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Research paper

Evaluation of selected 3D virtual screening tools for the prospective identification of peroxisome proliferator-activated receptor (PPAR) γ partial agonists

T. Kaserer [a, 1], V. Obermoser [b, 1], A. Weninger [b], R. Gust [b], D. Schuster [a, *]

[a] Computer-Aided Molecular Design Group, Institute of Pharmacy/Pharmaceutical Chemistry and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria
[b] Pharmaceutical Chemistry and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 80-82, 6020 Innsbruck, Austria

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ABSTRACT

The peroxisome proliferator-activated receptor (PPAR) γ regulates the expression of genes involved in adipogenesis, lipid homeostasis, and glucose metabolism, making it a valuable drug target. However, full activation of the nuclear receptor is associated with unwanted side effects. Research therefore focuses on the discovery of novel partial agonists, which show a distinct protein-ligand interaction pattern compared to full agonists. Within this study, we employed pharmacophore- and shape-based virtual screening and docking independently and in parallel for the identification of novel PPARγ ligands. The ten top-ranked hits retrieved with every method were further investigated with external *in silico* bioactivity profiling tools. Subsequent biological testing not only confirmed the binding of nine out of the 29 selected test compounds, but enabled the direct comparison of the method performances in a prospective manner. Although all three methods successfully identified novel ligands, they varied in the numbers of active compounds ranked among the top-ten in the virtual hit list. In addition, these compounds were in most cases exclusively predicted as active by the method which initially identified them. This suggests, that the applied programs and methods are highly complementary and cover a distinct chemical space of PPARγ ligands. Further analyses revealed that eight out of the nine active molecules represent novel chemical scaffolds for PPARγ, which can serve as promising starting points for further chemical optimization. In addition, two novel compounds, identified with docking, proved to be partial agonists in the experimental testing.

1. Introduction

The peroxisome proliferator-activated receptors (PPARs) belong to the class of nuclear receptors. Upon activation, the receptor forms heterodimers with the retinoid X receptor (RXR) to control the expression of its targets genes [1]. PPARγ is highly expressed in adipose tissue [2], where it is involved in adipogenesis [3], lipid homeostasis, and glucose metabolism (reviewed in Ref. [4]). It gets activated by a variety of endogenous compounds such as eicosanoid 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 or the fatty acid arachidonic acid [5], and by a number of synthetic [6–11] and natural compounds [12]. Most prominently, PPARγ was identified as the molecular target of the thiazolidinedione (TZD) class of antidiabetic drugs [7,8], including the blockbuster drugs rosiglitazone and pioglitazone. TZDs, of which most are full agonists, promote increased insulin sensitivity. However, their administration has also been

Abbreviations: A, anion; Acc, accuracy; Ar, aromatic feature; AUC, area under the curve; C, cation; DMEM, Dulbecco's Modified Eagle Medium; EE, early enrichment; EF, enrichment factor; FN, false negatives; FP, false positives; FRET, fluorescence resonance energy transfer; GST, gluthatione S-transferase; H, hydrophobic feature; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; maxEF, maximum enrichment factor; maxFN, maximum false negative rate; maxFP, maximum false positive rate; maxTN, maximum true negative rate; maxTP, maximum true negative rate; MI, metal interaction; MNA, Multilevel Neighborhoods of Atoms; NI, negatively ionizable; OE, overall enrichment; PASS, Prediction of Activity Spectra for Substances; PI, positively ionizable; PPAR, peroxisome proliferator-activated receptor; R, ring feature; SD, standard deviation; SEA, Similarity Ensemble Approach; Tc, Tanimoto coefficient; TN, true negatives; TP, true positives; TZD, thiazolidinedione; XVol, exclusion volume.

* Corresponding author.
E-mail address: Daniela.Schuster@uibk.ac.at (D. Schuster).
[1] These authors contributed equally to the publication.

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associated with severe side effects such as gain of body weight and congestive heart disease [13]. These deleterious effects were found to be diminished whereas beneficial effects on insulin sensitivity were maintained, when PPARγ was only partially activated [6,14]. The design of several partial agonists of PPARγ with improved side effect profiles have been reported [11,15,16], including the selective angiotensin receptor blocker telmisartan [9] and its analogues [17]. Partial agonists were shown to display a different interaction mode in the ligand-binding domain compared to full agonists. Most prominently, other than full agonists, partial agonists do not stabilize helix H12 *via* hydrogen bonding with Tyr473 [18].

Virtual screening tools are nowadays well integrated in the drug development process to complement and support experimental high-throughput screenings in the selection of the most promising drug candidates [19]. Several different virtual screening methods are available for this purpose [20] (please refer to www.click2drug.org for a comprehensive list of virtual screening tools), and many of them have already been successfully applied for the identification of novel PPARγ ligands [21–28]. However, whether there are differences in their performances, and if so, which one is the most suitable for addressing nuclear receptor-related issues, and PPAR-related in particular, is still unclear. Several method comparisons have been published throughout the last years [29–38], which points out the raising interest in this topic. Unfortunately, the different methodical set-ups of these studies hamper their comparison on a larger scale. Therefore, a comprehensive and prospective evaluation of the same methods employing identical datasets still remains to be accomplished.

