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Discovery of a novel selective PPARγ modulator from (−)-Cercosporamide derivatives

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ABSTRACT

In an investigation of (−)-Cercosporamide derivatives with a plasma glucose-lowering effect, we found that *N*-benzylcarboxamide derivative **4** was a partial agonist of PPARγ. A SAR study of the substituents on carboxamide nitrogen afforded the *N*-(1-naphthyl)methylcarboxamide derivative **23** as the most potent selective PPARγ modulator. An X-ray crystallography study revealed that compound **23** bounded to the PPARγ ligand binding domain in a unique way without any interaction with helix12. Compound **23** displayed a potent plasma glucose-lowering effect in db/db mice without the undesirable increase in body fluid and heart weight that is typically observed when PPARγ full agonists are administrated.

Type 2 diabetes is one of the most serious health problems in the world. The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. This number is likely to more than double by 2030.[1] Although many therapeutic agents have already been used in clinical situations, it is still difficult to tightly control plasma glucose and prevent diabetic complications.[2,3] So there is great need for novel pharmacotherapy which is able to achieve tight glycemic control singly or to be used with existing agents.

We have previously reported that (−)-Cercosporamide had a plasma glucose-lowering effect in KK/Ta mice, but it was accompanied by a severe decrease in food intake and a loss of body weight.[4] In that report it was also shown that *N*,*O*-acetal type derivatives **1** and **2** (Fig. 1) lowered the plasma glucose in KK/Ta mice without notably affecting the food consumption or body weight. Although they showed an obvious and promising effect on hyperglycemia, the mechanism of action had not yet been made clear.

We designed benzylated compounds **3** and **4** to enhance the desirable effect because the fact that compound **2** was superior to **1** indicated that the phenyl ring near the carbamoyl group was important for the plasma glucose-lowering effect. The preparation of these compounds was accomplished in the following manner (Scheme 1). For the preparation of compound **3**, the carbamoyl nitrogen and 3-hydroxyl group of (−)-Cercosporamide were reacted with 2,2-dimethoxypropane to form a acetonide. The carbamyl nitrogen and 1-hydroxyl group were benzylated by benzylbromide using sodium hydroxide as a base. The benzyl group on the 1-hydroxy group was selectively deprotected by Pd/C catalyzed hydrogenolysis to give compound **3**. For the preparation of compound **4**, the 3-hydroxyl group of (−)-Cercosporamide was selectively methylated by iodomethane in the presence of potassium carbonate. The carbamoyl nitrogen was benzylated to compound **4** by reductive amination with benzaldehyde, triethylsilane and trifluoroacetic acid in toluene.[5]

The plasma glucose-lowering effects of these compounds were tested in hyperglycemic KK/Ta mice. The test compounds were mixed with their diet in a ratio of 0.1% (about 100 mg/kg/day if the food intake did not change). This mixture was fed to the mice

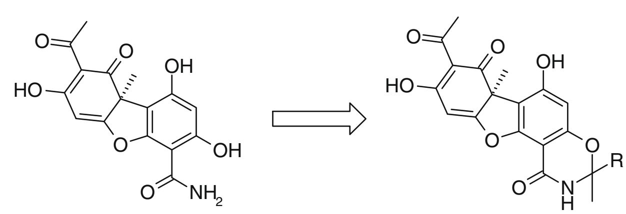

Figure 1. Previously reported (−)-Cercosporamide derivatives.

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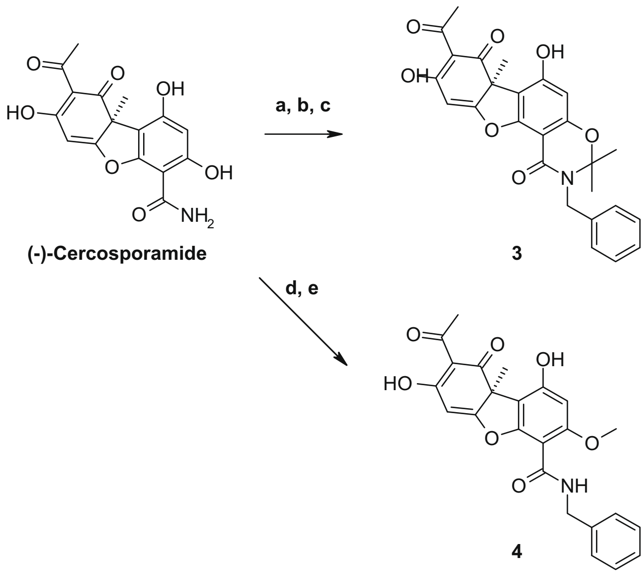


