DOI: 10.1002/cmdc.200800313

From Molecular Shape to Potent Bioactive Agents I: Bioisosteric Replacement of Molecular Fragments

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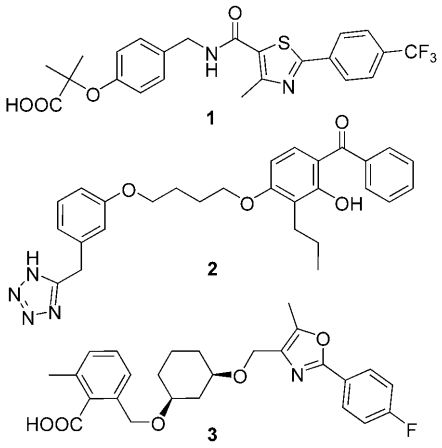
Ligand binding to a macromolecular receptor is based on complementarity of both molecular shape and receptor–ligand interaction points. With surprisingly few exceptions,[1] ligand-based virtual screening approaches consider only one of these two principles explicitly. To fill this gap, we have developed a method for comparison of both molecular shape and potential pharmacophore points (PPPs), termed SQUIRREL (Sophisticated QUantification of InteRaction RELationships). This ligand-based technique was applied in the design of a small, focused screening library with the aim to find novel agonists of peroxisome proliferator-activated receptors (PPARs). PPARs are pharmaceutically relevant members of the nuclear receptor superfamily.[2] Although agonists of PPARα and PPARγ have been approved for treatment of dyslipidemia and type-2 diabetes, novel leads with distinct selectivity profiles are required to improved safety and enhanced therapeutic efficacy.[3] Among several new bioactive chemotypes, we identified a potent PPARα-selective activator ($EC_{50} = 44 \pm 5$ nM) from a large compound collection with minimal experimental effort. This compound represents a scaffold-hop from known PPAR agonists and provides proof-of-concept for the potential utility of ligand-based virtual screening in early phases of drug discovery.

A necessary prerequisite for SQUIRREL is one or more active reference molecules, that is, known PPARα agonists in this study (compounds **1**–**3**, Scheme 1). The ligand binding site of all PPARs is large and deeply buried, and a great part of the ligand surface interacts with protein residues.[4] In particular, a potent PPAR agonist should interact with residues stabilizing the AF2 "activation" helix (S280, Y314, H440, Y464 in PPARα;[5] S289, H323, Y473, H449 in PPARγ[4]). Therefore, we decided to follow a two-step virtual screening protocol for shape-based matching and subsequent pharmacophore-based scoring of candidate compounds.

The first step was to obtain a multitude of possible shape-based alignments of the reference molecules and the screening compounds. For this task, we used our Shapelets approach.[6] The basic idea of Shapelets is to locate similar local shapes on molecular surfaces, and compare two molecules based on the pair-wise similarity of local shape descriptions (Figure 1 a). The method starts from a smooth triangulated molecular surface, which is obtained by isosurface extraction.[6] Isosurfaces are then decomposed into "knobs" and "planes" by fitting hyperbolic paraboloids. By representing two molecules as two sets of such hyperbolic paraboloids, pair-wise, shape-based alignments can be obtained by clique detection in association graphs.[7]



Scheme 1. Reference PPAR agonists for virtual screening. Compound **1** (GW590735): $EC_{50} = 4$ nM (PPARα), > 10 μM (PPARγ);[5] Compound **2** (Merck): $IC_{50} = 140$ nM (PPARα) $IC_{50} = 1.7$ μM (PPARγ)[14]; Compound **3** (Aventis): $EC_{50} = 0.3$ nM (PPARα),[15] activity on PPARγ not reported.

The second step was to assess the quality of the shape-based alignments in terms of a "fuzzy" pharmacophore function, which originates from the LIQUID concept.[8] This scoring function matches Gaussian representations of PPPs and computes their overlap for two aligned molecules. The sum of all PPPs can be interpreted as a pharmacophoric density field (Figure 1 b). The overlap of two fields is computed as a similarity score indicating the match of a molecule to a given reference molecule.

