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Structure-Based Pharmacophore Screening for Natural-Product-Derived PPARγ Agonists

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Peroxisome proliferator-activated receptors (PPARs) are transcription factors that play a critical role in lipid signalling and immunomodulation and functionally interact with other nuclear receptors, like PXR and NF-κB, in the regulation of lipid metabolism.[1] Therefore, agonists of PPARα and PPARγ serve as therapeutic agents for the treatment of dyslipidaemia, type II diabetes and artherosclerosis, while their effects on the regulation of cell proliferation are under investigation.[2] Several natural compounds have been identified that activate PPARs, including the tetrahydrocannabinol (THC) metabolite THC-11-oic acid,[3] carnosic acid and carnosol,[4] and resveratol.[5] These can provide a starting point for the combinatorial exploration of natural-product-derived compounds for lead discovery and development,[6] with the aim of substituting existing PPAR agonists with potentially safer drugs containing novel scaffolds.[7] Here, we present a virtual screening protocol that led to a PPARγ agonist from a combinatorial compound library that was derived from the scaffold structure of α-santonin, a natural sesquiterpene lactone found in mugwort.[8] We demonstrate that it is possible to find lead candidates in small combinatorial compound collections with minimal experimental effort by "fuzzy" pharmacophore screening.

For the generation of a pharmacophore query, we superimposed four high-resolution X-ray structures of the PPARγ ligand binding domain in complex with agonists (PDB[9] ID: 1nyx with Ragaglitazar,[10] 1knu with YPA,[11] 1i7i with Tesaglitazar,[12] 1zgy with Rosiglitazone,[13] Figure 1 A). The resulting ligand alignment served as the basis for pharmacophoric point assignment by our software LIQUID, as described previously.[14] Briefly, LIQUID represents potential pharmacophoric points (lipophilic, hydrogen-bonding) in a molecule by Gaussian densities. These densities are converted to probabilities for the pairwise matching of compounds. As a result of LIQUID matching and scoring, a screening library is sorted so that the best matching compounds appear at the top of the ranked list. From this list, the most promising screening candidates are picked. For the generation of the LIQUID descriptors from 3D molecular conformations, we used cluster radii of 1 Å for lipophilic centres and 2 Å for hydrogen-bonding centres (donors, acceptors, and donor + acceptor). No other pharmacophoric features were considered to obtain a coarse-grained model that allowed for scaffold hopping to occur. The resulting pharmacophore query is depicted in Figure 1 B. This procedure was performed in order to obtain a "receptor-relevant" pharmacophore model of PPARγ agonists instead of using a ligand-based spatial alignment of artificially generated conformers. This structure-based alignment of multiple ligands allowed us to compute "fuzzy" pharmacophoric feature points, so that we obtained a probability-weighted consensus model. It is noteworthy that the explicit consideration of "voids" or "forbidden regions" is not required, as the probabilities for the presence of a pharmacophoric feature adopt values close to zero in the vicinity of the model.

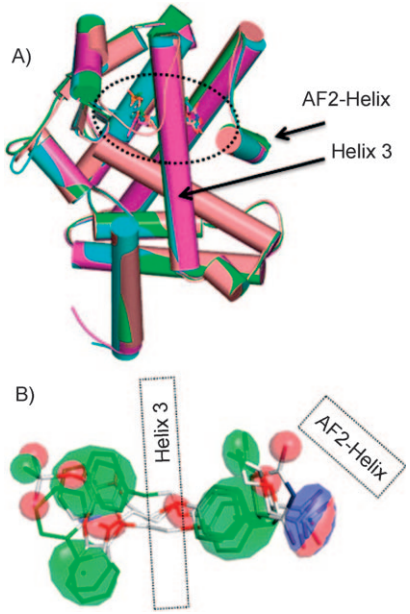

Figure 1. A) Structural superimposition of four PPARγ-agonist complexes; B) The alignment-derived LIQUID pharmacophore query. In the pharmacophore model, lipophilic centres are shown in green, potential hydrogen-bond donor sites are shown in blue, and potential hydrogen-bond acceptor sites are shown in red. Approximate locations of helix 3 and the AF2-helix are indicated. The trivariate Gaussians are shown with widths of one standard deviation in each direction.

We then searched the AnalytiCon Discovery collection of natural-product-derived combinatorial compounds (version 01/2007, 15 590 entries) for hits matching the LIQUID pharmacophore query. A single 3D conformation was computed for each compound by using Corina v3.2 (Molecular Networks GmbH, Erlangen). This concept was shown to be sufficient for first-

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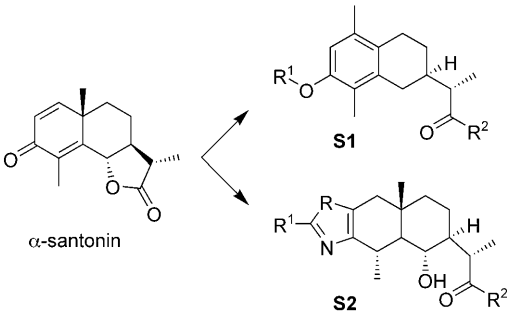
pass virtual screening, in comparison to more demanding multiconformer approaches.[15] LIQUID descriptors were generated from these conformers and compared to the query descriptor with the Euclidean distance metric. Among the top-scoring semisynthetic natural-product analogues, scaffolds **S1** and **S2** dominated (475 compounds in total; Scheme 1). Both scaffolds