Scheme 1. Reagents and conditions: (a) 2,2-dimetoxypropane, TsOH, acetone, reflux, 8 h, 80%; (b) BnBr, NaH, DMF 0–25 °C, 2 h, 79%; (c) Pd/C, EtOAc/EtOH, 6 h, 41%; (d) MeI, K_2CO_3, DMF, 25 °C, 24 h, 66%; (e) benzaldehyde, Et_3SiH, TFA, toluene, reflux, 8 h, 74%.

Table 1
Plasma glucose-lowering effect in KK/Ta mice

Compound number	Plasma glucose correction[a] (%)	Body Weight on 7th day[b] (%)	Food intake on 7th day[b] (%)
1	56	102	78
3	55	103	74
4	69	101	100

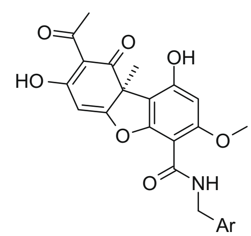
[a] The values are the % change in the plasma glucose concentration of the drug-treated mice relative to the vehicle controls. All the values are the means of 4 or 6 mice.
[b] The values are the % degree of the drug-treated mice relative to the vehicle controls.

for a week. The plasma glucose, body weight, and food intake data are summarized in Table 1. Compound **4** showed the most potent glucose-lowering effect and did not affect the body weight or food intake at all. On the other hand, compounds **1** and **3** slightly induced the decrease in food intake.

We were strongly interested in the mechanism of action of promising compound **4**. We thoroughly investigated the interaction between compound **4** and various targets which were related to diabetes. Finally, we found that compound **4** partially activated a peroxisome proliferator-activated receptor gamma (PPARγ (EC_{50}: 0.22 μM, E_{max}: 44%). PPARγ agonists promote adipocyte differentiation and improve insulin resistance.[6] The PPARγ full agonists Pioglitazone and Rosiglitazone have been clinically used and show beneficial effects in patients with type 2 diabetes. However, the use of these drugs has been limited because of their adverse effects, such as weight gain, edema, and anemia.[7] Recently, it was proposed that modulating PPARγ activity, rather than activating it fully, was effective in improving insulin resistance without undesirable adverse effects.[8] A number of compounds, such as Fmoc-L-Leucine,[9] FK614,[10] 5-substituted 2-benzoylaminobenzoic acids,[11] indole and azaindole derivatives,[12] T2384,[13] INT-131,[14] and MBX-102,[15] are reported to modulate PPARγ transactivation and improve insulin resistance in hyperglycemic rodent models. In some cases, it was observed that the adverse effects which accompany PPARγ activation were attenuated.

Accordingly, we embarked on a SAR study to acquire a more potent PPARγ modulator than compound **4**. First, we incorporated various substituents on the phenyl ring. Next, we changed the phenyl ring to a heteroaryl ring or a benzo-fused aryl ring. All these compounds were synthesized in the same way as compound **4** with the corresponding aromatic aldehyde in toluene or acetonitrile. We tested the PPARγ transactivation activities of these compounds. Then, for any compounds which showed potent transcriptional activities (EC_{50} <1 μM), we tested the potency of their effect as partial antagonists. Their structures and transcriptional activities are summarized in Table 2. In the various substituents on the phenyl ring, only chloride substitution on the *ortho*- or *para*-position (compounds **11** and **13**) sustained transactivation activity. In the heteroaryl or benzo-fused compounds, 2-furyl compound **16** and 1-naphtyl compound **23** had the potent PPARγ transactivation activity equal to that of compound **4**. As well compound **23** acted as a potent partial antagonist in comparison to compounds **4** and **16**. Then we tested the binding affinities to

