# STUDIES ON WF-3681, A NOVEL ALDOSE REDUCTASE INHIBITOR

## III. EFFECTS OF WF-3681 AND ITS DERIVATIVES ON SORBITOL ACCUMULATION IN DIABETIC RATS

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Aldose reductase (E.C.1.1.1.21), which catalyzes the conversion of glucose to sorbitol plays an important role in the pathogenesis of diabetic complications.[1] Aldose reductase inhibitors (ARIs) have been shown to reduce tissue sorbitol levels and to improve diabetic complications.[2] In the preceding papers, we reported the isolation,[3] structure elucidation and total synthesis of WF-3681, a novel aldose reductase inhibitor isolated from a cultured filtrate of *Chaetomella raphigera*.[4] Moreover, several derivatives of WF-3681 were synthesized and their structure-aldose reductase inhibitory activity relationship was also discussed.[5] In this paper, we report the effects of WF-3681 and its derivatives on sorbitol accumulation in the sciatic nerve of diabetic rats.

Fig. 1. Structure of WF-3681.

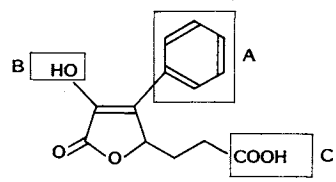
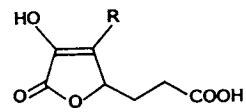
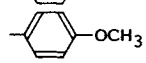
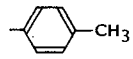
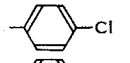
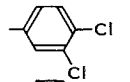
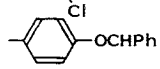
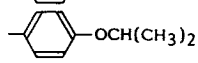
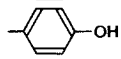
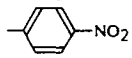
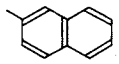
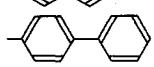
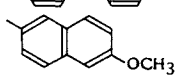


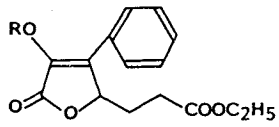

Table 1. Effects of 3-aryl derivatives on the sorbitol levels in the sciatic nerve of diabetic rats.




| Compound | R | Inhibition of the sorbitol level (%) | *In vitro* activity ($IC_{50}$)[5] (M) |
|---|---|---|---|
| **1** (WF-3681) |  | 23 | $2.5 \times 10^{-7}$ |
| **2** |  | 27 | $5.4 \times 10^{-8}$ |
| **3** |  | 0 | $8.4 \times 10^{-8}$ |
| **4** |  | 16 | $9.2 \times 10^{-8}$ |
| **5** |  | 11 | $9.8 \times 10^{-8}$ |
| **6** |  | 0 | $4.9 \times 10^{-8}$ |
| **7** |  | 5 | $5.2 \times 10^{-8}$ |
| **8** |  | 0 | $1.6 \times 10^{-7}$ |
| **9** |  | 14 | $1.1 \times 10^{-7}$ |
| **10** |  | 8 | $4.3 \times 10^{-8}$ |
| **11** |  | 20* | $1.5 \times 10^{-8}$ |
| **12** |  | 20 | $4.6 \times 10^{-8}$ |

*$P<0.05$ against untreated diabetic rats. n=6.

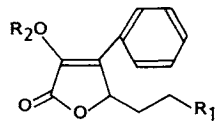
Table 2. Effects of 4-alkoxy and 4-aralkoxy derivatives on the sorbitol levels in the sciatic nerve of diabetic rats.




| Compound | R | Inhibition of the sorbitol level (%) | *In vitro* activity ($IC_{50}$)[5] (M) |
|---|---|---|---|
| **13** | $-C_3H_7$ | 0 | $1.5\times10^{-7}$ |
| **14** | $-C_4H_9$ | 31** | $1.6\times10^{-7}$ |
| **15** | $-C_5H_{11}$ | 30** | $5.0\times10^{-8}$ |
| **16** | $-C_7H_{15}$ | 23** | $5.5\times10^{-8}$ |
| **17** | $CH_2$— | 3 | $1.4\times10^{-7}$ |
| **18** | $CH_2$—Cl | 28** | $1.4\times10^{-7}$ |
| **19** | $CH_2$—$OCH_3$ | 27** | $1.5\times10^{-7}$ |
| **20** | $CH_2$—$CH_3$ | 1 | $1.3\times10^{-7}$ |

