Identification of novel multitargeted PPARα/γ/δ pan agonists by core hopping of rosiglitazone

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Abstract: The thiazolidinedione class peroxisome proliferator-activated receptor gamma (PPARγ) agonists are restricted in clinical use as antidiabetic agents because of side effects such as edema, weight gain, and heart failure. The single and selective agonism of PPARγ is the main cause of these side effects. Multitargeted PPARα/γ/δ pan agonist development is the hot topic in the antidiabetic drug research field. In order to identify PPARα/γ/δ pan agonists, a compound database was established by core hopping of rosiglitazone, which was then docked into a PPARα/γ/δ active site to screen out a number of candidate compounds with a higher docking score and better interaction with the active site. Further, absorption, distribution, metabolism, excretion, and toxicity prediction was done to give eight compounds. Molecular dynamics simulation of the representative Cpd#1 showed more favorable binding conformation for PPARs receptor than the original ligand. Cpd#1 could act as a PPARα/γ/δ pan agonist for novel antidiabetic drug research.

Keywords: PPARs, diabetes, docking, molecular dynamics simulation, ADMET

Introduction

Peroxisome proliferator-activated receptors (PPARs) are nuclear ligand-activated transcription factors and include three subtypes, namely PPARα, PPARγ, and PPARδ.¹⁻³ The drugs targeting PPARs mainly include: 1) PPARγ agonists such as rosiglitazone and pioglitazone, which are used as antidiabetic drugs and also possess anti-inflammatory or antineoplastic activities,⁴⁻⁶ and 2) PPARα agonists such as fenofibrate and bezafibrate, which are used as antilipemic drugs (Figure 1).⁷ Rosiglitazone and pioglitazone have shown side effects in clinical use, such as liver function abnormality, edema, and weight gain.⁷ Especially in 2007, Nissen and Wolski⁸ reported the cardiac safety of rosiglitazone, which showed that singly selective agonism of PPARγ not only enhanced insulin sensitivity and the therapeutic effect of insulin metabolism but also caused edema, weight gain, and the potential risk of heart failure.

In recent years, some novel PPARs concepts appeared in the antidiabetic drug research area, such as multitargeted cooperative PPARα/γ/δ dual agonists and PPARα/γ/δ pan agonists. These multitargeted agonists could cooperatively improve glucose and lipid metabolism. They could not only effectively control blood sugar but also reduce the content of triglyceride, free fatty acid, and low-density lipoprotein, as well as increase high-density lipoprotein concentration, thus having a preventive effect on cardiovascular complications of type 2 diabetes patients. Some of these multitargeted PPARs agonists have entered clinical trials and represent promising PPARs drug research.¹¹⁻¹³

The pharmacophore of PPARs agonists consists of the polar head, linker, and hydrophobic tail. The polar head of PPARs agonists could form a hydrogen bond with Tyr residue at the AF-2 region, producing a transactivation effect. The hydrophobic
tail of PPARs agonists could bind to the residues at the active site entrance, affecting subtype selectivity. This indicates that by modification of the polar head and hydrophobic tail, various pharmacological effects can be produced, such as PPARα/γ dual agonistic activity and PPARα/γ/δ pan agonistic activity.

In our previous research, using GW409544 as the starting point, by means of “core hopping” and “glide docking” techniques, a novel class of PPARα/γ dual agonists was discovered. In this paper, starting from rosiglitazone as the lead compound, using a core hopping approach, the polar head, linker, and hydrophobic tail of rosiglitazone were modified to produce various compounds. These compounds were then screened by docking and absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction to discover some excellent PPARα/γ/δ pan agonists. Molecular dynamics simulations of the representative Cpd#1 with PPARα/γ/δ were also done to study the binding details (Figure 2).