Table 2
PPARγ transactivation



Compound number	Ar	Transactivation			
		EC_{50}[a] (μM)	E_{max}[b] (%)	IC_{50}[c] (μM)	I_{max}[d] (%)
4	Phenyl	0.22	44	ND[e]	
5	*o*-MeO-phenyl	ND[e]			
6	*m*-MeO-phenyl	3.8	36		
7	*p*-MeO-phenyl	1.7	43		
8	*o*-NO_2-phenyl	1.6	42		
9	*m*-NO_2-phenyl	2.4	34		
10	*p*-NO_2-phenyl	6.0	46		
11	*o*-Cl-phenyl	0.28	19	ND[e]	
12	*m*-Cl-phenyl	6.7	56		
13	*p*-Cl-phenyl	0.77	50	Negative	
14	2-Pyridinyl	Negative			
15	3-Pyridinyl	11	51		
16	2-Furyl	0.56	34	13	70
17	3-Furyl	3.2	54		
18	2-Thiophenyl	1.2	40		
19	3-Thiophenyl	4.4	45		
20	2-Benzofuryl	2.3	93		
21	2-Benzothiophenyl	3.8	77		
22	3-Benzothiophenyl	ND[e]			
23	1-Naphthyl	0.18	47	0.62	45
24	2-Naphthyl	ND[e]			
Pioglitazone		0.088	118	Negative	
Rosiglitazone		0.011	106	Negative	

[a] EC_{50} was defined as the compound concentration at which 50% of a given compound's intrinsic maximal response had been reached.
[b] The transcriptional activity in the presence of an in-house potent PPARγ full agonist (3.3 nM) was defined as 100%, while that in the vehicle alone was defined as zero. The maximum transcriptional activity in the presence of the test compound was defined as E_{max} (%).
[c] IC_{50} was defined as the compound concentration at which 50% of a given compound's intrinsic maximal inhibition had been reached.
[d] The maximum inhibition of the test compound against the transcriptional activity in the presence of an in-house potent PPARγ full agonist (3.3 nM) was defined as I_{max} (%).
[e] ND means 'not determined' because the E_{max} or I_{max} was too low.

PPARγ. It was surprising that compound **23** showed a higher affinity (K_i: 14 nM) than Rosiglitazone (K_i: 82 nM), although the other compounds, even compound **4**, had relatively low affinities (data not shown). Moreover, we tested the selectivity of compound **23** and it did not transactivate either PPARα or PPARδ. We therefore concluded that compound **23** was a potent selective PPARγ modulator.

In order to reveal how compound **23** interacts with PPARγ, the crystal structure of PPARγ-LBD (ligand binding domain) with compound **23** and a peptide derived form coactivator SRC-1 was determined.[16] The ternary complex structure showed that compound **23** bound to the PPARγ ligand binding site similar to other PPARγ agonists. However, compound **23** lacks direct interaction with helix12, though most PPARγ full agonists, including Rosiglitazone, interact with Tyr473 in helix12 to stabilize PPARγ in the active conformation (Fig. 2, A).[17] The Cercosporamide scaffold of compound **23** is located between helix3 and β-sheet, while the 1-naphthyl group is embedded in a hydrophobic region which consisted of helix3, helix5, β-strand2 and helix7. The Cercosporamide scaffold also makes water-mediated hydrogen bonds with Leu340 and Ser342 (Fig. 2, B). Together with previous works[11,13,14] which reported PPARγ partial agonists bound with PPARγ without direct interaction to residues in helix12, the lack of direct interaction of compound **23** with helix12 might also be responsible for its partial agonist and partial antagonist activities.

