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Activity induced by two Steroid-Dihydropyrimidine derivatives on Glucose levels in a Diabetic Rat Model. Relationship between descriptors *logP* and π and its Antidiabetic activity

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Abstract: In this work was designed to investigate the effects of two steroid-dihydropyrimidine derivatives on glucose levels in a rat diabetic model. Additionally, to delineate the structural chemical requirements of the dihydropyrimidine derivatives as antidiabetic agents; several parameters such as, the descriptors *LogP* and π were calculated. The results showed that dihydrotestosterone-dihydropyrimidine derivative significantly decrease the blood glucose levels at doses of 3.5 mg/dL (490 to 124 **mg**/dL; ***p*** = 0.06) and 7.0 mg/dL (466 to 81 **mg**/dL; ***p*** = 0.05) in comparison with *progesterone-dihydropyrimidine* derivative at doses of 3.5 mg/dL (456 to 222 mg/dL; ***p*** = 0.05) and 7.0 mg/dL (487 to 232 **mg**/dL; ***p*** = 0.05). This phenomenon is conditioned mainly by the contribution of all substituent atoms involved in the chemical structure of the steroid derivatives which can induce several changes in both the lipofilicity and its activity on glucose levels.

Keywords. Dihydrotestosterone, dihydropyrimidine, *LogP*, π.

Introduction

Diabetes is a disease of metabolic disorders is associated with a number of chronic complications such as nephropathy[1], retinopathy[2] and cardiovascular[3]. Epidemiological and clinical studies suggest that sex hormones are associated with increased risk for diabetes and changes in glucose levels[4]. For example, there are reports which indicate that low plasma levels of testosterone are associated with increased risk for diabetes in men[5]. Other reports showed that low levels of testosterone derivative (dihydrotestosterone) are correlated with diabetic men in comparison with normal males[6]. Another type of steroids derivatives also can induce effect on glucose levels, for example, there are reports that nortestosterone may contribute to a greater extent than progesterone to glucose intolerance[7,8].

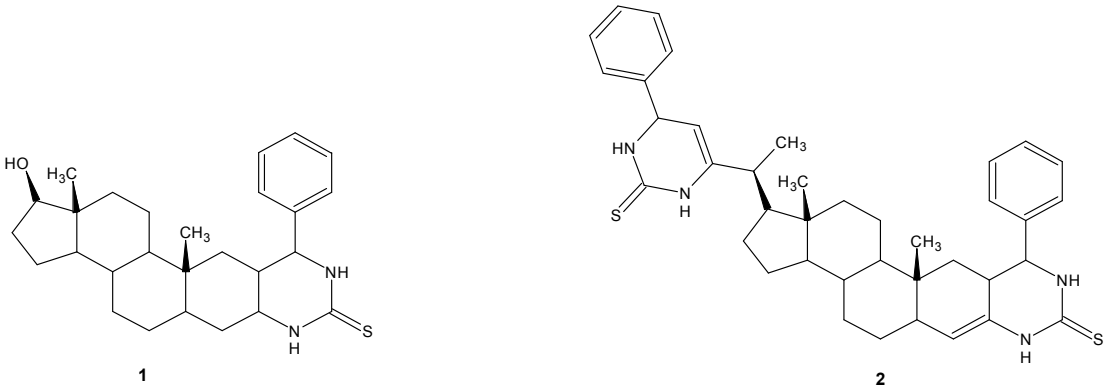

Figure 1. Chemical structures of *dihydrotestesterone-dihydropyrimidine* and *progesterone-dihydropyrimidine*.

Additionally, clinical data indicate that other progesterone derivative (levonorgestrel) can induce minimal effect on carbohydrate metabolism[9]. In order to evaluate the activity of some testosterone and progesterone derivatives on glucose levels have been used several animals models, in this sense there are reports which show that testosterone derivative (16 alpha-fluoro-5-androsten-17-one) induce an antihyperglucemic effect on diabetic mice[10]. Nevertheless, other reports showed that other androgen derivative (dihydrotestosterone) not modify the blood glucose levels in diabetic male Sprague-Dawley rats[11]. In addition, there are reports which showed that 17alpha-acetoxyprogesterone does not induce alterations in glucose metabolism in a model animal (rats, and monkeys)[12].

