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From Molecular Shape to Potent Bioactive Agents II: Fragment-Based de novo Design**

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Agonists of peroxisome proliferator-activated receptors (PPARs) are important drugs for dyslipidemia and type-II-diabetes.[1] Agonists of PPARα and PPARγ, like Bezafibrate, Fenofibrate, and Rosiglitazone, are already on the market, but novel PPAR agonists with improved pharmacological profiles are still required.[2] In this study, we present the successful de novo design of a PPAR agonist using a novel fragment-based compound assembly strategy.

Previously, we have demonstrated the superiority of methods that combine shape and pharmacophoric information for fully automated virtual screening of PPAR agonists.[3] For the present work, we built on this knowledge and developed a software tool that generates suggestions for bioisosteric replacements to be exploited by medicinal chemists for the generation of novel lead structures.

Our software, SQUIRREL (Sophisticated QUantification of InteRaction RELationships), uses decomposition of the molecular surface into regions with equal curvature ("Shapelets")[4] for shape-based molecular alignment of two molecules by an established subgraph matching routine implemented using a Bron–Kerbosch algorithm.[5] For the de novo design of a compound, we used this procedure to match molecular building-blocks, rather than complete molecules, which were obtained from pseudo-retrosynthetic decomposition of druglike bioactive agents. The computer program then suggests a ranked list of best-fitting building-blocks, and expert visual inspection was then used to identify the best fragments and gain ideas for bioisosteric replacement of molecular moieties.

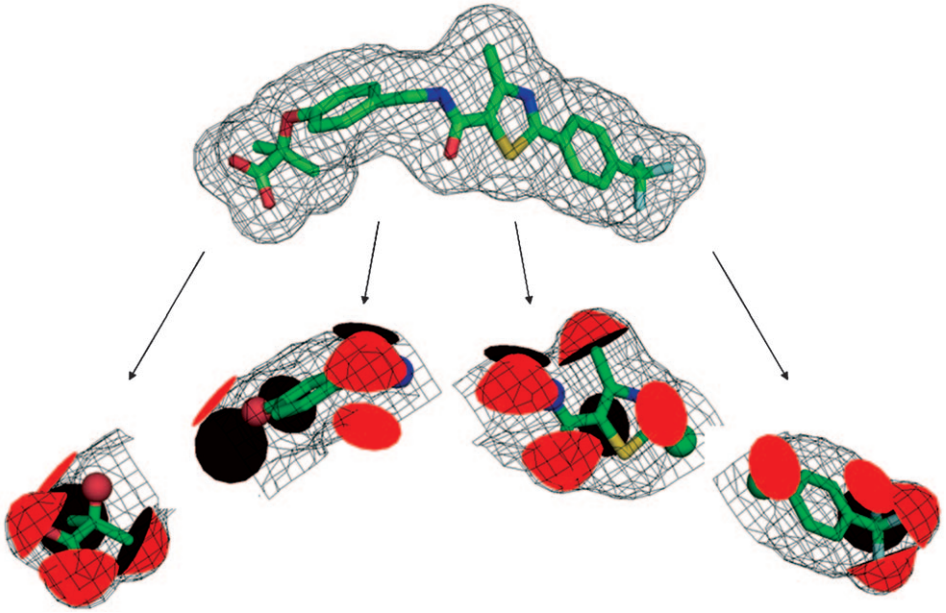

Figure 1. Pseudo-retrosynthetic fragmentation of PPARα agonist GW590735.[10] Shapelets are indicated in red, colored spheres show virtual attachment sites for fragment assembly.

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The first step of our de novo design concept is the generation of a multi-conformer database from a set of reference molecules. Here, we used our COBRA 6.1 collection of known drugs and lead structures.[6] Then, for each conformer, an isosurface is computed and decomposed into Shapelets, which are distinct surface patches representing the local curvature of the molecular surface. RECAP rules[7] are used for pseudo-retrosynthetic fragmentation as described previously.[3] Surface patches are assigned to each generated fragment (Figure 1). As a result, a library of 17934 drug-derived building-blocks is obtained, where each fragment is associated with information about its surface shape. Fragment conformations derived from the parent molecules were used, rather than fragmenting first and computing conformations independently, because the contribution of a fragment to the overall shape of a compound

differs depending on the linker moiety. As expected, the conformational freedom of the fragments is more restricted compared with the parent compounds (on average 2.3 conformations per fragment; 4.5 per compound).

