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Design, Synthesis and Evaluation of Substituted Phenylpropanoic Acid Derivatives as Peroxisome Proliferator-Activated Receptor (PPAR) Activators: Novel Human PPARα-Selective Activators

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Abstract—A series of substituted phenylpropanoic acid derivatives was prepared as part of a search for subtype-selective human peroxisome proliferator-activated receptor (PPAR) activators. Structure–activity relationship studies indicated that the substituent at the α-position of the carboxyl group plays a key role in determining the potency and the selectivity for PPAR transactivation.

Introduction

The peroxisome proliferator-activated receptor (PPAR) family of nuclear hormone receptors (NR1C's) is heterogeneous, and its members have been classified into three subtypes encoded by separate genes: PPARα (NR1C1), PPARδ (NR1C2), (also known as PPARβ, NUCI, FAAR) and PPARγ (NR1C3).[1] Each of the PPAR subtypes appears to be differentially expressed in a tissue-specific manner, and they play pivotal roles in lipid and lipoprotein homeostasis. Upon ligand binding, PPARs regulate specific gene expression by binding to specific consensus DNA sequences, termed PPRE (peroxisome proliferator responsive element),[2] which are located in the regulatory regions of the target genes, after heterodimerization with another nuclear receptor, retinoid X receptor (RXR).[3]

PPARα, the first isoform to be identified,[4] is expressed at high density in tissues that have high levels of fatty acid catabolism, such as liver, and regulates the expression of genes encoding for proteins involved in lipid and lipoprotein metabolism.[1] In addition, in mice lacking PPARα (PPARα−/−), inhibition of cellular fatty acid flux caused massive hepatic and cardiac lipid accumulation.[5] These results clearly indicate a pivotal role for PPARα in lipid homeostasis in vivo.

Fibrate-class antihyperlipidemic drugs, such as clofibrate, bezafibrate, and fenofibrate (Chart 1), decrease serum triglyceride and increase high-density lipoprotein in humans.[6] The exact molecular mechanism(s) of action of these drugs are not known, though recent molecular-pharmacological studies demonstrated that fibrates not only activate, but also bind to PPARs.[7] Although fibrates are ligands or activators of PPARs, their affinity is weak and their subtype-selectivity is poor.

Therefore, more potent and selective activators of PPARα, especially human PPARα (fibrates exhibit species-dependent PPARα transactivation, for example fibrates are more potent activators of murine PPARα than human PPARα[8]) are expected to have superior therapeutic utility for the treatment of altered lipid homeostasis in target organs, especially in the liver.[9]

In order to develop structurally new human PPARα-selective activators, we selected KRP-297 (Chart 1) as a lead compound. Although KRP-297 belongs structurally to the glitazones (thiazolidine-2,4-dione class insulin sensitizers), it binds directly to and activates both PPARγ and PPARα isoforms with almost equal affinity.[9] This character is interesting, because the classical glitazones, such as troglitazone,[10] pioglitazone,[11] and rosiglitazone,[12] were reported to bind to and activate the PPARγ isoform selectively.[13] The reason why KRP-297 exhibited co-ligand nature is not known, but we anticipated that replacement of the thiazolidine-2,4-

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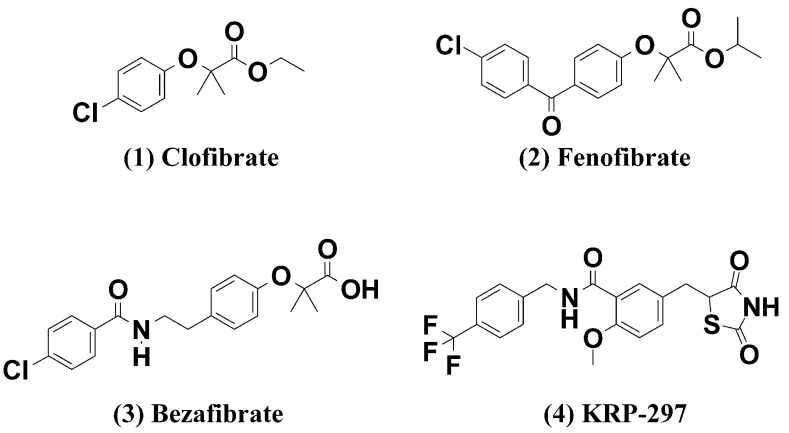


