

Evaluation of Biological Activity of Two Steroid Derivatives on Glucose Levels Using a Diabetic Model

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Abstract: In this study, three steroid derivatives (compounds 2-4) were prepared from an estrone derivative (compound 1) to evaluate their biological activity on glucose concentration using a diabetic model. Besides, the compounds 1 and 4 were bound to technetium-99m (Tc-99m) via a radioimmunoassay method to evaluate the biodistribution of either compounds 1 and 4 in different organs over time (15, 30, 45, and 60 min). The results showed that both compounds 1 and 4 increase glucose levels compared with either compounds 2 and 3. In addition, other data showed that the biodistribution of the Tc-99m-compound 4 conjugate in all organs was higher compared with Tc-99m-compound 1 complex. In conclusion, compound 4 had greater hypoglycemic effects, and its biodistribution was wider than 1. The data suggest that amino groups may be important to the hypoglycemic activity of compound 4, and this could be related to their higher lipophilicity degree compared with compound 1.

Keywords: Steroid; glucose; glibenclamide; metformin; biodistribution.

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1. Introduction

Diabetes mellitus is a risk factor in the development of cardiovascular diseases [1-3]; It is noteworthy that several drugs such as sulfonylureas [4], biguanides [5], α -glucosidase inhibitors [6], thiazolidinediones [7] have been used for the treatment of this clinical pathology. However, some of these drugs can produce side effects; for example, some studies have reported that the use of glibenclamide can be associated with severe hypoglycemia [8, 9] and ventricular arrhythmia in diabetic men [10]. Besides, other reports indicate that metformin can be associated with lactic acidosis in diabetic patients [11]. Other data indicate that rosiglitazone could exert an increased risk of myocardial infarction in type II diabetic patients [12]. Therefore, in the search for new therapeutic alternatives for treatment to diabetes, some compounds have been synthesized; for example, the preparation of an indole derivative from 2,3,5,6-tetrofluoroaniline and pyridine as an aldose reductase inhibitor for the treatment of

diabetes [13]. Other data showed the synthesis of a series of imidazoline derivatives with biological activity on glucose homeostasis in a rat model of type II diabetes [14]. In addition, a study showed the preparation of a pyran-3,4,5-triol derivative from xylose as a sodium-dependent glucose cotransporter 2 Inhibitor for the Treatment of Type 2 Diabetes [15]. Other reports showed the synthesis of a butanamide analog from 2-Methyl-5-bromobenzoic acid as sodium-dependent glucose cotransporter 1 Inhibitor in a diabetic model [16].

On the other hand, the pharmacological activity of some steroid derivatives on glucose levels has evaluated using some biological models; for example, a study has shown that either dihydroprogesterone or tetrahydroprogesterone can exert a neuroprotective effect against streptozotocin-induced diabetic neuropathy [17]. Besides, other reports have shown the synthesis of a steroid derivative with hypoglycemic activity via glucocorticoid receptor inhibition in a diabetic animal [18]. Other data showed that either 2-methoxyestradiol or 2-ethoxyestradiol could decrease the levels of glucose in an animal model [19]. Other studies showed the synthesis of a progesterone-dihydropyrimidine from progesterone, which induces a decrease in glucose levels in diabetic rats [20]. All these data indicate that several compounds have prepared for the treatment of diabetes; however, there are few data on the biological activity exerted by estrone derivatives on glucose concentration; in this way, the aim of this research was to synthesize some estrone derivatives from an estrone analog to evaluate their biological activity on glucose levels using a diabetic model.

2. Materials and Methods

2.1. General.

The compound **1** (4-(6-fluoro-)-2-nitroestrone-1,5-dinitro-3-azabicyclo-[3.3.1]non-6-en-9-yl)butan-2-one) was prepared using a previously method reported (21). In addition, the reagents used in this research were acquired from Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were evaluated with a Thermo Scientific iSOFT-IR spectrometer. ^1H and ^{13}C NMR spectra were recorded using a Varian VXR300/5 FT NMR spectrometer at 300 MHz in CDCl_3 using TMS as an internal standard. EIMS spectra were obtained with a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

2.1.1. Synthesis.

1-[6-fluoro-3-[2-[(Z)-[(16S)-16-methyl-5-oxapentacyclo[9.7.0.02,8.04,6.012,16] octadeca-2(8),3,6-trien-15-ylidene]amino]ethyl]-1,5-dinitro-3-azabicyclo[3.3.1]non-6-en-9-yl]propan-2-one (2)