All these studies suggest that some testosterone and progesterone derivatives can affect the glucose levels; nevertheless it is important to mention that this phenomenon could be dependent of the changes in the chemical structure of these steroids. To provide this information, the present study was designed to investigate the effects of two steroid-dihydropyrimidine derivatives on glucose levels in a rat diabetic model. Additionally, to delineate the structural chemical requirements of the steroid-dihydropyrimidine derivatives as antidiabetic agents; several parameters such as, the descriptors *LogP* and π were calculated.

Material and Methods

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)[13]. Male rats (Wisstar; weighing 200-250 g) were obtained from UAC.

Reagents

Two steroid-dihydropyrimidine derivatives (Figure 1) were prepared according to a previously reported method by previously reported method by Figueroa and coworkers[14]. Other reagents were obtained from Sigma-Aldrich Chemical Co. All drugs were dissolved in *methanol* and different dilutions were obtained using Krebs-Henseleit solution (≤ 0.01%, v/v).

Experimental induction of diabetes in rats

The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. intraperitoneally[15]. After 2 weeks, rats with hyperglycaemia (i.e. with a blood glucose ≥ 200 mg/dL) were used for the experiments.

Experimental design and treatment

In the experiment a total of 48 rats were used. Diabetes was induced in rats 2 weeks before starting the experiments. The rats were divided into eight groups after the induction of diabetes. In the experiments six rats were used in each group (42 diabetic surviving rats and six normal rats) as followed.

Group 1: Normal rats given with 2 ml of normal saline solution daily using an intragastric tube for 30 days.
Group 2: Diabetic control rats given 1 ml of normal saline solution daily using an intragastric tube for 30 days.
Group 3: Diabetic rats given *Glibenclamide* (600 µg/kg body wt.)[16] in 1 mL of aqueous solution daily was using an intragastric tube for 30 days.
Group 4: Diabetic rats given *Metformin* (350 mg/kg body wt.)[17] in 1 mL of aqueous solution daily was using an intragastric tube for 30 days.
Group 5: Diabetic rats given *dihydrotestesterone-dihydropyrimidine* (3.5 mg/kg body wt.) in 1 mL of aqueous solution daily was using an intragastric tube for 30 days.
Group 6: Diabetic rats given *dihydrotestesterone-dihydropyrimidine* (7.0 mg/kg body wt.) in 1 mL of

aqueous solution daily was using an intragastric tube for 30 days.

Group 7: Diabetic rats given *progesterone-dihydropyrimidine* (3.5 mg/kg body wt.) in 1 mL of aqueous solution daily was using an intragastric tube for 30 days.

Group 8: Diabetic rats given *progesterone-dihydropyrimidine* (7.0 mg/kg body wt.) in 1 mL of aqueous solution daily was using an intragastric tube for 30 days.

It is important to mention that dose reported of steroid-derivatives in this work were the product of curve dose-response (not showed).

Biochemical assays

Measured in acute form

Blood glucose was determined from tail blood with a rapid glucose analyzer (Accutrend Sensor Comfort; Roche, U.S.A)[18] every 24 hours.

Statistical analysis

All the experimental data were statistically evaluated and the significance of various treatments was calculated using Student's *t*-test. All the results were expressed as mean ± S.D.

QSAR. To estimate the logarithmic octanol-water partition coefficient (log P) and π of *steroid-dihydropyrimidine* derivatives the logKow method (atom/fragment contribution)[19], available as the KOWWIN software was used.

Table 1. Physicochemical parameters (Log*P* [LogKow] and π) of Dihydrotestosterone- dihydropyrimidine derivative DHTB

Compound	Fragment	Contribution
Dihydrotestosterone-DHTB	**-CH3 [aliphatic carbon**	**1.0946**
	-CH2- [aliphatic carbon]	**3.9288**
	-CH [aliphatic carbon]	**2.8912**
	-OH [hydroxy, aliphatic attach]	**-1.4086**
	-NH- [aliphatic attach]	**-2.9924**
	Aromatic Carbon	**1.7640**
	-NC(=S)N- [thiourea]	**1.2905**
	-tert Carbon [3 or more carbon attach]	**0.5352**
	Fused aliphatic ring unit correction	**-2.7368**
	Equation constant	**0.2290**
	Log Kow	**4.5955**
	π	**1.5296**

