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8(5), pp. 157-167, 8 February, 2014
DOI: 10.5897/AJPP2013.3908
ISSN 1996-0816 © 2014 Academic Journals
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African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Evaluation of activity exerted by a steroid derivative on injury by ischaemia/reperfusion

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Accepted 3 February, 2014

There are reports which indicate that some steroid derivatives have activity atcardiovascular level; nevertheless, there is scarce information about the effects exerted by the progesterone derivatives on cardiac injury caused by ischemia/reperfusion. In this study, a new steroid (progesterone derivative) was synthetized with the objective of evaluating its activity on ischemia/reperfusion injury. The Langendorff technique was used to evaluate the effect of progesterone derivative on ischemia/reperfusion injury. Additionally, molecular mechanism involved in the activity exerted by the progesterone derivative on perfusion pressure and coronary resistance was evaluated by measuring left ventricular pressure in absence or presence of following compounds; mifepristone, prazosin, metoprolol, indomethacin and nifedipine. The results showed that the progesterone derivative reduces infarct size compared with control. Other results showed that the progesterone derivative significantly increase (p = 0.05) the perfusion pressure and coronary resistance in isolated heart. Other data indicate that the progesterone derivative increase left ventricular pressure in a dose-dependent manner (0.001 to 100 nM); however, this phenomenon was significantly inhibited by nifedipine at a dose of 1 nM (p = 0.05). In conclusion, these data suggest that progesterone derivative exert acardioprotective effect via the calcium channels activation and consequently induces changes in the left ventricular pressure levels. This phenomenon results in decrease of myocardial necrosis after ischemia and reperfusion.

Key words: Steroid, progesterone, left ventricular pressure, indomethacin.

INTRODUCTION

There are several reports which indicate that myocardial infarction is a major cause of death and disability worldwide (Yusuf et al., 2005; Thygesen et al., 2007), this cardiovascular disease is due to cell death of cardiac-myocytes caused by prolonged myocardial ischaemia. Acute myocardial infarction can produce alterations in the topography of both the infarcted and noninfarcted regions of the ventricle (Pfeffer, 1995).

Some reports showed that the most effective method of limiting necrosis is restoration of blood flow; however, the

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effects of reperfusion itself may also be associated with tissue injury (Klone at al., 1989). In search of therapeutic alternatives to reduce myocardial necrosis, some steroid derivatives has been used; for example, a study showed that 17β-estradiol reduced injury by ischemia/reperfusion via production of nitric oxide in an animalmodel (Node et al., 1997). Other studies showed that a progestin (norethindrone acetate) can also reduce ischemia-reperfusion injury in ovariectomized monkeys receiving estrogen therapy previously (Suparto et al., 2005).

Recently, a study indicated that a progesterone derivative induce acardioprotective effect on injury by ischaemia/reperfusion via M2 muscarinic receptor and activation of nitric oxide synthase in an animal model (Figueroa-Valverde et al., 2012). Nevertheless, there are reports which indicate that the administration of medroxyprogesterone acetate can inhibit the effects of estradiol that lead to protection of the myocardium from reperfusion injury and that this involves both neutrophil-dependent and neutrophil-independent mechanisms (Jeanes et al., 2006). Apart from these experiments, which also do not show clearly the cellular site and actual molecular mechanisms of progesterone and its derivatives, data are needed for characterizing the activity induced by this steroid on ischemia-reperfusion injury.

To test this aspect, the present study was designed to investigate the effects induced by a new steroid (progesterone derivative) in a myocardial infarction/ reperfusion model. In addition, molecular mechanism involved in the activity exerted by the progesterone derivative on left ventricular pressure was evaluated using some pharmacological tools for blocking various biological systems such as; mifepristone (progesterone receptor antagonist) (Couzintet et al., 1986), metoprolol (selective β1 receptor blocker) (Bengtsson et al., 1975), prazosin (selective α1 receptor blocker) (Graham et al., 1977), indomethacin (prostanglandin synthesis blocker) (Owen et al., 1975), nifedipine (antagonist of calcium channel) (Henry, 1980).

METHODOLOGY

Chemical synthesis

The compound 1 (1-[(2-Amino-ethylamino)-phenyl-methyl]-naphthalen-2-ol) was prepared according to a previously reported method (Figueroa-Valverde et al., 2013a). The other compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting point for the progesterone derivative was determined on an Electrothermal (900 model). Infrared spectra (IR) were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz (megahertz) in $CDCl_3$ (deuterated chloform) using tetramethylsilane (TMS) as internal standard. Electron impact mass spectroscopy (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/0 2400 elemental analyzer.

Synthesis of N-[2-(1-{3-[2-(2,4-Dinitro-benzenecarbonylamino)-ethylimino]-10,13-dime-thyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl}-ethylideneamino)-ethyl]-2,4-dinitro-benzamide **(compound 3)**

A solution of compound 1 (83 mg, 0.32 mmol), progesterone (100 mg, 0.32 mmol) and boric acid (40 mg, 0.65 mmol) in 10 ml of methanol was stirred for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, the residue washed 3 times with water. Then the precipitate was separated and dried at room temperature.

Synthesis of N-{2-[[1-(3-(N-(3-Chloro-2-oxo-cyclobutyl)-N-{2-[(3-chloro-2-oxo-cyclo-butyl)-amino]-ethyl}-2,4-dinitro-benzencarbonilamino])-10,13-dimethyl-2,3,6,7,8,9, 10,11, 12,13,14,15, 16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-ethyl]-(3-Chloro-2-oxo-cyclo-butyl)-amino]ethyl}-N-(3-Chloro-2-oxo-cyclobutyl)-2,4-dinitro-benzamide (compound 5)

A solution of compound 3 (100 mg, 0.13 mmol), chloroacetyl chloride (30 µl, 0.40 mmol) and triethylamine (55 µl, 0.39 mmol) in 10 ml of methanol was stirred for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, and the residue washed 3 times with water. Then the precipitate was separated and dried at room temperature.

Biological method

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal care and use Committee of University Autonomous of Campeche (No. PI-420/12) and were in accordance with the guide for the care and use of laboratory animals (Bayne, 1996). Male Wistar rats, weighing 200 to 250 g were obtained from University Autonomous of Campeche.

Reagents

All drugs were dissolved in methanol and different dilutions were obtained using Krebs-Henseleit solution (≤ 0.01%, v/v).

