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ORIGINAL ARTICLE

Antihyperglycemic Effect of Novel Pyridazinone Derivative on the Alloxan-Induced Diabetes Model in Rats

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ABSTRACT

Type 2 diabetes mellitus represents a significant and escalating global health concern. Projections estimate that the number of individuals living with diabetes will increase dramatically, rising from 425 million in 2017 to approximately 629 million by 2045. While available antidiabetic medications are effective, their use is frequently accompanied by adverse effects, including low blood sugar and digestive disturbances, underscoring the need for safer alternatives. Pyridazinones, a class of six-membered nitrogen-containing heterocycles with adjacent nitrogen atoms, have emerged as promising scaffolds in medicinal chemistry. Specifically, pyridazine-3-one, which features a carbonyl group at the third position, is recognized for its wide spectrum of biological activities. By introducing a benzoxazole ring onto the pyridazinone framework, researchers aim to enhance and explore novel antidiabetic properties in these derivatives. Thus, the novel compound 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione has been synthesized and is proposed for evaluation as a potential antidiabetic agent.

Keywords: Diabetes mellitus; Pyridazinone; Benzoxazole

INTRODUCTION

In diabetes, hyperglycemia is the defining characteristic. Type 1 diabetes, in which pancreatic beta cells are destroyed, results in an absolute deficiency of insulin; and type 2 diabetes, in which insulin resistance can lead to hyperglycemia[1]. Diabetes type 2 is associated with obesity and is on the rise[2]. As well as obesity, research has revealed that a form of diabetes mellitus known as "lean diabetes mellitus" results from fundamental defects in insulin secretion, that is primarily triggered by a dysfunctional pancreas[3]. It is a major public health problem to have type-2 diabetes mellitus (T2DM). By 2045, 629 million people are predicted to have diabetes, up from 425 million in 2017[4]. The presence of T2DM also puts the individual at risk for cardiovascular diseases, which are among the leading causes of death and disability worldwide and fatalities in the world today[5]. In addition, the economic burden of T2DM contributes to approximately 12% of global health expenditures related to diabetic treatment and complications[4]. Clinical and epidemiologic research performed within the last two decades has led to the recognition that heart failure contributes significantly to cardiovascular morbidity and mortality in diabetes patients, in addition to myocardial infarction and other atherosclerosis-related cardiovascular events. Myocardial dysfunction has been reported in some diabetic patients in the absence of coronary artery disease, valve disease, and the consequences of related cardiovascular risk factors[6].

Hyperglycemia can be effectively controlled with hypoglycemic agents which exert clinical effects via different mechanisms like in the liver biguanides like metformin reduce gluconeogenesis, sulfonylureas stimulate the pancreas to secrete insulin, thialzolidinediones improves sensitivity of peripheral tissues to insulin, and in the form of recombinant insulin, insulin or its analogues can be provided exogenously[7]. However, despite the availability of a number of diabetic drugs, other drug monotherapies failed to manage blood glucose levels and other comorbidities satisfactorily and, as a result; therapeutic

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management is often achieved by pairing drugs with distinct mechanisms of action[8]. Yet they are known to cause side effects, such as hypoglycemia and gastrointestinal problems. Therefore, it is crucial to develop alternatives that reduce the complications of diabetes while reducing the side effects. It is therefore imperative to develop substitutes that reduce the complications of diabetes while also causing fewer side effects. Pyridazinones have six members heterocyclic compounds, and their nitrogen atoms are located adjacently. Pyridazine-3-one refers to a pyridazine ring bearing a carbonyl group at the third carbon and has often been highlighted as a privileged scaffold due to its wide range of biological activities. The pyridazine nucleus serves as a foundation for creating new pharmacologically active molecules. Among diazines—a family of nitrogen-containing heterocyclic compounds—pyridazine, pyrimidine, and pyrazine are notable representatives. These compounds are stable, colorless, and possess good water solubility[9,10]. Pyridazinones, in particular, have long been recognized for exhibiting numerous pharmacological effects shown in Figure 1.