In a round bottom flask (10 ml), compound **1** (200 mg, 0.37 mmol), potassium carbonate (50 mg, 0.36 mmol), and dimethyl sulfoxide (5 ml) were stirred to room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:water (3:1) system; yielding 52% of product; m.p. 156-158 °C; IR (V_{max} , cm^{-1}) 3322, 1542, 1222 and 1180: ^1H NMR (300 MHz, CDCl_3 -*d*) δ_{H} : 1.02 (s, 3H), 1.22-1.86 (m, 7H), 2.08 (s, 3H), 2.10-2.26 (m, 4H), 2.50 (m, 1H), 2.56 (m, 1H), 2.72 (m, 1H), 2.74 (m, 1H), 2.78-2.80 (m, 3H), 2.84-2.88 (m, 2H), 3.16-3.50 (m, 4H), 3.62-4.32 (m, 4H), 6.32 (m, 1H), 6.40 (m, 1H), 6.56 (m, 1H) ppm. ^{13}C NMR (300 Hz, CDCl_3) δ_{C} : 16.10, 21.94, 25.76, 26.00, 29.72, 29.84, 32.42, 32.47, 33.90, 37.62,

37.94, 39.64, 44.56, 50.72, 50.87, 54.22, 54.29, 55.16, 57.95, 89.78, 91.90, 108.92, 109.96, 110.37, 131.44, 135.81, 147.39, 147.67, 155.00, 166.38, 202.90 ppm. EI-MS m/z: 580.26. Anal. Calcd. for C₃₁H₃₇FN₄O₆: C, 64.12; H, 6.42; F, 3.27; N, 9.65; O, 16.53. Found: C, 64.10; H, 6.40.

(13S,17Z)-17-[2-(9-acetyl-6-fluoro-1,5-dinitro-3-azabicyclo[3.3.1]non-6-en-3-yl)ethyl-imino]-13-methyl-2-nitro-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3-carbaldehyde (3)

In a round bottom flask (10 ml), compound **2** (200 mg, 0.34 mmol), and dimethyl sulfoxide (5 ml) were stirred to reflux for 24 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:hexane:water (3:1:1) system; yielding 45% of product; m.p. 176-178 °C; IR (V_{max} , cm⁻¹) 3322, 1722, 1540 and 1182: ¹H NMR (300 MHz, CDCl₃-*d*) δ_H: 1.02 (s, 3H), 1.22-1.86 (m, 7H), 2.08 (s, 3H), 2.10-2.26 (m, 4H), 2.50-2.72 (m, 2H), 2.74 (m, 1H), 2.78 (m, 1H), 2.82 (m, 1H), 2.84-3.02 (m, 4H), 3.16-3.50 (m, 4H), 3.62-4.32 (m, 4H), 6.40 (m, 1H), 7.80-8.16 (m, 2H), 10.80 (s, 1H, J = 0.75 Hz) ppm. ¹³C NMR (300 Hz, CDCl₃) δ_C: 16.10, 21.94, 26.00, 27.04, 29.60, 29.72, 32.42, 32.44, 33.92, 37.62, 37.92, 39.64, 44.10, 50.74, 50.87, 54.22, 54.29, 55.16, 57.95, 89.78, 91.91, 110.34, 123.80, 126.24, 126.49, 146.67, 151.75, 152.24, 155.00, 166.36, 194.24, 202.90 ppm. EI-MS m/z: 639.27. Anal. Calcd. for C₃₂H₃₈FN₅O₈: C, 60.08; H, 5.99; F, 2.97; N, 10.95; O, 20.01. Found: C, 60.04; H, 5.96.

1-[1,5-diamino-3-[2-[(Z)-[(13S)-2-amino-3-hydroxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-17-ylidene]amino]ethyl]-6-fluoro-3-aza-bicyclo[3.3.1]non-6-en-9-yl]propan-2-one (4)

