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# Antidiabetic and Antioxidant Effects of Newly Synthesized Pyrimido[1,6-a] Pyrimidine Derivatives in Neonatal Streptozotocin-Induced Diabetic Rats

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**Abstract**

Synthesis of some novel pyrimido[1,6-*a*]pyrimidine derivatives **4, 8** and **10** was described through the respective reactions of sodium salts of formyl ketones **1**, **7** and **9** with 6-aminothiouracil. The characterization of the reaction products was confirmed by using the available elemental analysis and spectral data. One of these derivatives (**4b**), 1-thioxo-1,2,7,8,9,10-hexahydro-3*H*-pyrimido[1,6-*a*]quinazolin-3-one, was tested using sublethal dose level (10 mg/kg b. w./day for 3 weeks) and was found to have potent antihyperglycemic, antihyperlipidemic and antioxidant properties in neonatal streptozotocin-induced (n-STZ) diabetic male and female albino rats.

**Keywords**: Formyl salt; 6-aminothiouracil; 3*H*-pyrimido[1,6-*a*] pyrimidine; Neonatal streptozotocin-induced diabetic rats, Antidiabetic and antioxidant efficacies

## Introduction

Pyrimidopyrimidines, analogues of folic acid (one of the B vitamins that is a key factor in the synthesis of nucleic acids RNA and DNA) and an important class of annulated uracil and thiouracil, are pharmacologically useful as powerful inhibitor of aggregation of thrombocytes [1], hepatoprotective [2], bronchodilators, anticancer [3-5], vasodilators [6,7], antiallergic [8] and antihypertensive [9] agents. It was also reported that pyrimidopyrimidine derivatives inhibited lipid peroxidation in human and rat liver [10]. Here, an easy construction of some new and interesting pyrimidopyimidines, the ring systems that can be found in marine–derived natural products such as crambescidin [11] and batzelladine [12] alkaloids has been achieved.

Due to the high prevalence of diabetes mellitus worldwide, extensive research is still being performed to develop new antidiabetic agents and determine the mechanisms of action. As type 2 diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM) is much more prevalent form of diabetes and is responsible for 90% of the disease prevalence [13-15], experimental animal model representing type 2 diabetes, neonatal streptozotocin diabetic rats, is used to assess the antidiabetic as well as the antioxidant efficacy of the tested compound. This animal model develops most of the biochemical and pathological symptoms associated with type 2 diabetes in humans [16].

There are many classes of antidiabetic agents available and these drugs have different mechanisms of action and variable efficacy. Most of these drugs have many side effects. Thus, the continuous search for novel antidiabetic agents that are more effective and safe is a target of research by many investigators. 3*(H)*-quinazolinone derivatives have been shown as a group of compounds of broad medical interest [17-21]. It was reported that some 3*(H)*-quinazolinone derivatives exhibited potent antihyperlipidemic and antihyperglycemic activity in alloxan diabetic-hypercholesterolemic and streptozotocin diabetic rats respectively [22,23].

Thus, this study was designed to synthesize new pyrimido[1,6-*a*] pyrimidine derivatives and to assess the anthyperglycemic, antihyperlipidemic and antioxidant efficacies of one of these derivatives, 1-thioxo-1,2,7,8,9,10-hexahydro-3*H*-pyrimido[1,6-*a*]quinazolin-3-one (4b) in neonatal streptozotocin-induced type 2 diabetes in rats.

## Materials and Methods

### Chemistry

All melting points were determined on an electrothermal apparatus and are uncorrected. IR spectra were recorded (KBr discs) on a BRUKER IFS-25 FT-IR spectrophotometer at the region 400-4000 $cm^{-1}$. $^1H$ NMR spectra were recorded in $CDCl_3$ and $(CD_3)_2SO$ solutions on a Varian Gemini 300 MHz spectrometer and chemical shifts are expressed in δ units using TMS as an internal reference. Mass spectra were recorded on a GC-MS QP 1000 EX Shimadzu. Elemental analyses were carried out at the Microanalytical Center of the Cairo University, Giza, Egypt. Piperidine acetate was prepared by addition of 5 mL piperidine to a mixture of 4 mL acetic acid and 10 mL water [24,25].

### Synthesis of cyclocondensed pyrimido[1,6-*a*]pyrimidine derivatives (4a-d), (8) and (10)

**General procedure:** A mixture of equivalent amounts of sodium salts (**1**), (**7**) or (**9**) (0.012 mole) and 6-aminothiouracil was refluxed with a solution of piperidine acetate (1.5 mL) for 15-20 minutes. The reaction mixture is then diluted with 20 mL ethanol and refluxed for another 1 hr. The reaction was quenched by the addition of 1.5 mL acetic acid, then the mixture was cooled and the solid product was collected by filtration and recrystallized from the appropriate solvent (Tables 1 and 2).

### Biology

**Experimental animals:** Experimental albino mice (weighing 20-25 g) and pregnant female albino rats (weighing 180-200 g) were obtained from the Animal House of Ophthalmology Institute, Giza, Egypt. They kept under observation for 2 weeks in the department animal house

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**Table 1:** Characterization data of compounds 4a-d, 8 and 10.

| *Comp. No.* | *M.P.0C Solvent* | *Color Yield%* | *Mol. Formula ( M.Wt.)* | *Elemental analysis calc. / found%* | | | |
|---|---|---|---|---|---|---|---|
| | | | | C | H | N | S |
| **4a** | 276-277<br>EtOH | Pale brown<br>71.6 | $C_{10}H_9N_3OS$<br>(219) | 54.79<br>54.88 | 4.11<br>4.22 | 19.17<br>19.00 | 14.61<br>14.60 |
| **4b** | 230-232<br>EtOH | Yellow<br>75.2 | $C_{11}H_{11}N_3OS$<br>(233) | 56.65<br>56.66 | 4.72<br>4.66 | 18.02<br>18.22 | 13.73<br>13.71 |
| **4c** | 249-251<br>EtOH | Yellow<br>81 | $C_{13}H_{15}N_3OS$<br>(261) | 59.77<br>59.55 | 5.74<br>5.89 | 16.09<br>15.99 | 12.26<br>12.26 |
| **4d** | 230-231<br>EtOH | Yellow<br>70.3 | $C_{17}H_{23}N_3OS$<br>(317) | 64.35<br>64.34 | 7.25<br>7.45 | 13.24<br>13.33 | 10.09<br>10.11 |
| **8** | 266-268<br>EtOH | Pale yellow<br>61.1 | $C_8H_7N_3OS$<br>(193) | 49.74<br>49.58 | 3.63<br>3.61 | 21.76<br>21.65 | 16.58<br>16.65 |
| **10** | 288-289<br>EtOH/ DMF | Yellowish green 63 | $C_{13}H_9N_3OS$<br>(255) | 61.17<br>61.24 | 3.53<br>3.42 | 16.47<br>16.45 | 12.55<br>12.34 |

