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Enantio-Dependent Binding and Transactivation of Optically Active Phenylpropanoic Acid Derivatives at Human Peroxisome Proliferator-Activated Receptor Alpha

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Abstract—Optically active phenylpropanoic acid derivatives [(*S*)-**5**, and (*R*)-**5**] were prepared, and their affinities for peroxisome proliferator-activated receptor (PPAR)α and PPARγ were evaluated. Binding assay and cell-based reporter assay indicated that the activity of these compounds is enantio-dependent, and resides exclusively on the (*S*)-isomer.

Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptors, which include steroid receptor, thyroid receptor, retinoid receptor and others.[1] These receptors are ligand-dependent transcription factors. Upon ligand binding, the activated PPARs heterodimerize with another nuclear receptor, the retinoid X receptor (RXR),[2] and alter the transcription of the target genes after binding to specific peroxisome proliferator response elements (PPREs), which are a direct repeat of the hexameric AGGTCA recognition motif separated by one nucleotide (DR1).[3] Three subtypes of PPARs, termed PPARα, PPARδ (also known as PPARβ, NUCI, FAAR) and PPARγ have been identified so far in various speices, including humans.[4]

PPARα, the first isoform to be cloned, in 1990,[5] was found at high density in the liver and regulates the expression of genes encoding proteins involved in lipid and lipoprotein metabolism, such as acyl-CoA oxidase, bifunctional enzyme, liver fatty acid binding protein, apo A, apo C-III, and so on.[6] In addition to the above in vitro results, PPARα-deficient transgenic mouse (PPARα−/−) exhibited massive hepatic and cardiac lipid accumulation owing to the inhibition of cellular fatty acid flux.[7] These results clearly indicate a pivotal role for PPARα in lipid homeostasis in vivo.

Fibrate compounds, such as clofibrate and bezafibrate (Chart 1), have been used for the treatment of hypertriglyceridemia for more than 20 years,[8] and recently fenofibrate (Chart 1) was launched in Japan. Although the molecular target of these drugs remains to be definitively established, recent molecular pharmacological studies have demonstrated that fibrates activate PPAR's at high micromolar concentrations, with poor subtype-selectivity.[9]

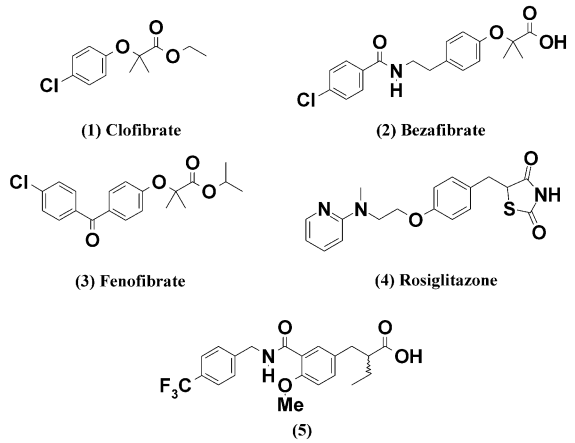

Chart 1. Chemical structures of the fibrate drugs, rosiglitazone and **5**.

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We considered that more potent and selective activators of PPARα, especially human PPARα, might have therapeutic utility for the treatment of altered lipid homeostasis in the target organs, especially in the liver.

Recently, we have reported the design and the synthesis of some novel phenylpropanoic acid derivatives as subtype-selective PPAR activators,[10] and we selected the 2-ethylphenylpropanoic acid derivative (**5**; Chart 1) for further pharmacological study. As a part of our continuing research directed toward the development of subtype-selective PPAR activators, we report here the enantio-dependency of the activity of **5**.

There is little information on the differences of binding and transactivation activity of the enantiomers of synthetic PPARα activators in the case of human PPARα. Although several optically active peroxisome proliferators that can activate PPARα, such as leucotriene D_4 receptor antagonist (MK-571)[11] and clofibric acid analogues,[12] are enantio-dependent, it is not clear whether these compounds are true PPARα ligands (that can bind directly to human PPARα).

Therefore, we decided to investigate the transactivation and binding activity of optically active **5**, using PPARα and PPARγ (PPARδ was not examined, since **5** shows very weak PPARδ transactivation activity). Compound **5** has an asymmetric center at the α-position of the carboxylic acid functionality, and it is important to determine which enantiomer is more potent.

Chemistry

Synthetic routes to optically active **5** [(+)-**5**, and (−)-**5**] are outlined in Chart 2. Racemic **5** was prepared as previously described.[10] Optically active **5** was obtained by the resolution of the corresponding 4-(*S*)-benzyloxazolidinone imide derivatives by silica-gel column chromatography and recrystallization, and subsequent deprotection of the 4-(*S*)-benzyloxazolidinone.[13] The enantiomeric excesses of both (+)-**5** and (−)-**5** were more than 95% ee.[14]

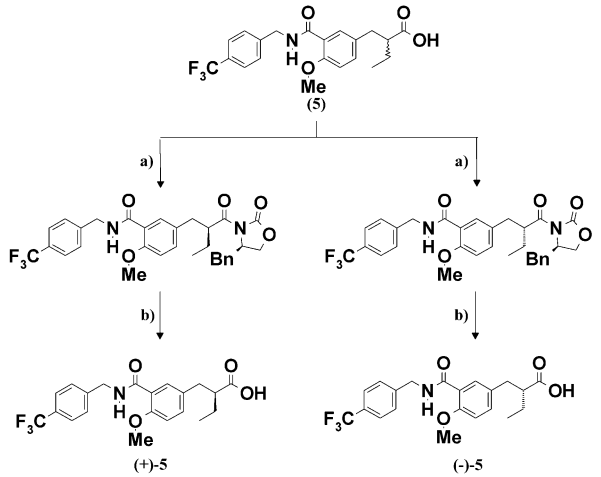

Chart 2. Synthetic routes to optically active **5.** Reagents and conditions: (a) (1) Pivaloyl chloride, NEt_3, THF; (2) 4-(*S*)-benzyloxazolidinone, *t*-BuOK, THF; (b) LiOH, 30% H_2O_2, MeOH.