In a round bottom flask (10 ml), compound **3** (200 mg, 0.31 mmol), sodium borohydride (60 mg, 1.59 mmol), and ethanol (5 ml) were stirred to room temperature for 72 h. Then, the solvent was evaporated under reduced pressure and following the product was purified via crystallization using the methanol:water (4:1) system; yielding 67% of product; m.p. 194-196 °C; IR (V_{max} , cm⁻¹) 3400, 3380, 3320 and 1182: ¹H NMR (300 MHz, CDCl₃-*d*) δ_H: 1.02 (s, 3H), 1.22-1.86 (m, 7H), 1.98-2.00 (m, 2H), 2.08 (s, 3H), 2.10-2.16 (m, 2H), 2.20-2.22 (m, 2H), 2.26 (m, 1H), 2.36 (m, 1H), 2.44 (m, 1H), 2.52 (m, 1H), 2.54 (m, 1H), 2.74 (m, 1H), 2.78 (m, 1H), 2.80 (m, 2H), 2.82 (m, 2H), 3.12 (m, 1H), 3.50 (m, 2H), 3.66 (broad, 7H), 5.66 (m, 1H), 6.36-6.82 (m, 2H) ppm. ¹³C NMR (300 Hz, CDCl₃) δ_C: 16.10, 21.96, 25.76, 26.00, 29.72, 29.82, 32.42, 33.91, 36.04, 37.44, 37.62, 39.64, 43.86, 49.54, 50.84, 54.29, 57.16, 57.43, 58.54, 61.55, 62.97, 109.26, 114.02, 116.77, 132.71, 133.85, 134.16, 142.83, 159.62, 166.36, 209.10 ppm. EI-MS m/z: 537.34. Anal. Calcd. for C₃₁H₄₄FN₅O₂: C, 69.24; H, 8.25; F, 3.53; N, 13.02; O, 5.95. Found: C, 69.21; H, 8.22.

2.2. Pharmacological/biological assays.

2.2.1. Induction of diabetes.

The animal was injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. intraperitoneally [22].

2.2.2. Glucose analysis.

After 2 weeks, rats with moderate diabetes having glycosuria* (indicated by Benedict's qualitative test) and hyperglycemia (i.e., with a blood glucose ≥ 200 mg/dl) were used for the experiment [21].

* Blood glucose was determined from tail blood with a rapid glucose analyzer (Accutrend Sensor Comfort; Roche, U.S.A.) every 48 h. The rats were divided into sixteen groups after the induction of diabetes. Six rats were used in each group (90 diabetic surviving rats, six normal rats) as follows.

2.3. Experimental design (I).

Group 1: Normal rats were given 2 ml of normal saline.

Group 2: Diabetic control rats given 2 ml of normal saline.

Group 3: Diabetic rats were given an aqueous solution of glibenclamide^ϕ (600 µg/kg body mass) daily with an intragastric tube for 30 days.

Group 4: Diabetic rats given an aqueous solution of metformin[Ⓞ] (350 mg/kg body mass) daily had an intragastric tube for 30 days.

Group 5: Diabetic rats were given an aqueous solution of compound 1 (20 mg/ml) daily with an intragastric tube for 30 days.

Group 6: Diabetic rats were given an aqueous solution of compound 2 (20 mg/ml) daily with an intragastric tube for 30 days.

Group 7: Diabetic rats were given an aqueous solution of compound 3 (20 mg/ml) daily with an intragastric tube for 30 days.

Group 8: Diabetic rats were given an aqueous solution of compound 4 (20 mg/ml) daily with an intragastric tube for 30 days.

^{ϕ,Ⓞ}Dose administered of either glibenclamide or metformin was determined using a previous method reported [22].

2.4. Experimental design (II).

Group 9: Diabetic rats were given an aqueous solution of compound 1 (2.5 mg/ml) daily with an intragastric tube for 30 days.

Group 10: Diabetic rats were given an aqueous solution of compound 1 (5 mg/ml) daily with an intragastric tube for 30 days.

Group 11: Diabetic rats were given an aqueous solution of compound 1 (15 mg/ml) daily with an intragastric tube for 30 days.

Group 12: Diabetic rats were given an aqueous solution of compound 1 ((20 mg/ml) daily with an intragastric tube for 30 days.

2.5. Experimental design (III).

Group 13: Diabetic rats were given an aqueous solution of compound 4 (2.5 mg/ml) daily with an intragastric tube for 30 days.

Group 14: Diabetic rats were given an aqueous solution of compound 4 (5 mg/ml) daily with an intragastric tube for 30 days.

Group 15: Diabetic rats were given an aqueous solution of compound 4 (15 mg/ml) daily with an intragastric tube for 30 days.

Group 16: Diabetic rats were given an aqueous solution of compound 4 (20 mg/ml) daily with an intragastric tube for 30 days.

^{ϕ,Ⓞ}Dose administered of either glibenclamide or metformin was determined using a previous method reported [22].

2.6. Statistical analysis.

The obtained values are expressed as average \pm SE. The results were put under an analysis of variance (ANOVA) with the Bonferroni correction factor using the SPSS 12.0 program [23]. The differences were considered significant when p was equal to or smaller than 0.05.

2.7. Radiochemical analysis.

The compounds 1 and 4 were bound to Tc-99m using previously methods reported [22]. In this way, a solution of either compounds 1 or 4 (20 mg) in ethanol (1.0 ml) was adjusted to pH 7.0 using 0.1M NaOH. The mixture was then added to another freshly prepared solution (75 ml) of stannous chloride (2mg/ml in 0.1M HCl), and following the pH was readjusted to 7.0. Then, 2 ml of a Tc-99m pertechnetate solution eluted from a sterile 99Mo-99m-Tc shielded generator was added to the mixture solution.

