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Conversion of human-selective PPARα agonists to human/mouse dual agonists: a molecular modeling analysis

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Abstract—To understand the species selectivity in a series of α-methyl-α-phenoxy carboxylic acid PPARα/γ dual agonists (**1**–**11**), structure-based molecular modeling was carried out in the ligand binding pockets of both human and mouse PPARα. This study suggested that interaction of both 4-phenoxy and phenyloxazole substituents of these ligands with F272 and M279 in mouse PPARα leads to the species-specific divergence in ligand binding. Insights obtained in the molecular modeling studies of these key interactions resulted in the ability to convert a human-selective PPARα agonist to a human and mouse dual agonist within the same platform.

1. Introduction

A reliable rodent model possessing the target receptor for a human disease is often an important tool for drug development. In certain cases, however, potential drugs may target a human protein for which the corresponding rodent protein differs thus making pre-clinical candidate evaluation difficult. The species-dependent transactivation of some PPARα agonists has been reported previously.[1,2] For example, the classical PPAR agonist WY-14643 and a recently disclosed ureidothioisobutyric acid derivative, GW9578 showed preferential transactivation for rodent over human PPARα receptor.[2,3] In contrast, ETYA (5,8,11,14-eicosatetraynoic acid) showed preferential transactivation for human PPARα.[1,2] Nomura et al. published substituted phenylpropanoic acid derivatives as another example of human-selective PPARα activators.[4] During our investigation of novel PPAR ligands for the treatment of dyslipidemia and type 2 diabetes, a discrepancy in the reduction of triglycerides in apoA-1 transgenic mice was noticed for a series of phenoxy propionic acids with significant difference in human and mouse PPARα binding.[5,6] Here we report the results of our SAR study illustrating the ability to enhance mouse activity in this class of ligands and an interpretation for SAR convergence in human and mouse PPARα receptors using the molecular modeling studies. Recently, Miyachi and Uchiki reported their observations on molecular determinant leading to human-selective PPARα activation using wild type and a point-mutated LBD.[8]

2. Chemistry

The synthesis of compounds **1**–**11** was accomplished in a convergent manner as outlined in Schemes 1 and 2. The substituted toluene-4-sulfonic acid 2-(5-methyl-2-phenyl-oxazol-4-yl)-ethyl esters **12** were prepared in seven

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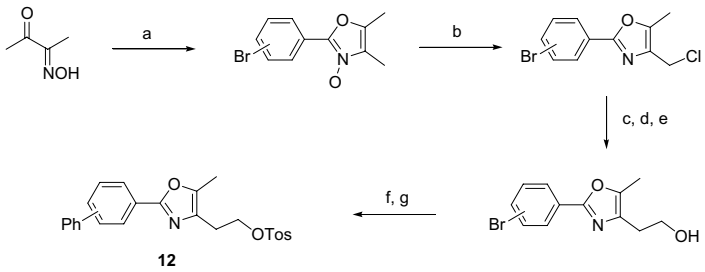
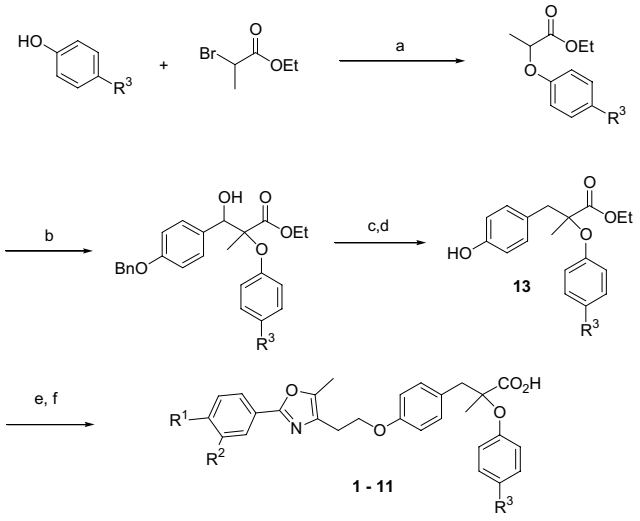