**Table 2:** The spectral data of the newly synthesized compounds 4a-d, 8 and 10.

| | |
|---|---|
| **4a** | IR ύ ($cm^{-1}$): 3325 (NH); 2967 (-CH); 1671 (C=O); 1609 (C=N) and 1243 (C=S).<br>$^1$H NMR $\delta_H$ (ppm): 1.89-1.96 (pentet, 2H, $CH_2$); 2.64-2.88 (m, 4H, $2CH_2$); 7.97 (s, 1H, pyrimidine HC=N); 8.11 (s, 1H, pyrimidine HC=CO) and 8.92 (s, 1 H , NH ).<br>Mass (m/z): 221 ($M^+$ +2, 6.6%); 219 ($M^+$, 100.0%); 161 (34.8 %); 131 (14.5%); 104 (19.6%) and 77 (18.8%). |
| **4b** | IR ύ ($cm^{-1}$): 3390 (NH); 2936 (-CH); 1658 (C=O); 1624 (C=N) and 1255 (C=S).<br>$^1$H NMR $\delta_H$ (ppm): 1.24-1.72 (m, 4H, $2CH_2$); 2.60-2.81 (m, 4H, $2CH_2$); 7.97 (s, 1H, pyrimidine HC=N); 8.14 (s, 1H, pyrimidine HC=CO) and 12.10 (s, 1H, SH)<br>Mass (m/z): 235 ($M^+$ +2, 6.7%); 233 ($M^+$, 100.0%); 175 (18.1 %); 143 (31.7%); 119 (15.8%) and 68 (25.2%). |
| **4c** | IR ύ ($cm^{-1}$): 3400 (NH); 2927 (-CH); 1680 (C=O); 1613 (C=N) and 1245 (C=S).<br>$^1$H NMR $\delta_H$ (ppm): 1.29-1.67 (m, 4H, $2CH_2$); 2.42-2.92 (m, 4H, $2CH_2$); 3.22-3.41 (m, 4H, $2CH_2$), 7.99 (s, 1H, pyrimidine N=C-H); 8.01 (s, 1H, pyrimidine O=C=CH) and 12.91 (br s, 1H, SH)<br>Mass (m/z): 261 ($M^+$, 100.0%); 323 (15.0%); 173 (7.4%); 103 (3.6%) and 77 (11.8%). |
| **4d** | IR ύ ($cm^{-1}$): 3352 (NH); 2923 (-CH); 1679 (C=O); 1621 (C=N) and 1246 (C=S).<br>Mass (m/z): 319 ($M^+$ +2, 19.8%); 317 ($M^+$, 94.0%); 246 (25.5 %); 207 (100.0%); 147 (21.2%) and 91 (19.0%). |
| **8** | IR ύ ($cm^{-1}$): 3368 (NH); 2970 (-CH); 1627 (C=O); 1591 (C=N) and 1243 (C=S).<br>$^1$H NMR $\delta_H$ (ppm): 1.44 (s, 3H, $CH_3$); 8.00 (s, 1H, pyrimidine N=C-H); 8.16 (s, 1H, pyrimidine C=CH); 8.26 (s, 1H, pyrimidine HC=C=O and 12.87 (s, 1H, SH).<br>Mass (m/z): 193 ($M^+$, 37.4%); 189 (100.0%); 161 (25.2%); 91 (20.3%); and 75 (42.3%). |
| **10** | IR ύ ($cm^{-1}$): 3325 (NH); 2970 (-CH); 1630 (C=O); 1592 (C=N) and 1243 (C=S).<br>$^1$H NMR $\delta_H$ (ppm): 6.89-7.89 (m, 5H, Ar); 8.10 (s, 1H, pyrimidine N=C-H); 8.21 (s, 1H, pyrimidine C=CH); 8.26 (s, 1H, pyrimidine HC=C=O and 12.68 (s, 1H, SH).<br>Mass (m/z): 255 (2.9%); 151 (65.5); 95 (81.5%); 80 (17.3 %) and 67 (100.0). |

(Zoology Department, Faculty of Science, Beni-Suef University, Egypt) to exclude any intercurrent infection. They were supplied standard diet and tap water ad libitum, and maintained under suitable living conditions in good aerated cages at natural daily 12 hours dark-light cycle and at room temperature 20-25°C. All animal procedures are in accordance with the recommendations of the Canadian Committee for Care and Use of Animals (CCAC) [26].

## Acute toxicity study and determination of $LD_{50}$

$LD_{50}$ of the studied compound **4b** was determined as described by Afifi et al. [27]. In this experiment, six groups each of 8 male albino mice weighing 20-25 g were used. One group serves as control and other groups of mice were orally administered the tested compound by gastric tube in gradual increasing doses (200, 400, 600, 800 and 1000 mg/ kg b. w.). After 48 hours of administration, the number of dead animals in each group, the mean of dead animals in two successive doses (z) and the constant factor between two successive doses (d) were recorded and $LD_{50}$ was calculated as follow:

$LD_{50}$ = the biggest dose which kill all animals - $\Sigma$(z.d)/n

Where n: number of animals in groups = eight animals in each group.

$LD_{50}$ of albino rats was calculated from that of mice by using the conversion table of Paget and Barnes [28] and the therapeutic dose used for the subsequent studies was chosen based on the obtained $LD_{50}$.

## Induction of type 2 diabetic rat model and treatment

Type 2 diabetic (NIDDM) rats was induced by intraperitoneal injection of streptozotocin (Sigma Chemical Company, USA), at dose level of 120 mg/kg b. w. (dissolved in citrate buffer, pH 4.5) to 8-hour fasted five-day-old albino rat pups according to Takada et al. [29]. The control counterpart rat pups were injected the equivalent volume of citrate solution by the same route. After 14 weeks post-STZ injection, the streptozotocin-injected rats were deprived of food and screened for hyperglycemia. Animals with serum glucose level after 2 hours of oral glucose loading (3 g/kg b. w.) ranging from 180-300 mg/dl were considered mild diabetic and included in the experiment. Both mild diabetic male and female rats were separately divided into two groups each of 8 animals. One diabetic group was treated with pyrimido[1,6-*a*] quinazoline derivative **4b** at dose level of 10 mg (dissolved in dimethyl sulfoxide, DMSO)/kg b. w./day by oral gavage for 3 weeks. The other diabetic group was daily administered the equivalent volume of the vehicle (DMSO) for the same period by the same route. The normal control was administered DMSO similarly to the diabetic control. At the day before sacrifice, animals of each group were deprived of food and oral glucose tolerance was performed by taking blood samples from lateral tail vein after 0, 1, 2 and 3 hours of oral glucose load (3 g/ kg b. w.). At the next day, animals were sacrificed. Blood was obtained from cervical vein, left to coagulate and then centrifuged at 3000 r.p.m. for 15 minutes to obtain serum. Animals were rapidly dissected, and liver and pancreas were excised from each animal. Part of liver (0.5 g) from each rat was homogenized in 0.9% saline at 10% w/v and homogenate supernatant was separated by centrifugation at 3000 r.p.m. for 15 minutes. Serum and homogenate supernatant were kept in deep freezer at -30°C till use for the determination of biochemical and oxidative stress markers. Another part of liver (1g) was used for

glycogen determination. Pancreas was kept in 10% neutral buffered formalin for histological examination.

