g., 5-oxoeicosatetraenoic acid) [48,49], as described in the latter mechanism, the alternate binding site could be a target for allosteric modulators [47].

The above-mentioned new findings have not yet been applied into *in silico* VS, but constitute very useful information for understanding the mechanism of action of full and partial agonists of PPAR γ . Finally, incorporation of this new knowledge in VS may be a stepping-stone for the identification of new and more efficient ligands for PPAR γ .

Peroxisome Proliferator-Activated Receptor γ Agonists From Natural Sources Discovered by Virtual Screening

Considering that plants have a long history in the traditional treatment of diabetes [50], natural product libraries represent a very promising source of novel PPAR γ ligands. Natural sources that contain PPAR γ agonists have been described in a few reviews [51–53]. Although these reviews do not represent an exhaustive and updated source of information about natural sources for

PPAR γ agonists, they contain useful information that may be helpful in building up natural product libraries to be used in VS. To date, several VS studies have been used successfully to identify PPAR γ agonists, including partial agonists from botanical sources. The first VS of a natural product library to identify novel PPAR γ agonists was performed by Salam et al. [54]. They used a structure-based docking method and screened an in-house natural product library consisting of 200 compounds extracted from botanical sources. The compounds were examined for their potential to engage several residues in a binding mode typical for PPAR γ agonists [40–42]. Their screening resulted in identifying several flavonoids, isoflavonoids, gingeroids, and ginkgolides as potential PPAR γ full agonists, with flavonoids/isoflavonoids being the most promising. Flavonoids/isoflavonoids of particular interest were apigenin, chrysin, pseudobaptigenin, biochanin A, genistein, and hesperidin (● Fig. 2). When tested *in vitro* for cell-based transcriptional factor activity, all compounds demonstrated significant activation of PPAR γ . The above screening also selected the flavanone naringenin (● Fig. 2) among the best scoring docked compounds, which was later confirmed to activate PPAR γ *in vitro* by Christensen et al. [55].

Using a structure-based pharmacophore screening of the AnalytiCon Discovery collection (AnalytiCon Discovery GmbH) of natural products and derivatives, Tanrikulu et al. [56] were able to identify two semisynthetic derivatives of the sesquiterpene lactone α -santonin (α -santonin-derivative 1 and α -santonin-derivative 2; ● Fig. 3) as potential PPAR γ agonists. The testing of these compounds in a cellular reporter gene assay revealed that α -santonin-derivative 1 activated PPAR γ with 110% relatively to the PPAR γ full agonist pioglitazone while it activated PPAR α with 16% relatively to the PPAR α agonist GW7647. This indicates that this compound acts as a PPAR α/γ dual agonist. In comparison, α -santonin-derivative 2 only activated PPAR γ with 33% relatively to pioglitazone without activation of PPAR α , indicating that this compound is a selective PPAR γ partial agonist. Docking of α -santonin-derivative 1 into a PPAR γ structure supported its function as a PPAR γ full agonist as it formed hydrogen bonds to several residues, including Tyr473 [40–42].

In aiming to identify PPAR γ partial agonists, several studies have been performed using pharmacophore-based VS on different natural product libraries (DIOS database [57], Chinese Herbal Medicine database [58], Chinese Herbal Constituents database [59], Natural product subset of the ZINC database [60], and in-house natural products databases). Fakhrudin et al. [58] identified three neolignans (dieugenol, tetrahydrodieugenol, and magnolol; ● Fig. 4) as PPAR γ partial agonists. The results were also confirmed by a PPAR γ luciferase reporter gene transactivation assay, as the maximal fold activation by all three compounds was several folds lower than the full agonist pioglitazone. The three compounds were docked into the X-ray structure of PPAR γ . X-ray crystallography experiments later confirmed the computationally predicted bivalent binding (● Fig. 5) and a conformation typical for partial agonists, including a hydrogen bond to the residue Ser342 [35,36]. Petersen et al. [61] identified oleanolic acid (● Fig. 4), which also shows a binding mode different from full agonists.