As an initial test of SQUIRREL, we performed retrospective virtual screening. The task was to retrieve known PPAR agonists from a large collection of druglike compounds. Virtual screening methods that use a combination of shape and pharmacophore information performed better on subtype-selective PPAR agonists than methods that exclusively used only shape- or pharmacophore-based matching (see Supporting Information). SQUIRREL was shown to be well-suited to the task of PPAR ligand retrieval, with high success rates for the top-ranking compounds.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200800313.

Notably, the SQUIRREL virtual screening procedure is completely independent from the underlying chemotype of the reference ligand, enabling scaffold hopping.[9] In Figure 2, an example is shown for the fibrate head group of selective PPARα agonists.[5] SQUIRREL screening of a library containing 17934 molecular fragments —obtained from retrosynthetic decomposition of the COBRA6.1 database[10]—automatically identified several fibrate bioisosters with known bioactivity.

Encouraged by the retrospective results, we then applied the SQUIRREL virtual screening procedure to identify prospective PPARα agonists in the SPECS compound library (199272 compounds, v.06.2007; www.specs.net).

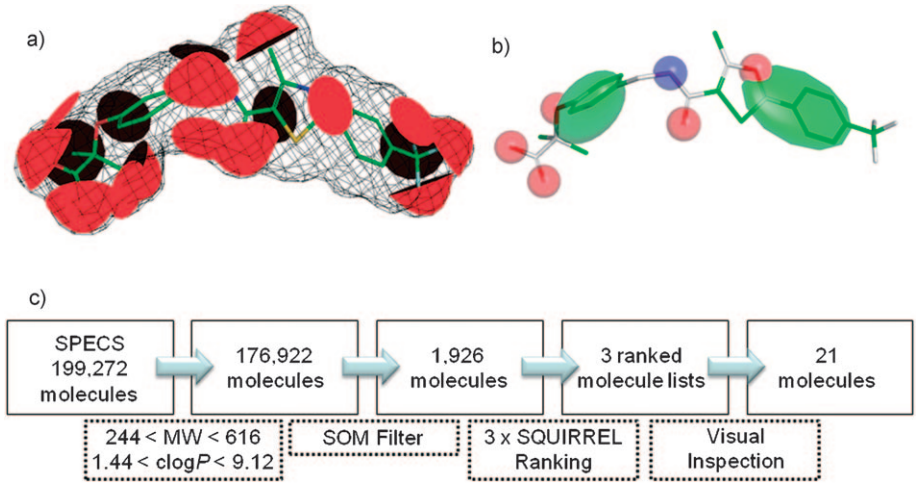


Figure 1. Surface decomposition of the selective PPARα activator GW590735 (**1**; PDB code: 2p54[5]). a) Molecular isosurface as mesh, with fitted paraboloids (Shapelets); b) Gaussian pharmacophore density field (red: potential H-bond acceptor, blue: potential H-bond donor, green: lipophilic). c) Virtual screening work-flow.