Scheme 1. Reagents and conditions: (a) Br-benzaldehyde, HOAc, HCl (74%); (b) $POCl_3$, $CHCl_3$ (72%); (c) KCN, KI, DMF (100%); (d) KOH, 2-MeO–ethanol (60%); (e) BH_3–THF, MeOH (72%); (f) Br–$PhB(OH)_2$, $Pd(OAc)_2$, PPh_3 (95%); (g) $(Tos)_2O$, Pyr, DMAP (95%).



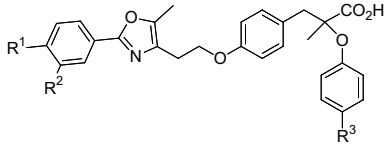
Scheme 2. Reagents and conditions: (a) Cs_2CO_3, DMF, 90 °C (90%); (b) LDA, −78 °C, then *p*-benzoloxy benzaldehyde (70%); (c) TFAA, Pyr., CH_2Cl_2 (quant.); (d) H_2, 5–10% Pd/C, EtOAc (90%); (e) 12, Cs_2CO_3, DMF, 55 °C (70%); (f) 5 N NaOH, EtOH, reflux (>99%).

steps from commercially available butanedione monooxime according to the literature procedure.[9,10] The substituted 3-(4-hydroxy-phenyl)-2-methyl-2-phenoxy-propionic acid ethyl esters **13** were prepared as previously described.[6] Treatment of tosylates **12** with phenol esters **13** in DMF with Cs_2CO_3, followed by hydrolysis afforded ligands **1**–**11**. The enantiomers of phenols **13** were separated by chiral chromatography using a Chiralpak AD 4.6 × 250 mm column. The (−)-enantiomer of **13** was utilized to prepare compound **2** while the (+)-enantiomer was utilized to prepare compound **3**.

3. Results and discussion

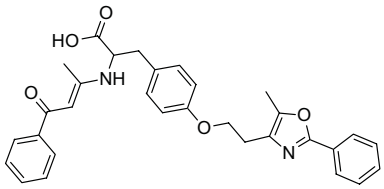
The in vitro binding affinity and transactivation activity of compounds **1**–**11** toward human and mouse PPARα are summarized in Table 1. To explain the structure–activity relationship in the mouse PPARα receptor, a homology model was prepared using the crystal structure of human PPARα with GW409544 (PDB: 1k7l, Fig. 1).[7,11] The root-mean square deviation (RMSD)

Table 1. In vitro binding IC_{50}[a] and cotransfection EC_{50}[b] data[c] in human and mouse PPAR receptor subtypes



Entry	R^1	R^2	R^3	Enantiomer	hPPARα[d]		mPPARα[d]	
					IC_{50} (nM)[a,c]	EC_{50} (nM)[b,c]	IC_{50} (nM)[a,c]	EC_{50} (nM)[b,c]
1	H	H	H	S/R	42 ± 6	12 ± 2	2563 ± 389	1625 ± 197
2	H	H	H	S	21 ± 4	5 ± 1	1543 ± 136	1299 ± 106
3	H	H	H	R	1546 ± 36	390 ± 23	6764 ± 844	Eff < 20
4	Ph	H	H	S/R	503 ± 41	69 ± 6	7020 ± 3744	2343 ± 175
5	H	Ph	H	S/R	167 ± 25	7 ± 1	404 ± 25	173 ± 11
6	H	H	Me	S/R	64 ± 8	7 ± 1	1396 ± 167	585 ± 151
7	Ph	H	Me	S/R	375 ± 26	39 ± 3	1461 ± 44	1041 ± 192
8	H	Ph	Me	S/R	306 ± 52	23 ± 5	530 ± 41	151 ± 22
9	H	H	^{t}Bu	S/R	149 ± 28	11 ± 2	515 ± 56	211 ± 26
10	Ph	H	^{t}Bu	S/R	196 ± 21	260 ± 28	700 ± 44	1167 ± 152
11	H	Ph	^{t}Bu	S/R	133 ± 12	49 ± 5	146 ± 66	91 ± 15