Lately, Guasch et al. [62,63] performed a VS study to identify potential PPAR γ partial agonists in extracts with known antidiabetic activity. They developed a structure-based pharmacophore and anti-pharmacophore (i.e., a 3D interaction pattern that matches those molecules, which describes the binding to undesired PPAR γ isoforms or binding modes) to identify potential PPAR γ partial

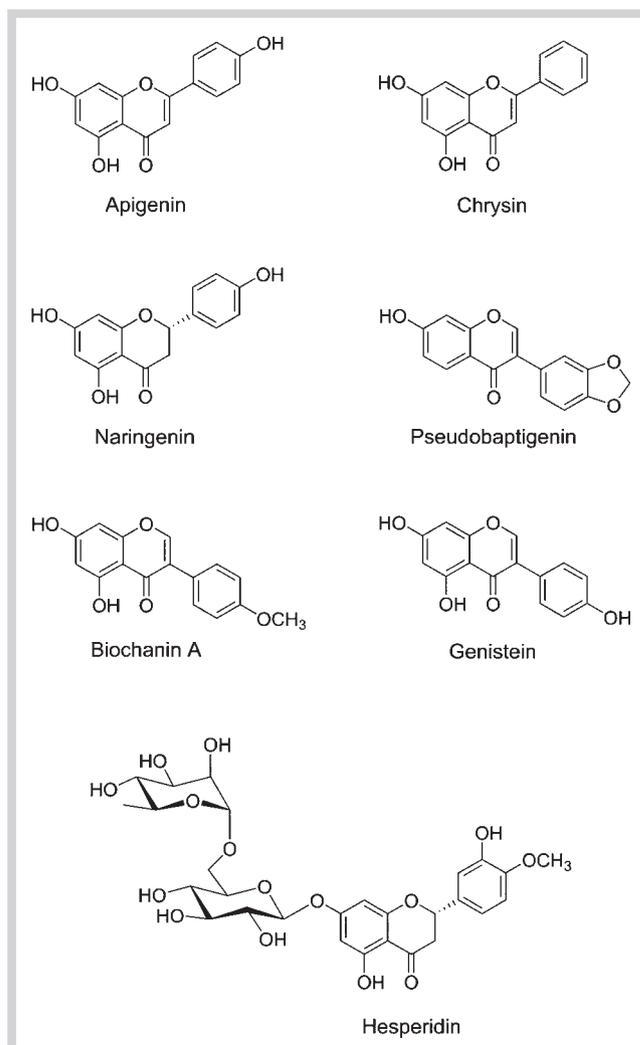


Fig. 2 Chemical structures of naturally occurring flavonoids (apigenin, chrysin, naringenin, hesperidin) and isoflavonoids (pseudobaptigenin, biochanin A, genistein) discovered by virtual screening as PPAR γ agonists.

agonists. The anti-pharmacophore was used to exclude possible full agonists because they present more clearly defined features than partial agonists. They applied their VS workflow to a group of PPAR γ partial agonists known from the literature and some decoys. Sixty-five compounds were predicted by the VS workflow to be potential PPAR γ partial agonists and have all been isolated from 74 natural sources. Among the identified compounds, some were found in extracts that exert antidiabetic activities while others were related to extracts never recorded for antidiabetic activity.

In silico screening has thus proven to be a highly effective enhancement to bioassay screening and thus renders the search for novel potential antidiabetic plant extracts and compounds faster and cheaper than the usual bioassay-guided approach.

Conflict of Interest



The authors declare no conflict of interest.