The absolute configuration of the optically active **5** was determined by the preparation of the (*R*)-form of **5** starting from optically active (*R*)-2-benzylbutanoic acid[15] (Chart 3). (*R*)-**5** exhibited levo rotation, so the absolute configuration of the (−)-**5** was deduced to be (*R*) and the antipodal (+)-form of **5** was deduced to be (*S*).[16]

In Vitro Studies

Transient transactivation assay

Analysis of the transient transactivation activity of the above compounds on human PPARα and human PPARγ was performed using the reported method.[17] The activity was expressed as EC_{50}, which is the concentration of the test compound that affords half-maximum transactivation activity.

Binding assay

Binding affinity to PPARα and γ was determined by competitive binding assay as previously described.[17]

Results and Discussions

Under the assay conditions used, no apparent decrease in optical purity of either enantiomer was observed.[18] As can be seen from Table 1, a clear enantio-dependence of the transactivation and binding activity to human PPAR isoforms was found. In the case of human PPARα isoform, only the (*S*)-form exhibited potent transactivation and binding activity and the corresponding (*R*)-form shows much weaker activities.

As for human PPARγ isoform, both enantiomers of **5** exhibited weak activities, and no apparent enantio-dependence was observed. The (*S*)-form exhibited high PPARα selectivity in both transactivation and binding assays, but the degree of subtype-selectivity of the antipodal (*R*)-form was low.

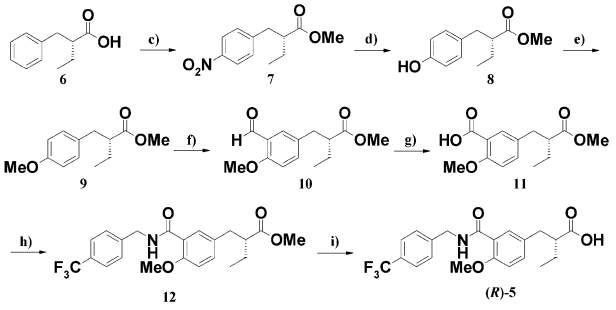

Chart 3. Synthetic routes to the (*R*)-form of **5.** Reagents and conditions: (c) (1) c. H_2SO_4, MeOH; (2) c.HNO_3; (d) (1) 10% Pd/C, EtOH; (2) $NaNO_2$, dil H_2SO_4; (3) Na_2SO_4, dil H_2SO_4; (e) MeI, K_2CO_3, DMF; (f) $CHCl_2OMe$, $TiCl_4$, CH_2Cl_2; (g) CrO_3–H_2SO_4, acetone; (h) 4-(trifluoromethyl)benzylamine, 2-chloro-1,3-dimethylimidazolium chloride, NEt_3, CH_2Cl_2; (i) dil NaOH, MeOH.

Table 1. Transactivation and binding activities of the enantiomers of **5** with human PPARs

Compd	Config.	Transactivation (EC_{50} μM)[a]		Binding (IC_{50} μM)[a]	
		PPARα	PPARγ	PPARα	PPARγ
(−)-**5**	(*R*)	1.5	>15	24	>50
(+)-**5**	(*S*)	0.06	14	0.74	44

[a]Transient transactivation activity and binding activity of the above compounds on human PPARα and human PPARγ were performed using the previously reported method.[17]

In this study, we investigated the enantio-dependency of the human PPARα-selective agonist **5** and found that the PPARα transactivation and binding activities reside almost exclusively in the (*S*)-enantiomer.

In the case of rosiglitazone (Chart 1), a potent and selective PPARγ agonist used for the treatment of non-insulin dependent diabetes mellitus (type II diabetes), enantio-dependency was also noted, and (*S*)-rosiglitazone exhibited much more potent binding to PPARγ than (*R*)-rosiglitazone.[19] However, the enantio-dependency of (*S*)-rosiglitazone decreased time-dependently, probably due to rapid racemization at the C-5 position of the thiazolidine-2,4-dione ring at physiological pH.[20]

A recent X-ray crystallographic analysis of the rosiglitazone–human PPARγ complex indicated that the sulfur atom of (*S*)-rosiglitazone is positioned in a hydrophobic region of the PPARγ ligand-binding pocket, surrounded by the side chains of the amino acids F363, Q286, F282, and L469.[21] Because the three-dimensional structures of the ligand-binding domains of members of the nuclear receptor superfamily are thought to be well conserved,[22] we speculate that the side chain ethyl group of (*S*)-**5** interacts hydrophobically with the corresponding hydrophobic region of the PPARα ligand-binding pocket, although the three-dimensional structure of human PPARα is not known.[23]

In conclusion, **5** shows enantio-dependent transactivation and binding activities to human PPARα. The activities were found to reside almost exclusively in the (*S*)-enantiomer. Further in vivo pharmacological evaluation of the (*S*)-form of **5** and related compounds, and a more detailed structure–activity relationship study in combination with computer-aided molecular modeling are in progress.

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