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(−)-Cercosporamide derivatives as novel antihyperglycemic agents

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ABSTRACT

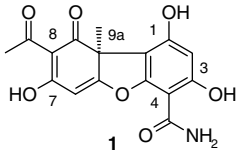
In our exploratory campaign for an antihyperglycemic agent with a novel mechanism of action, (−)-Cercosporamide **1**, which is known as an antifungal agent, showed a potent plasma glucose lowering effect in hyperglycemic KK/Ta mice. The trouble was that it was accompanied by a decrease in food intake and a loss of body weight. We synthesized some (−)-Cercosporamide derivatives and succeeded to separate these actions. N,O-ketal type derivatives, especially compound **10**, had the most potent plasma glucose lowering effect without affecting the food consumption or body weight.

Type II Diabetes is one of the most serious health problems in the world.[1] Despite the fact that many oral therapeutic agents, such as metofolmin, sulfonylureas, thiazolizine-diones and DPPIV inhibitors, 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 the existing agents. To discover antihyperglycemic agents with novel mechanisms of action, we chose an in vivo screening method. Various bioactive compounds were mixed with the diet in certain ratios. The mixture was fed to hyperglyemic KK/Ta mice for a week. Then (−)-Cercosporamide **1** was found to show a potent plasma glucose lowering effect. The more the ratio of (−)-Cercosporamide was increased, the more powerfully the plasma glucose was lowered. However, at the same time, the food intake and body weight also decreased (Table 1).

(−)-Cercosporamide was originally isolated in 1991 as an antifungal agent and phytotoxin from a fungal plant pathogen of cassava, *Cercosporidium henningsii*.[4] The absolute configuration was established as (9a*S*)-configuration by correlation with a synthetic analog.[5,6] In 2004 it was shown that (−)-Cercosporamide was a selective and highly potent fungal Pkc1 kinase inhibitor (IC_{50}: 0.044 μM).[7] The Pkc1-mediated cell wall integrity-signaling pathway was essential for fungal growth. So the antifungal activity of (−)-Cercosporamide was attributed to the inhibition of Pkc1 kinase. Fungal Pkc1 kinase shared a high homology with mammalian PKCs in their catalytic domain.[8] (−)-Cercosporamide showed moderate inhibitory activities against several human PKCs (IC_{50} PKCα: 1.02 μM, PKCβ: 0.35 μM, PKCγ: 5.8 μM) as well.[7] The activation of PKCs was associated with hyperglycemia induced cardio- and microvascular complications in diabetes patients.[9] Ruboxistaurin, a PKCβ selective inhibitor, showed efficacy in the treatment of diabetic retinal and renal abnormalities in both diabetic patients and animals.[10] But no compounds have ever been reported to improve the plasma glucose level of diabetic animals through inhibiting PKCs activation. Accordingly, we speculated that (−)-Cercosporamide lowered the plasma glucose of KK/Ta mice independently of PKCs inhibition, though we have not yet determined a plausible mechanism for the plasma glucose lowering effect.

For applying (−)-Cercosporamide to pharmacological therapy for type II diabetes, it was problem that the plasma glucose lowering was accompanied by toxic effects such as a decrease in food intake and a loss of body weight. Consequently, we began to synthesize some (−)-Cerocosporamide derivatives for the purpose of separating the plasma glucose lowering effect from the toxic ef-

Table 1
Plasma glucose lowering effect of (−)-Cercosporamide **1**



1

Ratio of Cercosporamide	Plasma glucose[a] (mg/dL)	Body weight on 7th day[a] (g)	Food intake on 7th day[a] (g)
Vehicle	602 (±28)	46.95 (±0.37)	5.61 (±0.24)
0.003%	571 (±13)	48.37 (±0.53)	5.78 (±0.37)
0.01%	408 (±45)	46.96 (±1.01)	4.81 (±0.27)
0.03%	272 (±22)	45.88 (±0.90)	3.65 (±0.19)
0.1%	176 (±15)	39.10 (±0.75)	1.89 (±0.46)

[a] (−)-Cercosporamide was mixed with the diet in a certain ratio. The mixture was fed to hyperglycemic KK/Ta mice for a week. The values are the means of 6 mice, the standard deviation is given in parentheses.