**$P<0.01$ against untreated diabetic rats. n=6.

Table 3. Effects of derivatives with modified carboxylic side chain on the sorbitol levels in the sciatic nerve of diabetic rats.




| Compound | $R_1$ | $R_2$ | Inhibition of the sorbitol level (%) | *In vitro* activity ($IC_{50}$)[5] (M) |
|---|---|---|---|---|
| **21** | $COOC_2H_5$ | $CH_3$ | 58*** | $2.2\times10^{-7}$ |
| **22** | $CH_2OH$ | $CH_3$ | 0 | $>1.0\times10^{-5}$ |
| **23** | $CONH_2$ | H | 28 | $>1.0\times10^{-5}$ |

***$P<0.001$ against untreated diabetic rats. n=6.

Seven-weeks-old Sprague-Dawley male rats were made diabetic by a single ip injection of streptozotocin with a dose of 75 mg/kg. After 7 days of the streptozotocin injection, plasma glucose levels of all rats were analyzed by sampling blood from the tail vein. The rats with a plasma glucose level of above 400 mg/deciliter were grouped at random, so that the mean body weights and blood glucose levels of each group were not significantly different. Each of the derivatives[5] of WF-3681 suspended in 0.5% methyl cellulose was given to rats orally once daily from day 8 to 12. At day 12, the animals were killed and sciatic nerve were separated from the animals. The nerves were weighed immediately and frozen at −20°C until determination of sorbitol levels. Sorbitol was measured enzymatically by the modified method of CLEMENTS *et al.*[6] Briefly, the sciatic nerves were homogenized in 1 N perchloric acid in a glass homogenizer and centrifuged at 900 × *g* for 10 minutes at 4°C. The supernatants were neutralized with 5 M $K_2CO_3$ and centrifuged. Sorbitol concentration in the supernatants was determined by an enzymatic method using sorbitol dehydrogenase (SDH). The reaction mixture contained glycine buffer 50 mM (pH 9.6), $MgCl_2$ 2 mM, $NAD^+$ 4 mM, SDH 0.7 U and 0.6 ml of the sample in a total volume of 2.0 ml. Sorbitol levels in the extract were determined fluorometrically. The change of fluorescence due to NADH after incubation for 30 minutes at 37°C was monitored by a fluorometer (excitation; 360 nm, emission; 460 nm).

WF-3681 and its derivatives which were obtained by addition of phenyl (A), hydroxy (B) and carboxy (C) groups[5] as depicted in Fig. 1 were evaluated in this model. Inhibitory effects of WF-3681 and its derivatives at a dose of 32 mg/kg on sorbitol accumulation in the sciatic nerve of diabetic rats are shown in Tables 1, 2 and 3. The data were expressed as inhibitory percent. Each value was calculated as follows: Inhibition $(\%)=(DS-IS)/(DS-NS)\times 100$, where DS and NS are sorbitol levels in the nerve of diabetic and normal control rats, respectively and IS is that of diabetic rats treated with each compound. *In vitro* aldose reductase inhibitory activities of these compounds were also shown as reference.

The effects of WF-3681 and 3-aryl derivatives on the sorbitol accumulation in the sciatic nerve of diabetic rats are shown in Table 1. WF-3681 showed 23% inhibition on sorbitol accumulation but the value was not statistically significant. The 3-aryl derivatives tested did not inhibit the sorbitol accumulation, except for compound **11**. Previously, the introduction of lypophilic substituents at the 4-position of the benzene ring, compounds **2**, **6** and **7**, enhanced the *in vitro* activity by about 5-fold as compared with the mother compound[5] but they were not effective in this model. *In vivo* efficacy of each compound might depend on its pharmacokinetic property. Table 2 shows the effects of the alkylether derivatives in this model. Compounds **14**, **15**, **16**, **18** and **19** were more effective than the parent compound. The carbon chain length of these compounds is important for activity. The effects of the alterations of the carboxylic acid are shown in Table 3. The compound with the ethyl ester (**21**) considerably increased the *in vivo* activity. The compound showed most potent activity among the WF-3681 derivatives that we examined in this model. The results presented here encourage the further development and further evaluation of the compound **21**.

## References

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