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Phenylacetic Acid Derivatives as hPPAR Agonists

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Abstract—Beginning with the weakly active lead structure **1**, a new series of hPPAR agonists was developed. In vivo glucose and triglyceride lowering activity was obtained by homologation and oxamination to **3**, then conversion to substituted benzisoxazoles **4** and **5**. Further manipulation afforded benzofurans **6** and **7**. Compound **7** was of comparable potency as a glucose and triglyceride lowering agent in insulin resistant rodents to BRL 49653.

The thiazolidinedione (TZD) class of insulin sensitizing agents for the treatment of Type II diabetes is well documented.[1,2] A mechanism has been advanced for their glucose lowering action invoking PPARγ nuclear receptor agonist activity, primarily in adipose tissue.[3,4] This is supported by the correlation of in vitro and in vivo structure–activity relationships.[5,6] Interested in discovering novel non-TZD PPARγ agonists, we screened in-house samples for hPPARγ binding activity using 2D similarities to various TZD's as a searching algorithm. Acetyl phenol **1** (Fig. 1) displayed modest in vitro hPPARγ agonist activity. A directed synthesis program was initiated in a bid to both improve the observed hPPARγ activity and increase the probability of eliciting in vivo antidiabetic efficacy.

The preparation of propionyl phenol **2**, oxime **3** and benzisoxazoles **4** and **5** is shown in Scheme 1. The thio halide **11** was prepared by thiocarbamoylation of **9**, thermal rearrangement to **10** and alkylation of the derived sodium thiolate. Resorcinols **13** were prepared by *O*-allylation of **12**, Claisen rearrangement and reduction. Combination of **11** and **13** under basic conditions afforded acyl phenols **14**. Saponification of **14** (R = Et) gave **2**, whereas oxamination and saponification gave **3**. Oxamination followed by acylation, cyclization and hydrolysis gave benzisoxazoles **4** and **5**. The preparation of benzofurans is depicted in Scheme 2. 3-Methoxyphenol was alkylated with the appropriate α-bromoketones, giving ketones **15**. Acid catalyzed cyclization cleanly gave benzofurans **16**, which underwent demethylation and propylation as above to give **17**. Alkylation with **11** and hydrolysis afforded **6** and **7**.

Recombinant hPPARγ receptor affinity was determined according to our new protocol.[7] Agonist activity was established using a cell-based transactivation assay.[8] Similar assays using Gal-4-PPARδ and Gal-4-PPARα chimers characterized activity on related PPAR isoforms. In vivo glucose and triglyceride lowering activities were obtained using either male *db*/*db* mice or Zucker diabetic fatty (ZDF) rats as previously described.[6,8] The in vitro activity of compounds **1** through **7** along with that of rosiglitazone (**8**) is shown in Table 1. Table 2 summarizes the in vivo data for compounds **3**–**7** with rosiglitazone.

Unlike rosiglitazone and TZD's in general, acids **1** through **7** were non-selective full hPPAR agonists, selectivity being defined here as a difference in affinity or activity equal to three orders of magnitude. By contrast, none of these compounds were agonists of mPPARα. In vitro activity against hPPARδ was comparatively con-