### Biochemical determinations

Serum glucose was measured according to the method of Trinder [30] using reagent kit obtained from Chronolab AG (Switzerland). Serum insulin and C-peptide levels were measured in Radioactive Isotopes Unit, Middle Eastern Regional Radioisotope Center (Dokki, Giza) by radioimmunoassay kits of DPC (Diagnostic Products Corporation, Los Angeles, USA) according to the methods of Marschner et al. [31] and Beyer et al. [32] respectively. Hepatic glycogen content and glycogen phoshorylase, glucose-6-phosphatase and hexokinase activities were estimated according to the methods of Seifter et al. [33], Stalmans and Hers [34], Begum et al. [35] and Brastrup et al. [36] respectively. Hepatic hydroxymethylglutaryl Co-A reductase activity was determined based on the method of Venugopala Rao and Ramakrishan [37]. Serum total lipid concentration was detected according to the method of Frings et al. [38] using reagent kits purchased from Diamond Diagnostics (Egypt). Serum triglycerides, total cholesterol and HDL-cholesterol levels were determined according to the methods of Fossati and Prencipe [39] , Allain et al. [40] and Burnstein et al. [41] respectively, using reagent kits obtained from Reactivos Spinreact (Spain). Serum LDL-cholesterol level was calculated from Friedewald [42] formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol). Serum vLDL-cholesterol concentration was calculated according to Nobert [43] formula (vLDL-cholesterol = triglycerides/5). The ratios of total cholesterol and LDL-cholesterol to HDL-cholesterol were also calculated.

Hepatic levels of total thiol, glutathione and lipid peroxidation peroducts were estimated according to the methods of Koster et al. [44], Beutler et al. [45] and Preuss et al. [46] respectively. Hepatic glutathione reductase, glutathione peroxidase, glutathione-S-transferase and catalase activities were assayed according to the methods of Goldberg and Spooner, [47] Pinto and Bartley [48], Mannervik and Guthenberg [49] and Cohen et al. [50] respectively.

### Histological investigation

The fixed specimens were routinely processed for embedding in paraffin wax and sectioning. Prepared sections were stained with modified aldehyde fuchsin stain method [51].

### Statistical analysis

Data were analyzed by one way-ANOVA followed by LSD test to compare various groups with each other using statistical program PC-STAT (University of Georgia, USA) [52]. F-probability expresses the general effect between groups.

## Results and Discussion

### Chemistry (synthesis)

In this research work, our synthetic strategy commences from the easily available compound, 6-aminothiouracil and the sodium salts of formyl ketones, which led to the direct construction of the novel fused pyrimido[1,6-*a*]pyrimidine nucleus. Thus, fusion of 6-aminothiouracil with the formyl salts (**1**) in piperidine acetate and acetic acid afforded in considerable yields the cyclocondensed pyrimido [1,6-*a*]pyrimidines **4a-d** as outlined in chart (1) [53,54,55].

The reaction mode for the formation of the products is suggested to proceed through the initial nucleophilic attack by the exocyclic amino group of 6-aminothiouracil at the formyl group of compound (**2**), that

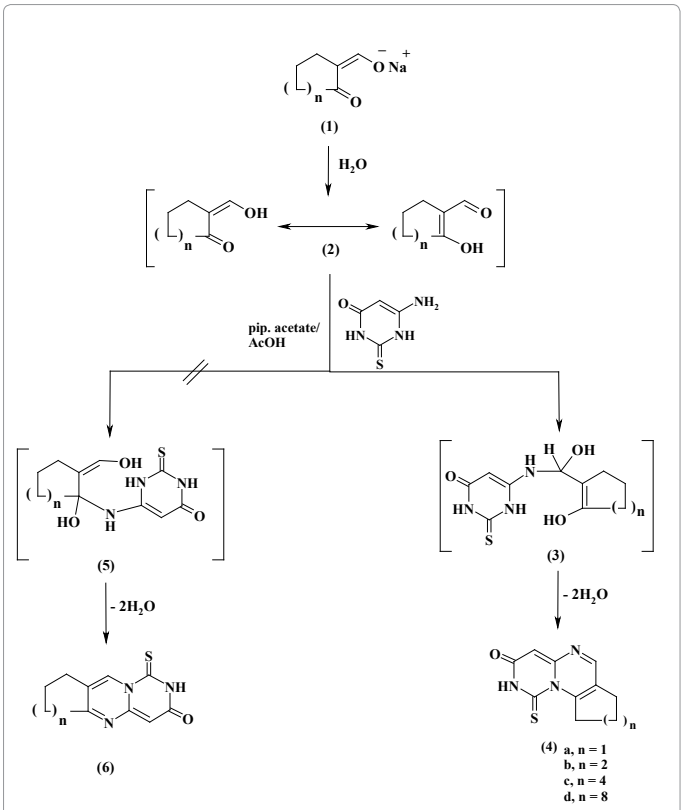
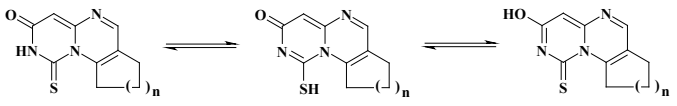



**Chart 1:**

formed *in situ* due to the reaction of the formyl salts (**1**) with water, followed by cyclization through the elimination of two water molecules leading to the formation of the non-planer products (4) rather than the planner products (**6**) [56-58].

The identity of compounds (**3**) was proven on the basis of their elemental analysis and spectral data. However, the nucleus of pyrimido [1,6-*a*] pyrimidine has more than one resonating forms must be taken in consideration while we discuss their spectral data. This ring system may be found in the following three resonating forms:



Thus, the IR of **4c** revealed bands at ύ = 3400 $Cm^{-1}$ (NH); 2927 (paraffinic CH); 1680 (C=O); 1613 (C=N) and 1245 (C=S). The $^1H$ NMR spectrum showed signals at δ = 1.29-1.67 ppm (m, 4H, $2CH_2$); 2.42-2.92 (m, 4H, $2CH_2$); 3.22-3.41 (m, 4H, $2CH_2$), 7.99 (s, 1H, pyrimidine N=C-H); 8.01 (s, 1H, pyrimidine C=CH) and 12.91 (br s, 1H, SH). The mass spectrum of this compound showed a molecular ion peak at m/z = 261 (100%), coincident with the molecular weight of the compound (261.35).