2.8. Quality control.

The labeling efficiencies with Tc-99m were evaluated via a chromatographic analysis on Silica-gel 60 F254 plate using the acetonitrile:water (4:1) system. The plates were counted by images in a gamma camera equipped with a high-resolution collimator with a digital computer (VP450). In addition, reference factor value was evaluated using as control Tc-99m pertechnetate and the hydrolyzed Tc-99m colloid. The purities of the Tc-99m-conjugates was evaluated by paper electrophoresis. The paper strips were run at a constant voltage of 600 V for 30 min using a buffer solution (0.1M, pH 7.4). The paper strips were counted by images in a gamma camera equipped with a high-resolution collimator with a digital computer. The movement was determined relative to Tc-99m pertechnetate and hydrolyzed Tc-99m colloid [24].

2.9. Pharmacocinetic analyses.

Each diabetic animal received 0.3 ml of either Tc-99m-compound **1** conjugate or Tc-99m-compound **4** complex (200 μ Ci, 1.3mg) via tail vein administration. It is noteworthy that sequential scintigrams were taken at 15, 30, 45, and 60 min with a gamma camera equipped with a high-resolution collimator with a digital computer. Then, the diabetic rats were sacrificed, and the organs were removed, and the radioactivity was counted by images in a gamma camera equipped with a high-resolution collimator with a digital computer. Beside, percentages of the injected dose per organ were determined by comparison of tissue radioactivity concentration with the total radioactivity. Following, the blood (ml) in the heart was collected to evaluate the radioactivity with the same equip.

3. Results and Discussion

Several compounds with hypoglycemic activity have been synthesized; however, several protocols use some reagents which require special conditions. In this research, some steroid derivatives were prepared to evaluate their biological activity on glucose levels using a diabetic model. The first stage was achieved as follows:

3.1. Chemistry.

3.1.1. Preparation of an ether derivative.

Several reagents such as Me_3SiCN , $\text{K}_2\text{CO}_3/\text{acetone}$, tetrahydrofuran/lithium/aluminum hydride, Me_3SiCl , $\text{K}_3\text{PO}_4/\text{DMF}$, ethyl cinnamate have used for the preparation of ether derivatives [25]. In this research, a previous method reported [26] was used to preparation of compound 2; in this way, compound 1 reacted with dimethyl sulfoxide in middle conditions to form 2 (Figure 1).

The ^1H NMR spectrum from 2 showed several signals at 1.02 ppm for methyl group bound to steroid nucleus; at 2.08 ppm for methyl group linked to ketone group; at 1.22-1.86, 2.10-2.26, 2.56, 2.78-2.80, 6.32 and 6.56 ppm for steroid moiety; at 2.50, 2.72, 3.62-4.32 and 6.40 ppm for 3-Aza-bicyclo[3.3.1]non-6-ene fragment; at 2.74 and 2.84-2.88 ppm for methylene groups bound to both 3-Aza-bicyclo[3.3.1]non-6-ene fragment and ketone group; at 3.16-3.50 ppm for methylene groups bound to both amino and imino groups. ^{13}C NMR spectra showed chemical shifts at 16.10 ppm dor methyl group bound to steroid nucleus; at 29.72 ppm for methyl group linked to ketone group; at 21.94-26.00, 29.84-32.42, 33.90-37.62, 29.64-44.56, 108.92-109.96 and 131.44-147.67 ppm for steroid moiety; at 32.47, 50.72, 55.16-110.37 and 155.00 ppm for 3-Aza-bicyclo[3.3.1]non-6-ene fragment; at 37.94 methylene groups bound to both 3-Aza-bicyclo[3.3.1]non-6-ene fragment and ketone group; at 50.87-54.22 ppm for methylene groups linked to both amino and imino groups; at 166.36 ppm for imino group; at 209.90 ppm for ketone group. Besides, the mass spectrum from 2 showed a molecular ion (m/z) 580.26.