Materials and methods
Preparation of PPAR receptors structures
The crystal structures of PPARα, PPARγ, and PPARδ receptors were downloaded from the Protein Data Bank (PDB) with PDB identification numbers 1I7G, 2PRG, and 2ZNP, respectively. The preparation of these receptors was performed on the Protein Preparation Wizard embedded in Schrodinger 2009. The process of preparing receptors included assigning bond orders, adding hydrogen, treating metals, treating disulfides, deleting waters, alleviating potential steric clashes, adjusting formal charges, minimizing proteins with the OPLS (Optimized Potentials for Liquid Simulation) 2005 force field, and refining the protein by limiting value of root mean square deviation (RMSD) to 0.50 Å as the constraint. Then, the original ligand was centered and redocked into the binding site to generate a docking box for molecular docking.

Core hopping and docking
The Core Hopping module in Schrodinger 2009 software was used to modify the polar head, linker, and hydrophobic tail of rosiglitazone (Figure 2). Core hopping is a docking algorithm that has the functions of fragment-based replacing and molecular docking. The first step of core hopping was to define the points at which the cores were attached to the scaffold. It was performed in the Define Combinations Step from the Combinatorial Screening panel. The second step was to define “the receptor grid file”, which was done in the Receptor Preparation panel. The third step was to prepare the cores attached to the scaffold using fragment database derived from ZINC. The fourth step was to align and dock the entire molecular structure built up by the core and scaffold. The cores were sorted and filtered by goodness of alignment and then redocked into the receptor after attaching the scaffold, followed by using the docking scores to sort the final molecules. The original ligand AZ242, rosiglitazone, and TIPP204 were used as positive control compounds.

ADMET prediction
The ADMET module of Discovery Studio 3.1 was used to predict pharmacokinetics and toxicity of the compounds (Figure 2). Taking rosiglitazone as control, the compounds as a mol2 file were imported into the ADMET Descriptors module and the Toxicity Prediction Extensible and Toxicity Prediction TOPKAT modules, respectively, obtaining pharmacokinetics and toxicity parameters.

Molecular dynamics simulation
In order to study the binding stability of compounds with PPARs active site, the 10ns molecular dynamics simulations were performed using the open GROMACS 4.0 package for Linux (Figure 2). Before the simulations, the coordinate file and topology file were prepared and the water box was constructed and filled with simple point charge water solution.
The Linear Constraint Solver algorithm was used for all of the bond restriction. 

### Results and discussion

**Ligand binding domains of the PPAR receptors**

The X-ray crystallography studies showed that the ligand binding domain of the PPARs was composed of 12α-helix and 4 antiparallel β-sheet. The three subtypes of PPARs were 60%–70% sequence similarities with RMSD between Cα atoms <1 Å. In addition, the ligand binding domain of the PPARα/γδ formed a Y-shaped hydrophobic pocket with a volume of about 1,300 Å³. The AF-2 region of H12 helix played an important role in the process of the activation of PPARs. As for PPARδ, the AF-2 region was significantly narrower, which was not suitable for binding ligands with a larger polar head.

**Core hopping and docking**

A total of 42,000 compounds were obtained by core hopping of rosiglitazone. These compounds were docked into PPARα (pdb 1I7G), PPARγ (pdb 2PRG), and PPARδ (pdb 2ZNP), respectively, screening out 23 compounds with higher docking scores and better binding poses than the original ligands. Further ADMET prediction studies produced the top eight compounds (Table 1). The docking scores of these compounds with PPARα and PPARγ were higher than the original ligand AZ242 and rosiglitazone, respectively. The docking scores of these compounds with PPARδ were somewhat lower than the original ligand TIPP204. In addition, the hydrogen bond distances between compounds and PPARs were <3.20 Å and the values were equal to the original ligand.

The docking mode of the representative Cpd#1 with the active site of PPARα/γδ receptors is shown in Figure 3. The carboxyl acidic head of Cpd#1 formed hydrogen bonds with the key residues of PPARα (Ser280, Tyr314, Tyr464, and His440), PPARγ (Ser289, His323, Tyr473, and His449), and PPARδ (His323, His449, and Tyr473) receptors, respectively. The aromatic hydrophobic tail and the linker of Cpd#1 bound to PPARα/γδ with similar conformations to the original ligand AZ242, rosiglitazone, and TIPP204, respectively.