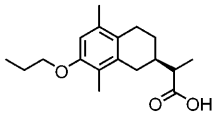
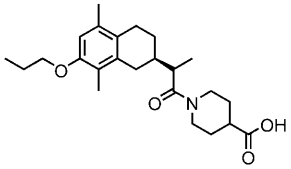
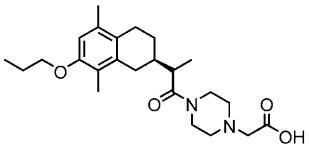
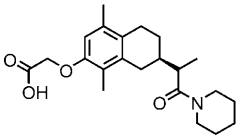
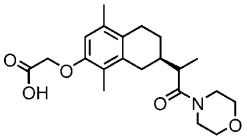
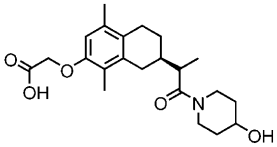
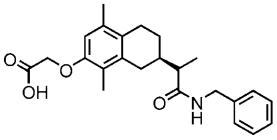
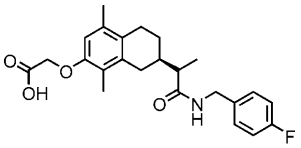
Scheme 1. Chemical structures of two molecular scaffolds derived from α-santonin. Both scaffolds were found among the top-scoring virtual hits obtained by LIQUID pharmacophore searching in the AnalytiCon Discovery compound collection.

were derived from α-santonin by means of chemical modifications.[8] Notably, α-santonin itself was inactive in our PPAR assays. We decided to focus on scaffold **S1**, which had the higher ligand efficiency[16] of the two prevalent scaffolds, and manually selected eight compounds from two small series for activity testing (Table 1). The manual selection aimed at compounds containing carboxyl groups in positions R^1 or R^2, as an acidic ligand moiety has been shown to be important for PPAR activation.[1]

Two compounds exhibited PPAR activation in a cell-based reporter gene assay (Table 1). Compound **1** was the more potent, activating PPARγ with an EC_{50} value of approximately 15 μM and only slightly but measurably activating PPARα (Figure 2). Compound **2**, stemming from the same series, was a weak PPARγ agonist, but did not activate PPARα due to the additional piperidine linker. It is noteworthy that compounds containing the tetrahydronaphtole **S1** are known to inhibit 5-lipoxygenase (5-LO).[17] This observation supports the hypothesis that natural-product-derived scaffolds might represent "biophores", that is, preferred molecular frameworks for protein binding.[18] Different side-chain decorations can be explored to yield ligands with an affinity towards different protein targets. The scaffold **S1** seems to orient side chains in such a way that lipid-binding receptors are preferably addressed. It is important to realise that biophores represent a general theoretical framework for promiscuous binding behaviour, but the individually decorated compounds might well be selective ligands.

Based on EC_{50} values as a very crude approximation of K_d values, the computed ligand efficiency[16] of compound **1** is $\Delta g = (\Delta G^\circ/\text{number of non-hydrogen atoms}) \approx -1.3\ \text{kJ mol}^{-1}$ per non-hydrogen atom, and for Farglitazar, $\Delta g \approx -1.2\ \text{kJ mol}^{-1}$ per non-hydrogen atom. This finding leaves room for the optimisation of **1** as a favourable lead structure, bearing in mind

Table 1. Activity of compounds tested in a cellular reporter gene assay[7] at a concentration of 30 μM. Values give PPARα activation relative to the selective agonist GW7647 (EC_{50} = 6 nM) and PPARγ activation relative to the selective agonist Pioglitazone (EC_{50} = 0.27 μM). Structures were aligned according to the α-santonin-derived scaffold.

No.	Chemical structure	PPARα	PPARγ
1		16 ± 3%	110 ± 31%
2		0%	33 ± 8%
3			inactive
4			inactive
5			inactive
6			inactive
7			inactive
8			inactive

that the maximally achievable affinity per non-hydrogen atom has been estimated to be $-4.2\ \text{kJ mol}^{-1}$.[19]

As a final test and to gain a preliminary understanding of the ligand-receptor interaction, we performed automated docking of **1** into the binding site of PPARγ using the software GOLD (version 3.2).[20] Figure 3 shows the docking pose of **1** suggested by the best score (*GoldScore* = 59) in comparison to the cocrystallised binding conformation of Farglitazar (K_i = 1 nM[21]), which was chosen as an independent reference (not part of the pharmacophore query). The carboxylic head group forms hydrogen bridges to amino acid side chains that are known to be involved in agonist binding, including Tyr473,