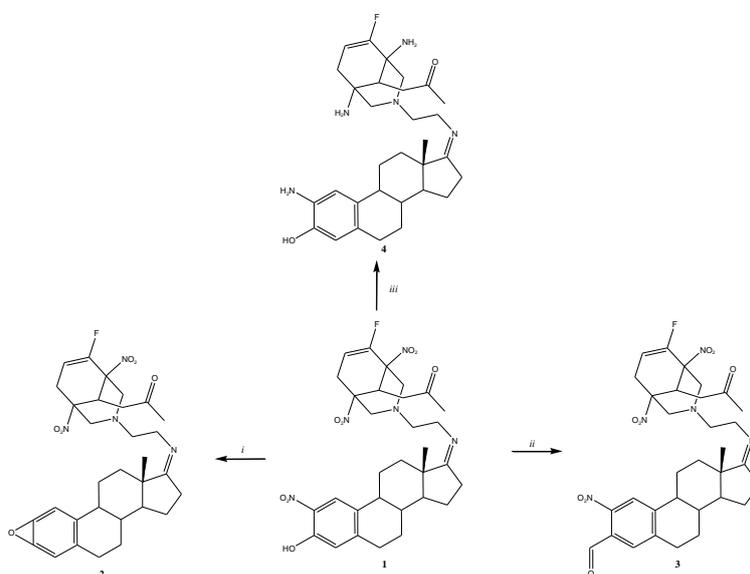


Figure 1. Synthesis of steroid derivatives (2-4). Reagents and conditions; *ii* = dimethyl sulfoxide, K_2CO_3 , room temperature, 72 h; *ii* = dimethyl sulfoxide, reflux 24 h; *iii* = sodium borohydride/ Zn , room temperature, 48 h.

3.1.2. Synthesis of an aldehyde-steroid derivative.

There are several reports for the preparation of some aldehyde derivatives using some reagents such as chromium(VI), palladium, rhodium/ruthenium, and hydrogen peroxide. However, these reagents require special conditions and may produce toxic effects by the generation of some products involved in the reaction mixtures [27]. Analyzing these data and other reports for the preparation of some aldehyde derivatives in the presence of dimethyl

sulfoxide [28]; in this study, an aldehyde-steroid derivative was prepared *via* reaction of compound **2** with dimethyl sulfoxide (Figure 1). The ¹H NMR spectrum from **3** showed several signals at 1.02 ppm for methyl group bound to steroid nucleus; at 2.08 ppm for methyl group linked to ketone group; at 1.22-1.86, 2.10-2.26, 2.78, 2.84-3.02 and 780-8.16 ppm for steroid moiety; at 2.50 and 3.62-6.40 ppm for 3-Aza-bicyclo[3.3.1]non-6-ene fragment; at 2.74 and 2.82 ppm for methylene groups bound to both 3-Aza-bicyclo[3.3.1]non-6-ene fragment and ketone group; at 3.16-3.50 ppm for methylene groups bound to both amino and imino groups; at 10.80 ppm for aldehyde group. ¹³C NMR spectra showed chemical shifts at 16.10 ppm for methyl group bound to steroid nucleus; at 29.72 ppm for methyl group linked to ketone group; at 21.94-29.60, 32.42, 33.92-37.62, 39.64-44.10, 54.29 and 123.80-152.24 ppm for steroid moiety; at 32.44, 50.74, 55.16-110.34 and 155.00 ppm for 3-Aza-bicyclo[3.3.1]non-6-ene fragment; at 37.92 ppm for methylene groups bound to both 3-Aza-bicyclo[3.3.1]non-6-ene fragment and ketone group; at 50.87-54.22 ppm for methylene groups bound to amino and imino groups; at 166.36 ppm for imino group; at 194.24 ppm for aldehyde group; at 202.90 ppm for ketone group. In addition, the mass spectrum from **3** showed a molecular ion (m/z) 639.27.

3.1.3. Reduction of nitro groups.

Several protocols have used to reduction of nitro groups using some reagents such as TiO₂ [29], CuSO₄ [30], phthalocyanine-cobalt(I) anion [31], Iron(II) sulfate [32], palladium [33]. In this investigation, compound **4** was prepared via the reduction of nitro groups in the presence of sodium borohydride (Figure 1). The ¹H NMR spectrum from **4** showed several signals at 1.02 ppm for methyl group bound to steroid nucleus; at 2.08 ppm for methyl group linked to ketone group; at 1.22-1.86, 2.10-2.16, 2.26, 2.52, 2.74, 2.80 and 6.36-6.82 ppm for steroid moiety; at 1.98-2.00, 2.20-2.22, 2.36, 2.78, 3.12 and 5.66 ppm for 3-Aza-bicyclo[3.3.1]non-6-ene fragment; at 2.44 and 2.54 ppm for methylene groups bound to both 3-Aza-bicyclo[3.3.1]non-6-ene fragment and ketone group; at 2.82 and 3.50 ppm for methylene groups bound to amino and imino groups; at 3.66 ppm for either hydroxyl and amino groups. ¹³C NMR spectra showed chemical shifts at 16.10 ppm for methyl group bound to steroid nucleus; at 29.72 ppm for methyl group linked to ketone group; at 21.96-26.00, 29.82-33.91, 37.62-43.86, 54.29 and 114.02-142.83 ppm for steroid moiety; at 36.04, 49.54, 57.16, 58.54.109.26 and 159.62 ppm for 3-Aza-bicyclo[3.3.1]non-6-ene fragment; at 37.44 ppm for methylene groups bound to both 3-Aza-bicyclo[3.3.1]non-6-ene fragment and ketone group; at 50.84 and 57.43 ppm for methylene groups bound to both amino and imino groups; at 166.36 ppm for imino group; at 209.10 ppm for ketone group. Finally, the mass spectrum from **4** showed a molecular ion (m/z) 537.34.