**ADMET prediction**

The development of the PPARα/γ dual agonist mura-glitazar has been discontinued during clinical trials because of danger and mortality rate of cardiovascular interaction.

*Figure 2* Discovery of multitargeted peroxisome proliferator-activated receptor agonists by core hopping of rosiglitazone.

**Abbreviation:** ADMET, absorption, distribution, metabolism, excretion, and toxicity.

which was then neutralized by sodium ions or chloride ions. The 1,000-step energy minimization of the system was performed using the steepest descending method. The NVT (constant number, volume and temperature) ensembles were used with temperature being maintained at 300 K. The cutoff radius of van der Waals interaction was 1.4 nm, and particle mesh Ewald algorithm was used for the electrostatic interaction.
Table 1 The docking results of rosiglitazone analogues with peroxisome proliferator-activated receptor (PPAR)α/γ/δ receptors

| Entry | Structure | Docking score – lg (Kd) | Hydrogen bond distance (Å) |
|-------|-----------|-------------------------|-----------------------------|
|       |           | PPARα | PPARγ | PPARδ | PPARα | PPARγ | PPARδ |
| Cpd#1 | ![Structure](image1.png) | 12.51 | 12.61 | 13.19 | Ser280:1.919 | Ser289:1.533 | Thr289:1.808 |
|       |           |       |       |       | Tyr314:1.881 | His323:2.384 | His323:1.865 |
|       |           |       |       |       | His440:2.029 | His449:2.808 | His449:1.963 |
|       |           |       |       |       | Tyr464:1.648 | Tyr473:2.877 | Tyr473:1.559 |
| Cpd#2 | ![Structure](image2.png) | 12.38 | 12.39 | 13.34 | Ser280:2.101 | Ser289:1.583 | Thr289:1.970 |
|       |           |       |       |       | Tyr314:1.934 | His323:2.389 | His323:1.935 |
|       |           |       |       |       | His440:1.949 | His449:1.981 | His449:1.653 |
|       |           |       |       |       | Tyr464:1.742 | Tyr473:2.694 | Tyr473:1.735 |
| Cpd#3 | ![Structure](image3.png) | 13.08 | 12.26 | 12.49 | Ser280:1.796 | Ser289:1.581 | Thr289:1.912 |
|       |           |       |       |       | Tyr314:2.010 | His323:2.253 | His323:1.882 |
|       |           |       |       |       | His440:2.042 | His449:2.925 | His449:1.693 |
|       |           |       |       |       | Tyr464:1.758 | Tyr473:2.684 | Tyr473:1.769 |
| Cpd#4 | ![Structure](image4.png) | 12.67 | 12.15 | 13.15 | Ser280:2.085 | Ser289:2.405 | Thr289:1.849 |
|       |           |       |       |       | Tyr314:1.951 | His323:3.011 | His323:1.896 |
|       |           |       |       |       | His440:2.101 | His449:1.753 | His449:1.891 |
|       |           |       |       |       | Tyr464:1.580 | Tyr473:2.823 | Tyr473:1.590 |
| Cpd#5 | ![Structure](image5.png) | 11.61 | 12.16 | 14.39 | Ser280:2.053 | Ser289:1.574 | Thr289:1.903 |
|       |           |       |       |       | Tyr314:2.154 | His323:2.212 | His323:1.707 |
|       |           |       |       |       | His440:2.711 | His449:3.071 | His449:1.817 |
|       |           |       |       |       | Tyr464:1.943 | Tyr473:2.667 | Tyr473:1.637 |
| Cpd#6 | ![Structure](image6.png) | 11.97 | 11.44 | 12.42 | Ser280:1.665 | Ser289:1.663 | Thr289:1.895 |
|       |           |       |       |       | Tyr314:1.948 | His323:2.253 | His323:2.031 |
|       |           |       |       |       | His440:2.344 | His449:2.915 | His449:2.019 |
|       |           |       |       |       | Tyr464:2.019 | Tyr473:2.662 | Tyr473:1.537 |
| Cpd#7 | ![Structure](image7.png) | 11.40 | 11.11 | 11.87 | Ser280:1.638 | Ser289:2.186 | Thr289:1.910 |
|       |           |       |       |       | Tyr314:2.133 | His323:2.742 | His323:2.041 |
|       |           |       |       |       | His440:1.866 | His449:2.186 | His449:1.996 |
|       |           |       |       |       | Tyr464:2.026 | Tyr473:2.610 | Tyr473:1.545 |
| Cpd#8 | ![Structure](image8.png) | 11.61 | 10.62 | 11.57 | Ser280:1.917 | Ser289:2.751 | Thr289:1.994 |
|       |           |       |       |       | Tyr314:2.498 | His323:2.745 | His323:2.974 |
|       |           |       |       |       | His440:2.158 | His449:2.659 | His449:1.717 |
|       |           |       |       |       | Tyr464:1.815 | Tyr473:2.052 | Tyr473:2.146 |
| AZ242 | ![Structure](image9.png) | 10.63 |       |       | Ser280:1.640 | Ser289:1.943 | His323:2.021 |
|       |           |       |       |       | His440:1.884 | His449:2.202 | His449:1.929 |
|       |           |       |       |       | Tyr464:2.040 | Tyr473:2.484 |                 |
| Rosiglitazone | ![Structure](image10.png) | 10.38 |       |       | Ser280:1.640 | Ser289:1.943 | His323:2.021 |
|       |           |       |       |       | His440:1.884 | His449:2.202 | His449:1.929 |
|       |           |       |       |       | Tyr464:2.040 | Tyr473:2.484 |                 |
| TIPP204 | ![Structure](image11.png) | 15.59 |       |       | Ser280:1.640 | Ser289:1.943 | His323:2.021 |
|       |           |       |       |       | His440:1.884 | His449:2.202 | His449:1.929 |
|       |           |       |       |       | Tyr464:2.040 | Tyr473:2.484 |                 |