Chart 1. Chemical structures of the fibrate drugs and KRP-297.

dione ring of KRP-297 with other acidic functionalities, such as the carboxyl group usually used in fibrates, would decrease the affinity for PPARγ and thereby afford PPARα-selective ligands. In this paper, we present our discovery of human PPARα-selective activators.

Synthesis

The compounds prepared in this study were synthesized by standard procedures as outlined in Chart 2, and were characterized by ^{1}H NMR, mass spectral, and elemental analyses (details and physicochemical data of the compounds will be published elsewhere).[14]

In Vitro Studies

Analysis of transient transactivation activity towards human PPARα and human PPARγ was done by using the previously described method.[9] The activity was expressed as EC_{50}, which is the concentration of the test

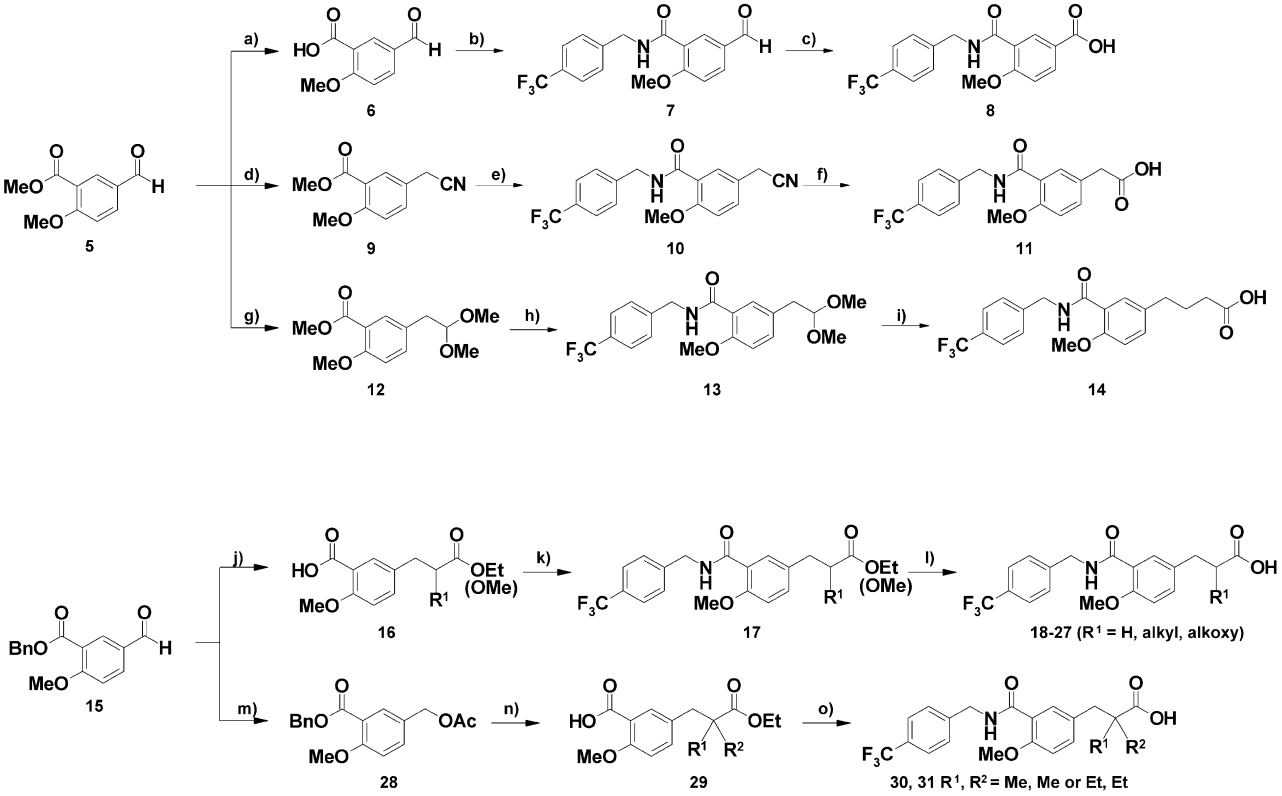


Chart 2. Synthetic routes to the phenylpropanoic acids and related compounds. Reagents and Conditions: (a) OH^-, MeOH; (b) 4-$(CF_3)PhCH_2NH_2$, $ClCO_2Et$, NEt_3, THF; (c) CrO_3–H_2SO_4, acetone; (d) (1) $NaBH_4$, MeOH; (2) $SOCl_2$; (3) KCN, *n*-Bu_3N^+ Cl^-, $CHCl_3$; (e) (1) same as in (a); (2) same as in (b); (f) (1) 30% H_2O_2, 0.1 N NaOH, EtOH,; (2) 1 N NaOH, EtOH; (g) (1) $(Ph)_3P^+CH_2OMeCl^-$, LDA, THF; (2) *p*-TosOH, MeOH; (h) (1) same as in (a); (2) same as in (b); (i) (1) 6 N HCl, AcOEt; (2) $(Ph)_3P{=}CHCO_2Me$, CH_2Cl_2; (3) H_2, 10% Pd/C, EtOH; (4) same as in (a); (j) (1) $(EtO)_2POCH(R^1)CO_2Et$, NaH, THF [or $(Ph)_3P{=}CHCO_2Me$, toluene]; (2) same as in (i) (3); (k) same as in (b); (l) same as in (a); (m) (1) same as in (d) (2), Ac_2O, pyridine, DMAP, CH_2Cl_2; (n) (1) $(R)_2C{=}C(OTMS)OMe$ (R=Me or Et), $Mg(ClO_4)_2$, CH_2Cl_2; (2) same as in (i) (3); (o) (1) same as in (b); (2) same as in (a).

