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Research Article

IN VITRO ANTI-INFLAMMATORY AND ANTIDIABETIC THIAZOLIDINEDIONE DERIVATIVE OF BENZODIOXOLE

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ABSTRACT

Thiazolidinedione derivative of benzodioxole, (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2, 4-dione (S1801), was synthesized in the laboratory and was evaluated for the anti-inflammatory and antidiabetic property *in vitro*. During the observation for the antidiabetic property, at the dose levels of 4 μg/ml and 40 μg/ml, S1801 offered 9% and 28% of inhibition of the α-amylase enzyme respectively, which appears to be dose dependent. Similarly, during the observation for the anti-inflammatory property S1801 offered 18% and 21% inhibition of the hemolysis at the dose levels of 100 μg/ml and 500 μg/ml. Thus, S1801 was found to have anti-diabetic as well as anti-inflammatory potential when tested *in vitro*. Due to the reported correlation between the chronic inflammation and diabetes, this scaffold may pose an interesting pharmacophore suitable for lead generation for managing diabetes mellitus.

Keywords: *Anti-inflammatory, antidiabetic, thiazolidinedione, benzodioxole.*

Introduction

Diabetes mellitus is a metabolic disorder resulting from defective insulin secretion and/or poor response of the cells to insulin action. This complex metabolic disorder is going to affect 300 million people by the year 2025 (Chang *et al.*, 2012; Zhu *et al.*, 2010 and Dixit *et al.*, 2007). In absence of proper measures, this will effect on 366 million individuals worldwide within the next 30 years (Kaveeshwar *et al.*, 2014). The major form of diabetes mellitus is Type II accounting for more than 90% of all diabetics (Zimmet *et al.*, 2001). Long-term hyperglycemia results in various microvascular complications including neuropathy, nephropathy, cardiovascular and cerebro-vascular diseases. Though, various oral treatment options are there including biguanides, sulfonylurea, glycosidase inhibitors etc. for managing this disease (Zimmet *et al.*, 2001 and Defronzo *et al.*, 1992), full recovery from diabetes has not been yet reported (Li *et al.*, 2004). Thus there is continuous search for better management of this lifestyle disease. Rather most of the

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existing treatment options lead to wait gain thereby posing additional problem for the obese persons who are especially more likely to be diabetic because of their excess body weight and food intake. However, among the various classes of antidiabetic drugs, thiazolidinediones have been found to affect glucose and lipid metabolism in insulin-sensitive tissues, thereby reducing the hepatic lipid content by modulating several mediators (Eugene *et al.*, 2014). Another approach is to reduce the availability of glucose from the intestine, which shows no relation with insulin. This action is more suitable for the obese diabetic patients who are usually habitual to have large carbohydrate intakes.

According to Kathryn *et al*. (2005), insulin affects cells through binding with receptor and then the stimulated insulin receptor phosphorylates itself thereby initiating downstream signaling events (White 1997 and Saltiel *et al.*, 2002). Inhibition of signaling downstream is a primary mechanism for inflammatory signals leading to insulin resistance. Thus better target can be compounds having both the anti-inflammatory and antidiabetic property. The thiazolidinedione derivatives are now commercially available as the oral therapy for managing the type II diabetes (Phillips *et al.*, 2001). Similar to other available agents these are also incapable of curing this autoimmune and progressive disease. Considering these, as a continuous search for better drugs, new scaffold having both the anti-inflammatory and antidiabetic potential were searched and the results have been reported here.

Material and Methods

Chemicals

The necessary reagents were purchased from Sigma-Aldrich (USA) and TCI (Japan). Methanol and dichloromethane were collected from Duksan Pure Chemicals Co. Ltd, South Korea. Starch and solvents were collected from Daejung Chemical & Metal Co. Ltd.