A successful trying for establishment of the cyclocondensation reaction has been carried out by the reaction of 6-aminothiouracil with the sodium salts of acyclic ketones **7** and **9** under the same reaction conditions and following the same reaction mechanism to afford 4-methyl-6-thioxo-6,7-dihydro-8*H*-pyrimido[1,6-*a*]pyrimidin-8-one (**8**) and 4-phenyl-6-thioxo-6,7-dihydro-8*H*-pyrimido[1,6-*a*] pyrimidin-8-one **(10)** respectively Chart (2).

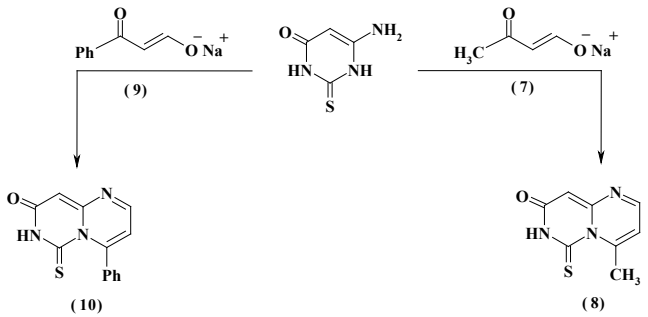



**Chart 2:**

The structure compound **8** was established by its correct elemental analysis and the IR spectrum which revealed bands at ύ = 3368 $Cm^{-1}$ (NH); 1627 (C=O) and 1243 (C=S). The mass spectrum showed a molecular ion peak at m/z = 193 (37.4%) coincident with its molecular weight (193.23) and the base peak appeared at m/z = 189 (100 %). The IR of compound **10** showed bands at ύ = 3325 $Cm^{-1}$ (NH); 1630 (C=O) and 1243 (C=S). The mass spectrum showed the molecular ion peak at m/z = 255 (1.7%) coincident with its molecular weight (255.30).

## Biological studies

For determination of lethal dose $(LD)_{50}$ of 1-thioxo-1,2,7,8,9,10-hexahydro-3*H*-pyrimido[1,6-*a*]quinazolin-3-one (**4b**), single gradual increasing doses were administered to various groups of normal albino mice. The number of dead animals in each group was determined after 48 hours of compound administration and $LD_{50}$ was calculated. $LD_{50}$ of **4b** was found to be 775 mg/kg b.w. for albino mice (Table 3). By using conversion table of Paget and Barnes [26], $LD_{50}$ for rats was found to be 542 mg/kg b.w. Based on this toxicity study, the orally therapeutic dose for subsequent *in vivo* study was chosen to be 10 mg/kg b. w. (about 1/50 of $LD_{50}$) which is so far from $LD_{50}$.

To assess the antihyperglycemic, antihyperlipidemic and antioxidant effects of pyrimido[1,6-*a*]quinazoline derivative (**4b**), neonatal streptozotocin (n-STZ)-induced type 2 diabetic rats were used. This n-STZ rat model was chosen because it was previously reported that it has several advantages over other models and is considered to be one of the suitable experimental models of type 2 diabetes mellitus [16,59].

The male and female adult n5-STZ rat model of the present study after 17 weeks of neonatal day 5 of STZ injection showed profound elevated basal serum glucose concentration, impaired glucose tolerance, decreased basal serum insulin, decreased number of islets and β-cells, lack of insulin response to glucose (due to β-cell glucose insensitivity), decreased liver glycogen content and hyperlipidemidia associated with increased serum levels of triglycerides, total cholesterol, LDL-cholesterol, vLDL-cholesterol and higher ratios of LDL-cholesterol and total cholesterol to HDL-cholesterol in addition to the increase in the oxidative stress (Figures 1-4 and Tables 4-11). These results are in accordance with various several publications [60-66].

The treatment of male and female n5-STZ diabetic rats with pyrimido[1,6-*a*] quinazoline derivative **4b** for three weeks beginning from the 14th week to the 17th week post-STZ injection at dose level of 10 mg/kg b. w. (1/50 $LD_{50}$) produced significant improvement ($p<0.01$, LSD) of the impaired oral glucose tolerance (Figures 1 and 2). While the effect of treatment with **4b** on glucose concentration at fasting state seemed to be more potent in male than female, the reverse was true at

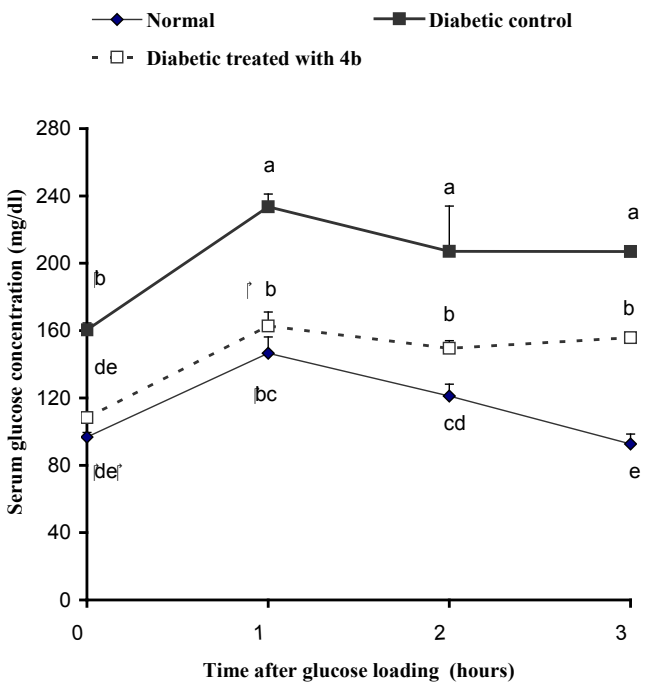



**Figure 1:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on oral glucose tolerance of n5-STZ-induced diabetic male rats.

**F-probability: p<0.001;** LSD at the 5% level: 27.42; LSD at the 1% level: **36.93.**
Values, which share the same superscript symbol(s), are not significantly different.