The actual design of candidate molecules begins with the alignment of fragments from the building-block database to a reference compound that is meant to undergo bioisosteric replacements. The alignment was performed by means of a graph matching algorithm as described,[4] and each alignment was scored by the LIQUID "fuzzy" pharmacophore function.[8] This step guarantees that not only the shape but also functional properties and pharmacophoric features of the replacement group are similar to the reference molecule.

In this case study, we focused on the de novo design of a PPARα agonist. Their generic structure is shown in Figure 2.[9]

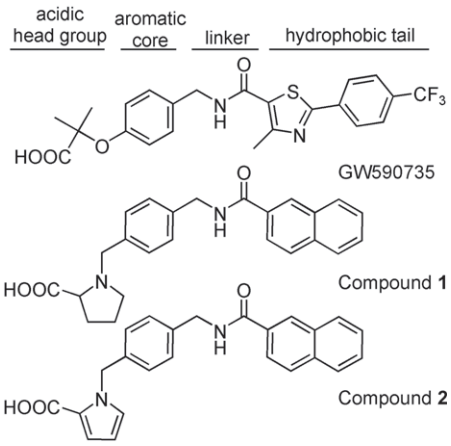


Figure 2. PPARα agonist GW590735 and designed compounds **1** and **2**.

Taking the potent and selective PPARα agonist GW590735 ($EC_{50} = 4$ nM)[10] as a reference structure, we decided to replace the acidic head group and the hydrophobic tail with suitable bioisosteric groups, and keep the central portion unchanged. To do so, we aligned the fragment library to GW590735 and obtained 77 head group and 90 hydrophobic tail fragment suggestions. Fragment matching was performed by SQUIRREL.[3] The resulting collection of chimeric molecules provided new PPARα agonist candidates. For scaffold-hopping, we selected proline from the structural suggestions as the new head group, with the attachment point located at the amine nitrogen, as it is a comparably rigid druglike building block, and coupling to the benzyl moiety is feasible in a single synthesis step (Figure 3a). From the various suggestions for the hydrophobic tail replacement, we selected naphthalene-2-carboxylate with an amide attachment site for ease of synthesis (Figure 3b). The complete lists of fragment suggestions can be found in the Supporting Information.

Compound **1** (1-(4-((2-naphthamido)methyl)benzyl)pyrrolidine-2-carboxylic acid) was synthesized

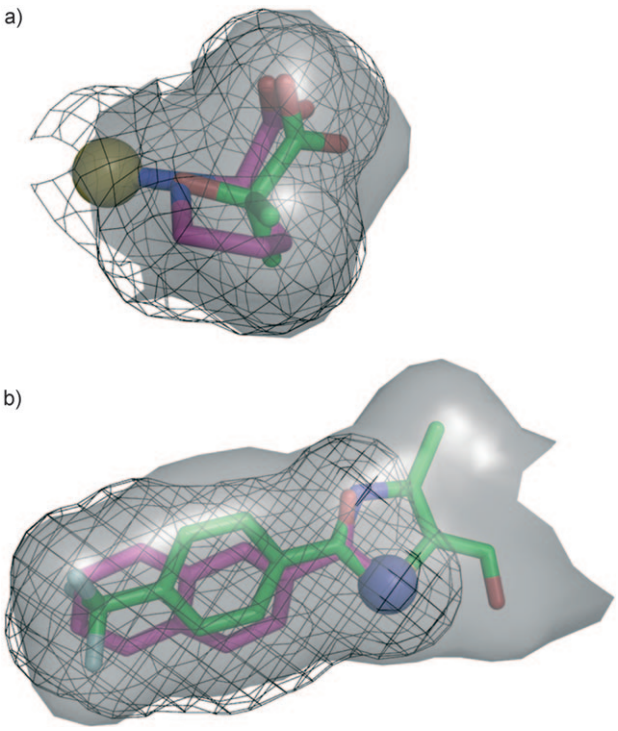
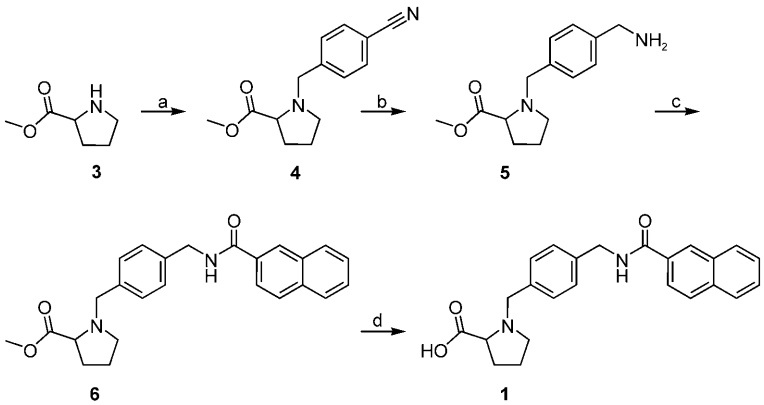


