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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2359–2362

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Benzoxazinones as PPARγ Agonists. Part 1: SAR of Three Aromatic Regions

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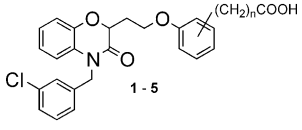
Received 28 February 2003; revised 15 April 2003; accepted 16 April 2003

Abstract—A series of benzoxazinones was synthesized as PPARγ agonists. The compounds were obtained in seven steps, and SAR was developed by variations to the core shown below. The compounds were tested as functional agonists in the induction of the aP2 gene in preadipocytes, and the most potent compound in the series has an $EC_{50} = 0.51$ μM. The potency was further confirmed through a PPAR-Gal4 construct. Efficacy has been demonstrated in the *db*/*db* mouse model of hyperglycemia.

PPARγ is a member of the peroxisome proliferator-activated receptor family and has been the subject of extensive research for mechanistic importance in glucose and lipid homeostasis.[1] The receptor is widely distributed in the spleen, the colon, adipose tissue and macrophages, and found to a lesser extent in the liver, the pancreas and skeletal muscle.[1a] Target genes that are upregulated or downregulated have been identified from white and brown adipose tissue, skeletal muscle and the liver.[1c] The details of how receptor activation leads to glucose homeostasis are not fully understood. Studies suggest that adipogenesis provides increased lipid metabolism and free fatty acid uptake in adipose tissue, leading to increased insulin sensitivity and glucose metabolism in muscle and liver.[1b,d,g] Recent evidence supporting this mechanism is that a PPARg agonist induces glycerol kinase gene expression in adipocytes, thus promoting triglyceride formation in that tissue, and reducing circulating free fatty acids.[1h] PPARγ is also a target protein for a growing number of agonists useful in the treatment of Type 2 diabetes.[2] These include Rosiglitazone® and Pioglitazone®, both marketed for this utility.

A PPARγ discovery program was initiated with a search of the corporate compound library. In the search paradigm, a phenyl substituted with carboxylic acid was used in place of the TZD-substituted phenyl typically seen in the literature. Select compounds were screened at 1 μM for aP2 gene induction in pooled human preadipocytes. The aP2 gene is essential for the maturation of preadipocytes adipocytes, and offered a large window of activation for primary screening and EC_{50} determinations.[3] The series of racemic benzoxazinones[4] in Table 1 emerged from this assay. Results indicated that the preferred substitution pattern on the aryl ether is 1,2-, while PPARγ agonists in the literature typically show a 1,4- substitution pattern (Rosiglitazone®,[5] Pioglitazone®,[6] Farglitazar®,[7] JTT-501[8] and others). This difference provided impetus to develop SAR in the series with respect to the position and spacing of the acidic moiety, amide substitution, and substitution on the aromatic portion of the benzoxazinone. Also, in the early stages of the program, after identifcation of the

Table 1. PPARγ aP2 Induction screening of library compounds[9]



1 - 5

Compd	COOH pos(n)	Functional assay[a] (Fold induction)
1	2(1)	6.2
2	3(1)	5.5
3	3(2)	5.6
4	4(1)	3.2
5	4(2)	3.6

[a]Values are the mean of two experiments for activation of aP2 gene in pooled human preadipocytes.

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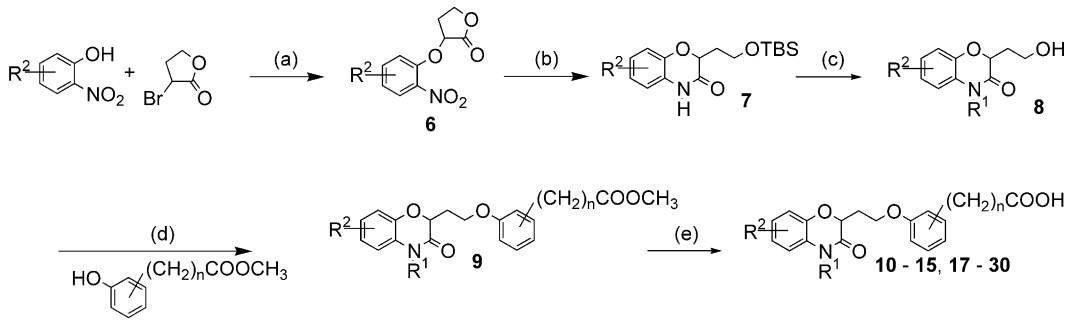
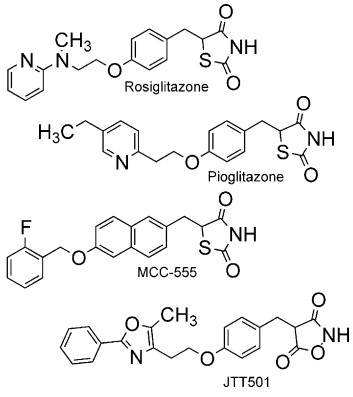
doi:10.1016/S0960-894X(03)00401-3