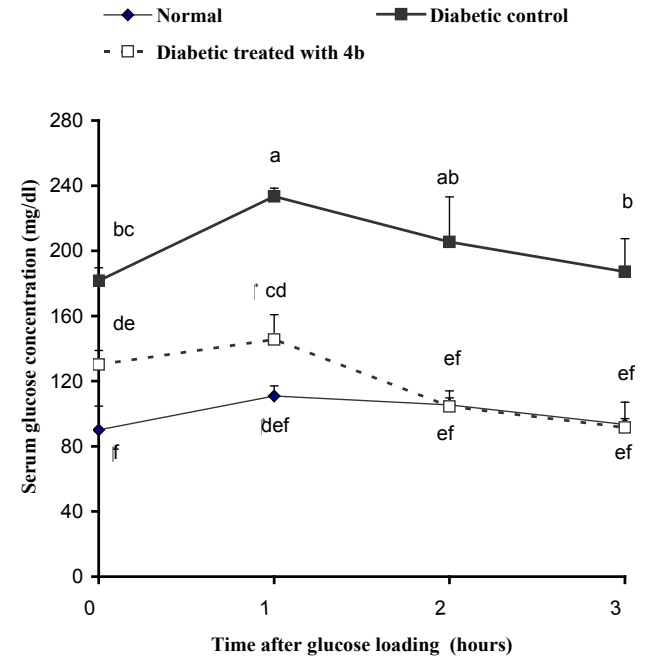
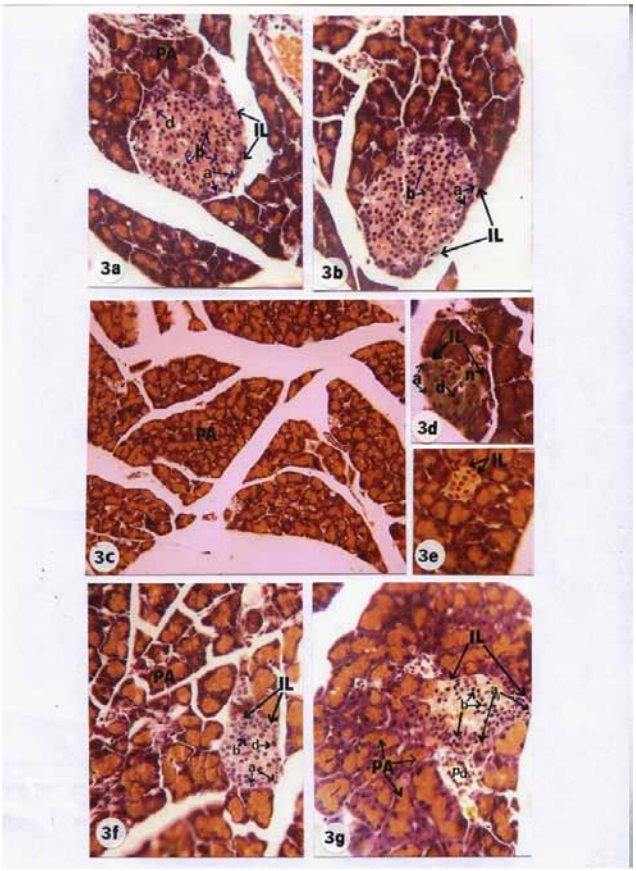


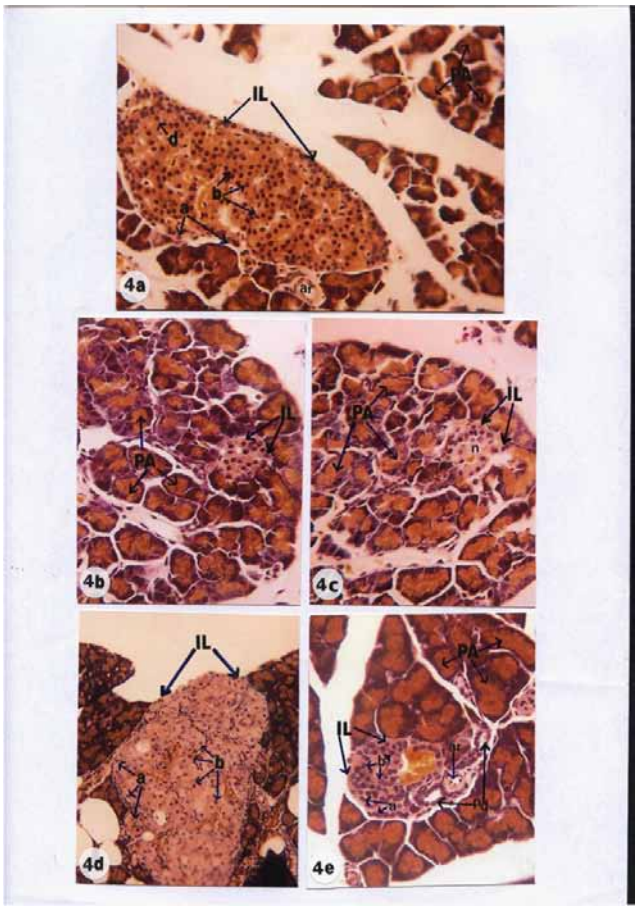

**Figure 2:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on oral glucose tolerance of n5-STZ-induced diabetic female rats.

**F-probability: p<0.001;** LSD at the 5% level: 38.97; LSD at the 1% level: **52.49.**
Values, which share the same superscript symbol(s), are not significantly different.

2 and 3 hours post-oral glucose loading. This amelioration of glucose tolerance was associated with potential amelioration of the depleted basal and postprandial serum levels of insulin and C-peptide which is a marker of β-cells function and insulin secretion; the effect seemed to be more potent in male than female as a result of treatment. The increase of serum insulin and C-peptide levels as a result of treatment could be attributed to the remarkable amendment of the perturbed histological architecture of islets of Langerhans and increase in the diminished number of β-cells of diabetic rats (Figures 3 and 4) in addition to the improvement of the impaired insulin response to glucose (Tables 4 and 5), which is one of the main characteristics in n-STZ diabetic rats [67]. The present study indicated that the insulin and C-peptide concentrations of n5-STZ diabetic control rats was not significantly affected after 2 hours of oral glucose loading as compared with the corresponding basal concentrations, while in n5-STZ diabetic rats treated with **4b**, they were potentially increased. This reflects that the compound **4b** may enhance the deteriorated insulin secretion in response to glucose in n-STZ diabetic rats. Our results are in concurrence with those of other publications [22,28] which revealed that the treatment with 3H-quinazolinone derivatives induced potential hypoglycemic and secretogouge effects in diabetic rats.