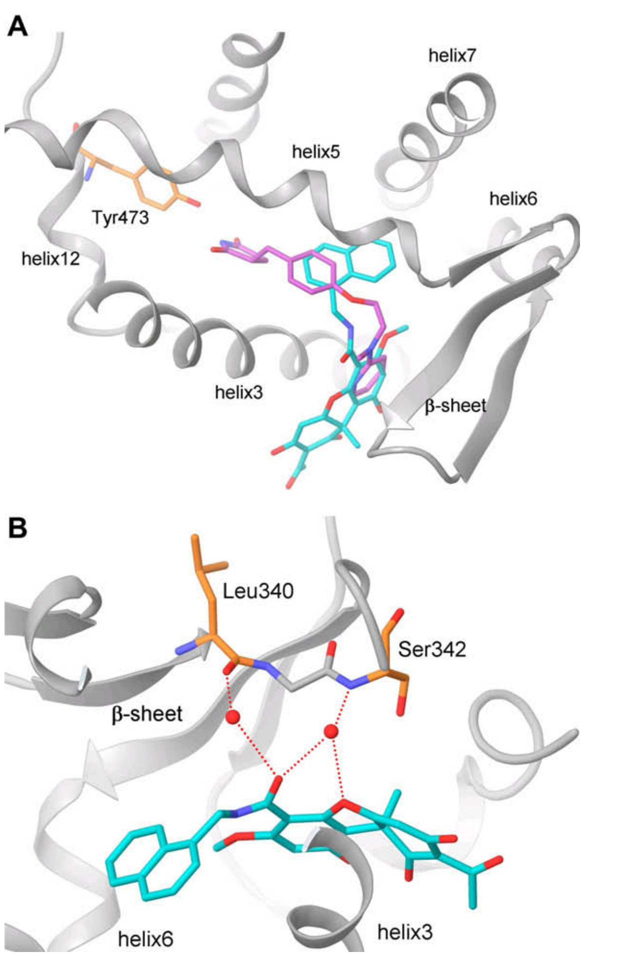


Figure 2. Crystal structure of the complex of PPARg-LBD and compound **23**. (A) The complex structure of PPARγ-LBD (shown as gray ribbon) with compound **23** (shown in cyan stick representation) is superimposed on that of Rosiglitazone (shown in magenta stick representation, derived from 2PRG.pdb[17]). (B) The Cercosporamide scaffold makes water-mediated hydrogen bonds with Leu340 and Ser342.

Table 3
Anti-diabetic effect and adverse effects of compound **23** in db/db mice

Compound	The ratio in the diet	Plasma glucose (mg/dL)	Body weight gain for 7 days (g)	Red blood cells ($\times 10^4$ cells/μL)	Heart weight (mg)
Vehicle	None	627 ± 72	3.19 ± 0.51	863 ± 11	106.6 ± 4.8
23	0.01%	357 ± 42	4.83 ± 0.38	846 ± 21	102.8 ± 1.0
	0.1%	223 ± 21	5.28 ± 0.45	812 ± 7	103.8 ± 2.3
Farglitazar	0.01%	206 ± 12	6.37 ± 0.41	746 ± 19	124.5 ± 4.3

The values are expressed as means ± SEM from four mice.

Finally, we tested whether the novel selective PPARγ modulator **23** showed an anti-diabetic effect and attenuated adverse effects in comparison with the PPARγ full agonist Farglitazar (GI262570).[18] The test compounds were mixed with the diet in a certain ratio. The mixture was fed to hyperglycemic male db/db mice[19] for a week (Table 3). Compound **23** lowered the plasma glucose levels in a dose-dependent manner and a high dose (0.1%) was as effective as Farglitazar (0.01%). Compound **23** also increased the body weight in a dose-dependent manner, but the increases in body weight tended to be less than the increase by Farglitazar. In the group treated with Farglitazar, it was also observed that the number of red blood cells decreased and the heart weight increased. The decrease in red blood cells indicated the fluid retention which is associated with edema and anemia. As well, cardiac hypertrophy was considered to be compensation for this fluid retention. On the other hand, in the groups treated with compound **23**, the tendency of fluid retention was slightly observed and cardiac hypertrophy was not recognized at all.

In conclusion, we succeeded in acquiring a potent selective PPARγ modulator **23** from (−)-Cercosporamide. An X-ray crystallography study revealed unique interaction between compound **23** and PPARγ-LBD. Furthermore, compound **23** showed a potent anti-diabetic effect in db/db mice. In comparison with the PPARγ full agonist Farglitazar, the adverse effects such as body weight gain and fluid retention were attenuated and no increase in heart weight was observed at all. Further investigations to enhance these desirable profiles are ongoing and the results will be reported elsewhere.

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19. Db/db mice are more suitable in a simultaneous evaluation of plasma glucose-lowering effect and undesirable adverse effect, such as the decrease of red blood cells. Incidentally, compound **23** also showed a potent plasma glucose-lowering effect in KK/Ta mice.