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Amphipathic 3-Phenyl-7-propylbenzisoxazoles; Human PPaR γ, δ and α Agonists

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Abstract—A series of amphipathic 3-phenylbenzisoxazoles were found to be potent agonists of human PPARα, γ and δ. The optimization of acid proximal structure for in vitro and in vivo potency is described. Results of po dosed efficacy studies in the *db*/*db* mouse model of type 2 diabetes showed efficacy equal or superior to Rosiglitazone in correcting hyperglycemia and hypertriglyceridemia. Good functional receptor selectivity for PPARα and γ over PPARδ can be obtained.
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The utility of the peroxisome proliferator activated receptor γ (PPARγ, NR1C3) nuclear hormone receptor agonists in the treatment of hyperglycemia associated with type 2 diabetes[1,2] (DM2) and of PPARα (NR1C1) agonists as hypolipidemics has been amply demonstrated.[3] Three thiazolidinedione PPARγ or PPARγ and α agonist insulin sensitizers have been marketed for treatment of DM2 over the past 5–7 years. The search for better PPAR agonist drugs is driven by limited efficacy and side-effects including instances of lethal hepatotoxicity associated with the first marketed drug Troglitazone.[4] The development of an amphipathic carboxylate lead **1** from a Merck Frosst Ltd_4 antagonist program into a series of benzisoxazole PPARα/δ/γ agonists has been reported recently.[5] The phenylacetic acid **2** is a potent but nonselective PPARγ, δ and α agonist and an efficacious insulin sensitizer in insulin resistant *db*/*db* ($lepr^{db\text{-}3J}$/$lepr^{db\text{-}3J}$) mice. Further optimization of **2** and the optimization of the series for PPARα/γ selectivity are reported here (Fig. 1).

Typical SAR studies of PPAR agonists hold an acid or heterocyclic head fragment relatively fixed and explore the SAR for a lipophilic tail. This study examines the opposite relation, holding a lipophilic 3-phenyl-7-propylbenzisoxazole tail fixed and studying minimum requirements for acid structure with the objective of maximum in vivo potency and determination of best attainable selectivity among the three known PPAR receptors for this series. Several varied acid structures give good potency and widely varied selectivities in this series. Good, but not complete, functional selectivity for PPARγ and α dual agonist activity over PPARδ (NR1C2) can be obtained while retaining reasonable potency.

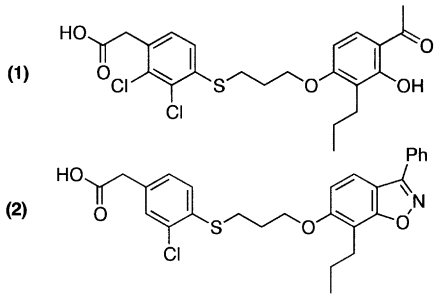

Figure 1.

Retaining the lipophilic anchor of the lead **2**, the total separation from the distal acid residue, as well as structure and topology of the connecting chain were varied to study effects on affinity and selectivity among the three PPAR receptors. The position of the acid proximal phenyl residue of the lead was shifted both in terms of its position in the linking tether and regio-isomerism. Synthesis of some benzisoxazole fragments and

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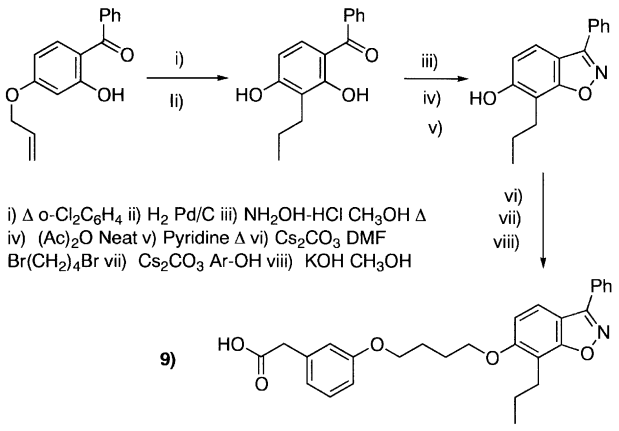


Figure 2.

elaboration to the typical carboxylate products have been previously reported.[5,6]

The synthesis of the 6-hydroxy-3-phenylbenzisoxazole proceeds from the commercially available allyl ether as illustrated in Figure 2 for example **9**. Alkylation of this benzisoxazole with the appropriate dibromide followed by O-alkylation of a phenolic acid and subsequent ester cleavage led to the analogues reported below. The required phenolic acid fragments were prepared by known methods. These various substrates include benzoic, phenylacetic, phenylpropionic, phenoxyacetate, cinnamic and phenylbutyric acid residues. Reasonably potent PPAR ligands could be found in all series with widely varying selectivity for the three PPAR subtypes. Results are reported in Table 1.