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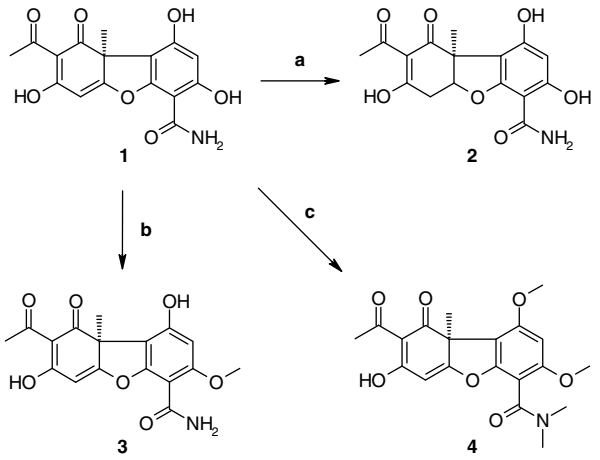
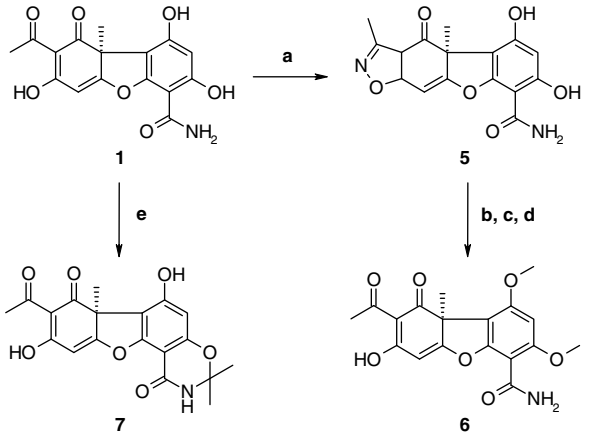


Scheme 1. Reagents and conditions: (a) Pd–C, THF–MeOH, 25 °C, 9 h, 71%; (b) MeI, K_2CO_3, DMF, 25 °C, 24 h, 66%; (c) MeI, NaH, DMF, 0–25 °C, 1 h, 42%.



Scheme 2. Reagents and conditions: (a) NH_2OH–HCl, CH_3CO_2Na, EtOH, reflux, 3 h, 20%; (b) MeI, K_2CO_3, DMF, 70 °C, 4 h; (c) PtO_2, EtOAc–EtOH, 25 °C, 3h; (d) aq NaOH, 24 h, 37% 3 steps; (e) 2,2-dimetoxypropane, TsOH, acetone, reflux, 8 h, 80%.

fects (see Schemes 1 and 2). As well, we postponed our investigation of the mechanism until we got a promising derivative.

To the best of our knowledge, there are few studies on the derivatization of (−)-Cercosporamide. A patent applied for by the BASF research group claimed some derivatives,[11] but the detailed experimental procedures and physical data were not mentioned. None of the derivatives reported here, except for compound **6**, were exemplified in the BASF patent.

The C5a-C6 double bond was selectively reduced to compound **2** by Pd–C catalyzed hydrogenation and the 3-hydroxyl group was selectively methylated to compound **3** by iodomethane in the presence of potassium carbonate. Because of strong intramolecular hydrogen bonds, the other hydroxyl groups had low reactivity.[12] When sodium hydride was used as a base for methylation, the 1,3-dihydroxyl groups and carbamyl nitrogen were methylated to compound **4**.