Notes: The original ligands AZ242, rosiglitazone, and TIPP204 were used as positive control. Hydrogen bond distance (N-H···O and so on) is acceptable under 3.20 Å.
Figure 3. The docking mode of representative Cpd#1 with the active site of peroxisome proliferator-activated receptor (PPAR)α (A), PPARγ (B), and PPARδ (C). Note: The original ligands AZ242 (left), rosiglitazone (middle), and TIPP204 (right) are colored in purple.

Table 2. The ADMET prediction results of rosiglitazone analogues

| Entry  | MW (g/mol) | AlogP<sub>98</sub> | PSA-2D  | QplogS | nON | nOHNH | SL3 computed probability of mutagenicity (%) |
|--------|------------|------------------|---------|--------|-----|-------|---------------------------------------------|
| Cpd#1  | 464.487    | 1.277            | 131.13  | -5.058 | 7   | 2     | 0                                           |
| Cpd#2  | 478.514    | 1.312            | 131.14  | -3.537 | 7   | 2     | 0                                           |
| Cpd#3  | 477.486    | 0.512            | 142.21  | -4.106 | 7   | 2     | 0                                           |
| Cpd#4  | 478.494    | 0.889            | 145.24  | -4.397 | 8   | 2     | 0                                           |
| Cpd#5  | 480.491    | 0.706            | 123.84  | -5.810 | 6   | 1     | 0                                           |
| Cpd#6  | 499.530    | 5.490            | 117.69  | -6.132 | 5   | 3     | 0                                           |
| Cpd#7  | 468.501    | 4.638            | 130.57  | -6.249 | 6   | 3     | 0                                           |
| Cpd#8  | 470.513    | 4.517            | 108.74  | -6.469 | 6   | 2     | 0                                           |
| AZ242  | 408.465    | 3.367            | 107.51  | -4.295 | 7   | 1     | 0                                           |
| Rosiglitazone | 357.427 | 3.268           | 96.83   | -4.289 | 6   | 1     | 0.001                                        |
| TIPP204 | 469.469   | 6.121            | 75.63   | -6.919 | 4   | 2     | 0                                           |