Experimental design

Briefly, the male rat (200 to 250 g) was anesthetized by injecting them with pentobarbital at a dose rate of 50 mg/kg body weight. Then the chest was opened, and a loose ligature passed through the ascending aorta. The heart was then rapidly removed and immersed in ice cold physiologic saline solution. The heart was trimmed of non-cardiac tissue and retrograde perfused via a non-circulating perfusion system at a constant flow rate. The perfusion medium was the Krebs-Henseleit solution (pH = 7.4, 37°C) composed of (mmol); 117.8 NaCl; 6 KCl; 1.75 $CaCl_2$; 1.2 NaH_2PO_4; 1.2 $MgSO_4$; 24.2 $NaHCO_3$; 5 glucose and 5 sodium pyruvate. The solution was actively bubbled with a mixture of O_2/CO_2 (95:5/5%). The coronary flow was adjusted with a variable speed peristaltic pump. An initial perfusion rate of 15 ml/min for 5 min was followed by a 15 min equilibration period at a perfusion rate of 10 ml/min. All experimental measurements were done after this equilibration period.

Perfusion pressure

Evaluation of measurements of perfusion pressure changes induced

by drugs administration in this study were assessed using a pressure transducer connected to the chamber where the hearts were mounted and the results entered into a computerized data capture system (Biopac).

Inotropic activity

Contractile function was assessed by measuring left ventricular developed pressure (LV/dP), using a saline-filled latex balloon (0.01 mm, diameter) inserted into the left ventricle via the left atrium (Figueroa-Valverde et al., 2011a). The latex balloon was bound to cannula which was linked to pressure transducer that was connected with the MP100 data acquisition system.

First stage

Ischemia/reperfusion model

After 15 min of equilibration time, the hearts were subjected to ischemia for 30 min by turning off the perfusion system (Booth et al., 2005). After this period, the system was restarted and the hearts were reperfused by 30 min with Krebs-Henseleit solution. The hearts were randomly divided into 2 major treatment groups with n = 9:

Group I: Hearts were subjected to ischemia/reperfusion but received vehicle only (Krebs-Henseleit solution).
Group II: Hearts were subjected to ischemia/reperfusion and treated with the progesterone derivative (compound 5) at a dose of 0.001 nM before ischemia period (for 10 min) and during the entire period of reperfusion.

At the end of each experiment, the perfusion pump was stopped, and 0.5 ml of fluoresce in solution (0.10%) was injected slowly through a side arm port connected to the aortic cannula. The dye was passed through the heart for 10 s to ensure its uniform tissue distribution. The presence of fluorescein was used to demarcate the tissue that was not subjected to regional ischemia, as opposed to the risk region. The heart was removed from the perfusion apparatus and cut into two transverse sections at right angles to the vertical axis. The right ventricle, apex and atrial tissue were discarded. The areas of the normal left ventricle non risk region, area at risk, and infarct region were determined using the technique reported by Boot and coworkers (2005). Total area at risk was expressed as the percentage of the left ventricle.

Second stage

Effect induced by the progesterone derivatives (compound 3 and 5) on perfusion pressure

Changes in perfusion pressure as a consequence of increases in time (3 to 18 min) in absence (control) or presence of the progesterone derivatives at a concentration of 0.001 nM were determined. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min.

Evaluation of effects exerted by the progesterone derivatives (compound 3 and 5) on coronary resistance

The coronary resistance in absence (control) or presence of the progesterone derivatives at a concentration of 0.001 nM was evaluated. The effects were obtained in isolated hearts perfused at a constant flow rate of 10 ml/min. Since a constant flow was used, changes in coronary pressure reflected the changes in coronary resistance (Figueroa-Valverde et al., 2012).

Third stage

Effects induced by the progesterone derivative (compound 5) on left ventricular pressure through progestagen receptors

Intracoronary boluses (50 µl) of the compound 5 (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of mifeprestone at a concentration of 1 nM (duration of preincubation with mifeprestone was by a 10 min equilibration period).

Effects induced by the progesterone derivative (compound 5) on left ventricular pressurethrough β1-adrenergic receptor

Intracoronary boluses (50 µl) of the compound 5 (0.001 to 100 nM) were administered and the corresponding effect on the left ventricular pressure was determined. The dose-response curve (control) was repeated in the presence of metoprolol at a concentration of 1 nM (duration of preincubation with metoprolol was by a 10 min equilibration period).

Effect exerted by the progesterone derivative (compound 5) on left ventricular pressure inthe presence of indomethacin

The boluses (50 µl) of the compound 5 (0.001 to100 nM) were administered and the corresponding effect on the left ventricular pressure was evaluated. The bolus injection administered was done in the point of cannulation. The dose response curve (control) was repeated in the presence of indomethacin at a concentration of 1 nM (duration of the pre-incubation with indomethacin was for a period of 10 min).

Effects of the progesterone derivative (compound 5) on left ventricular pressure throughthe calcium channel

Intracoronary boluses (50 µl) of the compound 5 (0.001 to100 nM) were administered and the corresponding effect on the left ventricular pressure was evaluated. The dose-response curve (control) was repeated in the presence of nifedipine at a concentration of 1 nM (duration of the pre-incubation with nifedipine wasfor a period of 10 min).

Statistical analysis

The obtained values are expressed as average ± SE, using each heart (n = 9) as its own control. The data obtained were put under analysis of variance (ANOVA) with the Bonferroni correction factor using the SPSS 12.0 program (Hocht et al., 1999). The differences were considered significant when *p* was equal or smaller than 0.05.

RESULTS

Chemical synthesis

The yield of the reaction product (compound 3) (Figure 1) was 64% with melting point of 172 to 174°C. In addition, the spectroscopic analyses show signals for IR (V_{max}, cm^{-1})

Table 1. 1H NMR (300 MHz, CDCl3) data for the compound 3.

Parameter
0.85 (s, 3H), 0.98-1.02 (m, 2H), 1.19 (s, 3H), 1.28-1.56 (m, 5H), 1.58 (s, 3H), 1.66-2.39 (m, 12H), 3.47 (t, 2H, *J* = 6.54), 3.67 (t, 2H, *J* = 6.54), 3.97 (t, 2H, *J* = 6.54), 3.99 (t, 2H, *J* = 6.54), 5.74 (m, 1H), 7.70 (t, 2H), 8.10-8.80 (m, 6H) ppm

Table 2. 13C NMR (300 MHz, CDCl3) data for the compound 3.