**Figures 3:** Photomicrographs of sections of pancreas of normal (Figures 3a and 3b), diabetic control (Figures 3c, 3d and 3e) and diabetic treated (Figures 3f and 3g) male rats. Normal rats showed intact islets of Langerhans (IL) with alpha cells (a) at the periphery, beta cells (b) in the core and delta cells (d) adjacent to beta cells with enlarged size. Diabetic rats showed absence of islets at almost all area of pancreas parenchyma (PA) (Figure 3c) or presence of little number of small-sized islets (Figures 3d and 3e) with necrosis (n). Diabetic rats treated with 4b exhibited marked increase in the number and size of islets with relatively increased number of islet cells (a, b and d). Pancreatic ductule (bd) is also visible.




**Figures 4:** Photomicrographs of sections of pancreas of normal (Figures 4a), diabetic control (Figures 4b and 3c) and diabetic treated (Figures 4d and 4e) female rats. Normal rats showed intact islets of Langerhans (IL) with alpha cells (a) at the periphery, beta cells (b) in the core and delta cells (d) adjacent to beta cells with enlarged size. Diabetic rats showed a deleterious decrease in the size of islets (Figures 4d and 4e) with necrosis (n). Diabetic rats treated with 4b exhibited marked increase in the size of islets and number of islet cells (a and b). Pancreatic ductule (bd) and pancreatic artery (ar) are also visible.

To elucidate the probable mechanism of hypoglycemic action of **4b** in diabetic rats, the activities of some hepatic enzymes (Tables 6 and 7), which may be concerned with glucose metabolism as well as uptake and production of glucose by the liver, were detected. The study revealed that the elevated liver glycogen phosphorylase and glucose-6-phosphatase activities in diabetic rats were detectably decreased in n5-STZ as a result of **4b** treatment. In contrast, the hepatic hexokinase activity was significantly increased in n5-STZ diabetic rats treated with **4b** as compared with the diabetic control counterparts. Concomitant with these changes, liver glycogen content which may also be considered as one of the best markers of antihyperglycemic effect of any drug [68] was detectably ameliorated after treatment of n5-STZ diabetic rats with **4b** (Tables 6 and 7). These previous changes led us to suggest that the tested pyrimido[1,6-*a*]quinazoline derivative (**4b**) may produce hypoglycemic effects in n5-STZ type 2 diabetic rats *via* decreasing hepatic glucose production and increasing glucose uptake which possibly due to enhancing blood insulin level and tissue insulin action. In concurrence with the present study, Mukherjee et al. [69] found that centpiperalone, 3H-quinazolinone derivative, enhanced glucose uptake into rat hemidiagrams.

**Table 3:** Determination of $LD_{50}$ of pyrimido[1,5-a]quinazoline derivative (4b) in albino mice.

| Dose (mg/kg b. w.) | Total number of animals | Number of dead animals | z | d | ∑(z.d) |
|---|---|---|---|---|---|
| **200** | 8 | 0 | - | - | - |
| **400** | 8 | 1 | 0.5 | 200 | 100 |
| **600** | 8 | 2 | 1.5 | 200 | 300 |
| **800** | 8 | 4 | 3 | 200 | 600 |
| **1000** | 8 | 6 | 5 | 200 | 1000 |
| **1200** | 8 | 8 | 7 | 200 | 1400 |

z: mean number of dead animals in two successive doses
d: constant factor between two successive doses
$LD_{50}$ = the biggest dose which kill all animals - ∑(z.d)/n = 1200-3400/8 = 775 mg /kg b. w.
By using the conversion table of Paget and Barnes (1964), LD50 for rats was calculated from that of mice and was found to be 542.5 mg /kg b. w.
1/50 of $LD_{50}$ is about 10 mg /kg b. w. which was considered as sublethal dose that used as therapeutic dose in the subsequent work

**Table 4:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on serum insulin and C-peptide levels at fasting state and after 2 hours of oral glucose loading in n5-STZ induced type 2 diabetic male rats.

| Parameters / Groups | Insulin (µIU/mL) | | C-peptide (pmol/mL) | |
|---|---|---|---|---|
| | **Fasting** | **2 hours** | **Fasting** | **2 hours** |
| **Normal control** | 25.23±1.83$^{b}$ | 36.43±2.26$^{a}$ | 160.67±6.41$^{bc}$ | 147.65±5.74$^{a}$ |
| **Diabetic control** | 10.01±0.76$^{c}$ | 10.80±0.54$^{c}$ | 58.05±13.85$^{d}$ | 64.05±12.33$^{d}$ |
| **Diabetic treated with 4b** | 31.36±3.30$^{ab}$ | 36.88±6.64$^{a}$ | 141.97±2.04$^{c}$ | 194.14±23.23$^{b}$ |
| F-probability | P<0.001 | | P<0.001 | |
| LSD at the 5% level | 9.46 | | 36.56 | |
| LSD at the 1% level | 12.74 | | 49.24 | |

- Data are expressed as mean ± standard error. Number of animals in each group is eight.
- For each parameter, means, which share the same superscript symbol(s), are not significantly different.
- If the difference between two means is higher than LSD at 5%, the effect will be significant (p<0.05).
- If the difference between two means is higher than LSD at 1%, the effect will be highly significant (p<0.01).

**Table 5:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on serum insulin and C-peptide levels at fasting state and after 2 hours of oral glucose loading in n5-STZ induced type 2 diabetic female rats.

| Parameters / Groups | Insulin (µIU/mL) | | C-peptide (pmol/mL) | |
|---|---|---|---|---|
| | **Fasting** | **2 hours** | **Fasting** | **2 hours** |
| **Normal control** | 30.73±4.83$^{b}$ | 41.91±0.64$^{a}$ | 158.60±6.26$^{b}$ | 209.23±21.88$^{a}$ |
| **Diabetic control** | 13.66±1.54$^{c}$ | 14.40±1.79$^{c}$ | 47.85±3.46$^{d}$ | 58.74±3.95$^{cd}$ |
| **Diabetic treated with 4b** | 29.90±5.25$^{b}$ | 34.41±5.60$^{ab}$ | 65.19±3.40$^{cd}$ | 172.17±10.03$^{b}$ |
| F-probability | P<0.001 | | P<0.001 | |
| LSD at the 5% level | 11.07 | | 29.91 | |
| LSD at the 1% level | 14.91 | | 40.29 | |

**Table 6:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on liver glycogen content and glycogen phosphorylase, glucose-6-phosphatase and hexokinase activities in n5-STZ induced type 2 diabetic male rats.