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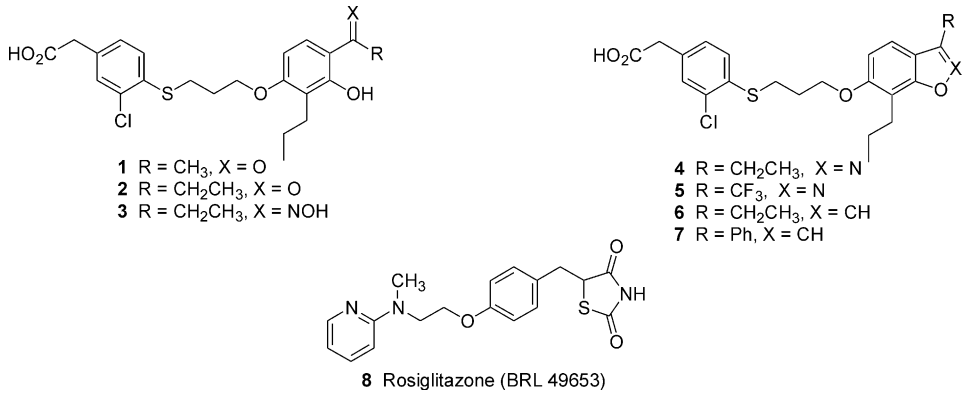
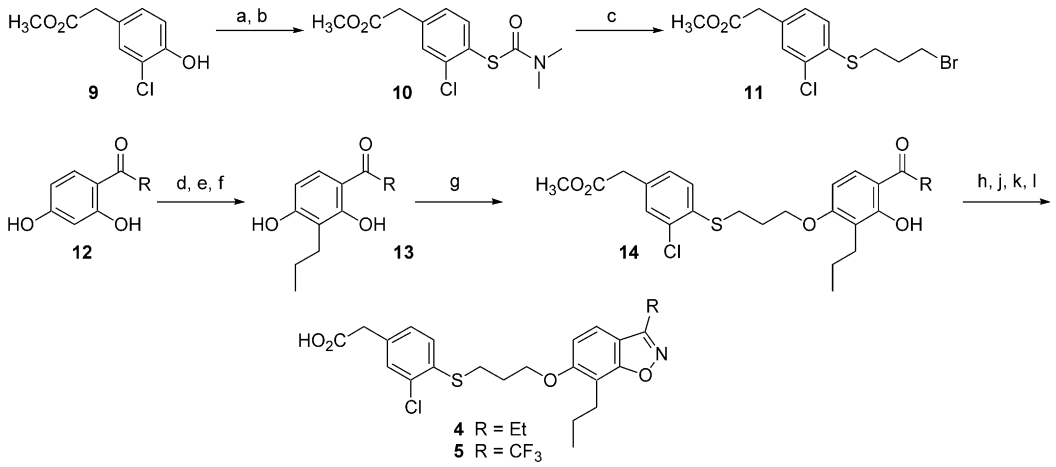
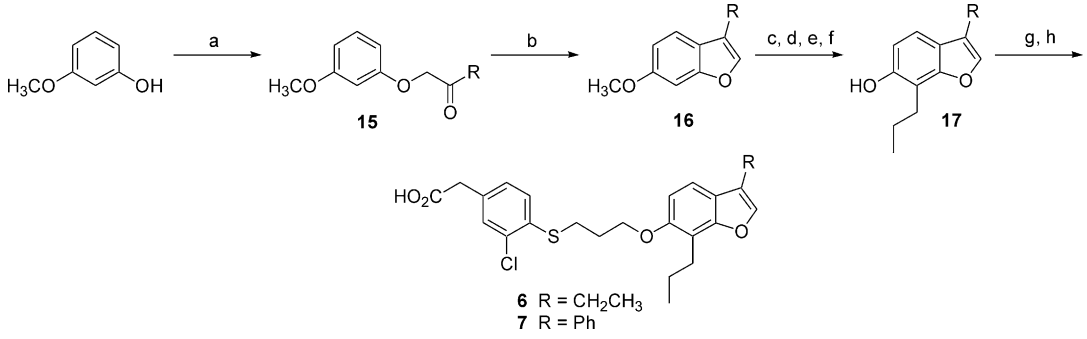


Figure 1.



Scheme 1. (a) 1.4 equiv $(CH_3)_2NC(S)Cl$, pyridine, reflux, 4 h, 80–85%; (b) sulfolane, reflux, 15 min, 45–60%; (c) (i) 1.2 equiv $NaOCH_3$, CH_3OH, reflux, 2 h; (ii) 4 equiv $Br(CH_2)_3Br$, CH_3OH, 0–20 °C, inverse addition, 70–75%; (d) 1.1 equiv each allyl bromide, Cs_2CO_3, DMF, 20 °C, 95–98%; (e) *ortho*-dichlorobenzene, reflux, 20–24 h, 75–80%; (f) H_2, 1 atm, 5% Pd/C, MTBE, 100%; (g) 1.05 equiv **11**, 1.1 equiv Cs_2CO_3, DMF, 65–70%; (h) 5 equiv each $NH_2OH{\cdot}HCl$, NaOAc, CH_3OH, reflux, 4 h, 95–99%; (j) Ac_2O (as solvent), 20 °C, 30 min; (k) pyridine, reflux, 3 h, 85–90% (two steps), (l) 1.3 equiv aq KOH, *i*PrOH, 50 °C, 100%.



Scheme 2. (a) 0.9 equiv crude $RC(O)CH_2Br$, 1.1 equiv Cs_2CO_3, DMF, 70–75%; (b) 15 vol% CH_3SO_2OH/CH_2Cl_2, −10–20 °C, 65–70%; (c) 1.1 equiv BBr_3, CH_2Cl_2, −10 to 20 °C, 1 h, 85–90%; (d) 1.1 equiv each allyl bromide, Cs_2CO_3, DMF, 95–98%; (e) *ortho*-dichlorobenzene, reflux, 75–80%; (f) H_2, 1 atm, 5% Pd/C, MTBE, 30 min, 90–95%; (g) 1.05 equiv **11**, 1.1 equiv Cs_2CO_3, DMF, 65–70%; (h) 1.3 equiv aq KOH, *i*PrOH, 50 °C, 100%.

stant across most of the compounds, but hPPARα and γ activity were more dependent on structural changes. The weak in vitro activity of **1** was markedly improved by simple homologation to **2,** most notably in hPPARα binding (12×) and, to a lesser extent (3–8×) in the functional activation assays. Further potentiation was obtained by converting **2** to oxime **3**, which was much more potent than **1** as an hPPARα and γ agonist (12–75×). Oxime **3** was also a more potent hPPARγ agonist than rosiglitazone, albeit clearly less active in vivo (ZDF rat). The derived benzisoxazole **4** was similar to **3** as an hPPARγ agonist in vitro, but appreciably less potent against hPPARα. Benzisoxazole **4** was also noticeably less active in vivo (*db*/*db* mice) than rosiglitazone. Pharmacokinetic profiling of **3** and **4** indicated low systemic exposure following oral dosing. Benzisoxazole **4**,