Parameter
13.20 (**C**-18), 17.12 (**C**-32), 21.16 (**C**-5), 22.52 (**C**-33), 25.28 (**C**-9), 26.32 (**C**-8), 30.40 (**C**-15), 31.13 (**C**-16), 31.31 (**C**-11), 31.60 (**C**-10), 35.26 (**C**-17), 35.61 (**C**-3), 35.84 (**C**-28), 36.42 (**C**-22), 38.04 (**C**-6), 42.80 (**C**-1), 51.97 (**C**-4), 54.20 (**C**-21), 54.38 (**C**-27), 56.52(**C**-2), 56.63 (**C**-7), 115.50 (**C**-13), 120.66 (**C**-36, **C**-42), 128.04 (**C**-38, **C**-44), 128.18 (**C**-39, **C**-45), 141.48 (**C**-34, **C**-40), 143.43 (**C**-35, **C**-41), 147.50 (**C**-37, **C**-43), 158.02 (**C**-12), 162.60 (**C**-19), 163.34 (**C**-24, **C**-30), 165.90 (**C**-14) ppm

Table 3. ^{1}H NMR (300 MHz, CDCl3) data for the compound 5.

Parameter
0.67 (s, 3H), 0.69 (m, 1H), 0.83 (m, 1H), 0.84 m, 1H), 0.93 (s, 3H), 1.07-1.67 (m, 11H), 1.66-1.70 (m, 2H), 1.71-1.94 (m, 4H), 2.00 (m, 1H), 2.07-2.22 (m, 3H), 2.37 (m, 2H), 2.64 (m, 1H), 2.65 (m, 2H), 2.67-2.75 (m, 4H), 3.49 (m, 1H), 3.60-3.62 (m, 2H), 3.64 (m, 1H), 3.65 (t, 2H,*J* = 6.25 Hz), 3.67-4.66 (m, 8H),4.86 (m, 1H), 8.30-8.80 (m, 6H) ppm

Table 4. ^{13}C NMR (300 MHz, CDCl3) data for the compound 5.

Parameter
14.30 (**C**-33), 15.20 (**C**-18), 19.00 (**C**-32), 21.12 (**C**-5), 28.25 (**C**-15), 28.36(**C**-8), 31.45 (**C**-11), 31.50 (**C**-10), 31.95 (**C**-39, **C**-53), 32.40(**C**-49), 32.66 (**C**-35), 34.40 (**C**-16), 36.16 (**C**-22), 38.24 (**C**-17), 39.10 (**C**-6), 42.80 (**C**-28), 43.05 (**C**-1), 50.70 (**C**-21), 50.76 (**C**-27), 54.58 (**C**-19), 54.70 (**C**-2), 55.70 (**C**-4), 56.22(**C**-7), 59.00 (**C**-14), 60.50 (**C**-40, **C**-54), 62.88 (**C**-36, **C**-50), 69.50 (**C**-38, **C**-52), 69.70 (**C**-48), 70.00 (**C**-34), 120.70 (**C**-44,**C**-58), 121.40 (**C**-13), 127.68 (**C**-47,**C**-61), 127.90 (**C**-46, **C**-60), 138.97 (**C**-42, **C**-56), 143.10 (**C**-57), 146.76 (**C**-12), 147.44 (**C**-45,**C**-59), 164.90 (**C**-24, **C**-30), 199.80 (**C**-41, **C**-55), 20.56(**C**-51), 202.12 (**C**-37) ppm

at 3338, 1648 and 1350. In addition, the chemical shifts of the spectroscopic analyses of ^{1}H NMR and^{13}C NMR for the compound 3 are shown in Tables 1 and 2. Finally, the results of mass spectroscopy (MS) (70 eV) was shown as m/z 786.30. Additionally, the elementary analysis data for the compound 3 ($C_{39}H_{46}Cl_4N_6O_{10}$) were calculated (C, 59.53; H, 5.99; N, 14.24; O, 20.33) and found (C, 59.50; H, 5.96). On the other hand, the yield obtained for the compound 5 (Figure 2) was 74% with melting point of 144 to 146°C. The spectroscopic analyses show signals for IR (V_{max}, cm^{-1}) at 3338, 1712, 1654, 1352 and 1200. In addition, the chemical shifts of the spectroscopic analyses of ^{1}H NMR and^{13}C NMR for the compound 5 are shown in Tables 3 and 4. Finally, the results of mass spectroscopy (MS) (70 eV) was shown as m/z 1198.30. Additionally, the elementary analysis data for the compound 3 ($C_{55}H_{62}Cl_4N_8O_{14}$) were calculated (C, 55.01; H, 5.20; Cl, 11.81; N, 9.33; O, 18.55) and found (C, 55.00; H, 5.18).

Biological activity

First stage

Effect of progesterone derivative (compound 5) on ischemia/reperfusion injury: The results (Figure 3) showed that the benzamide derivative reduces infarct size expressed as a percentage of the area at risk compared with vehicle-treated hearts (control).

Second stage

The activity exerted by the progesterone and its derivatives (compound 3 and 5) on perfusion pressure and coronary resistance in the isolated rat hearts was evaluated. The results obtained from changes in perfusion pressure as a consequence of increases in the time (3 to18 min) in absence (control) or in presence of the progesterone derivatives (Figure 4), showed that compound 5 (0.001 nM) significantly increase the perfusion pressure (p = 0.05) in comparison with the control conditions, progesterone and the compound 3 at a dose of 0.001 nM. In addition, another result (Figure 5) showed that coronary resistance calculated as the ratio of perfusion pressure at coronary flow assayed (10 ml/min) was higher in the presence of the compound 5 (p = 0.05) in comparison with the control conditions and progesterone and the compound 3 at a dose of 0.001 nM.