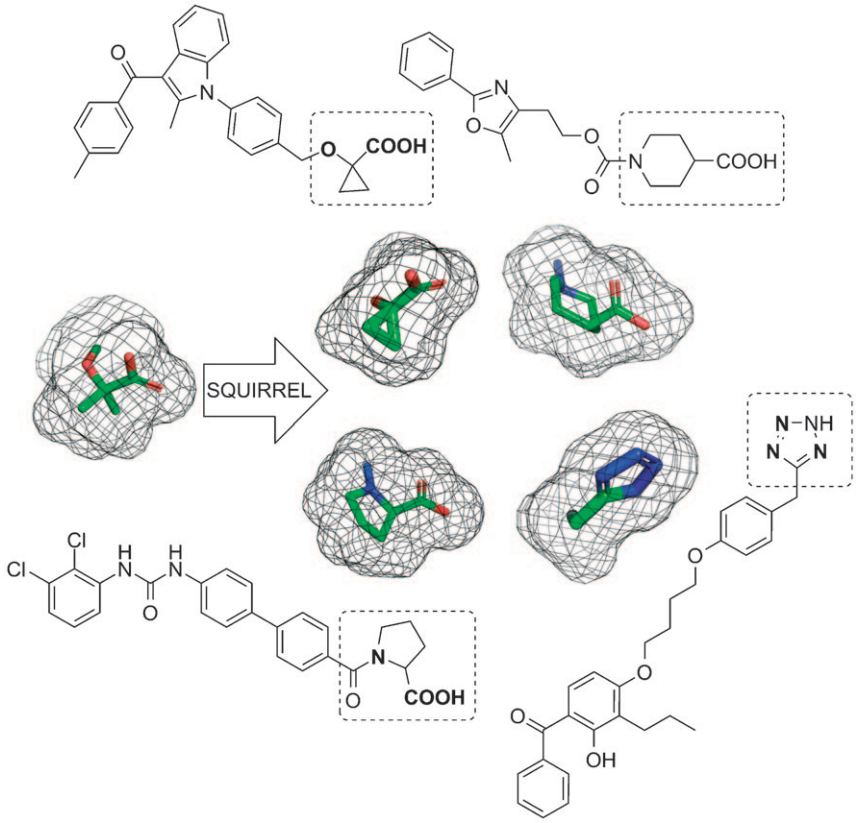
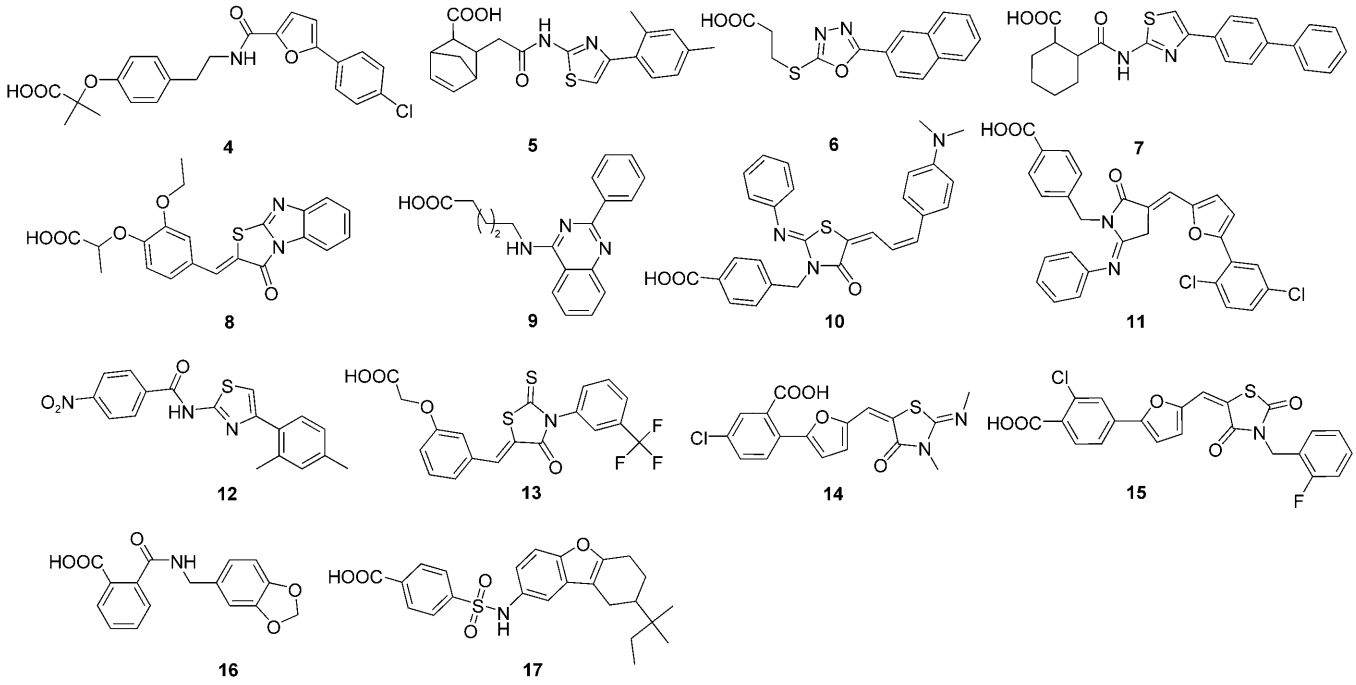