The initial set of analogues **5**–**12** retained the lead structure phenyl acetic acid terminus with the metabolically vulnerable sulfur replaced by oxygen. A fairly sharp affinity maximum was found for compound **9** with the *m*-butyloxy link to the benzisoxazole fragment. The apparently isosteric replacement of the sulfur in this linking residue with a methylene oxy unit, yielding a four carbon linking chain, is consistent with the many known examples of substitution of sulfur for two carbon units.[10] A substantial sensitivity to regioisomeric substitution was observed. The difference in affinity for the PPARγ receptor implies an apparent ΔΔG of approximately −1.3 kcal[7] comparing **9** with *meta* substitution versus **6** with *para*. While **9** is modestly more potent than **2** it is still nonselective. Some indication of PPAR γ/α selectivity over PPARδ was seen in the *para* analogue **6**, but much more credible selectivity results from *alpha* methylation as in **7** and **10**.

Agonist competence was assayed in the COS cell transfected with a GAL 4-PPAR ligand binding domain chimer expression vector and 5X-UAS-luciferase reporter plasmid as described in Berger et al.[9] Most of the analogues in these series were competent full agonists in this GAL4-PPAR transactivation assay. Where the titration curves cannot be fit to generate an EC_{50}, maximum observed activation at the highest nontoxic dose is reported. Apparent intrinsic potency varies within the γ, δ and α receptor subtypes, with generally better intrinsic potency demonstrated in the PPARγ transfectant, resulting in apparent functional selectivity superior to the affinity selectivity (Fig. 3).

A second set of analogues retaining a similar range of overall lengths was prepared based on alkyloxy-linked dihydrocinnamic and cinnamic acids. A fairly sharp and slightly greater magnitude (ΔΔG −1.5 kcal[7] for **15** vs **23**) affinity maximum for the PPARγ receptor was observed but now favoring the *para* isomer. The cinnamate analogues **27** and **28** showed relatively small penalties in PPARγ and α affinity for the restriction of rotation in the acid proximal chain. The *para*-cinnamate isomer **28** shows, in fact, the best PPARγ/α dual agonist selectivity of the analogues studied here based on affinity. Both α,α- and β,β-dimethylation in the dihydrocinnamate proved to be most effective in generating PPARγ/α receptor subtype selectivity. These result in 10- to 15-fold selectivity for PPARγ/α over PPARδ with good functional selectivity in the GAL4-PPAR transactivation assay.

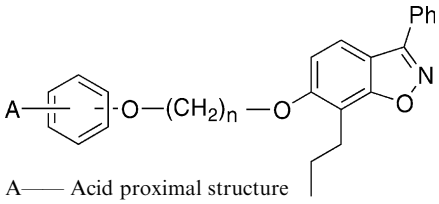
Incorporation of one of the best known PPAR agonist acid residues, a phenoxyacetate,[11] also yielded potent agonists on all three receptors. Some modest 7- to 9-fold selectivity is evident for PPARγ/α over PPARδ affinity in analogues **20** and **21**. Functional selectivity found in this series is similar to that for the α,α- and β,β-dimethyl analogues above.

The tolerance for changes in the carbon skeleton proximal to the acid residue is limited as demonstrated by the 4-phenylbutyrate analogues **29** and **30**. These compounds, with a rigid aryl element nearer the center of the molecule, showed substantially poorer affinity for PPARγ and PPARδ and begin to show some functional selectivity for PPARα.

To verify whether binding affinity in this SAR series is still driven by the chosen anchor residue, the magnitude of the 7-propyl-3-phenylbenzisoxazole fragment contribution to the binding energy for the optimized analogue **9** was assessed. Simplification of the distal heteroaryl to 2-propylphenyl by the complete removal of the fused 5-phenylisoxazole ring resulted in titratable affinity for only the PPARδ (K_i 830 nM) and PPARγ ($K_i \sim 5000$ nM) subtypes. Further truncation of the distal heteroaryl to unsubstituted phenyl resulted in loss of titratable affinity for all PPAR subtypes. The fused phenylisoxazole ring accounts for some 4.6 kcal of apparent binding energy on PPARδ and >5.5 kcal on PPARγ. Given a contribution of this magnitude, it is reasonable to assume that these compounds have in common a binding mode largely determined by the distal heteroaryl binding affinity.[12]