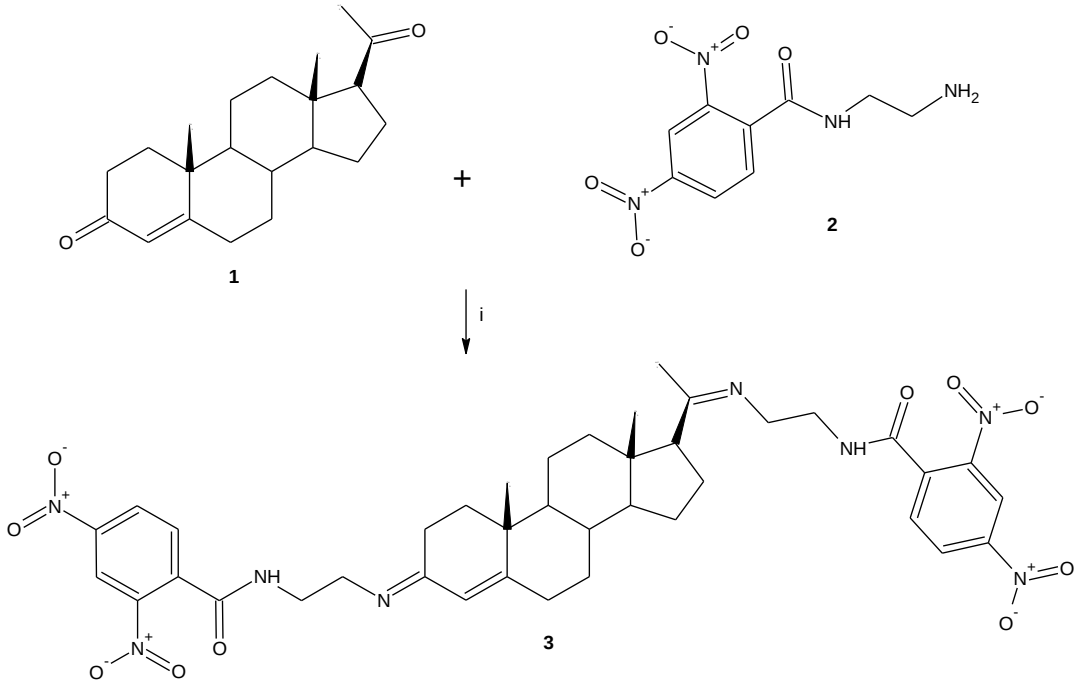

Figure 1. Synthesis of *N*-[2-(1-{3-[2-(2,4-Dinitro-benzenecarbonylamino)-ethylimino]-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phe-nanthren-17-yl}-ethylideneamino)-ethyl]-2,4-dinitro-benzamide (3). Reaction ofprogesterone (1) with 1-[(2-Amino-ethylamino)-phenyl-methyl]-naphthalen-2-ol (2) to form the compound (3) i = boric acid

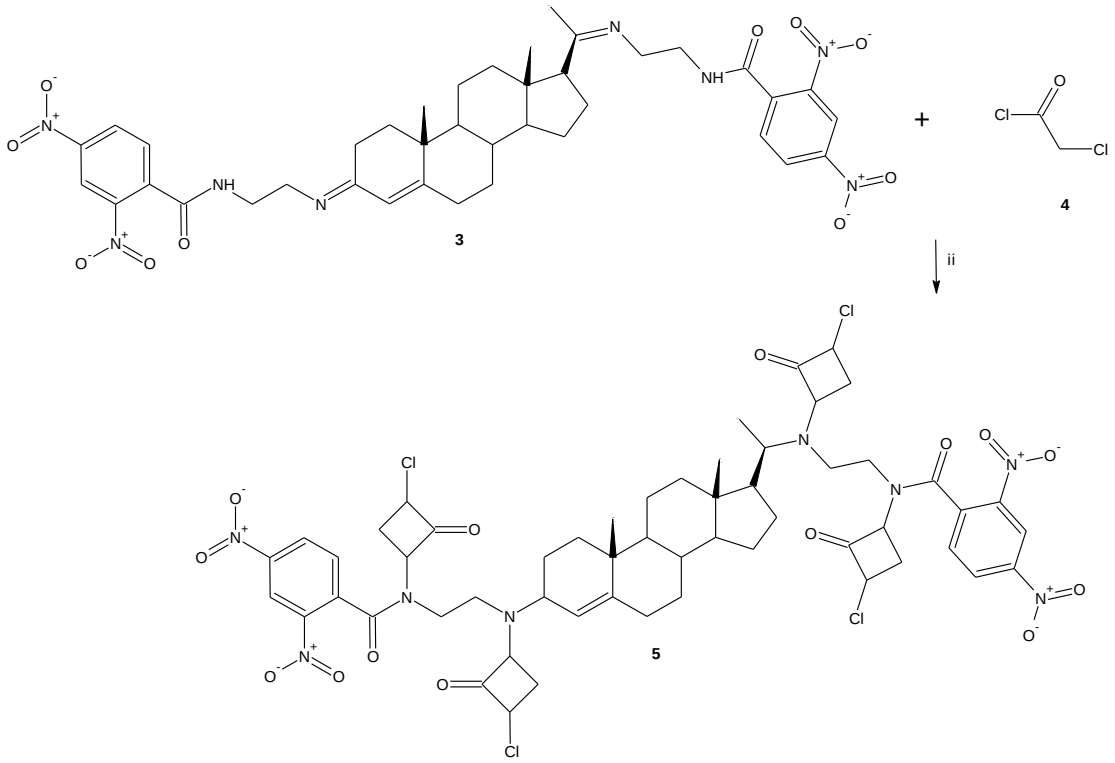

Figure 2. Synthesis of *N*-{2-[[1-(3-(*N*-(3-Chloro-2-oxo-cyclobutyl)-*N*-{2-[(3-chloro-2-oxo-cyclobutyl)-amino]-ethyl}-2,4-dinitro-benzencarbonilamino])-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15, 16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-ethyl]-
(3-Chloro-2-oxo-cyclo-butyl)-amino]ethyl}-*N*-(3-Chloro-2-oxo-cyclobutyl)-2,4-dinitro- benzamide(5). Reaction of 3 with chloroacetyl chloride to form 5.ii = triethylamine.