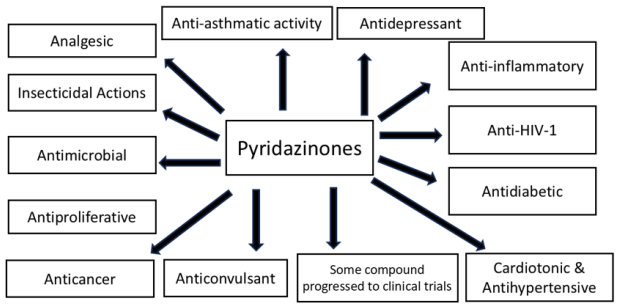

Fig. 1: Pharmacological effects of Pyridazinones[11–18]

Notably, specific pyridazinone derivatives have displayed potent antihyperglycemic effects and have been shown to inhibit aldose reductase in oral glucose tolerance tests conducted in normal rats as well as in enzyme assays[19].

Likewise, benzoxazole derivatives are also highly valued in medicinal chemistry, owing to their broad spectrum of pharmacological activities. The benzoxazole scaffold forms the core structure for various bioactive molecules and can be chemically modified to yield compounds with novel therapeutic properties. Research in this area has identified numerous benzoxazole analogues with significant pharmaceutical potential, functioning as antibacterial and antifungal agents, inhibitors of HIV-1 reverse transcriptase, topoisomerase-I inhibitors, anticancer drugs, and as antidiabetic agents[20–24], among others. Due to their complementary bioactivities, integrating a benzoxazole moiety with a pyridazinone core may enhance or unveil new antidiabetic capabilities in the resulting hybrid molecules. Therefore, the test compound—identified as novel pyridazinone derivative 1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione—is hypothesized to possess antidiabetic properties. The current study is designed to evaluate the antidiabetic efficacy of this newly synthesized pyridazinone derivative in a rat model of alloxan-induced diabetes.

MATERIALS AND METHODS

1-(5-chloro-1,3-benzoxazol-2-yl)-4-methyl-1,2-dihydropyridazine-3,6-dione synthesised according to the standard procedure[25].

Selection and Acclimatization of Experimental Animals

After taking approval from the institutional animal ethical committee selected male albino Wister rats weighing 180 to 220 grams. The rats were housed in spacious cages and gotten a water and standard diet of commercial pellets all the time. All rats were acclimated to laboratory conditions prior to the start of the experiments, which are maintaining a temperature of 22 $\pm 5^{\circ}$ C, relative humidity of 55 ± 5 %, and persistent light and dark cycle of 12 hours each to ensure that animals adapt well to the environment throughout the study period.

Induction of Diabetes Mellitus

Diabetes mellitus was induced in male Wistar rats by administering a single intraperitoneal injection of a freshly prepared alloxan solution at a dose of 150 mg/kg body weight, following an overnight fast of 12 hours[26]. Alloxan was chosen for its well-established capacity to selectively destroy pancreatic β-cells, primarily through the generation of excessive reactive oxygen species such as hydrogen peroxide, superoxide, and hydroxyl radicals. Seventy-two hours after alloxan administration, the development of hyperglycemia was verified by measuring plasma glucose levels. Rats exhibiting fasting plasma glucose concentrations exceeding 180 to 220 mg/dl were selected for inclusion in this study.

Experimental Procedure

A total of 25 rats were utilized for this study, comprising 20 alloxan-induced diabetic rats that survived induction and five healthy control rats. Diabetes was induced three days prior to the commencement of the experimental treatments. Following confirmation of hyperglycemia, all animals were randomly assigned to five groups, each containing five rats.

Treatment Protocol

The experimental rats were allocated into five groups, each consisting of five animals, and received the following treatments:

Group 1 (Normal Control): Non-diabetic rats administered normal saline orally at a dose of 10 ml/kg.

Group 2 (Diabetic Control): Diabetic rats, induced with alloxan (150 mg/kg, intraperitoneally), received no further treatment.