Figure 2. Bioisosteric replacement of the fibrate head group, 2-hydroxy-2-methyl-propionic acid, by SQUIRREL. These fragments (highlighted) were found as partial matches in known PPAR agonists.[11,15]

First, we selected molecules with PPAR agonist-like properties ($244 < MW < 616$ Da; $1.44 < clogP < 9.12$)[12] leaving 176922 compounds. Then, a self-organizing map (SOM) was trained, and we focused on the candidate compounds located on the PPARα "activity islands" on the SOM (Supporting Information).[13] These 1926 candidates were screened using SQUIRREL; compounds **1**–**3** with nanomolar agonistic activity on PPARα and at least tenfold selectivity for PPARγ were used as reference agonists. Preparation of reference compounds for virtual screening is described in the Supporting Information. From the three resulting ranked lists (Supporting Information), 21 molecules were manually selected and tested in a cell-based functional assay. This inspection was performed to ensure that different chemotypes were selected and molecules with potentially reactive groups were avoided. Manual inspection was deliberately performed as the last step of the screening process to keep potential bias introduced by a human expert at a minimum.[16]

With regard to the ab initio expected low affinity of putative PPAR ligands[17] we chose an initial screening concentration of up to 100 μM. Seven of the tested 21 compounds activate PPARα at a ligand concentration below 100 μM.[17] In total, 14 compounds (shown) were active on either PPARα or PPARγ. Hit compound **4** is a typical representative of the fibrate chemotype with expected high potency and selectivity. The remaining hits belong to remarkably different chemotypes. Compound **10**,



in particular, exhibits nanomolar activity and marked selectivity for PPARα. A putative receptor binding mode of compound **10** was obtained by automated docking (Figure 3). In this model, the carboxylic moiety of compound **10** interacts with residues S280, Y314, H440 and Y464, while the phenyl moiety occupies the right distal pocket. The long hydrophobic tail occupies the hydrophobic (left distal)[18] pocket of PPARα, which is likely to be the reason for selectivity as the analogous pocket in PPARγ is lined with bulky residues. The left proximal pocket, which is smaller in PPARα, remains unoccupied.

Summarizing, these results demonstrate the ability of sophisticated ligand-based approaches, like SQUIRREL, to find potent and selective ligands of different chemotypes, where a combination of shape and pharmacophore information proved particularly suited for scaffold hopping.

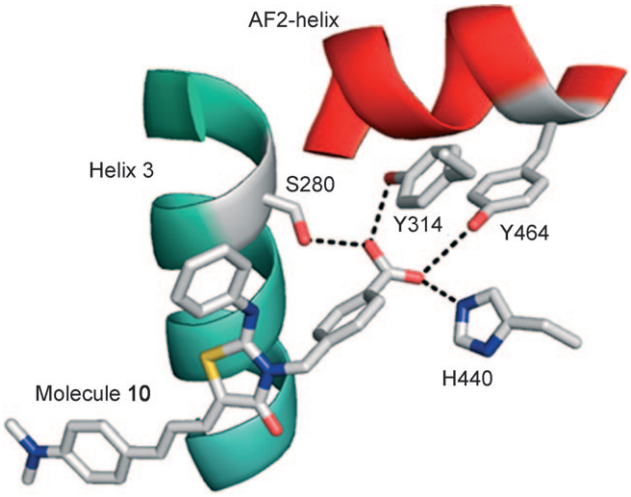


Figure 3. Docking mode of the novel nanomolar PPARα agonist **10**. For automated ligand placement and scoring, the software GOLD (version 3.2)[19] was used. The docking was performed with the X-ray structure of PPARα (PDB code: 2p54[5]). The binding site was defined within a 10 Å radius around the co-crystallized ligand GW590735. Ten docking solutions (ChemScore function) were analyzed, and the best-scoring binding mode (Score = 49.5) is shown.