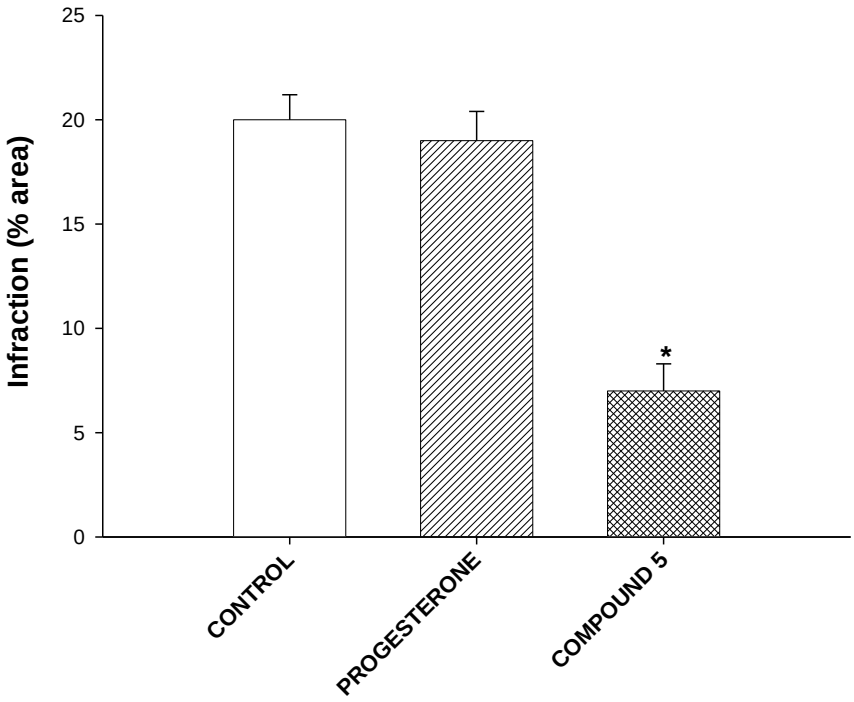

Figure 3. Effect exerted by the progesterone derivative (compound 5) on cardiac ischemia/reperfusion.The results showed that the progesterone derivative (0.001 nM) significantly reduced (p = 0.05) infarct size expressed as a percentage of the area at risk compared with progesterone (0.001 nM) and the vehicle-treated hearts. Each bar represents the mean ± SE of 9 experiments.

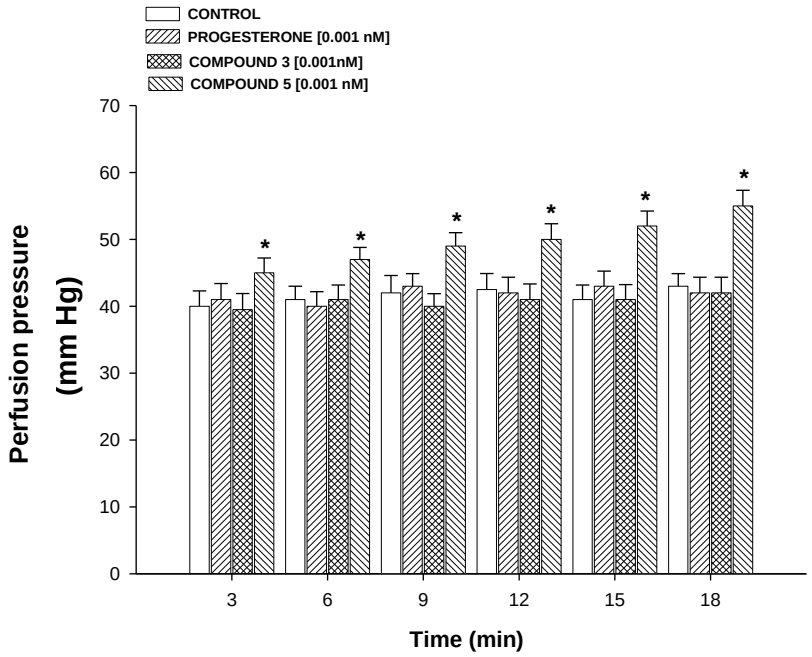

Figure 4. Effect induced by the progesterone derivatives (compound 3 and 5) on perfusion pressure. The results showed that the progesterone derivative significantly increase perfusion pressure (p = 0.05) through time in comparison with progesterone, the compound 3 and the control conditions. Each bar represents the mean ± SE of 9 experiments.

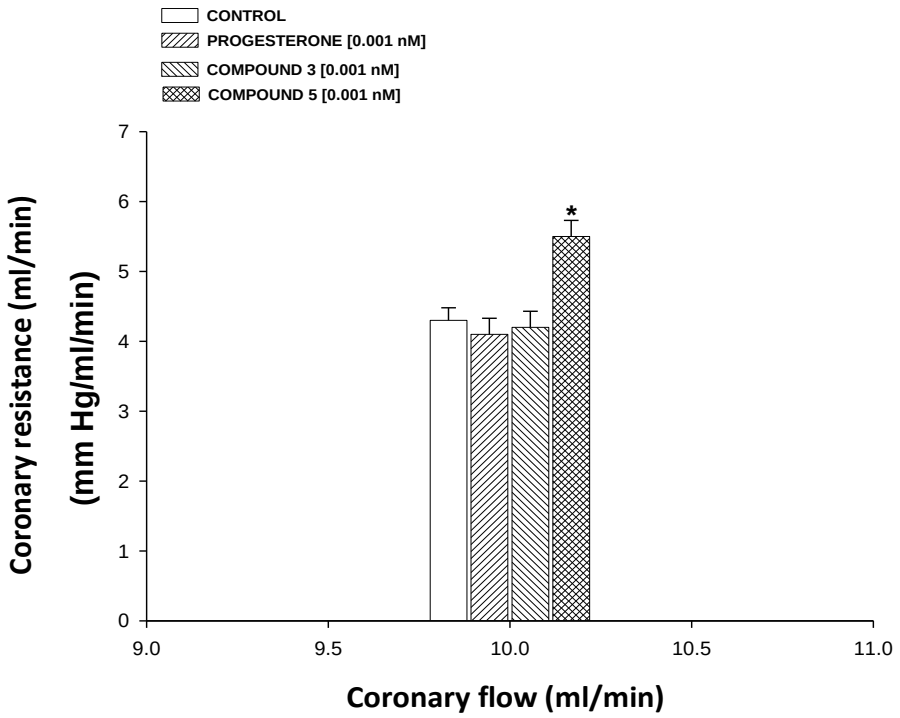

Figure 5.Activity exerted by the progesterone derivatives (compounds 3 and 5) on coronary resistance. The results show that coronary resistance was higher (p = 0.05) in the presence of the compound 5 in comparison with the control conditions, progesterone and the compound 3. Each bar represents the mean ± SE of 9 experiments.

Third stage

Other results showed that activity exerted by the compound 5 (0.001 to 100 nM) increased the left ventricular pressure and this effect was not inhibited in presence of mifepristone (Figure 6), metoprolol, prazosin (Figure 7) or indomethacin (Figure 8) drugs at a concentration of 1 nM. Finally, other data obtained (Figure 9) indicate that the progesterone derivative (compound 5) induces an increase in left ventricular pressure in a dose dependent manner (0.001 to 100 nM) and this effect was significantly inhibited by nifedipine (p = 0.05) at a dose of 1 nM.