Group 3 (Standard Drug Treated): Diabetic rats treated orally with Glipizide at a dose of 10 mg/kg daily for 28 days.

Group 4 (Low Dose Test Compound): Diabetic rats given the novel pyridazinone derivative at 30 mg/kg body weight via intraperitoneal injection once daily for 28 days.

Group 5 (High Dose Test Compound): Diabetic rats administered the novel pyridazinone derivative at a higher dose of 60 mg/kg body weight intraperitoneally once daily for 28 days.

Estimation of Blood Glucose: Blood glucose levels were measured using a commercially available glucose monitoring kit (One Touch Ultra, Johnson & Johnson) that employs the glucose oxidase method.

Plasma insulin: Plasma insulin was determined by Enzyme-linked Immunosorbent Assay (ELISA) method using a Boehringer Mannheim kit[27] with an Boehringer Mannheim ES 300 immunoassay analyzer.

Estimation of total haemoglobin and glycosylated haemoglobin: Total haemoglobin was determined by the method of Drabkin and Austin[28] and glycosylated haemoglobin was determined by the method of Sudhakar Nayak and Pattabiraman[29].

Statistical Analysis

Data obtained from the biochemical assays were subjected to analysis of variance (ANOVA) to assess differences among the experimental groups. For multiple comparisons between group means, the Newman-Keuls multiple range test was applied. A p-value of less than 0.01 was regarded as indicative of statistical significance.

RESULTS

Table 1 presents data on the initial and final blood glucose levels as well as changes in body weight across the various groups of normal and experimental rats. Diabetic control rats (Group 2) exhibited a marked reduction in mean body weight compared to the normal control group. Treatment of diabetic rats with the novel pyridazinone derivative at both 30 mg/kg and 60 mg/kg doses resulted in an increase in body weight, though this change was not statistically significant relative to the normal controls.

Fasting blood glucose was found to be significantly elevated in diabetic animals (222.35 ± 5.10 mg/dl) compared to the normal control group. Administration of the novel pyridazinone derivative at both tested dosages (30 mg/kg and 60 mg/kg, intraperitoneally) effectively reduced fasting blood glucose levels, bringing them closer to those observed in normal animals.

Table 2 summarizes the results for total haemoglobin, glycosylated haemoglobin, and plasma insulin in the different groups. In diabetic rats, there was a significant decrease in total haemoglobin and plasma insulin, while glycosylated haemoglobin levels were markedly increased when compared to the normal controls. Treatment with the pyridazinone derivative at 30 mg/kg and 60 mg/kg doses restored the levels of total haemoglobin, glycosylated haemoglobin, and plasma insulin towards normal values in diabetic rats.

DISCUSSION

Diabetes mellitus, encompassing a group of metabolic disorders, is primarily distinguished by chronic hyperglycemia resulting from inadequate insulin secretion or impaired insulin function. Clinically, diabetes is categorized into two main types: type 1 and type 2. The alloxan-induced diabetic rat model utilized in this study replicates characteristics of type 1 diabetes, including an autoimmune-like destruction of pancreatic β-cells. This progressive β-cell loss mirrors the gradual decline in early-phase insulin secretion, ultimately culminating in reduced insulin availability and elevated blood glucose levels[30].

In this research, male albino Wistar rats, each weighing between 180 and 220 grams, were employed. Diabetes was established via a single intraperitoneal injection of freshly prepared alloxan at a dose of 150 mg/kg body weight, which effectively induced significant hyperglycemia. Post-induction, diabetic animals received intraperitoneal administrations of the novel pyridazinone derivative at varying dosages for 28 consecutive days. At the start and conclusion of the treatment period, key parameters including blood glucose, body weight, total haemoglobin, glycosylated haemoglobin, and plasma insulin were recorded and evaluated in both control and experimental groups.