| Parameters / Groups | Glycogen (mg/g tissue) | Glycogen phosphorylase (mU/100mg tissue) | Glucose-6-phosphatase (mU/100mg tissue) | Glucokinase (mU/100mg tissue) |
|---|---|---|---|---|
| **Normal control** | 6.96±0.98$^{a}$ | 11.39±0.32$^{b}$ | 21.97±0.56$^{b}$ | 32.52±0.17$^{b}$ |
| **Diabetic control** | 4.56±0.29$^{b}$ | 21.46±3.64$^{a}$ | 31.32±2.37$^{a}$ | 32.82±0.83$^{b}$ |
| **Diabetic treated with 4b** | 5.56±0.28$^{ab}$ | 11.10±0.91$^{b}$ | 29.05±1.07$^{a}$ | 44.65±2.07$^{a}$ |
| F-probability | P<0.05 | P<0.01 | P<0.01 | P<0.001 |
| LSD at the 5% level | 1.84 | 6.56 | 4.61 | 4.84 |
| LSD at the 1% level | 2.55 | 9.07 | 6.38 | 6.69 |

It is also interesting, in the present study, to find that pyrimido [1,6-*a*] quinazoline derivative **4b** produced antihyperlipidemic efficacy which was indicated by potential decreases in the levels of serum total lipids, triglycerides, total cholesterol, LDL-cholesterol and vLDL-cholesterol in both male and female diabetic rats. Hepatic hydroxymethylglutaryl-CoA (HMG-CoA) reductase, the key enzyme responsible for cholesterol synthesis [70], indicated by the ratio of HMG-CoA to mevalonate, was non-significantly affected (p>0.05) or significantly increased (p<0.01) respectively in female and male n5-STZ diabetic rats treated with the tested compound as compared with the diabetic counterparts (Tables 6 and 7). Based on these results, it can be concluded that the decrease in serum cholesterol levels after treatment of n5-STZ was not attributed to the effects of **4b** on cholesterol synthesis, but may be due to the enhancement of the clearance rate of cholesterol from the blood through upregulation of LDL-receptor and tissue lipoprotein lipase (LPL) activity [71,72] and/or decreases in intestinal cholesterol absorption. In support to the latter postulation, Refaie et al. [23] suggested that antihypercholesterolemic effects of quinazolinone compound was brought about by inhibition of dietary cholesterol absorption.

Cadiovascular risk indices, represented by the ratios of LDL-cholesterol and total cholesterol to HDL-cholesterol, were significantly increased (p<0.01) in the n5-STZ diabetic male and female rats. The treatment with 4b solely induced significant (p<0.01) improvement of

the elevated total cholesterol: HDL-cholesterol ratio in diabetic male rats (Tables 8 and 9).

To evaluate the effect of pyrimido[1,6-*a*]quinazoline derivative **4b** on oxidative stress and antioxidant defense system in type 2 diabetic male and female rat model, liver levels of total thiol, glutathione, glutathione reductase, glutathione peroxidase, glutathione-S-transferase, lipid peroxidation products and catalase were investigated and presented in tables 10 and 11. The treatment of diabetic male and female rats with 4b induced a profound increase in hepatic total thiol and glutathione concentration as well as glutathione peroxidase activity. Moreover, the elevated lipid peroxidation in diabetic male and female rats was significantly alleviated as a result of 4b treatment. This result was in consonance with that obtained by de la Cruz et al. [10] who revealed that 3 pyrimido-pyrimidine derivatives inhibited lipid peroxidation in human liver membranes. It is noteworthy to recognize that in diabetes, protein glycation and glucose auto-oxidation can lead to the formation of free radicals and induction of lipid peroxidation in several organs [73]. Thus, the improvement in the elevated levels of blood glucose and glycated proteins secondary to amelioration of insulin level and action may in turn lead to decrease of the formation of free radicals and attenuation of lipid peroxidation [64]. However, the glutathione reductase, glutathione-S-transferase and catalase activities were not significantly altered after treatment of diabetic rats with 4b. Overall, based on these results, it can be suggested that the tested pyrimido[1,6-*a*]quinazoline derivative **4b** may have antioxidant activities in n5-STZ diabetic male and female rats.

## Conclusions

In this investigation, it has been found that the novel synthetic 1-thioxo-1,2,7,8,9,10-hexahydro-3*H*-pyrimido[1,6-*a*]quinazolin-3-one has antihyperglycemic and antihyperlipidemic potentials in n5-STZ induced type 2 diabetic male and female rats. These effects may be

**Table 7:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on liver glycogen content and glycogen phosphorylase, glucose-6-phosphatase and hexokinase activities in n5-STZ induced type 2 diabetic female rats.

| Parameters / Groups | Glycogen (mg/g tissue) | Glycogen phosphorylase (mU/100mg tissue) | Glucose-6-phosphatase (mU/100mg tissue) | Glucokinase (mU/100mg tissue) |
|---|---|---|---|---|
| **Normal control** | 8.20±0.32$^{a}$ | 14.15±1.14$^{b}$ | 28.04±1.01$^{b}$ | 50.93±6.09$^{a}$ |
| **Diabetic control** | 4.63±0.39$^{c}$ | 29.36±3.29$^{a}$ | 32.89±1.53$^{a}$ | 56.89±8.49$^{a}$ |
| **Diabetic treated with 4b** | 6.74±0.41$^{b}$ | 12.79±1.04$^{b}$ | 28.97±1.21$^{b}$ | 63.65±8.96$^{a}$ |
| F-probability | P<0.001 | P<0.001 | P<0.05 | P>0.05 |
| LSD at the 5% level | 1.14 | 6.32 | 3.82 | - |
| LSD at the 1% level | 1.58 | 8.74 | 5.29 | - |

**Table 8:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on serum lipid profile in n5-STZ induced type 2 diabetic male rats.

| Parameters / Groups | Total lipids (g/L) | Triglycerides (mg/dl) | Total cholesterol (mg/dl) | LDL-cholesterol (mg/dl) | HDL-cholesterol (mg/dl) | vLDL-cholesterol (mg/dl) | LDL/ HDL | Total chol./ HDL | HMGCo-A/ mevalonate |
|---|---|---|---|---|---|---|---|---|---|
| **Normal** | 3.16±0.19$^{c}$ | 42.75±1.37$^{a}$ | 72.76±1.24$^{b}$ | 29.90±0.63$^{b}$ | 34.44±1.04$^{a}$ | 8.55±0.27$^{a}$ | 0.87±0.02$^{b}$ | 2.11±0.05$^{b}$ | 1.31±0.01$^{b}$ |
| **Diabetic control** | 4.71±0.20$^{a}$ | 46.07±2.38$^{a}$ | 76.76±1.17$^{a}$ | 37.49±2.79$^{a}$ | 30.05±1.22$^{a}$ | 9.21±0.07$^{a}$ | 1.27±0.12$^{a}$ | 2.56±0.06$^{a}$ | 1.30±1.01$^{b}$ |
| **Diabetic treated with 4b** | 4.03±0.08$^{b}$ | 42.28±0.54$^{a}$ | 67.89±0.90$^{c}$ | 25.77±1.55$^{b}$ | 33.67±2.33$^{a}$ | 8.46±0.30$^{a}$ | 1.31±0.06$^{a}$ | 2.06±0.12$^{b}$ | 1.59±2.10$^{a}$ |
| F-probability | P<0.001 | p>0.05 | P<0.001 | P<0.01 | p>0.05 | p>0.05 | P<0.01 | P<0.01 | P<0.001 |
| LSD at 5% level | 0.49 | - | 3.36 | 5.65 | - | - | 0.236 | 0.25 | 0.042 |
| LSD at 1% level | 0.68 | - | 4.65 | 7.82 | - | - | 0.326 | 0.35 | 0.058 |