DISCUSSION

Chemical synthesis

In this work we report some straight forward routes for the preparation of compound 5 (*N*-{2-[[1-(3-(*N*-(3-Chloro-2-oxo-cyclobutyl)-*N*-{2-[(3-chloro-2-oxo-cyclobutyl)-amino]-ethyl}-2,4-dinitro benzencarbonilamino])-10,13-dimethyl-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-ethyl]-(3-Chloro-2-oxo-cyclo-butyl)-amino] ethyl}-*N*-(3-Chloro-2-oxo-cyclobutyl)-2,4-dinitro-benzamide). The first stage involve the synthesis of the compound 3 (*N*-[2-(1-{3-[2-(2,4-Dinitrobenzenecarbonylamino)-ethylimino]-10,13-dimethyl 2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclo- penta[*a*]phenanthren-17-yl}-ethylideneamino)-ethyl]-2,4-dinitrobenzamide) which have an imino group (Schiff base) involved in their chemical structure (Figure 1). There are several procedures for the synthesis of imino groups which are described in the literature (Shirayev et al., 2005; Uppiah et al., 2009; Figueroa-Valverde et al., 2013b).

In this study, the synthesis of the compound 3 was developed by the reaction of compound 1 (1-[(2-Amino-ethylamino)-phenyl-methyl]-naphthalen-2-ol) with progesterone using boric acid as catalyst to form the compound 3. The structure of 3 was confirmed using IR and NMR spectroscopy (Tables 1 and 2). The IR spectra contained characteristic vibrations at 3338 for imino group; at 1648 for amide group and 1350 for nitro groups. The ^{1}H NMR spectrum of the compound 3 shows signals at 0.85 and 1.19 ppm for methyl groups bound to steroid nucleus; at 1.58 ppm for methyl group bound to methanimine group; at 0.98 to 1.02, 1.28 to 1.56, 1.66 to 2.39 and 5.74 ppm for steroidnucleus; at 3.47 to 3.99 ppm for methylene groups bound to both imino and amide groups. Finally, other signals at 7.70 ppm for both amide groups; at 8.10 to 8.80 ppm for phenyl groups were found.

On the other hand, the ^{13}C NMR spectra displays chemical shifts at 13.20 and 17.12 ppm for both methyl groups bound to steroid nucleus; at 22.52 ppm for methyl group bound to imino group; at 21.16, 22.28 to 35.61,

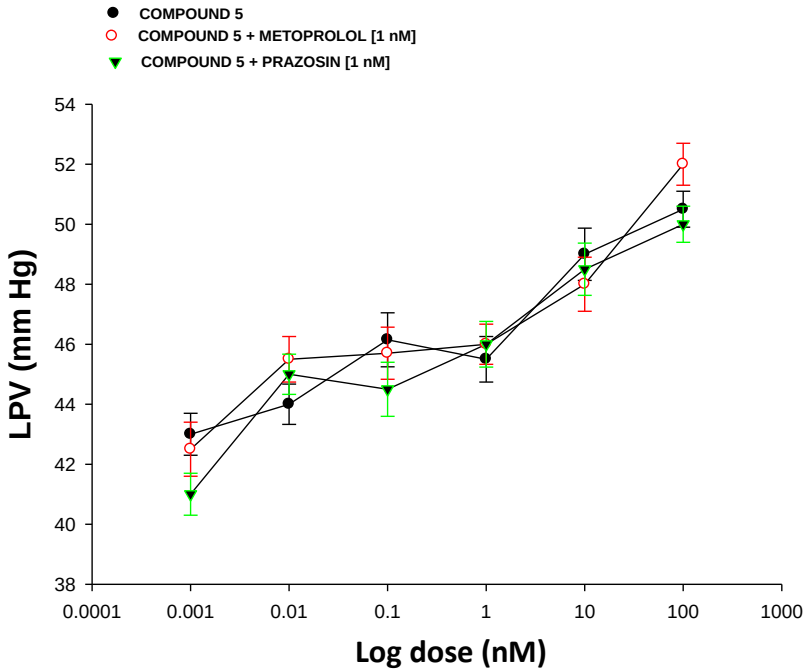

Figure 7. Activity exerted by the progesterone derivative (compound 5) on LVP through of adrenergic receptors. Progesterone derivative [0.001 to 100 nM] was administered (intracoronary boluses, 50 µl) and the corresponding effect on the LVP was evaluated in the absence and presence of prazosin or metoprolol. The results showed that activity induced by the progesterone derivative on LVP was not inhibited in the presence of prazosin ormetoprolol. Each bar represents the mean ± SE of 9 experiments. LVP = left ventricular pressure.

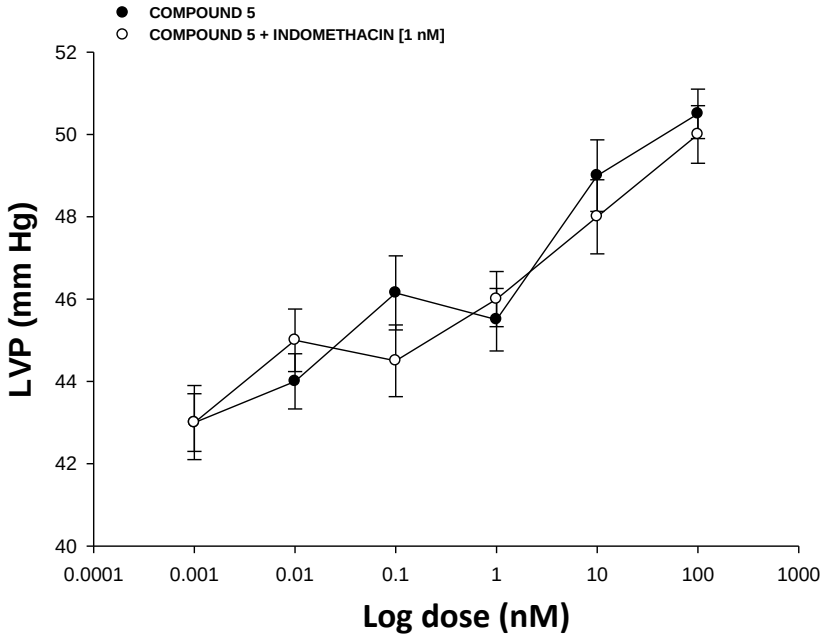

Figure 8. Effects induced by the progesterone derivative (compound 5) on LVP through prostaglandins synthesis. Intracoronary boluses (50 µl) of the progesterone derivative [0.001 to 100 nM] were administered and the corresponding effect on the LVP was determined in the absence and presence of indomethacin. The results showed that progesterone derivative increase the LVP in a dependent dose manner and this effect was not inhibited in the presence of indomethacin.
Each bar presents the mean ± SE of 9 experiments. LVP = left ventricular pressure.