One obvious avenue open to affect selectivity in this series is offered by the introduction of rigidifying unsaturation into the center of the four methylene spacer of the highest affinity analogues. Both 2′,3′ olefin isomers and a 2′,3′ acetylenic analogue of **9** were prepared to investigate this question. Retention of PPARγ and PPARδ binding is

Table 1. PPAR binding, K_i: agonist efficacy in the transfected COS Cell, EC_{50}

		Isomer	Tether length	PPAR binding apparent K_i nM[a]			Transfected COS-1 cell EC_{50} nM or Max% at 3 μM [b]		
			n	γ	δ	α	γ	δ	α
	Rosiglitazone			136	> 33,300	> 33,300	21	0%	3%
2	Lead Phenylbenzisoxazole	*para*	3	11	3.7	14.0	4	3	3
	 A—— Acid proximal structure								
3	–COOH	*para*	4	> 5300	> 14,000	920.0	ND	ND	ND
4	–COOH	*meta*	4	100.0	508.0	5.2	193	34%	8
5	$–CH_2COOH$	*para*	3	32	5	3	194	147	3
6	$–CH_2COOH$	*para*	4	30	93	7	60	86%	8
7	$–C(CH_3)_2COOH$	*para*	4	**17**	**203**	**23**	10	59%	2
8	$–CH_2COOH$	*meta*	3	662	26	76	ND	ND	ND
9	$–CH_2COOH$	*meta*	4	**3.1**	**0.3**	**7.2**	**6**	**20**	**5**
10	$–C(CH_3)_2COOH$	*meta*	4	26	165	46	52	400	50
11	$–CH_2COOH$	*ortho*	3	498	62	489	ND	ND	ND
12	$–CH_2COOH$	*ortho*	4	348	47	86	ND	ND	ND
13	$–CH_2CH_2COOH$	*para*	2	304	11	11	ND	ND	ND
14	$–CH_2CH_2COOH$	*para*	3	32	2	7.3	99%	74%	42%
15	$–CH_2CH_2COOH$	*para*	4	8.9	37.5	7.6	485	74%	429
16	$–C(CH_3)_2CH_2COOH$	*para*	4	**52**	**529**	**213**	37	18%	53
17	$–CH_2C(CH_3)_2COOH$	*para*	4	**20**	**317**	**13**	20	860	4
22	$–CH_2CH_2COOH$	*meta*	3	448	21	53	ND	ND	ND
23	$–CH_2CH_2COOH$	*meta*	4	115	52	329	ND	ND	ND
26	$–CH_2CH_2COOH$	*ortho*	3	1,330	3,520	> 33,330	ND	ND	ND
27	–CH = CHCOOH	*para*	3	152	18	50	254	148	56%
28	–CH = CHCOOH	*para*	4	**13**	**265**	**18**	29	640	13
18	$–O–CH_2COOH$	*para*	3	34	3.7	8.1	75	16	4
19	$–O–CH(CH_3)COOH$	*para*	3	22	4.6	9.3	27	13	2
20	$–O–CH(CH_3)COOH$	*para*	4	20	136	47	16	117%	10
21	$–O–CH(CH_3)_2COOH$	*para*	4	**2.4**	**23**	**17**	2	172	2
24	$–O–CH_2COOH$	*meta*	4	52	47	133	87	127%	248
25	$–O–CH(CH_3)_2COOH$	*meta*	4	41	110	255	100	85%	92
29	$–CH_2CH_2CH_2COOH$	*para*	2	616	156	305	72%	69%	33
30	$-CH_2CH_2CH_2COOH$	*para*	3	145	38	121	156	183	3

ND, Not run.

[a]The apparent K_i was calculated from the Cheng–Prusoff equation[7] using the IC_{50} determined in the previously described SPA binding assay at 15 °C.[8] The radioligand for the PPARα and γ determination was tritiated **9**.

[b]The EC_{50} refers to the concentration yielding a 50% response relative to the standard in the previously described PPAR GAL4 chimer COS-1 cell transactivation assay.[9]

highest in the same *cis* olefin conformation offering little encouragement for further analogues. All three analogues retain very good PPARδ affinity.[5] The remarkable tolerance toward ligand structure observed for the PPARδ receptor here and above may be explained by X-ray structure observations of ligands bound in multiple conformations in the PPARδ binding pocket.[13]