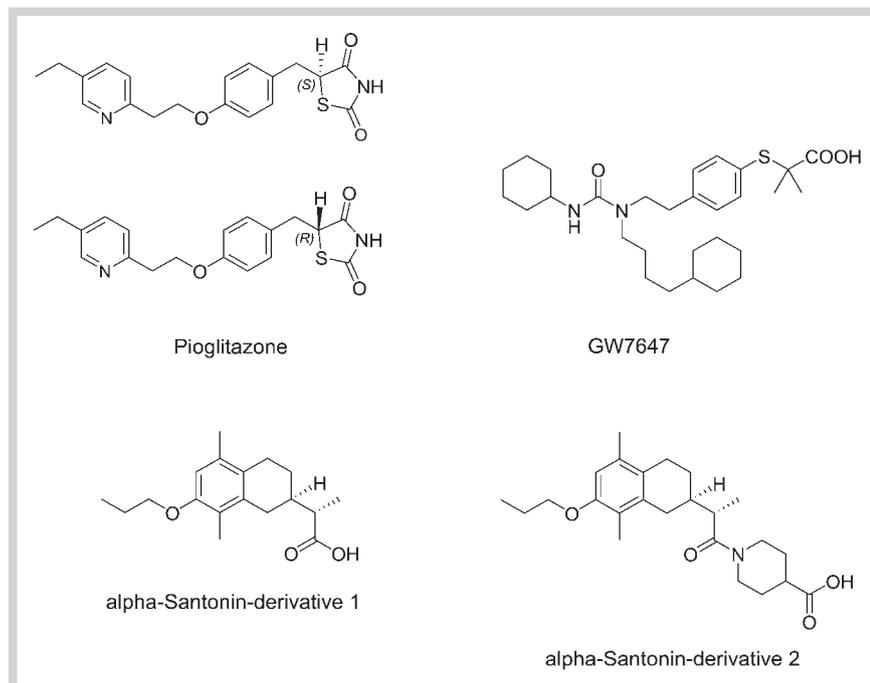


Fig. 3 Chemical structures of pioglitazone, GW7647, α -santonin-derivative 1, and α -santonin-derivative 2. Pioglitazone has one chiral center situated in the thiazolidinedione ring and the active substance is an equimolar mixture of the (R)- and (S)-enantiomers of pioglitazone (racemic mixture) [73].

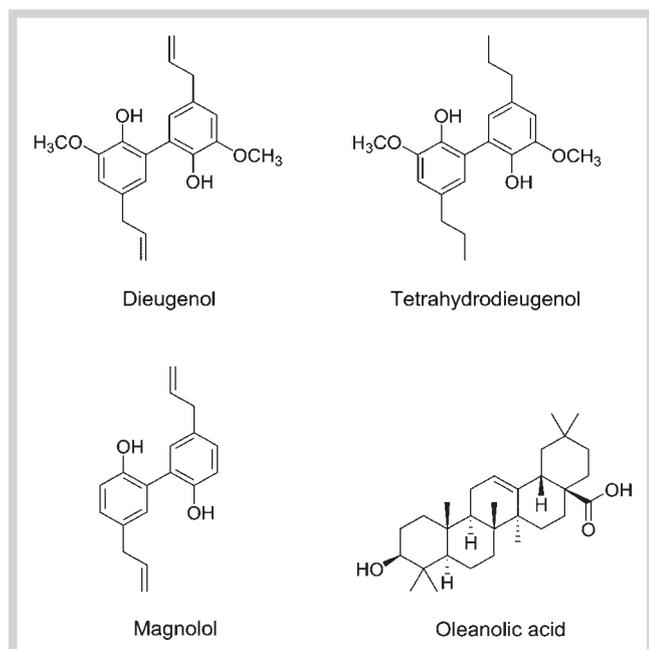


Fig. 4 Chemical structures of neolignans (dieugenol, tetrahydrodieugenol, magnolol) and the triterpenoid oleanolic acid isolated from natural sources and discovered by *in silico* screening as being potential PPAR γ partial agonists.

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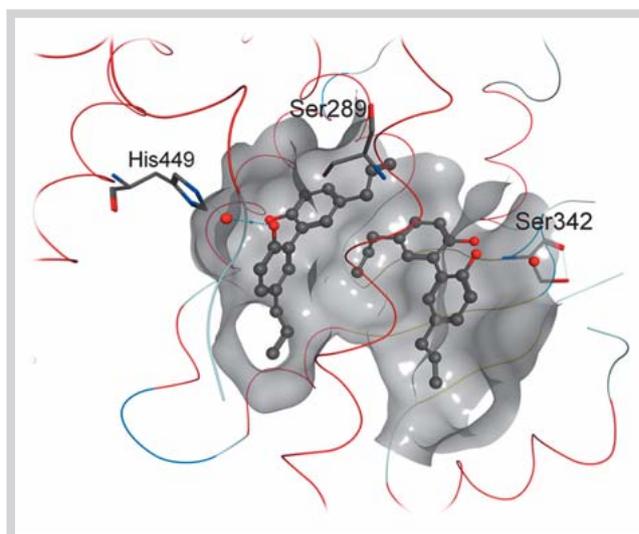


Fig. 5 Binding mode of magnolol to PPAR γ . Two magnolol molecules bound to the ligand-binding site of PPAR γ are shown in ball and stick depiction (PDB code 3R5N [74]). Key interacting residues are shown in stick mode. (Color figure available online only.)

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