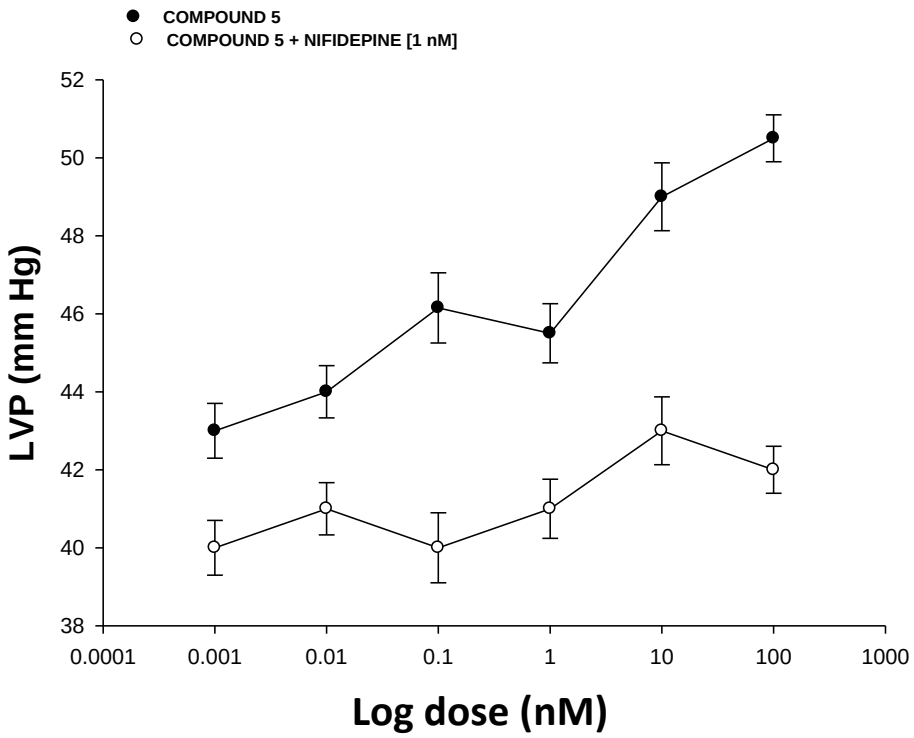

Figure 9. Effects induced by the progesterone derivative (compound 5) on LVP through calcium channel activation. Intracoronary boluses (50 µl) of the progesterone derivative (0.001 to 100 nM) were administered and the corresponding effect on the LVP was determined in the absence and presence of nifedepine. The results showed that the progesterone derivative increase the LVP in a dependent dose manner and this effect was significantly inhibited (p = 0.05) in the presence of nifedipine.
Each bar represents the mean ± SE of 9 experiments. LVP = left ventricular pressure.

38.04 to 51.97, 56.52 to 115.50, 158.02 and 165.90 ppm for steroid nucleus; at 36.42 to 54.20 for methylene groups bound to both imino and amide groups; at 120.66 to 147.50 ppm for both phenyl groups; at 162.60 ppm for carbon bound to both imino and methyl groups; at 163.34 ppm for both amide groups. Finally, the presence of benzamide derivative was further confirmed from mass spectrum which showed a molecular ion at m/z 786.30.

The second stage was achieved by the reaction of the compound 3 with chloroacetylchloride in presence of triethylamine to form the compound 5; this compound had characteristic of a cyclobutanone group in its chemical structure (Figure 2). The IR spectra contained characteristic vibrations at 1200 for amino group; at 1654 for amide group; at 1712 for carbonyl groups and 1352 for nitro groups. The ^{1}H NMR spectrum of the compound 5 (Tables 3 and 4) shows signals at 0.67 and 0.95 ppm for both methyl groups bound to steroid nucleus; at 0.83 for methyl group bound to methanoamino group; at 0.69, 0.83, 1.07 to 1.67, 1.77 to 1.94, 2.07 to 2.22, 2.64, 3.64 and 4.86 ppm for steroid nucleus; at 1.66 to 2.00, 2.37, 2.65, 3.67 to 4.66 ppm for cyclobutanone groups; at 2.67 to 2.75 and 3.60 to 3.62 and 3.65 to 3.67 ppm for methylene groups involved in the arms bound to both amide and amine groups; at 3.48 ppm for methylene group bound to both methyl and amino group; at 8.30 to 8.80 ppm for phenyl groups.

In addition, the ^{13}C NMR spectra displays chemical shifts at 14.30 ppm for methyl bound to methanoamino group; at 15.20 to 19.00 ppm for methyl groups bound to steroid nucleus; at 21.12 to 31.50, 34.40 to 39.10. 43.70, 54.70 to 59.00, 121.40 and 146.70 ppm for steroid nucleus; at 31.95 to 32.66 and 60.50 to 70.00 ppm for cyclobutanone groups; at 42.80 to 50.76 ppm for methylene groups involved in the arms bound to both amide and amine groups; at 54.58 ppm for carbon bound to both methyl and amino groups; at 164.90 ppm for both amide groups; at 127.68 to 143.10, and 147.44 for phenyl groups; at 164.90 for both amide groups; at 199.80 to 202.12 ppm for ketone groups. Finally, the presence of compound 5 was further confirmed from mass spectrum which showed a molecular ion at m/z 1198.30.

Biological evaluation

In this study, the activity of progesterone and its derivative (compound 5) on injury byischaemia/reperfusion in an isolated heart model was evaluated. The results showed that this progesterone derivative reduced

infarct size (expressed as a percentage of the area at risk) compared with both progesterone and vehicle-treated hearts. This effect can be conditioned by structured chemical difference between the progesterone and the compound 5 which may consequently bring activation of some biological structure (p.e. ionic channels or specific receptors) involved in the endothelium of coronary artery (Bouïs et al., 2000) or by the influence exerted by the progesterone derivative on blood pressure which consequently bring reduction in the infarct size, and decrease the myocardial injury after ischemia/reperfusion similar to other reports for other compounds such as estrogens (Beer et al., 2002).

In order to evaluate this hypothesis, the activity induced by the compound 5 on blood vessel capacity and coronary resistance translated as changes in perfusion pressure was evaluated in an isolated rat heart model. The results showed that the compound 5 significantly increase the perfusion pressure over time (3 to 18 min) compared with progesterone and the control conditions. Analyzing this data and evaluating the possibility of that cyclobutanone group involved in the chemical structure of compound 5 could be by itself responsible for their activity; in this study, the compound 3 was used such pharmacological tool for evaluate its effects exerted on perfusion pressure.