Experimental Section

In-vitro transactivation assays, cell culture and transfection: Plasmids and propagation of the luciferase assay were described previously.[20,21] Cos7 cells were grown in DMEM supplemented with FCS, sodium pyruvate, penicillin/streptomycin and glutamine at 10% CO_2 and 37 °C. Cells were seeded in 96-well plates at a density of 30 000 per well the day before transfection. Cells were transfected with Lipofectamine 2000 according to the manufacturer's protocol with pFR-Luc (Stratagene, La Jolla, CA, USA), pRL-SV40 (Promega, Mannheim, Germany) and the respective receptor expression plasmid pFA-CMV-hPPARγ-LBD or pFA-CMV-hPPARα-LBD. After transfection, the medium was changed to DMEM without phenol red and FCS, containing appropriate concentrations of test compounds. Each concentration was tested in triplicate wells and each determination was repeated at least three times.

Luciferase assay: After overnight incubation with the test compounds, the luciferase assay was carried out using Dual-Glo Luciferase Assay System (Promega, Mannheim, Germany) according to the manufacturer's protocol. Luminescence was measured using a GENios Pro luminometer (Tecan, Crailsheim, Germany).

Compounds: Compounds were dissolved in DMSO and diluted 1:1000 upon addition to the cells. Normalization for transfection efficacy and cell growth was done by Renilla luciferase data. Activation factors were obtained through dividing by the DMSO control.

Table 1. Experimentally confirmed PPAR agonists from virtual screening (**4**–**17**).[a] For comparison, reported activities of reference compounds **1**–**3** are given.

Cmpd	PPARα agonism[b]	PPARγ agonism[b]
1	0.004 μM[5]	>10 μM[5]
2	0.140 μM (IC_{50})[14]	1.7 μM (IC_{50})[14]
3	0.0003 μM[15]	not reported
4	1.75±0.24 μM	13±2.2 μM
5	inactive	47±12%[c]
6	inactive	26±8%[c]
7	inactive	47±12%[c]
8	inactive	34±18%[c]
9	inactive	7±0.5 μM
10	0.044±0.005 μM	4.9±0.4 μM
11	80±17%[c]	inactive
12	40±6%[c]	inactive
13	inactive	56±28%[c]
14	63±6%[c]	106±37 μM
15	inactive	10.5±0.7 μM
16	34±14%[c]	70.5±8.1 μM
17	20.2±25.6 μM	19.5±0.1 μM

[a] The highest compound concentration was 100 μM; [b] EC_{50} values obtained from triplicate measurement with at least five ligand concentrations in a cell-based reporter gene assay, or maximum effect given as percentage of the positive control (GW7647 for PPARα, Pioglitazone for PPARγ); [c] PPAR agonism observed at 100 μM.

EC_{50} values and the standard deviations were calculated from at least three determinations by SigmaPlot 2001 (SPSS Inc.) using four parameter logistic regressions. All active compounds were evaluated to act as full agonists by comparison of the achieved maximum effect to that of reference compounds GW7647 (PPARα) and Pioglitazone (PPARγ).

Acknowledgements

This work was supported by the Beilstein-Institut zur Förderung der Chemischen Wissenschaften, and the LOEWE Lipid Signaling Forschungszentrum Frankfurt (LiFF).

Keywords: bioisosters · molecular modeling · PPAR agonists · scaffold hopping · virtual screening

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Received: September 25, 2008
Published online on December 4, 2008