We have already investigated the performances of selected common virtual screening tools for the identification of novel bioactive molecules for cyclooxygenases-1 and -2 as representatives of classical enzymes [39], and for members of the cytochrome P450 (CYP) superfamily involved in metabolic turnover [40]. Intriguingly, we could observe quite distinct performances of the tools, suggesting that different tools might be better suited to meet the requirements of the various target classes. Nuclear receptors display different properties concerning the structure of the proteins and the ligands compared to the two examples investigated so far, which might be attributed to their different biological functions. Therefore we assumed that our findings so far may not be extrapolated to nuclear receptors. To investigate the advantages and limits of selected common virtual screening tools also for this target class, we selected PPARγ as a case study representing nuclear receptors and applied the same study design in line with our previous investigations [39]. As mentioned above, research efforts concerning PPARγ shifted towards the identification of partial agonists rather than full agonists. Besides the identification of the most suitable virtual screening method for the identification of novel PPARγ ligands, we therefore additionally aimed to investigate the ability of the applied methods to reflect this specific binding mode that results in the partial activation of the receptor.

2. Methods

2.1. Study design

Analogous to our previous study [39], we generated PPARγ partial agonist pharmacophore- and shape-based models, and established a docking protocol that could discriminate between PPARγ partial agonists, PPARγ full agonists, and inactive compounds. All optimized models and the docking workflow were used for virtual screening of the commercial Maybridge database (www.maybridge.com). The ten top-ranked hits from each of the three methods were selected for further investigations and merged to the "overall hit list". In the next step, we analyzed whether these compounds were also predicted by the other two virtual screening methods above the activity cut-off defined during the model generation and theoretical validation. In addition, all compounds were independently and in parallel investigated with external 2D- and 3D bioactivity profiling tools. All generated *in silico* predictions were summarized in a prediction matrix. After biological testing, the performances of all the applied tools were evaluated and compared. The workflow is depicted in Fig. 1.

2.2. Hardware specification

All processes and predictions were performed on a multi-core workstation with 2.4 + GHz, 8 GB of RAM, a 1 + TB fast mass storage, and a NVIDIA graphical processing unit. All programs run on the Windows 7 platform.

2.3. Datasets

Known active partial agonists of PPARγ were manually assembled from the literature. Only compounds that activated the receptor from 15% up to a maximum of 80% compared to the full agonist control in a transactivation assay, and where direct binding was either shown by scintillation proximity assay or crystallographic data were included. In total, 51 known partial agonists were included in the "partial agonist" dataset (a detailed list is provided in Table S1 of the supporting information).

To investigate whether the generated models and the docking workflow can discriminate between full and partial agonists, also a "full agonist" dataset was included. This dataset contained 14 known full agonists from the literature for which direct binding was shown either *via* X-ray crystallography or a scintillation proximity assay and that activated the receptor >80% in a transactivation assay (for a detailed list of compounds please refer to Table S2 of the supporting information). Some of these compounds originated from the same chemical series as known partial agonists and were therefore especially useful for investigating the structural characteristics determining the biological activity.

PPARγ was included as target in the ToxCast dataset and evaluated in a fluorescence polarization assay [41]. The CAS-numbers of all compounds that were inactive in this dataset against PPARγ were extracted and converted to a sd-file using Pipeline Pilot version 9.1 [42] script "Search PubChem for CAS Number". Thereby, a database of 799 unique structures (Table S3 in the supporting information) was generated. A detailed description of this protocol is provided in Fig. S1 of the supporting information. In addition, 13 compounds which proved to be inactive in *in vitro* binding assays were manually assembled from the literature (a detailed list is provided in Table S4 of the supporting information). In total, this led to an "inactives" dataset containing 812 known inactive compounds.

A cdx-file was created for all literature-derived compounds using ChemBioDraw Ultra 11.0 [43]. These cdx-files were then converted to sd-files using the ChemDraw Reader and SD Writer components in Pipeline Pilot 8.5 [44].

For the prospective screening, the Maybridge_HitDiscover database was downloaded from the Maybridge homepage (www.maybridge.com, access date 27 February 2014). This compound collection contained about 52,000 diverse molecules that are commercially available. According to the information on the homepage of the provider, the majority of compounds does fulfill generally acknowledged criteria of drug-likeness (http://www.maybridge.com/portal/alias__Rainbow/lang__en/tabID__146/DesktopDefault.aspx). As a consequence, we did not apply additional pre-screening filters, which would have introduced additional bias to this study: The application of an additional filter