[a] Concentration of test compound required to displace 50% of tritiated ligand.
[b] Concentration of test compound that produced 50% of the maximal reporter activity.
[c] $n = 3$.
[d] Tritium-labeled PPARα agonist 2-(4-{2-[3-(2,4-difluorophenyl)-1-heptylureido]ethyl}phenoxy)-2-methylbutyric acid was used as radioligand for generating displacement curves and IC_{50} values.



GW409544

Figure 1. Structure of GW409544.

for the 267 Cα atom pairs is 0.25 Å. The four differences in the ligand binding pocket of mouse PPARα as compared to the human receptor, I272F (helix 3), T279M (helix 3), V324L (helix 5), and V332I (β-strand), are on helixes 3 and 5, and the β-strand. The compounds were docked into both human and mouse LBDs using CDocker.[12,13] The docking mode was selected according to the molecular overlay with GW409544 as it appeared in the crystal complex among the top 10 poses.

3.1. Effect of the stereochemistry at the α-position of the α-aryloxy-α-methylhydrocinnamic acid

The enantiomer selectivity in human PPARα transactivation activity was previously reported for α-alkoxy substituted carboxylic acids.[4,14] We chose compound **1** to investigate the enantiomer-dependent binding affinity and transactivation activity on human PPARα. The (*R*)- and (*S*)-enantiomers of **1** were made from the pure enantiomers of **13**.[6] The racemic mixture **1** showed a similar profile to the (*S*)-enantiomer **2** in both binding and transactivation activities, therefore, only racemates were tested in the study.

The docking of **2** and **3** into the human PPARα receptor suggests that the (*S*)-enantiomer **2** assumes a similar binding mode to GW409544 in which the α-methyl of the α-aryloxy-hydrocinnamic acid head piece points toward the space formed by helix 3 and the loop between helix 10 and the AF2 helix. The carboxylic acid group formed a good hydrogen bond network with Y464, Y314, and S280 as GW409544. Nε of H440 was within 2.8 Å distance to the phenyl ether oxygen of **2**. However, the methyl group on the (*R*)-enantiomer **3** would bump F138 and H440 on helixes 5 and 10 assuming the same binding mode. As a result, the (*S*)-enantiomers were docked in the subsequent SAR rationalization study.

3.2. Effect of substitution at the aryloxy ring

Substitution of the *para* position of the aryloxy ring (R^3 in Table 1) with lipophilic substituents, such as methyl, or *tert*-butyl (**6** and **9**) led to a two- to fivefold increase in mouse PPARα binding with a two- to eightfold increase in functional activity relative to the nonsubstituted **1**. We successfully converted a human-selective PPARα agonist to a more potent mouse PPARα agonist by the addition of the *tert*-butyl group to provide **9**.

The impact of R^3 on the binding to mouse PPARα can be explained by the interaction with the side chain of F272 according to the docking to the mouse PPARα