Figure 3. a) The bioisosteric replacement of the acidic head group in GW590735 with Proline: 2-hydroxy-2-methyl-propionic acid, green with transparent surface; proline, purple with mesh surface and attachment site represented by a sphere. b) The bioisosteric replacement of the hydrophobic tail moiety in GW590735 with naphthalene-2-carboxylate: *p*-triflouromethylbenzene, green with a transparent gray surface; naphthalene-2-carboxylate, purple with mesh surface and amide attachment site represented by a sphere.

using the proposed fragments (Scheme 1). Proline methyl ester and 4-formylbenzonitrile were coupled via reductive amination.[12] After hydrogenation of the nitrile to a benzyl amine, amide bond formation took place under microwave irradiation.[13] Ester cleavage led to the desired product. After purification, compound **1** was tested in a cell-based reporter gene



Scheme 1. Synthesis of compound **1**. *Reagents and conditions*; a) 4-formylbenzonitrile, TEA, $NaBH(OAc)_3$, DCE, 3 h, RT; b) Raney-Ni, NH_3, MeOH, 24 h, RT, 10 bar; c) 2-naphthoyl chloride, DCE, μw, 15 min, 90 °C, 50 W; d) KOH, H_2O/MeOH/THF (2:1:1), μw, 15 min, 70 °C, 35 W.

assay for activation of PPARα and PPARγ.[14,15] To our surprise, this compound did not show any significant effect, even at a concentration of 100 μM. Further modeling revealed a plausible reason for this apparent lack of activity; the receptor pocket formed by residues S280, Y314, H440, and Y464 accommodates the acidic head group of PPARα agonists.[10] The surface of this pocket exhibits a marked positive electrostatic potential. As the tertiary amine of the proline moiety present in compound **1** is positively charged under assay conditions, repulsive Coulombic forces prevent the tight binding of compound **1** to PPARα, leading to a weaker than expected biological activity.

To probe this hypothesis we decided to remove the basic centre, replacing the proline moiety with pyrrole-2-carboxylic acid. Ethyl 1*H*-pyrrole-2-carboxylate was coupled with 4-(bromomethyl)benzonitrile in a phase-transfer reaction, resulting in compound **8**,[16] which was treated analogously to compound **4**. After hydrogenation, amidation, and deprotection steps, compound **2** (1-(4-((2-naphthamido)methyl)benzyl)-1*H*-pyrrole-2-carboxylic acid) was obtained in an overall yield of 65% (Scheme 2).

Subsequent testing confirmed the hypothesis; compound **2** exhibits high potency in the cellular activity assay with EC_{50} values of 0.51 ± 0.12 and 0.63 ± 0.03 μM against PPARα and PPARγ, respectively (see Supporting Information for synthesis and assay conditions). The alignment of suggested binding poses of compounds **1** (Figure 4a) and **2** (Figure 4b) with a PPARα-bound conformation of GW590735[10] supports the hypothesis that loss of the positive charge in compound **2** contributes to PPAR binding. In compound **1**, the amine nitrogen is located at the same place as the ether oxygen of GW590735,[10] which might provide a highly unfavorable interaction with H440 in PPARα. In addition, the almost perpendicular position of the central aromatic ring in compound **1** could hinder edge-to-face arene–arene interaction with F318 (not shown).

The lack of selectivity of compound **2** between the two PPAR receptor types is likely to be due to the absence of subtype-specific substituents at the hydrophobic tail, in particular, filling the left distal pocket of PPARα.[17] Future lead optimization could follow this concept to achieve receptor subtype selectivity.

