Supporting Information

New Biguanides as Anti-diabetic Agents Part I: Synthesis and Evaluation of 1-Substitutedbiguanide Derivatives as Anti-diabetic Agents of Type II Diabetes Insulin Resistant

Authors

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**Figure 1:** Comparison of blood glucose level fasting between our synthesized compounds and metformin

**Figure 2:** Comparison of the AST, ALT, ALP enzyme activities between our synthesized compounds and metformin as standard anti-diabetic reference drug
**Figure 3:** Comparison of the lipid profile TG, TC and TL between our synthesized compounds and metformin as standard anti-diabetic reference drug

**Figure 4:** Comparative effects of different organic compounds and metformin on oxidative stress markers, Lipid peroxide (MDA) and Nitric oxide (NO) as well as enzymatic and nonenzymatic antioxidant (Glutahione (GSH), Superoxide dismutase (SOD) and Glutathione Reductase (GR)
HPLC analysis:

Samples were dissolved in 1 mL of HPLC grade MeOH/H2O (50:50) and centrifuged for 10 min at 4000 rpm before HPLC analysis. Samples were analyzed on a Shimadzu LC-8A liquid chromatography system (Shimadzu) with LC solution software, SPD-M20A photodiode array detector (Shimadzu). A RESTEK (5 μm) C18 column was used (4.6 mm i.d. × 150 mm). Elution was carried out with a gradient solvent system at a flow rate of 1 mL min⁻¹. The mobile phase consisted of MeOH (B) and 1 % formic acid in water (A). The LC time program was as follows: 5–5 % B (2 min), 5–20 % B (5 min), 20–30 % B (3 min), 30–50 % B (5 min), 50–98 % B (8 min), 98–98 % B (5 min) and 98–5 % B (5 min), 5–5 % B (2 min). The sample was injected into a volume of 40 μL and the eluate was monitored at 210 nm.

Evaluation of pharmacological activity:

Experimental Design

Seventy male rats with an average weight of 220-250 g were obtained from animal house lab., National Research Centre, Dokki, Giza were used in this study. Animals were housed under normal laboratory condition for one week before initiation of the biological experiments (adaptation period), housed in a well-ventilated box (22 ± 20 °C) on a twelve hours light and dark cycle. Animals were fed with natural basal diet. Diets and water were supplied ad libitum and has free access of water. The animals were divided randomly into eight main groups of ten rats each to study the effect of the synthesized products as well as metformin on the blood glucose level, liver function enzyme activities and lipid profile levels in STZ-induced diabetic rats as follows:

Groups 1: Normal control rats.
Groups 2: diabetes was induced by STZ. Each rat was injected intraperitoneally with a single dose of STZ (45 mg/kg body weight dissolved in 0.01 M citrate buffer immediately before use. After injection, animals have free access for food and water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock. Animals were checked daily for the presence of glycosuria [1]. Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. After 3 days of STZ injection fasting blood samples were obtained and fasting blood sugar was determined (>300 mg/dl). Hyperglycemic rats were used for the experiment and classified as follows:

Groups 3-7: Diabetic rats orally administered 150 mg/kg body weight synthetic organic compounds for 30 days respectively [2].
Group 8: Diabetic rats orally administered antidiabetic metformin reference drug 150 mg/kg body weight daily for 30 days [3].

Sample Preparations

After 30 days of treatments, rats were fasted overnight (12-14 hours), anesthetized by diethyl ether and blood collected by puncture of the sublingual vein in clean and dry test tube, left 10
minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was used for biochemical analysis of blood glucose level, liver function enzyme activities, alanine and aspartate aminotransferases (AST, ALT) and alkaline phosphatase (ALP), lipid profile including, triglycerides (TG), total cholesterol (TC) and total lipid (TL). Liver in each exponential group weighed and homogenized in 5-10 volumes of appropriate medium using electrical homogenizer, centrifuged at 3000 rpm for 15 min, the supernatants (10%) were collected and placed in Eppendorff tubes then stored at -80 °C and used for determination of oxidative stress markers (NO and MDA) as well as non-enzymatic antioxidant (GSH), glutathione reductase (GR) and superoxide dismutase (SOD). After blood collection, rats of each group were sacrificed, the liver, kidney and pancreas were removed immediately (a part was fixed in 10% formalin for histopathological examination).

**Biochemical analysis**

Biochemical parameters were determined in serum using Biodiagnostic kits (Bio diagnostics Co., Egypt).

**Methods**

Blood glucose level was measured in fasting blood serum according to the method of Trinder [4]. Liver function enzyme activities, alanine and aspartate aminotransferases (AST and ALT) as well as alkaline phosphatase (ALP) were determined in mice serum according to the Reitman [15] and Belfield [6] methods. Serum total lipids concentration was determined according to the method of Zollner [7], serum triglyceride (TG) concentration was determined according to the method of Fassati and Prencipe [8] and serum total cholesterol concentration was estimated according to the method of Allain [9]. Liver nitrite (NO) level was estimated [10]. GSH level was assayed in liver homogenate according to Beutler method [11]. Liver MDA level was estimated according to the method of Satoh [12]. GR was determined according to the method of Goldberg and Spooner [13] and SOD according to the Nishikimi et al. [14].

**Histopathological Analysis**

Liver and kidney slices were fixed instantaneously in buffer neutral formalin (10%) for 24 hours for fixation then processed in automatic processors, embedded in paraffin wax (melting point 55-60 °C) and paraffin blocks were obtained. Sections of 6 μm thicknesses were prepared and stained with Haematoxylin and Eosin (H & E) stain [15]. The cytoplasm stained shades of pink and red and the nuclei gave blue color. The slides were examined and photographed under a light microscope at a magnification power of x400.

**References**

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