3.2. Pharmacology/biology.

3.2.1. General methods.

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of Universidad Autonoma de Campeche (UAC) and were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996) (30). Female rats (Wistar; weighing 200–250 g) were obtained from UAC.

3.2.2. Glucose analysis.

The biological activity exerted by the compounds 1 to 4 on glucose levels was evaluated using a diabetic animal model. In this way, alloxan was administered to male rats to induce diabetes; it is noteworthy that alloxan reagent can cause a massive reduction in insulin release, via the destruction of β -cells of the islets of Langerhans, which resulting as an indirect increase in the glucose levels [34].

The results showed that both compounds 1 and 4 lower glucose levels at doses of 20 mg/ml compared to compounds 2 and 3 (Figure 2). These data indicate that the biological activity exerted by compounds 1 and 4 may depend on the functional groups involved in its chemical structure.

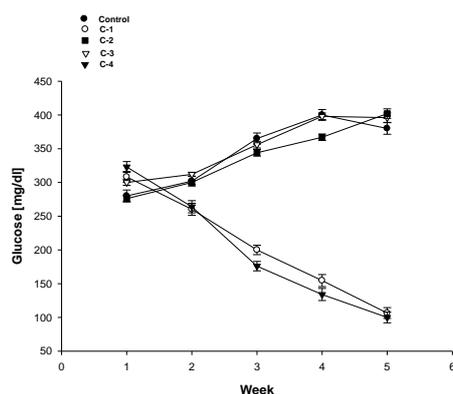


Figure 2. Biological activity was exerted by both compound 1 (C-1) and compound 4 (C-4) on glucose concentration in a diabetic rat model. The results showed that either compounds 1 or 4 significantly decreases ($p=0.05$) the blood glucose concentration at a dose of 20 mg/ml compared with both compounds 2 or 3. The effects are expressed as mean \pm S.E. $n = 6$.

However, it is important to mention that alternative experiments were carried out to find the minimum effective dose by which these compounds could lower the glucose concentration. The results showed that compound 1 significantly decrease the glucose concentration at a dose of 20 mg/dl in a dose-dependent manner (Figure 3); however, compound 4 significantly decrease the glucose levels at a dose of 10 and 20 mg/dl. This phenomenon could depend on effect exerted of amino groups involved in the chemical structure of 4 (Figure 4).

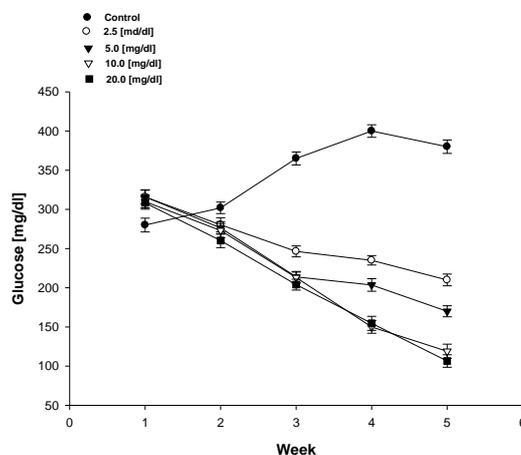


Figure 3. Effects exerted by compound 1 on glucose levels in a diabetic rat model. The results showed that 1 significantly decrease ($p=0.05$) in a dose-dependent manner, the blood glucose concentration. The effects are expressed as mean \pm S.E. $n = 6$.