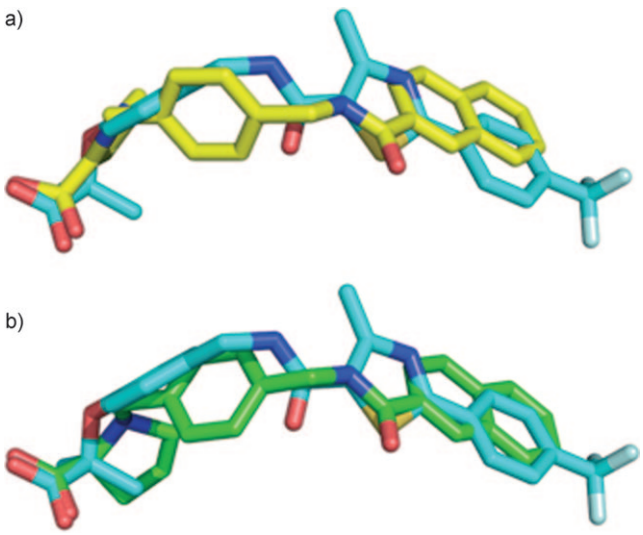
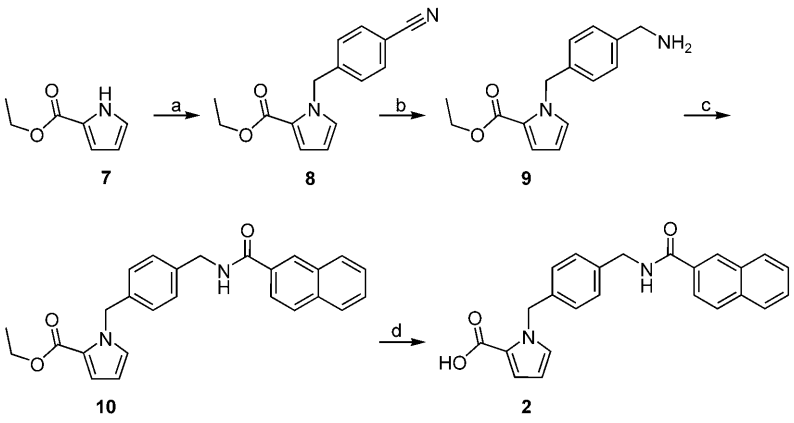


Figure 4. Superposition of reference compound GW590735 (blue) with a) designed compound **1** (yellow) and b) designed compound **2** (green). The conformation of GW590735 was taken from a co-crystal structure (PDB: 2p54[10]), conformations of compounds **1** and **2** were obtained by automated ligand docking into the PPARα binding site with the software GOLD v3.2.[11] The ChemScore values of the best poses shown are 34 (**1**) and 41 (**2**), indicating a better fit of compound **2** to PPARα.



Scheme 2. Synthesis of compound **2**. *Reagents and conditions*; a) 4-(bromomethyl)benzonitrile, TBAI, NaOH, DCM, 24 h, 0 °C?RT; b) Raney-Ni, NH_3, MeOH, 24 h, RT, 10 bar; c) 2-naphthoyl chloride, DCE, μw, 15 min, 90 °C, 50 W; d) KOH, H_2O/MeOH/THF (2:1:1), μw, 15 min, 70 °C, 35 W.

Summarizing, we developed a molecular design tool, which is able to suggest bioisosteric replacement groups for ligand-based de novo design. The applicability of this shape-based technique was demonstrated in a preliminary study on PPAR agonists. Expert knowledge of the target and the synthesis route were shown to be indispensable prerequisites for successful de novo design, which is in perfect agreement with earlier reports.[18–20] Although fully automated de novo design based on different techniques can lead to potent compounds,[21–24] we are still far from designing ready-to-use drugs exhibiting low nanomolar activity and a desired selectivity profile from scratch. As shown here, de novo design should rather be considered as a valuable technique for lead candidate generation and identification of bioisosters in the early phase of drug discovery projects. By visual inspection of bioisosteric replacements the molecular designer can interactively add human expertise and avoid computer-generated artefacts.

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Keywords: bioisosters · de novo design · drug discovery · molecular modeling · scaffold hopping

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