Pharmacokinetics and Efficacy Studies

Compound **9** showed promising PK parameters with 76% bioavailability and dose normalized AUC of 4.2 μM-Hr for a 2 mpk po dose of the sodium salt in rats.[14] The isomeric *para* phenylacetic acid analogue **6**, evaluated in a high throughput nonquantitative PK screen, showed similar behavior.[15] Both were chosen as good candidates for efficacy studies. Several analogues including the most selective, β,β-dimethyl analogue **16**, and the cinnamate analogue **28** could not be evaluated in the *db*/*db* mouse model due to very poor oral bioavailability. Efficacy studies for **6** and **9** were performed in 7–8 week old highly insulin resistant leptin receptor defective *db*/*db* mice as described by Berger et al.[9] Efficacy was assessed relative to Rosiglitazone which shows variable (40–67%) correction of hyperglycemia (degree

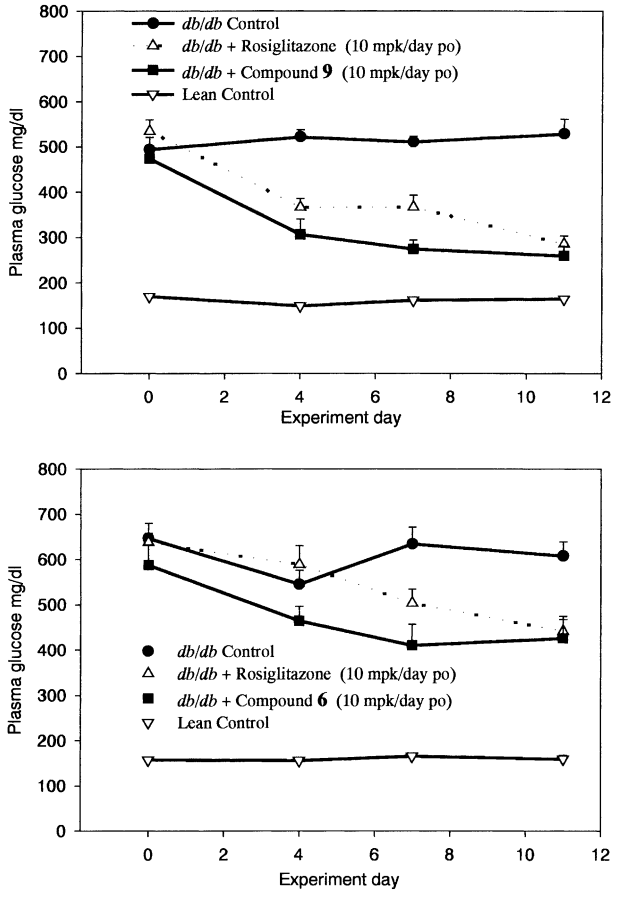


Figure 3.

of mean glucose lowering as percentage of the difference between vehicle treated *db*/*db* mice vs lean control mice) at a dose of 10 mpk for 11 days. The partially optimized lead compound **2** dosed at 30 mpk showed approximately 80% of the correction observed with Rosiglitazone dosed at 30 mpk. Both **6** and **9**, dosed at 10 mpk/day po, showed 110% of the observed Rosiglitazone 10 mpk correction for a significant improvement in in vivo potency over the lead compound **2**.

The fine adjustments of structure between the anchor benzisoxazole and the carboxylate residue generated high affinity PPARγ agonists possessing binding affinity surpassing the initial lead **2** on PPARγ in series with 6 atom spacers with any of the original phenylacetic, dihydrocinnamic or fibric acid residues. Functional and binding selectivity was most effectively influenced by introduction of either α-methyl substitution or β-substitution. Good oral bioavailability and efficacy equal or superior to the benchmark, Rosiglitazone, can be obtained in this series. The most potent analogues in this series showed good antihyperglycemic efficacy at 10 mpk/day orally. The *para* substituted compounds showing the highest PPARγ/α selectivity in this series showed a consistent trend toward poor bioavailability which precluded efficacy testing. The observed in vivo potency for this series in the mouse reflects only the PPARγ agonist potency as the series showed poor or no agonist activity on the mouse PPARα receptor in a PPAR homogenous time resolved fluorescence (HTRF) assay.[16] There is evidence to indicate that the added human PPARα mediated potency would enhance efficacy in humans.[17] Complete receptor binding selectivity over PPARδ was not obtained in this series, and remains the most challenging problem.

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expressed as inflection points calculated by a four-parameter logistic equation. K_is are calculated by the equation of Cheng and Prusofff, ref 7. Typical precision is represented by Rosiglitazone or compound **9**. Four independent determinations for rosiglitazone with PPARγ, in duplicate, yield an average K_i of 136 nM, Stdev 46, SEM 23. More than 400 determinations of compound **9** with PPARγ, in duplicate, yield an average K_i of 3.1 nM, Stdev 1.1. For compound **9** on PPARα, K_i 7.2 nM, Stdev 2.7.

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