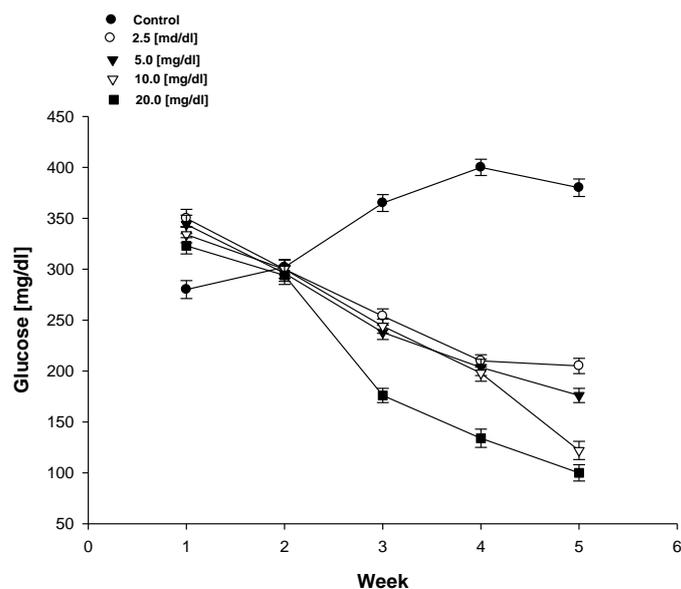


Figure 4. Biological activity induced by compound 4 on glucose levels in a diabetic rat model. The results showed that 4 significantly decrease ($p=0.05$) in a dose-dependent manner, the blood glucose concentration. The effects are expressed as mean \pm S.E. $n = 6$.

On the other hand, the biological activity exerted by metformin (insulin receptor activator) [35] and glibenclamide (potassium-channel inhibitor) [36] on glucose concentration was evaluated. The results showed that both metformin and glibenclamide decreases glucose levels (Figure 5).

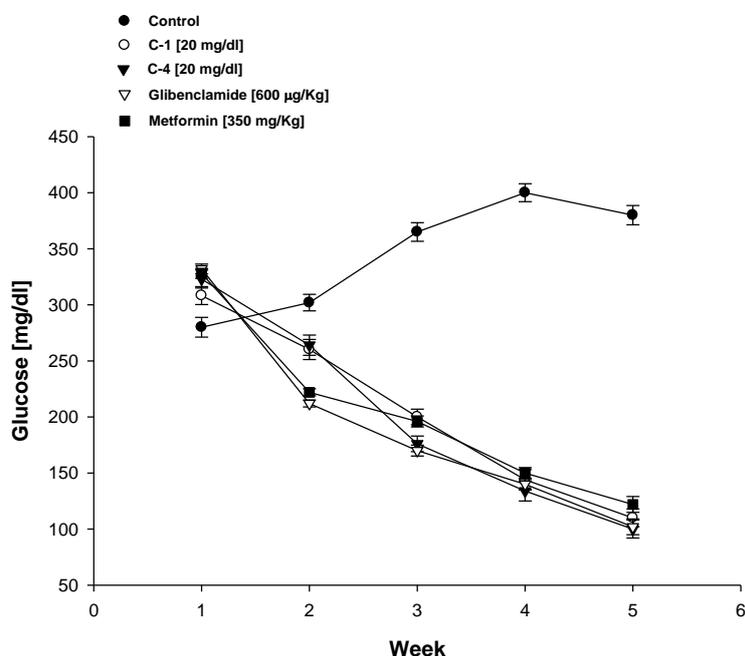


Figure 5. Effects are exerted by the compounds 1, 4, glibenclamide, and metformin on glucose levels in a diabetic rat model. The results showed that compounds 1, 4, glibenclamide and metformin significantly decrease ($p=0.05$) in a time-dependent manner in the blood glucose levels. The effects are expressed as mean \pm S.E. $n = 6$.

It is noteworthy that these effects were compared with biological activity exerted by either compounds 1 or 4. The data indicate that the effect exerted by either compounds 1 and 4 was similar to biological activity induced by metformin on glucose concentration. This

phenomenon could also be associated with some pharmacokinetic parameters involved in the biological activity of compounds 1 or 4 on glucose concentration. To evaluate this hypothesis, this research was evaluated the biodistribution of either compound 1 or 4 using a previously radioimmunoassay method reported [22]. In this way, both compounds 1 and 4 were bound to Tc-99m with the conventional stannous chloride method; it is important to mention that steroid conjugates were excellent chelating agents, *via* the hydroxyl groups binding to Tc-99m.

On the other hand, the biodistribution of either Tc-99m-compound 1 and Tc-99m-compound 4 conjugates as a consequence of increases in time (15, 30, 45, and 60 min) was evaluated. The results showed that the biodistribution of the Tc-99m-Compound 4 conjugate was significantly higher in each organ evaluated compared with the values for the Tc-99m-compound 1 complex (Figure 6, Tables 1 and 2).