To methylate the 1-hydroxyl group without the methylation of carbamyl nitrogen, it was necessary to change the intramolecular hydrogen bonds. For that purpose, we reacted the 8-acetyl group with hydroxylamine to form hydroxime and then the following cyclization led to isoxazole **5**. This was similar to derivatization of Usnic acid.[13] The 1,3-dihydroxyl groups of isoxazole **5** were successfully methylated using an iodomethane and potassium carbonate system. The N–O bond of isoxazole was catalytically cleaved by PtO_2. The resulting imine was hydrolyzed to compound **6**. Meanwhile, the carbamyl nitrogen and 3-hydroxyl group formed an acetonide **7** by reaction with 2,2-dimethoxypropane, similar to a salicylamide.[14]

Table 2
Effect of (−)-Cercosporamide derivatives in a ratio of 0.1%

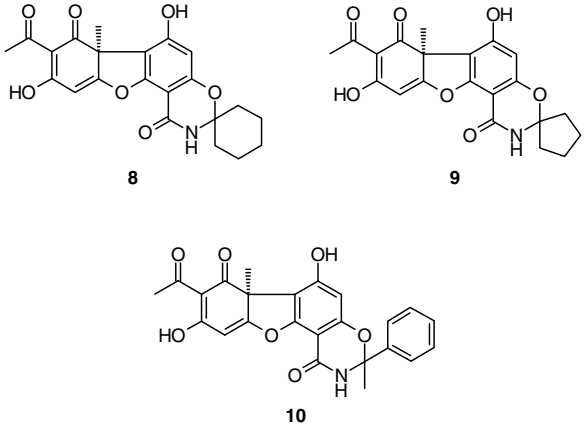
Compound	Plasma glucose correction[a] (%)	Body weight on 7th day[b] (%)	Food intake on 7th day[b] (%)
1	71	83	34
2	29	101	111
3	70	83	37
4	14	96	95
5	1	102	126
6	26	100	92
7	56	102	78

[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 6 mice.
[b] The values are the % degree of the drug-treated mice relative to the vehicle controls.

The plasma glucose lowering effects of these compounds were tested in hyperglycemic KK/Ta mice. The test compounds were mixed with the 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 for a week. The plasma glucose, body weight, and food intake data are summarized in Table 2.

The derivatives with left side ring modification (compounds **2** and **5**) did not lower the plasma glucose. Among the methylated derivatives (compounds **3, 4** and **6**), only compound **3** lowered the plasma glucose. These results indicated that the left side ring structure and the 1-hydroxyl group were essential for the plasma glucose lowering effect. But compound **3** induced a decrease in food intake and a loss of body weight. On the other hand, acetonide derivative **7** powerfully lowered the plasma glucose, as well as (−)-Cercosporamide **1**, without a loss of body weight, although food intake slightly decreased. As well, compound **7** did not inhibit any PKCs (PKCα, PKCβ1, PKCβ2, PKCδ, PKCε, PKCη, PKCζ) at 1 μM. These results showed, as first speculated, that the plasma glucose lowering effect of (−)-Cercosporamide was independent of PKCs inhibition.

Table 3
Effect of N,O-ketal type derivatives in a ratio of 0.03%



Compound	Plasma Glucose correction[a] (%)	Body weight on 7th day[b] (%)	Food intake on 7th day[b] (%)
7	35	102	92
8	38	104	104
9	7	102	106
10	54	100	94

[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.

Although the mechanism was still unclear, we succeeded in separating the plasma glucose lowering effect from the toxic effects. For the purpose of further increasing the efficacy and reducing the toxic effects, we synthesized N,O-ketal type derivatives **8–10** by *p*-toluenesulfonic acid catalyzed condensation with corresponding dimethylketal in chloroform.

These compounds were similarly tested in a ratio of 0.03% (about 30 mg/kg/day if the food intake did not change). The results are summarized in Table 3. Though cyclization of the dimethyl moiety was not so effective (compounds **8** and **9**), incorporation of the phenyl ring (compound **10**) was useful to enhance the plasma glucose lowering effect. Moreover, compound **10** caused no decrease in food intake or loss of body weight whatsoever.

In conclusion, we synthesized a number of derivatives of (−)-Cercosporamide and clarified that the left side structure and the 1-hydoroxyl group were necessary for the plasma glucose lowering effect. We also succeeded in separating the plasma glucose lowering effect from the decrease in food intake and loss of body weight by forming N,O-ketal. Compound **10** showed the most potent glucose lowering effect without any toxic effect. Further derivatizations are under investigation and the results will be reported elsewhere.

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