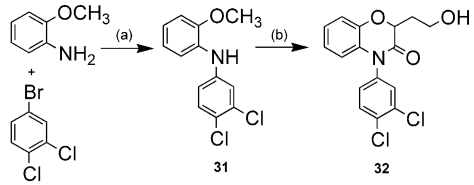


Scheme 1. (a) K_2CO_3, DMF, 0 °C to rt overnight, 70%. (b) (i) H_2, Pd/C, EtOH. (ii) TBSCl, imidazole, DMF, 60% for 2 steps. (c) (i) NaH, benzyl or phenethyl halide, DMF. (ii) CH_3SO_3H, MeOH, H_2O, 60–80% for 2 steps. (d) Bu_3P, ADDP, PhH, 60–95%. (e) NaOH, MeOH, H_2O, 80–95%.

benzoxazinone series, the TZD-substituted naphthyl compound MCC-555 was licensed from Mitsubishi Chemical as an early development candidate. Clinical data was not yet available on MCC-555, so there was no data indicating incorporation or avoidance of a particular structural feature in the lead compound. In the interest of maintaining a diverse set of clinical compounds, the benzoxazinone series was explored as a backup series.



The compounds were synthesized as outlined in Schemes 1 and 2. 2-Nitrophenol was alkylated with α-bromo-γ-butyrolactone to provide **6**, followed by nitro reduction, cyclization to the benzoxazinone and protection of the primary alcohol (**7**). Alkylation of the amide with either a benzyl or phenethyl halide and deprotection provided Mitsunobu substrate **8**. Formation of the phenyl ether (**9**) and saponification provided target compounds **10**–**15**, **17**–**30**. The route was altered to obtain *N*-aryl amides, shown in Scheme 2. Palladium mediated coupling of the aryl bromide and aniline provided **31**.[10] Deprotection and cyclization to **32** yielded a product that was elaborated to target compound **16** in a manner identical to the conversion of **8**–**10**.



Scheme 2. (a) $Pd(dba)_2$, BINAP, $PhCH_3$, 66%. (b) (i) BBr_3, xylene, CH_2Cl_2. (ii) NaH, α-bromo-γ-butyrolactone, DMF, 55% for 2 steps.

Functional potency was determined in pooled human preadipocytes by measurement of aP2 gene induction. Induction was quantified with a branched DNA technique previously described.[3] A PPARγ-Gal4Luciferase cotransfection assay[11] confirmed that the compounds exert their agonist activity through PPARγ. The compounds showed weak PPARα activity in a screening assay (data not shown) and were not tested for PPARδ activity. The data for a representative number of compounds tested in this manner are shown in Table 2. Compounds **1**, **2**, **10**–**13** explored the position and spacing of the carboxylic acid with both 3- or 4-chlorobenzyl amides. 1,2- disposal on a phenylacetic acid provided the best results. The amide substituent was varied as the 3,4-dichlorobenzyl-, 3,4-dichlorophenethyl- and 3,4-dichlorophenylamides (**14**, **15**, **16**). Extending the linkage by one atom as in **15** provided no advantage. Direct linkage as in **16** improved the binding value but decreased the potency in the functional assay. In compound **17** the heterocycle was converted to the corresponding benzoxazine and suffered tremendously in both assays (compare to **14**). Turning to fluorinated derivatives (**18**–**20**), increased potency was observed relative to the chloro and dichloro analogues while trifluoromethyl analogue had the highest potency in both the binding and functional assays. Emphasis was next placed on electron rich or neutral benzyls (**21**–**24**). Binding and functional data was modest in all cases.