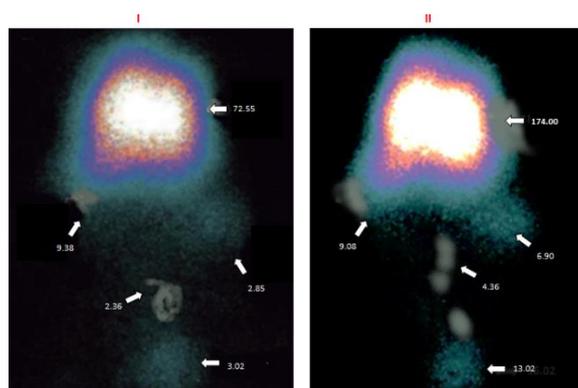


Figure 6. Scintigrams (μCi) were taken 60 min after the administration of the Tc-99m-steroid derivatives. The Tc-99-compound 1 conjugate (I) shows different values in each organ compared with Tc-99-compound 4 complex. The paper strips were counted by images in a gamma camera equipped with a high-resolution collimator with a digital computer.

Table 1. Biodistribution of Tc-99m-compound 1 (μCi). Each value is mean \pm S.E. n = 6.

Organ	15 min	30 min	45 min	60 min
Brain	69.02 \pm 2.67	70.28 \pm 2.24	71.34 \pm 2.34	72.55 \pm 2.68
Spleen	10.09 \pm 2.98	10.26 \pm 2.45	9.56 \pm 2.12	10.31 \pm 2.14
Stomach	9.02 \pm 1.68	9.09 \pm 1.53	9.22 \pm 1.87	9.38 \pm 2.34
Intestine	3.16 \pm 1.28	2.96 \pm 2.12	2.87 \pm 1.24	2.36 \pm 1.24
Liver	2.35 \pm 1.56	2.50 \pm 1.44	2.42 \pm 1.76	2.85 \pm 1.34
Kidney	2.04 \pm 1.62	3.22 \pm 1.54	2.82 \pm 1.34	3.02 \pm 1.28
Gonads	4.10 \pm 1.44	3.88 \pm 1.42	3.66 \pm 1.55	3.02 \pm 1.44
Blood	2.55 \pm 1.45	2.12 \pm 1.06	1.66 \pm 1.45	1.43 \pm 1.34

Table 2. Biodistribution of Tc-99m-compound 1 (μCi). Each value is mean \pm S.E. n = 6.

Organ	15 min	30 min	45 min	60 min
Brain	190.02 \pm 1.89	178.09 \pm 2.22	170.00 \pm 2.28	174.00 \pm 2.45
Spleen	12.45 \pm 2.43	12.26 \pm 2.67	11.00 \pm 2.02	10.98 \pm 2.14
Stomach	10.90 \pm 1.68	10.56 \pm 2.22	9.28 \pm 1.67	9.08 \pm 1.22
Intestine	5.16 \pm 2.02	6.06 \pm 1.67	5.66 \pm 1.24	4.36 \pm 1.67
Liver	7.76 \pm 1.86	7.70 \pm 1.44	6.98 \pm 1.36	6.90 \pm 1.34
Kidney	3.04 \pm 1.44	4.28 \pm 1.14	3.82 \pm 1.15	3.22 \pm 1.27
Gonads	15.05 \pm 1.56	15.44 \pm 1.42	14.32 \pm 1.12	13.02 \pm 1.22
Blood	3.98 \pm 1.22	3.12 \pm 1.35	2.22 \pm 1.45	1.89 \pm 1.14

Table 3. Lipophilicity degree of compounds 1 and 4.

Log P method	Compound 1	Compound 4
iLOGP	3.08	2.96
XLOGP3	3.80	1.11
WLOGP	4.84	3.88
MLOGP	1.21	2.18
SILICOS-IT	-0.38	3.90
Consensus	2.51	2.81

The results indicate the following: 1) both compounds 1 or 4 were distributed to all tissues; 2) the concentration of either compounds 1 or 4 in the brain was higher than in other organs. This phenomenon may be conditioned by the interaction between the compounds 1 or 4 with some endogenous substances involved in the brain; 3) differences in the biodistribution of either compound 1 or 4 could depend on their lipophilicity degree. Analyzing these data, in this study was carried out a theoretical test to evaluate the lipophilicity degree of either compound 1 or 4 using SwissADME software. The results showed that the lipophilicity degree was higher for 4 compared with compound 1 (Table 3).

4. Conclusions

Compound 4 exerts a higher hypoglycemic activity, and its biodistribution was greater compared with compound 1. These data suggest that amino groups involved in the chemical structure of 4 may be important to their hypoglycemic, and this phenomenon could be related to higher lipophilicity in comparison with 4.

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Conflicts of Interest

The authors declare no conflict of interest.

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