Smiles : C14(C5CCC6(C(CCC6C5CCC4CC2NC(NC(C2C1)c3ccccc3)=S)O)C)C

Mol. For.: C_{27} H_{38} N_2 O_1 S_1

Mol. WT : 438.68

Table 2. Physicochemical parameters (Log*P* [LogKow] and π) of *Progesterone*- dihydropyrimidine derivative (*Progesterone- DHTB).*

Compound	Fragment	Contribution
Progesterone-DHTB	**-CH3 [aliphatic carbon]**	**1.6419**
	-CH2- [aliphatic carbon]	**3.4377**
	-CH [aliphatic carbon]	**3.2526**
	=CH- or =C< [olefinc carbon]	**1.5344**
	-NH- [aliphatic attach]	**-5.9848**
	Aromatic Carbon	**3.5280**
	-NC(=S)N- [thiourea]	**2.5810**
	-tert Carbon [3 or more carbon attach]	**0.5352**
	Fused aliphatic ring unit correction	**-2.7368**
	Equation constant	**0.2290**
	Log Kow	**8.0182**
	π	**4.3517**

Smiles: c1cc(ccc1)C2/C=C(\NC(N2)=S)[C@H](C3CCC4C5CCC6/C=C7/NC (NC(C7CC6 (C5CCC34C)C)c8ccccc8)=S)C

Mol For: C_{39} H_{48} N_4 S_2

Mol WT : 636.96

Results and discussion

In this work the activity of two steroid-dihydropyrimidine derivatives on glucose was evaluated. In this sense, changes of plasma glucose levels were determinate (Figure 2) after of oral administration of *progesterone-dihydropyrimidine* and *dihydrotestesterone-dihydropyrimidine* in diabetic rats, using *metformin, glibenclamide* as control. It is important to mention, that diabetes was induced with alloxan. There are reports that alloxan causes massive reduction in insulin release, through the destruction of *β*-cells of the islets of Langerhans[20]. The results obtained in a acute form, showed that *glibenclamide* (470 to 112 mg/dL; $p = 0.05$) and *metformin* (458 to 148 mg/dL; $p = 0.05$) significantly reduced the blood glucose levels. Additionally, other results showed that dihydrotestosterone-dihydropyrimidine derivative (Figure 2) significantly decrease the blood glucose levels at doses of 3.5 mg/dL (490 to 124 **mg**/dL; $p = 0.06$) and 7.0 mg/dL (466 to 81 **mg**/dL; $p = 0.05$) in comparison with *progesterone-dihydropyrimidine* derivative (Figure 3) at doses of 3.5 mg/dL (456 to 222 mg/dL; $p = 0.05$) and 7.0 mg/dL (487 to 232 **mg**/dL; $p = 0.05$). It is important to mention that experiments control with normal rats the level of glucose showed an average of 112 ± 1.45 (data not shown). These data indicate that dihydrotestosterone-dihydropyrimidine had potency similar in comparison with *glibenclamide* and high effect that metformin and progesterone-dihydropyrimidine.