Synthesis

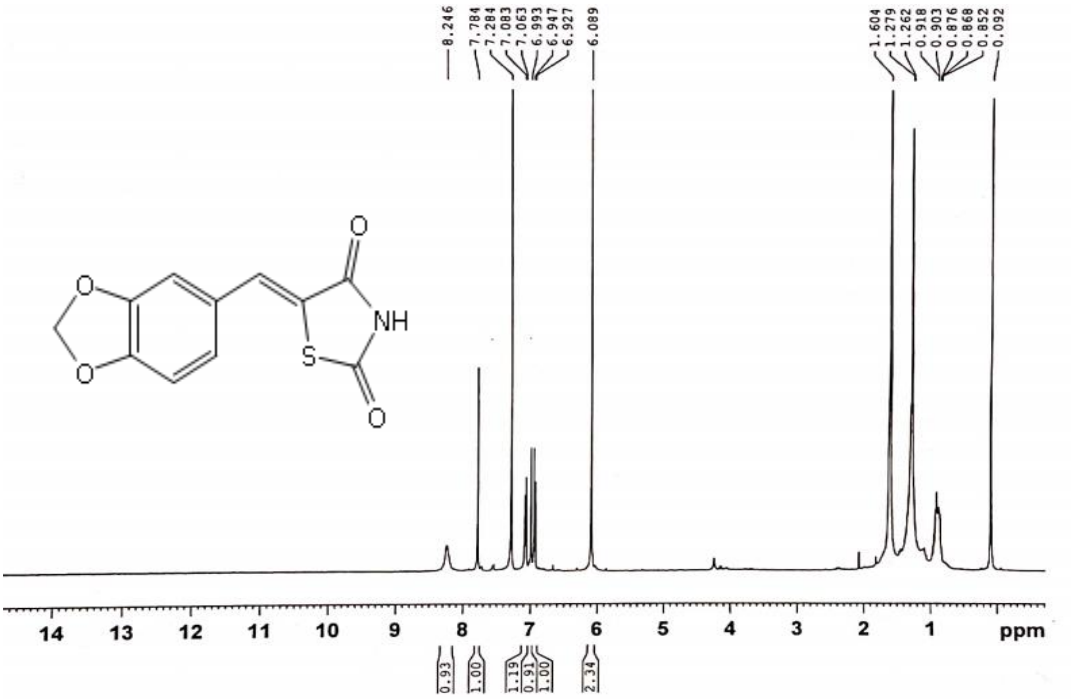
Derivative was synthesized and then was purified by flash column chromatography using silica gel (45-100μ). The reaction endpoints were checked by the TLC using Sigma-Aldrich Glass plates having silica gel coated with fluorescent indicator F254. The synthesized compound was characterized by ^{1}H NMR by Bruker 400 MHz.

Experimental

Synthesizing (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene)thiazolidine-2,4-dione

Thiazolidindione (2 equivalents) was added to the solution of piperonal in acetic acid. The mixture was refluxed for 12 hours. The completion of reaction was monitored by TLC. After subsequent neutralization by water under an ice-bath system and addition of EtOAc, the organic layer was collected, dried and evaporated to get the crude product which was then purified by column chromatography using increasing polarity gradients of Dichloromethane/Ethylacetate to get the (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione (88% yield).

^{1}H NMR spectrum of (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene)thiazolidine-2,4-dione



Spectral data from (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene)thiazolidine-2,4-dione:

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 (s, 2H), 6.93 (d, *J* = 8 Hz, 1H), 6.99 (s, 1H), 7.07 (d, *J* = 8 Hz, 1H), 7.78 (s, 1H), 8.24 (br s, 1H)

Observation of the in vitro α- amylase inhibitory (antidiabetic) activity

For the *in vitro* antidiabetic effects, reported method (Sudha *et al.*, 2011) was applied with minor modification. The mixture of 6.0 ml phosphate buffer (pH 6.9), 0.2 ml of enzyme solution (10 mg of α-amylase in 100 ml of phosphate buffer), and 0.2 ml of the desired test compound dissolved in distilled water were incubated at 37 ℃ for 10 mins. After subsequent addition of 0.2 ml starch solution (1% w/w) the mixture was incubated for 15 mins. After subsequent addition of 0.2 ml of 1M HCl, was added 0.3 ml iodine reagent (635 mg iodine and 1 gm potassium iodide in 250 ml distilled water). Absorbance was taken at 620 nm. Enzyme solution was made by replacing test drug with vehicle. The control was made by replacing both the test drug and enzyme with vehicle. Enzyme activity was calculated by the following equation-

Enzyme activity (EA) = Absorbance of control – Absorbance of sample/Test

The % inhibition of the enzyme activity was calculated by the following equation-

$$\% \text{ inhibition of the enzyme activity} = (EA_{[Enzyme]} - EA_{[Test]}) / EA_{[Enzyme]} \times 100$$