compound that affords half-maximum transactivation activity. PPARδ transactivation activity was not examined, since the lead compound (**4**) shows very weak PPARδ transactivation activity.

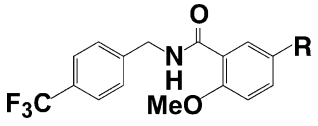
Results and Discussion

The transactivation activity of the present series of compounds is summarized in Table 1, together with the results for a representative fibrate, bezafibrate. Bezafibrate exhibited weak and non-selective PPAR activation. These results are consistent with reported data.[8]

The distance between the carboxyl group and the right-side benzene ring is important for human PPARα transactivation potency. The benzoic acid and phenylacetic acid derivatives (**8** and **11**) were inactive at the concentration of 10 μM, but the phenylpropanoic acid derivative (**18**) exhibited potent activity, comparable to that of the lead compound (**4**). Further elongation of the methylene chain to give the phenylbutanoic acid derivative (**14**) decreased the activity to some extent.

On the other hand, these compounds (**8**, **11**, **18**, and **14**) were inactive (**8**, **11**, **18**) or weak activators (**14**) of human PPARγ compared with the lead compound (**4**). These results are consistent with the prevailing hypothesis that the thiazolidine-2,4-dione ring structure is important for potent PPARγ transactivation activity.[15]