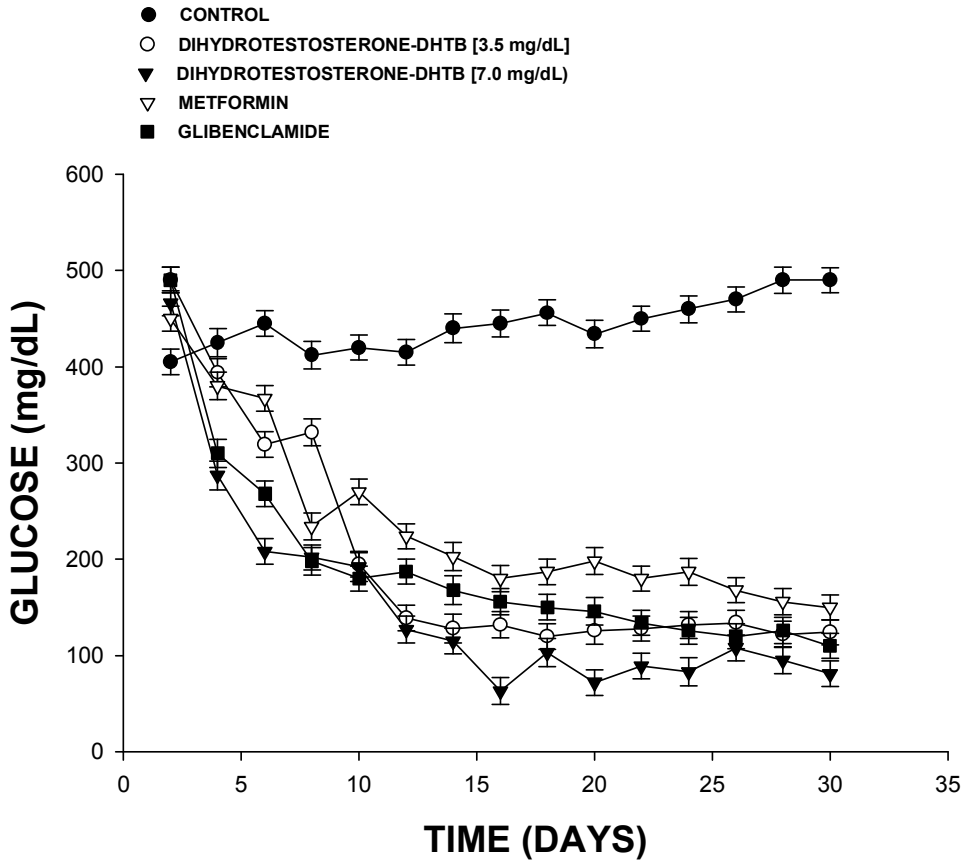

Figure 2. Antidiabetic activity induced by *dihydrotestesterone-dihydropyrimidine* (*dihydrotestesterone-DHTB)* in diabetic rat. The results showed that *glibenclamide* ($p = 0.05$) and metformin ($p = 0.05$) significantly reduced the blood glucose levels in comparison with control conditions. Additionally, other results showed that *dihydrotestesterone-DHTB* significantly decrease the blood glucose levels in a manner dose dependent. The effects are expressed as mean ± S.D.

These experimental data suggest that differences in the chemical structure of two steroid-dihydropyrimidine derivatives (Figure 1) induce different activity on glucose levels. In order to delineate the structural chemical requirements of both steroid-dihydropyrimidine derivatives for evaluate their activity, we calculate other parameters such as, the descriptors ***LogP*** and ***π***.[21] The *LogP* estimates the logarithmic octanol-water partition coefficient; therefore the *LogP* represents the *lipophilic* effects of a molecule which includes the sum of the *lipophilic* contributions of the parent molecule and its substituent[22]. The difference between the substituted and unsubstituted *LogP* values is condicionated by the π value for the particular substituent. Hammett showed that π values measure the free energy change caused by particular substituent to relate to biological activity[23,24]. Therefore, the *Log P* and π parameters were calculated by the method proposed by Mannhold and Waterbeemd et al[19]. The results (Figure 4, Table 1 and 2) showed an increase in both *Log P* and π values of *progesterone-dihydropyrimidine* with respect to *dihydrotestosterone-dihydropyrimidine*. This phenomenon is conditioned mainly, by the contribution of all substituent atoms involved in the chemical structure of progesterone-derivative, as is showed in the Table 1 and 2. The results showed that aliphatic carbons (-CH_3 and -CH), olefinic carbon (=CH), thiourea and aromatic carbons involved in the chemical structure of *progesterone-dihydropyrimidine* contribute to high lipophilicity in comparison with *dihydrotestosterone-dihydropyrimidine*. This result is supported by the QSAR studies on structurally for others substances which indicate that substituent involved in their chemical structure induced changes in both the lipofilicity and its activity on glucose levels.[25] In conclusion, the activity induced by the *steroid-dihydropyrimidine* derivatives on glucose levels is conditioned mainly by the contribution of all substituent atoms involved in their chemical structure.