Alloxan is known to cause extensive destruction of pancreatic β-cells within the islets of Langerhans, leading to a substantial reduction in insulin secretion[31,32]. In this study, treatment of alloxan-induced diabetic rats with the pyridazinone derivative at both 30 mg/kg and 60 mg/kg doses resulted in a notable elevation in plasma insulin levels. This effect may be attributed to an enhancement of insulin secretion from residual β-cells or the facilitation of insulin release from existing stores.

In uncontrolled diabetes, an increase in glycation of various proteins, including haemoglobin and α-crystallin in the lens, has been documented[33]. Glycosylated haemoglobin (HbA1c) is frequently elevated in diabetic individuals, sometimes reaching values close to 16%[34], and this rise is closely linked to current fasting blood glucose levels[35]. During diabetes, the surplus glucose in the bloodstream reacts with haemoglobin, leading to reduced levels of total haemoglobin in alloxan-treated rats[36].

Table 1: Effect of novel pyridazinone derivative on initial and final body weight and blood glucose in normal and treated animals

GROUP	Body weight (g)		Blood glucose (mg / 100ml)	Blood glucose (mg / 100ml)
	Initial	Final	Initial	Final
G1	220 ± 4.20	245 ± 4.60	90.65 ± 2.15	93.80 ± 2.40
G2	230 ± 4.45	175 ± 3.85**(a)	88.40 ± 2.08	222.35 ± 5.10**(a)
G3	215 ± 4.10	240 ± 4.40	93.70 ± 2.55	126.50 ± 3.12*(b)
G4	225 ± 4.30	250 ± 4.70	91.75 ± 2.45	142.45 ± 3.65**(b)
G5	215 ± 4.05	242 ± 4.65	89.10 ± 2.22	136.20 ± 3.40**(b)

• Values are expressed as mean ± SEM.
• Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
** (a) Values are significantly different from normal control G1 at P<0.001.
** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Table 2: Effect of novel pyridazinone derivative on plasma insulin, Hemoglobin & Glycosylated hemoglobin in normal and treated animals

GROUPS	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA_1 (%)	Plasma Insulin (μU/ml)
G1	12.60 ± 1.15	0.40 ± 0.09	35.25 ± 2.30
G2	7.30 ± 0.30**(a)	0.91 ± 0.15**(a)	13.35 ± 1.60**(a)
G3	14.15 ± 1.55**(b)	0.44 ± 0.11**(b)	31.10 ± 2.05**(b)
G4	13.40 ± 1.30**(b)	0.51 ± 0.15**(b)	26.55 ±1.85**(b)
G5	13.85 ± 1.45**(b)	0.48 ± 0.13**(b)	29.45 ± 1.94**(b)

• Values are expressed as mean ± SEM.
• Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
** (a) Values are significantly different from normal control G1 at P<0.001.
** (b) Values are significantly different from Diabetic control G2 at P<0.01.

Administration of the test pyridazinone derivative at 30 mg/kg and 60 mg/kg over 28 days effectively prevented a significant increase in glycosylated haemoglobin and supported the restoration of total haemoglobin levels. This suggests that the compound contributed to improved glycemic regulation.

Additionally, a decline in body weight was observed in rats following induction of diabetes with alloxan. Treatment with the novel pyridazinone derivative at both administered doses attenuated the loss of body weight seen in diabetic rats. The ability of the compound to mitigate substantial weight reduction is likely related to its pronounced antihyperglycemic action, contributing to an improved metabolic state.

CONCLUSION

In summary, evaluation of the novel pyridazinone derivative in an alloxan-induced diabetic model using male Wistar rats demonstrated that administration of the compound at doses of 30 mg/kg and 60 mg/kg led to a modest, non-significant improvement in body weight compared to healthy controls. Diabetic rats showed a marked elevation in fasting blood glucose levels (222.35 ± 5.10 mg/dl) relative to non-diabetic animals. Furthermore, significant reductions in total haemoglobin and plasma insulin were observed, alongside a notable increase in glycosylated haemoglobin when compared to normal controls.

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Conflicts of Interest

There is no conflict of interest.

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