Finally the role of substitution on the benzoxazinone aromatic was explored, and data for a representative set of compounds is provided (**25**–**30**). The 4-chlorobenzyl amide and 2-phenylacetic acid were held constant for this portion of the study. Overall, incorporation of substituents at the 6- or 7-positions did not improve the potency of the target compounds. Additional substitution patterns had the same effect (data not shown).

In vivo efficacy with the series was required to demonstrate the potential for further exploration, although none of these analogues had the potency needed for preclinical evaluation. Compound **11** was chosen for this study early in the SAR work, and a 27% decrease in plasma glucose was observed after 5 days of oral dosing at 30 mg/kg in *db*/*db* mice. This compound also had satisfactory metabolic stability in both human liver microsomes and hepatic S9 fraction ($t_{1/2} > 50$ min in each), and good oral bioavailability in rats (30 mg/kg, $AUC_{0-24\ h} = 308$ μM-h, $t_{1/2} = 20$ h). A more robust effect would be anticipated from compound **20**, but the

Table 2. [9] PPARg aP2 Gene functional and PPARγ-Gal4 luciferase data

Compd	R^1	R^2	COOH pos(*n*)	aP2[a](μM)	PPARγ-Gal4[b] (μM)
1	3-ClBn	H	2(1)	2.4	1.9
2	3-ClBn	H	3(1)	>10	>5
10	4-ClBn	H	2(0)	>10	>5
11	4-ClBn	H	2(1)	2.1	1.3
12	4-ClBn	H	2(2)	>10	>5
13	4-ClBn	H	3(1)	>10	>5
14	3,4-Cl_2Bn	H	2(1)	2.6	0.96
15	3,4-Cl_2Ph$(CH_2)_2$	H	2(1)	>10	>5
16	3,4-Cl_2Ph	H	2(1)	5.9	0.57
17[c]	3,4-Cl_2Bn	H	2(1)	>10	>5
18	4-FBn	H	2(1)	3.2	1.1
19	3,4-F_2Bn	H	2(1)	1.3	0.59
20	4-CF_3Bn	H	2(1)	0.51	0.50
21	Bn	H	2(1)	4.6	>5
22	4-CH_3Bn	H	2(1)	5.0	2.0
23	4-CH_3OBn	H	2(1)	5.4	0.52
24	3,4-OCH_2OBn	H	2(1)	4.4	1.1
25	4-ClBn	7-F	2(1)	>10	>5
26	4-ClBn	7-CH_3	2(1)	4.2	2.1
27	4-ClBn	7-CH_3C(O)	2(1)	6.2	1.8
28	4-ClBn	6-COOH	2(1)	>10	>5
29	4-ClBn	6-CH_3O	2(1)	6.0	1.8
30	4-ClBn	6,7-CHCHCHCH	2(1)	>10	>5
Rosiglitazone				0.12	0.19

[a]Values are the mean of two experiments for activation of aP2 gene in pooled human preadipocytes.
[b]Values are the mean of two experiments with a PPARg-Gal4Luc construct.
[c]Benzoxazine in place of benzoxazinone.

purpose of this study was to prove that the series can potentially provide a backup. These results have encouraged the continued synthesis and evaluation of compounds in the chemical series with the goal of increased in vitro potency, while maintaining acceptable parameters of pharmaceutical suitability.

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11. Transfection assay materials and method for PPARγ:

HEK293 cells were grown in DMEM/F-12 Media supplemented with 10%FBS and glutamine (GIBCOBRL). The cells were co-transfected with DNA for PPARγ-Gal4 receptor and Gal4-Luciferase Reporter using the DMRIE-C Reagent. On the following day, the DNA-containing medium were replaced with 5% Charcoal treated FBS growth medium. After 6 h, cells were seeded in 96-well plate and incubated at 37 °C in CO_2 incubator overnight. Cells were challenged by test compounds and incubated for 24 h at 37 °C in 5%CO_2 incubator. Luciferase activity was assayed using the Steady-Glo Luciferase Assay Kit from Promega. DMRIE-C Reagent was purchased from GIBCO Cat. No.10459-014. OPTI-MEM I Reduced Serum Medium was purchased from GIBCO Cat. No. 31985. Steady-Glo Luciferase Assay Kit were from Promega Part# E254B.