Observation of the in vitro anti-inflammatory activity

For the anti-inflammatory activity was followed by reported methods (Seema *et al.*, 2011 and Althaf *et al.*, 2013) with minor modifications. Phosphate buffer (pH 7.4, 0.15 M, 1 ml), hypo saline (2 ml, 0.36%), HRBC suspension (0.5 ml, 10% v/v), Test drug (0.5 ml, 100 and 500 µg/ml) and standard drug diclofenac sodium (0.5 ml, 100 and 500 µg/ml) were mixed separately. The mixtures were incubated at 37 ℃ for 30 mins before centrifugation. The supernatant fluid was taken for measuring hemoglobin content by observing the absorbance at 560 nm using UV-vis spectrophotometer. For control, distilled water was taken instead of hypo saline to produce 100% hemolysis. Percentage of hemolysis was calculated by the following equations:

% Hemolysis = (Optical density of Test sample)/(Optical density of Control)×100

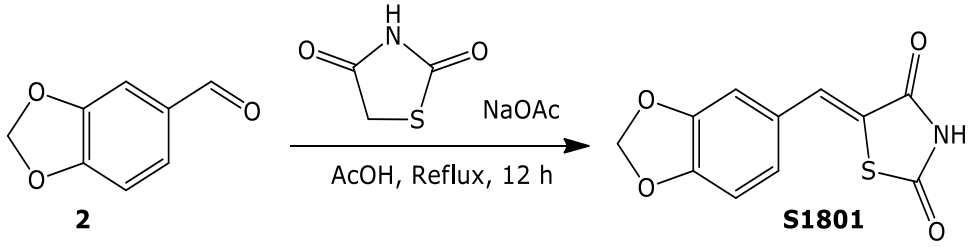
The percentage of HRBC membrane stabilization was calculated as follows:

% Inhibition= 100 – Percentage of Hemolysis

Results and Discussion

Synthesis of the Target Compound

In this study (Scheme 1), commercially available simple piperonal (**2**) was subjected for coupling with thiazolidinedione in presence of sodium acetate in acetic acid at refluxing temperature to get the desired (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione (**S1801**). This method was adopted with some minor modifications (Yang *et al*., 2012).



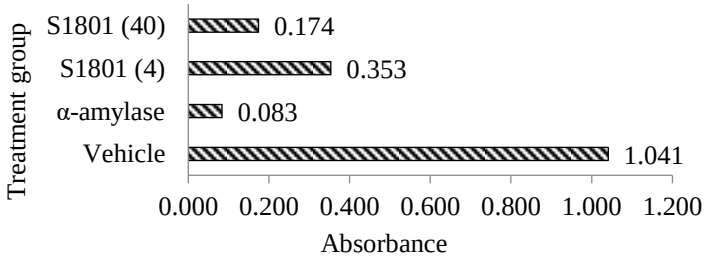
Scheme 1. Synthesis of (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione.

In Vitro Antidiabetic Activity

The average absorbance values observed from various samples have been represented in Graph1. While considering the *in vitro* antidiabetic activity of Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione, enzyme sample was found to show the absorbance value of 0.957 (Table 1). Taking this as 100% enzyme activity, there was only 9% reduction shown by **S1801** at the dose level of 4 µg/ml. But the 40 µg/ml dose showed 28% enzyme inhibition thereby indicating a dose dependent inhibitory potential.

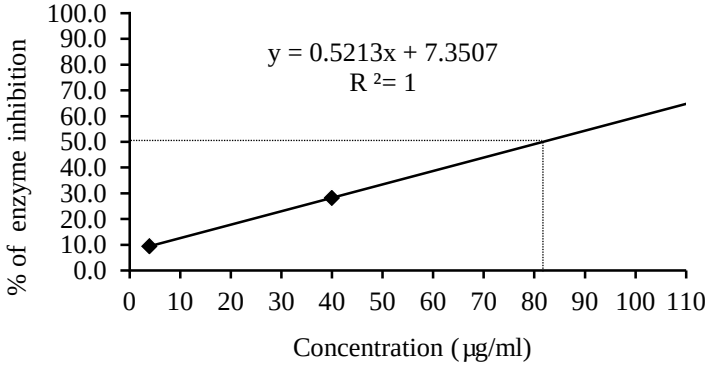
Table 1. α-amylase inhibition by test samples.