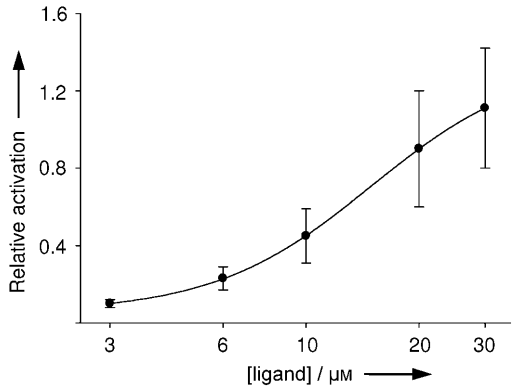


Figure 2. Dose response curve of **1** for the activation of PPARγ, determined in a Gal4-dependent luciferase reporter gene assay in Cos7 cells. The plot gives activities ±SD relative to the reference compound Pioglitazone ($n \geq 3$).

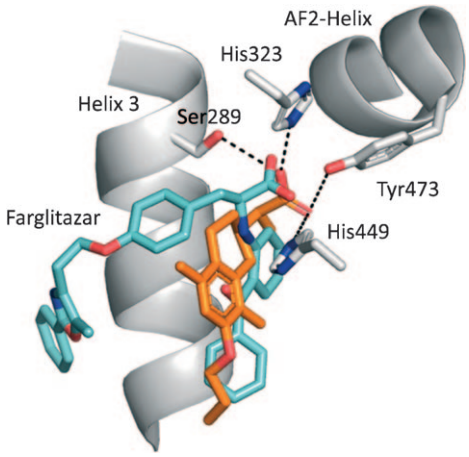


Figure 3. Docking pose of **1** (in orange) in a crystal structure of PPARγ in complex with Farglitazar (PDB ID: 1fm9[27]). Potential hydrogen bonds are drawn as dashed lines.

which is part of the activation function 2 (AF2-helix, helix 12), a critical structural domain for PPAR function.[1,22] The docking study supports our experimental findings and suggests that compound **1** is a full agonist of PPARγ, as binding to the AF2-helix of the ligand-binding site is required for full agonist activity, and partial agonists function via an independent mechanism.[23] Due to its comparably small lipophilic tail, compound **1** does not embrace helix 3 (Figure 3); this might explain its lower potency compared to classical full PPARγ agonists. Recently, a structure-based virtual screening approach was followed by Salam et al.,[24] who identified ψ-baptigenin (EC_{50} = 2.9 μM) and other flavonoids as PPARγ agonists by fully flexible, induced-fit docking of 200 natural products into the binding pocket of PPARγ. Compound **1** (290 Da) and ψ-baptigenin (282 Da) exhibit a similar pharmacophoric pattern and an apparently similar binding mode. The head groups of ψ-baptigenin (hydroxyl) and **1** (carboxyl) presumably interact with the same residues (Ser289, His323, Tyr473). Both docking methods place the compounds in the left proximal pocket of the PPARα binding site,[25] although in the rigid docking approach the reported π-π stacking interaction with Phe363[24] could not be observed. This observation additionally corroborates our fuzzy pharmacophore approach as a complementary technique to automated ligand docking, as it implicitly considers receptor flexibility. A main advantage of the pharmacophore technique is the fact that virtually millions of compounds can be screened for candidate compounds, which may either be tested directly—as in this study—or serve as input for flexible, structure-based docking as a second filtering step in a virtual-screening triage. In the case of PPARγ, the ligand-binding domain can bind a ligand in several slightly different conformations, as shown for the dual-acting PPARα/γ agonist Ragaglitazar by Ebdrup et al.[26] The authors also demonstrated that both PPARγ and PPARα contain a lipophilic interaction surface in the hydrophobic tail pocket, which is in agreement with our docking pose of **1** (Figure 3).

In summary, we have demonstrated that natural-product-derived combinatorial libraries can be efficiently screened by structure-based pharmacophore matching and scoring to give novel bioactive substances. For "fuzzy" pharmacophore query generation, we used the alignment of cocrystal structures of PPARγ with potent agonists, rather than a ligand-based alignment. This concept provides a straightforward route to finding novel bioactive agents with minimal experimental effort. Here, we have identified a new chemotype of PPAR agonists that could serve as a starting point for lead-structure generation and optimisation.

Experimental Section

Receptor activation was tested in a luciferase reporter gene assay as described.[4] In brief, Cos7 cells were seeded in 96-well plates at a density of 30 000 cells per well (100 μL) and transfected with lipofectamine (Gibco), the Gal4-dependent reporter vector pFR-luc, the Gal4-PPAR-LBD fusions pFA-CMV-PPAR of the respective subtype, and pRL-SV40 for normalisation. Four hours after transfection, cells were incubated with the test compound in triplicate wells. Luciferase activity was determined the following day with Dual-glo (Promega). Each experiment was repeated at least three times. The average relative activation was calculated relative to a reference compound (Pioglitazone (1 μM) for PPARγ and GW7647 (1 μM) for PPARα). The determination of EC_{50} values was carried out with SPSS2001 (SPSS, Chicago).

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Keywords: bioisosters · combinatorial chemistry · drug design · lipid metabolism · virtual screening

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