Table 1. PPAR transactivation activities of the present series of compounds



Structure			Transactivation (EC_{50}, μM)[a]	
No.	R	mp (°C)	PPARα	PPARγ
4	CH_2TZD[b]	177–178	1.0	0.8
8	CO_2H	236–237	ia[c]	ia
11	CH_2CO_2H	167–168	ia	ia
18	$(CH_2)_2CO_2H$	157–158	1.3	ia
14	$(CH_2)_3CO_2H$	107–108	2.2	3.0
19	$CH_2CH(Me)CO_2H$[d]	155–156	0.24	ia
20	$CH_2CH(Et)CO_2H$[d]	145–147	0.040	0.40
21	$CH_2CH(n\text{-}Pr)CO_2H$[d]	147	0.36	ia
22	$CH_2CH(i\text{-}Pr)CO_2H$[d]	174–175	0.29	ia
23	$CH_2CH(n\text{-}Bu)CO_2H$[d]	150	1.0	2.5
24	$CH_2CH(Ph)CO_2H$[d]	159–161	ia	ia
25	$CH_2CH(OMe)CO_2H$[d]	161–163	0.23	ia
26	$CH_2CH(OEt)CO_2H$[d]	146–148	1.6	2.8
27	$CH_2CH(OPh)CO_2H$[d]	142–143	ia	ia
30	$CH_2C(Me)_2CO_2H$	151–152	2.9	ia
31	$CH_2C(Et)_2CO_2H$	156–157	2.8	ia
	Bezafibrate		>78	>137

[a]Compounds were screened for agonist activity on PPAR-GAL4 chimeric receptors in transiently transfected CHO-K1 cells as described in the text. EC_{50} value is the molar concentration of the test compound that causes 50% of the maximal reporter activity ($n=3$).
[b]KRP-297, TZD means thiazolidine-2,4-dione ring. The data were taken from ref 9a.
[c]ia, inactive at 10 μM.
[d]Assayed as a racemate.

Since compound (**18**) exhibited potent and subtype-selective human PPARα activation, we selected **18** as the next lead compound and performed further chemical modification focused on the α-position of the carboxyl group of **18** (although compound **14** exhibited comparable PPARα transactivation activity, we were not interested in this compound because it is a dual activator of human PPARα and human PPARγ). We anticipated that the introduction of a hydrophobic substituent at the α-position of the carboxyl group of **18** might enhance the human PPARα transactivation activity, based on the result of an X-ray crystallographic analysis of human PPARγ–rosiglitazone complex.[16]

As also indicated in Table 1, the introduction of appropriate substituents at the α-position of the carboxyl group of **18** strikingly affected human PPARα transactivation activity and subtype-selectivity. Introduction of a methyl group (**19**) enhanced human PPARα transactivation activity, and the introduction of an ethyl group (**20**) afforded maximum activity. Bulkier substituents generally decreased the activity, that is the potency decreased in the order of *n*-pr (**21**) = *i*-pr (**22**) > *n*-bu (**23**) > Ph (**24**). These data suggest that there are distinct steric requirements to exhibit potent human PPARα transactivation activity, and the ethyl group appears to be the most favorable.

Introduction of a further substituent at the α-position of the α-monosubstituted phenylpropanoic acid derivatives is unfavorable, that is the α,α-dimethyl- and the α,α-diethyl-substituted derivatives (**30**, **31**) exhibited decreased human PPARα transactivation activity as compared to those of the corresponding α-monosubstituted compounds (**19**, **20**).

The same steric tendency was seen in a series of α-alkoxy-substituted derivatives (**25**–**27**), though to a lesser extent than a series of α-alkyl-substituted derivatives.

As regards human PPARγ transactivation, only compound **20**, which has an ethyl group at the α-position of the carboxyl group, exhibited more potent transactivation activity than the lead compound (**4**). Other α-substituted derivatives (**19**, **21**–**31**) exhibited weak activities. Thus, these α-substituted derivatives retained moderate or high human PPARα selectivity, except for the compounds **23** and **26**.

In conclusion, we have developed a potent human PPARα activator (**20**; KCL1998001079) with high selectivity for PPARα over PPARγ, as compared with the fibrates. The structure–activity relationship study indicated that the substituent at the α-position of the carboxyl group plays a key role in determining the potency of PPARα transactivation, and the ethyl group is the most effective.

Further pharmacological evaluation of KCL1998001079, and a more detailed structure–activity relationship study of the present series of α-substituted phenylpropanoic acid derivatives are in progress, including investigations of enantio-dependency and species-selectivity.

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