Group	Dose	Enzyme Activity (Mean Absorbance)	Enzyme inhibition (%)
Vehicle	-	-	-
α-amylase	-	0.957	-
S1801	40 μg/ml	0.687	28
S1801	4 μg/ml	0.867	9



Graph 1. Absorption by various samples solutions observed α-amylase inhibition assay.

From the two-point observations, the IC_{50} value was found to be 82 μg/ml (Graph 2). Though the value was high, further modification can be taken under consideration. According to this study, **S1801** possesses the antdiabetic activity as measured by amylase inhibitory potential by *in vitro* method. This property is especially important for the huge number of obese diabetic patients.



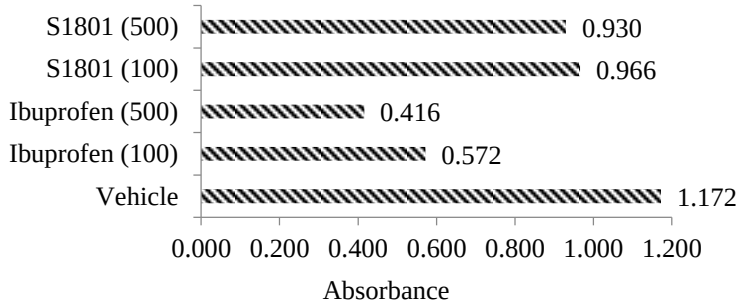
Graph 2. IC_{50} value of (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione against the α-amylase enzyme.

In Vitro Anti-Inflammatory Activity

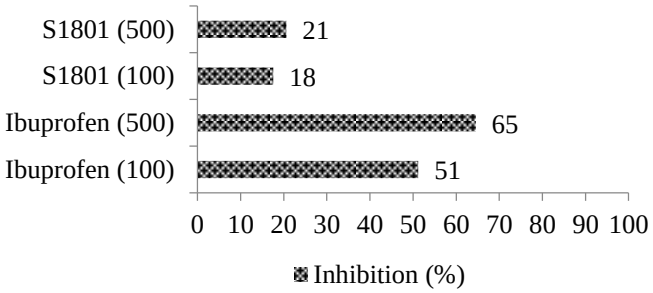
The average absorption values observed from various sample solutions have been represented in Graph 3. While considering the *in vitro* anti-inflammatory activity (Table 2), (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione (**S1801**) showed 82% and 79% of lysis of human red blood cell (RBC) from the doses of 100 μg/ml and 500 μg/ml respectively. The observations were compared with reference standard ibuprofen where the hemolysis was 49% and 35% from the doses of 100 μg/ml and 500 μg/ml respectively. However, the calculated percentage inhibition has been represented in Graph 4. Form this graph it is clear that **S1801** showed a dose dependent inhibition of the hemolysis.

Table 2. Percentage of hemolysis in samples containing S1801 and Ibuprofen.

Group	Dose (μg/ml)	Mean Absorbance	% Lysis
Vehicle	-	1.172	
Ibuprofen	100	0.572	49
Ibuprofen	500	0.416	35
S1801	100	0.966	82
S1801	500	0.930	79



Graph 3. Absorbance values observed from the inhibition of hemolysis.



Graph 4. Inhibition of hemolysis as observed from the tested samples.

While observing the anti-inflammatory potential (Graph 4), **S1801** showed 18% and 21% inhibition of hemolysis from the doses of 100 μg/ml and 500 μg/ml respectively. The observations were compared with reference standard ibuprofen where the inhibition of hemolysis was 51% and 65% from the doses of 100 μg/ml and 500 μg/ml respectively. Thus **S1801** appeared to possess also the anti-inflammatory property.

Conclusion

In this study, (Z)-5-(benzo[d][1,3]dioxol-5-ylmethylene) thiazolidine-2,4-dione (**S1801**) showed both the antidiabetic potential and anti-inflammatory potential. It is already known that the diabetes mellitus is a progressive autoimmune disease. Thus the anti-diabetic agents having anti-inflammatory potential might have better role in managing the diabetes mellitus. Thus **S1801** poses an interesting pharmacophore suitable for extended researches for lead generation in this clinical condition.

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