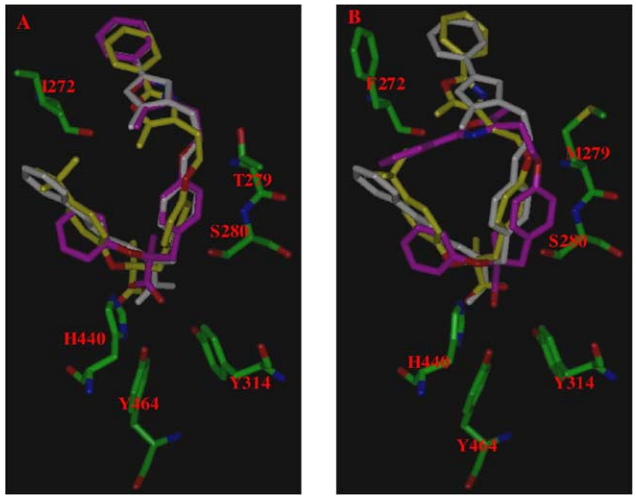


Figure 2. The proposed binding model for α-aryloxy-α-methylhydrocinnamic acid derivatives **1** (magenta) and **9** (yellow) in the human (A) and mouse (B) PPARα LBD. GW409544 is shown in white.

homology model. The R^3 substitution is buried in a hydrophobic cluster of F272, F273, F351, L344, and M355 in the mouse receptor. Increasing the size of R^3 would enhance hydrophobic interaction with the mouse receptor and therefore better affinity to the receptor. However, for the human isoform, the γ-CH_3 of I272 interferes with the binding of the *tert*-butyl in **9**, which may contribute to the threefold decrease in IC_{50} on human PPARα. It was also found that when R^3 is H as in compound **1**, the docked conformers in human and mouse receptors are very different in the orientation of hydrophobic tail piece (Fig. 2). Increasing the size of the R^3 group led to the convergence of docked conformations in the two species. The *t*-BuPhO group resembles the position of the side chain of GW409544. The docked conformation for **9** in both human and mouse PPARα LBD supports the in vitro results.

3.3. Effect of the substitution at the phenyloxazole

A comparison of compounds **1** and **5** showed that a significant improvement in mouse PPARα binding and transactivation activity was obtained by substitution of the phenyloxazole ring with an additional phenyl ring in the *meta* position (R^2 in Table 1). A sixfold improvement in mouse PPARα binding and a ninefold increase in mouse PPARα functional activity was achieved for **5** with this modification. In contrast, substitution of the phenyloxazole with a *para* phenyl group such as **4** (R^1 in Table 1) led to a two- to threefold decrease in mouse PPARα binding. The same trend was also observed when R^3 is methyl or *tert*-butyl (e.g., **7** and **10**).

F272 also plays an important role in understanding the impact of R^1 substitution on mouse PPARα binding. While I272 in human PPARα allows the extension of the R^1 from H to phenyl, it is difficult to dock **4** into the mouse PPARα receptor without changing the binding mode (the *p*-phenyl group would overlap with F272 otherwise). Instead, the *m*-phenyl (R^2) of **5** pushes the central phenyl ring out of its original position slightly

causing minimal changes to the interaction with the LBD. This rearrangement is also facilitated by the T279M mutation in the mouse receptor.

Furthermore, the combination of R^2 and R^3 exhibit greater mouse PPARα binding and transactivation activity than either alone as exemplified in compounds **8** and **11**. Compound **11** showed a 18-fold improvement in both assays compared to **1**, establishing an SAR convergence for this series on both human and mouse PPARα. When R^3 changes from hydrogen to *tert*-butyl, the binding modes in human and mouse converge and the additional van der Waals interaction from R^2 helps the ligand achieve the high affinity to both receptor isoforms. The convergence of human and mouse PPARα binding and transactivation potential within this series translated into improved rodent in vivo potency. Using triglycerides reduction as an indicator of in vivo PPARα activation, compound **1** reduced triglycerides by ~39% at a dose of 3 mg/kg in mice treated for 7 days, whereas compound **11** lowered triglycerides by ~56% at the same dose level as compare to the control vehicle.

In summary, the molecular modeling analysis for the SAR trends in human and mouse PPARα receptors for a novel series of α-aryloxy-α-methylhydrocinnamic acids was described. Two of the four differences in the human PPARα ligand binding pocket (F272 and M279) predominantly confer the noted species-specific selectivity. The SAR indicated the species selectivity can be overcome by 4-phenoxy and phenyloxazole substitutions of these ligands.

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