**Table 9:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on serum lipid profile in n5-STZ induced type 2 diabetic female rats.

| Parameters / Groups | Total lipids (g/L) | Triglycerides (mg/dl) | Total cholesterol (mg/dl) | LDL-cholesterol (mg/dl) | HDL-cholesterol (mg/dl) | vLDL-cholesterol (mg/dl) | LDL/ HDL | Total chol./ HDL | HMGCo-A/ mevalonate |
|---|---|---|---|---|---|---|---|---|---|
| **Normal** | 3.46±0.18$^{b}$ | 37.48±1.31$^{b}$ | 68.61±3.55$^{b}$ | 27.84±0.28$^{b}$ | 33.27±1.57$^{a}$ | 7.49±0.26$^{b}$ | 0.88±0.05$^{b}$ | 2.07±0.09$^{b}$ | 1.36±0.04$^{b}$ |
| **Diabetic control** | 4.82±0.34$^{a}$ | 46.35±2.14$^{a}$ | 80.12±1.77$^{a}$ | 42.83±1.76$^{a}$ | 28.14±1.24$^{a}$ | 9.27±0.43$^{a}$ | 1.54±0.11$^{a}$ | 2.87±0.18$^{a}$ | 1.59±0.02$^{a}$ |
| **Diabetic treated with 4b** | 3.47±0.07$^{b}$ | 36.50±081$^{b}$ | 76.13±2.47$^{ab}$ | 42.62±1.83$^{a}$ | 27.67±2.65$^{a}$ | 7.30±0.16$^{b}$ | 1.58±0.09$^{a}$ | 2.85±0.21$^{a}$ | 1.60±0.03$^{a}$ |
| F-probability | P<0.01 | P<0.001 | P<0.05 | P<0.001 | P>0.05 | P<0.001 | P<0.001 | P<0.01 | P<0.001 |
| LSD at 5% level | 0.68 | 4.59 | 8.13 | 4.45 | - | 0.92 | 0.256 | 0.511 | 0.103 |
| LSD at 1% level | 0.94 | 6.35 | 11.24 | 6.16 | - | 1.27 | 0.355 | 0.706 | 0.144 |

**Table 10:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on oxidative stress and antioxidant defense markers in liver of n5-STZ induced type 2 diabetic male rats.

| Parameters / Groups | Total thiol (nmole/100mg) | Glutathione (nmole/100mg) | Lipid Peroxidation (nmole MDA/100mg) | Glutathione reductase (U/g) | Glutathione peroxidase (mU/100mg) | Glutathione-S-transferase (U/100mg) | Catalase (k.10$^{2}$) |
|---|---|---|---|---|---|---|---|
| **Normal** | 247.13±0.94$^{b}$ | 56.78±2.39$^{ab}$ | 51.65±1.27$^{a}$ | 12.02±0.19$^{a}$ | 96.20±4.61$^{b}$ | 37.71±3.38$^{a}$ | 71.85±1.63$^{a}$ |
| **Diabetic control** | 203.50±0.90$^{c}$ | 53.50±0.90$^{b}$ | 54.65±0.02$^{a}$ | 11.20±0.31$^{ab}$ | 77.80±0.67$^{c}$ | 40.68±2.43$^{a}$ | 61.55±3.69$^{a}$ |
| **Diabetic treated with 4b** | 257.32±1.91$^{a}$ | 60.49±0.89$^{a}$ | 45.27±2.58$^{b}$ | 10.89±0.32$^{b}$ | 120.97±3.25$^{a}$ | 21.28±1.63$^{a}$ | 72.33±4.32$^{a}$ |
| F-probability | P<0.001 | P<0.05 | P<0.01 | P<0.05 | P<0.001 | p>0.05 | p>0.05 |
| LSD at the 5% level | 4.03 | 4.71 | 5.01 | 0.85 | 9.87 | - | - |
| LSD at the 1% level | 5.57 | 6.51 | 6.93 | 1.17 | 13.66 | - | - |

**Table 11:** Effect of pyrimido[1,6-*a*]quinazoline derivative 4b on oxidative stress and antioxidant defense markers in liver of n5-STZ induced type 2 diabetic female rats.

| Parameters / Groups | Total thiol (nmole/100mg) | Glutathione (nmole/100mg) | Lipid Peroxidation (nmole MDA/100mg) | Glutathione reductase (U/g) | Glutathione peroxidase (mU/100mg) | Glutathione-S-transferase (U/100mg) | Catalase ($k.10^2$) |
|---|---|---|---|---|---|---|---|
| **Normal** | 241.72±4.59$^{a}$ | 63.13±2.94$^{a}$ | 44.27±1.38$^{ab}$ | 10.88±0.90$^{a}$ | 96.30±1.18$^{b}$ | 33.52±23.78$^{a}$ | 60.27±0.99$^{b}$ |
| **Diabetic control** | 203.33±14.33$^{b}$ | 54.93±1.56$^{b}$ | 48.92±4.29$^{a}$ | 10.24±0.03$^{a}$ | 88.87±1.67$^{b}$ | 26.62±2.05$^{b}$ | 72.08±3.35$^{a}$ |
| **Diabetic treated with 4b** | 235.85±4.13$^{ab}$ | 59.85±0.83$^{ab}$ | 37.23±1.84$^{b}$ | 10.58±0.28$^{a}$ | 134.1±10.35$^{a}$ | 26.45±1.64$^{b}$ | 57.38±5.79$^{b}$ |
| F-probability | P<0.05 | P<0.05 | P<0.05 | P>0.05 | P<0.001 | P<0.05 | P<0.05 |
| LSD at the 5% level | 27.16 | 5.95 | 8.48 | - | 8.17 | 6.17 | 11.76 |
| LSD at the 1% level | 37.55 | 8.23 | 11.73 | - | 11.29 | 8.53 | 16.27 |

due insulinogenic action and extrapancreatic effects in addition to the enhancing action on the antioxidant defense system. However, further clinical studies are required to assess the safety and efficacy of the tested compounds in diabetic human beings.

## Declaration of interest

The authors report no conflicts of interest. The work was partially funded by the Faculty of Science, Beni-Suef University, Egypt.

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