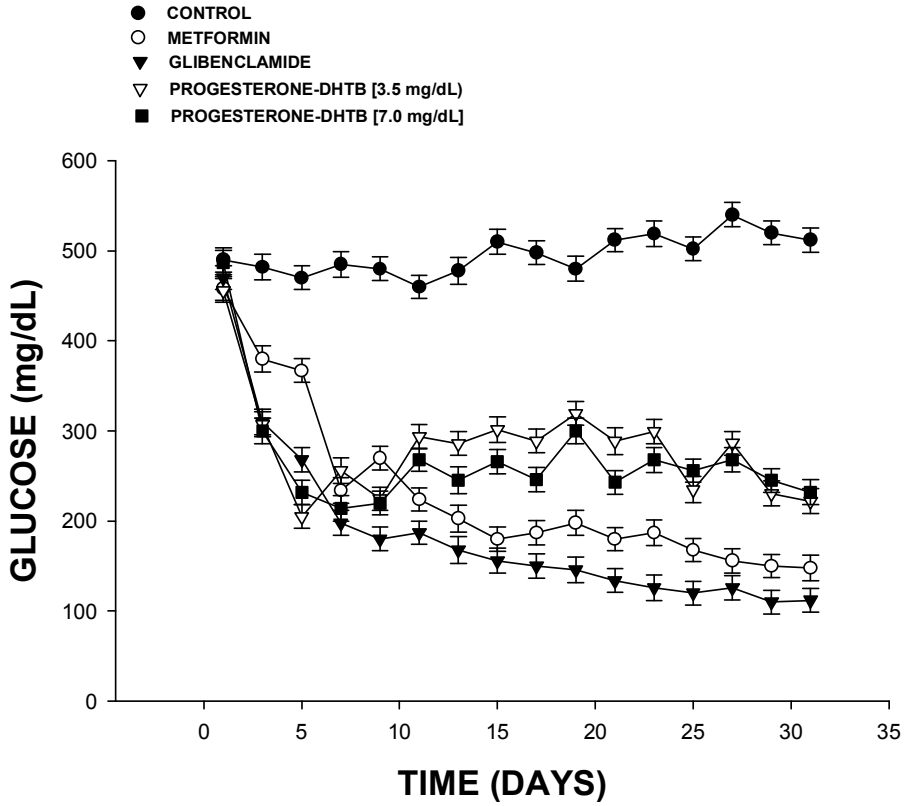

Figure 3. Effect induced by *progesterone-dihydropyrimidine* (*progesterone-DHTB)* on glucose levels in diabetic rat. The results showed that activity induced by *glibenclamide* and metformin on the blood glucose levels is high in comparison with the effect exerted by *progesterone-DHTB* at different doses. The effects are expressed as mean ± S.D.

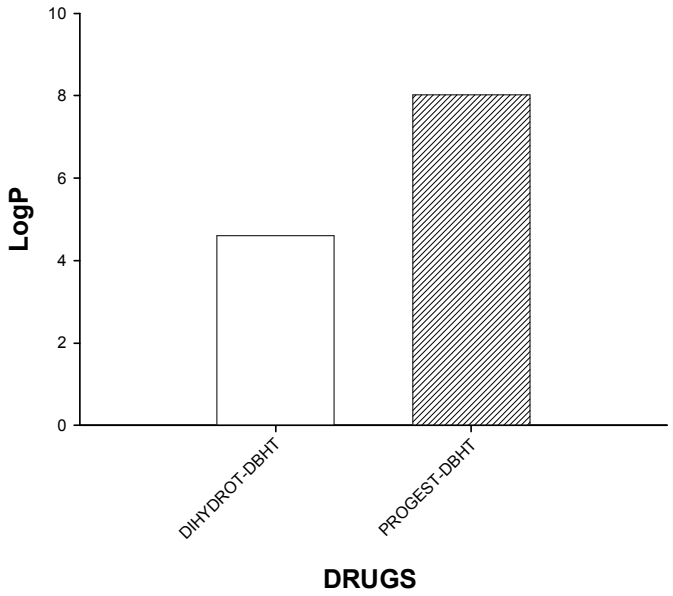

Figure 4. *LogP* of *dihydrotestesterone-dihydropyrimidine* (DIHYDROT-DBHT) and *progesterone-dihydropyrimidine* (PROGEST-DBHT)

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