Table 1. In vitro data for compounds 1–8

Compd	hPPAR Binding IC_{50} (nM)			hPPAR Activation EC_{50} (nM)		
	α	δ	γ	α	δ	γ
1	7400	20	580	1600	80	230
2	600	20	300	260	10	70
3	100	10	48	74	9	15
4	570	4	110	247	11	19
5	310	10	140	125	5	23
6	1730	40	560	1300	12	102
7	54	6	94	18	6	9
8[a]	$>5\times10^5$	$>5\times10^5$	210	NA[b]	NA[b]	10

[a]Binding IC_{50}'s are ±25%. All activation EC_{50}'s were reflective of full agonist activity (>80% activation).
[b]NA indicates <20% activation at all concentrations tested (max concentration = 3 μM).

Table 2. In vivo data for compounds 3–7

Compd		Zucker diabetic fatty rat			db/db mouse[a]		Pharmacokinetic profiles[b]			
	Dose (mpk/day)	Glu (% Corr)[c]	TG (% Dec)[d]	FFA (% Dec)[d]	Glu (% Corr)[c]	TG (% Dec)[d]	iv (1.5 mpk) Clp	$t_{1/2}$	po (10 mpk) nAUC	F (%)
3	100	89	80	54						
3	30	44	65	15			5.7	2.8	0.73	33
4	100	66	70	52	33	67				
4	30	29	42	−14			17.6	1.0	0.62	50
5	30	95	−65	74	96	81				
5	10	83	−53	52	71	46				
5	3	18	−34	45	30	46	2.6	4.7	2.52	100
6	30				74	60				
7	30	56	66	88	94	86				
7	10	27	13	67	77	72	3.3	2.1	3.14	84
8[e]	30	109	94	86						
8[e]	10	99	78	54	65±5	75±5	2.6	3.8	4.08	64

[a]Male db/db mice (12–13 weeks of age, $n=7$) and non-diabetic mice (lean control, $n=7$) were provided ad libitum access to rodent chow and water and received once-a-day oral dosing of the sodium-salts of tested compounds by gavage with vehicle (0.25% methylcellulose) for 11 days. Blood was collected from the tail for measurement of plasma levels of glucose and triglyceride. For further experimental details and a complete description of the related Zucker Diabetic Fatty Rat protocol, see refs 6 and 8.
[b]Fasted male Sprague–Dawley rats ($n=3$), which have been surgically cannulated in the femoral artery and vein, received an intravenous dose by bolus injection into the femoral vein, or an oral gavage dose. Blood samples were taken serially at selected time points from the femoral arterial cannula. Clp = clearance (mL/min/kg); $t_{1/2}$ = half life (h); nAUC = dose-normalized AUC (μg·h/mL); F, bioavailability. Variability of PK parameters as follows: Clp ±20%, $t_{1/2}$ ±25%, nAUC ±15%.
[c]Defined as the percentage of the difference between diabetic controls and lean controls (i.e., 100% correction = lean control).
[d]Defined as the percentage decrease in the absolute level of the indicated parameter (FFA, free fatty acids).
[e]Unless otherwise indicated, variability is ±20%. Missing entries under the ZDF rat and *db*/*db* mouse headings indicate experiments not carried out.

in particular, was the most rapidly cleared compound in this group. Replacement of the benzisoxazole ethyl substituent with a trifluoromethyl group gave a compound (**5**) that, although not much better than **4** in vitro, was superior to it and comparable to rosiglitazone as a glucose lowering agent in vivo (ZDF rat and *db*/*db* mouse). This was ascribed to the enhanced PK profile relative to **4**. Curiously, although **5** also effectively lowered triglycerides in mice (although less so than rosiglitazone), it raised them in rats. The origin of this effect is under active investigation and its cause will be disclosed in a separate report. Replacement of the benzisoxazole nitrogen of **4** with methine gave benzofuran **6**. Although appreciably less potent as an hPPARα/γ agonist than **4**,**6** was more effective in reduction of serum glucose and triglycerides in vivo (*db*/*db* mice).[8] Focusing on the apparently more efficacious benzofurans, a phenyl substituent was introduced in place of ethyl, affording **7**, the most potent in vitro agonist in this series. Benzofuran **7** displayed efficacy in *db*/*db* mice at least equal to rosiglitazone, although it was less robust in the ZDF rat. Its pharmacokinetic profile was also among the more favorable in this group and compared well with rosiglitazone (**8**). Incorporation of a phenyl substituent at the benzisoxazole 3-position led to a series that is being reported elsewhere.[9]

In summary, beginning with **1**, structural modifications afforded a number of potent but non-selective hPPAR agonists. The most notable of these, benzofuran **7**, displayed serum glucose and triglyceride lowering efficacy in *db*/*db* mice comparable to the clinical agent rosiglitazone. This compound can be used as a tool to further understand the pharmacology of pan-hPPAR agonists in vivo.

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