Abbreviations: ADMET, absorption, distribution, metabolism, excretion, and toxicity; MW, molecular weight; AlogP<sub>98</sub>, atom-based LogP (octanol/water); PSA-2D, 2D fast polar surface area; QplogS, predicted aqueous solubility; nON, number of hydrogen bond acceptors; nOHNH, number of hydrogen bond donors.
Thus, the prediction of drug ADME/Tox was crucial and could reduce the risk of the drug development. The pharmacokinetics and toxicity of the top eight compounds were predicted using the ADME module of Discovery Studio 3.1. The molecular weight (MW), octanol–water partition coefficient (AlogP), polar surface area (PSA-2D), aqueous solubility (QplogS), number of hydrogen bond acceptors (nON), number of hydrogen bond donors (nOH), and mutagenicity of rosiglitazone analogues are listed in Table 2, respectively. These compounds accorded with Lipinski’s rule of five (Mol_MW<500, 0.4<AlogP<5.6, nOH<5, nON<10, 7<PSA-2D<200, 0.5<QplogS<6.5), and the values were equal to the positive control AZ242, rosiglitazone, and TIPP204. The probabilities of mutagenicity of these compounds were also lower than for rosiglitazone.

Molecular dynamics trajectory analysis

In order to study the dynamics behaviors and the binding stability of the PPARs–Cpd#1 complex, the 10ns molecular dynamics simulations were performed on PPARs–apo, PPARs–original ligand complex, and PPARs–Cpd#1 complex.

The RMSD versus the simulation time was considered as a significant criterion to evaluate the stability of dynamic behavior. The final RMSD values for all the simulation systems were <0.8 nm (Figure 4). After 3ns, the RMSD values for Cpd#1–PPARs system (red) was the lowest one among these three simulation trajectories.

In order to study the dynamic details of key residues interacted with the ligand, the root mean square fluctuations (RMSF) of all the side chain residues were obtained. The RMSF curve of PPARs–Cpd#1 complex was similar to that of PPARs–original ligand complex (Figure 4). At the key residues of PPARα such as Ser280 (the pink area), Tyr314 (the conch area), Tyr464 (the cyan area), and His440 (the coral area), the RMSF values of the PPARα–Cpd#1 complex were somewhat lower than those of the PPARα–original ligand complex and PPARα–apo form. As for PPARγ/δ, similar circumstances existed just as with PPARα. These molecular dynamics simulation trajectories indicated that PPARs became more stable after binding Cpd#1.

Figure 4 The molecular dynamics simulation results of PPARs–Cpd#1 complex.
Notes: The black line indicates the outcome for the system of the receptor alone without any ligand; the blue line indicates the outcome for the system of the receptor with the original ligand; and the red line indicates the outcome for the system of the receptor with the ligand Comp#1.
Abbreviations: PPAR, peroxisome proliferator-activated receptor; RMSD, root mean square deviation; RMSF, root mean square fluctuations.
Conclusion
In this study, rosiglitazone was modified by core hopping strategy to produce various analogues. Using docking and ADME prediction techniques, eight novel compounds were identified as multitargeted PPARα/γ/δ pan agonists with excellent pharmacokinetic properties. Molecular dynamics simulations of the representative Cpd#1 showed that Cpd#1 bound steadily to PPARα/γ/δ active site and restricted the target movement. These compounds have not been reported in the literature and could act as novel PPARs multitargeted agonists for antidiabetic drug research.

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Disclosure
The authors report no conflicts of interest in this work.

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