The results obtained indicate that compound 3 did not exert significant effects on perfusion pressure in comparison with the compound 5. These data suggest that activity induced by the compound 5 on perfusion pressure could modify vascular tone and coronary resistance of heart. Therefore, in this study the activity exerted by the compound 5 on coronary resistance was evaluated. The results indicate that coronary resistance was increased in presence of this compound in comparison with progesterone and the compound 3. These data suggest that the compound 5 exerts effect on vascular tone through generation or activation of vasoactive substances such as intracellular nitric oxide, calcium and others, and other such happening with other type of compounds such as the carbamazepine-alkyne derivative (Figueroa-Valverde et al., 2011a).

In order to characterize the molecular mechanism of this phenomenon we analyzed the reports of some investigations which indicate that progesterone induces its effect on blood pressure via activation of the progesterone receptor (Bayliss et al., 1987; Williams and Sigler, 1998). In this study, mifepristone (a progesterone receptor blocker) was used to determine if the effects of progesterone derivative on perfusion pressure were via the progesterone receptor. It is important to mention that interaction of progesterone-derivative with the progesterone receptor may be a key requirement for the biological activity as in the case of other progesterone derivatives (Melcangi et al., 1999). Our results showed that the effect of progesterone derivative was not inhibited by mifepristone, suggesting that the molecular mechanism is not via the progesterone receptor.

Therefore, in search of molecular mechanism involved in the activity of the progesterone derivative (compound 5), another study was analyzed which indicates that progesterone may stimulate synthesis or release of catecholamines (Tollan et al., 1993) which has an important role in the development or maintenance of elevated blood pressure (Lilley et al., 1976). In this sense, the effect exerted by the progesterone derivative on left ventricular pressure was evaluated in the absence or presence of prazosin or metoprolol. The results showed that the effect induced by the compound 5 was not inhibited in the presence of prazosin or metoprolol. These data suggest that the molecular mechanism involved in the activity of progesterone derivative is not via adrenergic system. Therefore, in the search of the molecular mechanism involved in activity induced by the progesterone-derivative on left ventricular pressure and analyzing previous reports, which indicate that some steroid derivatives exert effects on perfusion pressure via prostaglandins synthesis (Sheillan et al., 1983) and to evaluate the possibility that the activities induced by the compound 5 involve stimulation and secretion of prostaglandins.

In this experimental study, the activity exerted by the progesterone derivative on left ventricular pressure in the absence or presence of indomethacin was evaluated. The results showed that effect induced by the progesterone derivative on left ventricular pressure was not blocked by indomethacin. These data indicate that the molecular mechanism involved in the effect exerted by the progesterone derivative was not via prostaglandins.

Analyzing these experimental data and other reports which indicate that some steroid derivatives can induce changes to blood pressure by increasing calcium levels (Figueroa-Valverde et al., 2011b) and analyzing some reports which indicates that some positive cardiotonic agents act by an increase in intracellular Ca^{2+} and consequently induce an increase in the sensitivity of contractile proteins to Ca^{2+} ions or by combinations of the two mechanisms (Bowman et al., 1999). Therefore, in this study, we also considered validating the effect induced by the progesterone-derivative (compound 5) on left ventricular pressure via the calcium channels activation using it as pharmacological tool to nifedipine. The results showed that the effect induced by the progesterone derivative was significantly inhibited in the presence of nifedipine. All these data suggest that the molecular mechanism involved in the activity of the progesterone derivative is via the calcium channels activation. This phenomenon is similar to activity exerted by other drugs on left ventricular pressure (Thiemermann et al., 1997) which may contribute to decrease cell death caused by ischemia/reperfusion in men.

Conclusion

The progesterone derivative is a particularly interesting

drug, because the activity induced for this compound on injury by ischemia/reperfusion involves a molecular mechanism different in comparison with other drugs. This phenomenon may constitute a novel therapy for ischemia/ reperfusion injury.

REFERENCES

Bayliss A, Millhorn D, Gallman E (1987). Progesterone stimulates respiration through a central nervous system steroid receptor-mediated mechanism in cat. Proc. Nat. Acad Sci. 84:788-7792.

Bayne K (1996). "Revised Guide for the Care and Use of LaboratoryAnimals Available," The Physiologist. 9:208-211.

Beer S, Reincke M, Kral M, Lie S, Schmidt W, Allolio B, Neubauer S (2002). Susceptibility to cardiac ischemia/reperfusion injury is modulated by chronic estrogen status. J. Cardiov. Pharmacol. 40:420-428.

Bengtsson C, Johnsson G, Regårdh C (1975). Plasma levels and effects of metoprolol on blood pressure and heart rate in hypertensive patients after an acute dose and between two doses during long-term treatment.Clin.Pharmacol.Ther. 17:400-408.

Booth E, Obeid N, Lucchesi B (2005). Activation of estrogen receptor-α protects the in vivo rabbit heart from ischemia-reperfusion injury. AJP-Heart. 289:5H2039-H2047.

Bouïs D, Hospers G, Meijer C, Molema G, Mulder N (2000). Endothelium *in vitro:* A review of human vascular endothelial cell lines for blood vessel-related research. Angiogenesis. 4:91-102.

Bowman P, Haikala H,Paul R (1999). Levosimendan, a calcium sensitizer in cardiac muscle, induces relaxation in coronary smooth muscle through calcium desensitization. J. Pharmacol. Exp. Ther. 288:316-325.

Couzinet B, Le-Strat N, Ulmann A, Baulieu E, Schaison G (1986).Termination of Early Pregnancy by the Progesterone Antagonist RU 486 (Mifepristone). N. Engl. J. Med. 315:1565-1570.

Figueroa-Valverde L, Díaz-Cedillo F, López-Ramos M (2011a). Inotropic Activity Induced by Carbamazepine-AlKyne Derivative in an Isolated Heart Model and Perfusedto Constant Flow. Biomedica 31:232-241

Figueroa-Valverde L, Díaz-Cedillo F, López-Ramos M, García-Cervera E, Pool-Gómez E (2011b). Design and synthesis of an estradiol derivative and evaluation of its inotropic activity in isolated rat heart. Afr. J. Pharm. Pharmacol. 5:1703-1712.

Figueroa-Valverde L, Diaz-Cedillo F, Garcia-Cervera E (2012). Effect of progesterone-carbachol derivative on perfusion pressure and coronary resistance in isolated rat heart: via activation of the M_2 muscarinic receptor. Biomed. Pap. 156:XX. DOI 10.5507/bp.2012.010.

Figueroa-Valverde L, Díaz-Cedillo F, García-Cervera E, Pool-Gómez E, López-Ramos M, Camacho-Luis A (2013a). Design and Synthesis of N-(2-{[(2-Hydroxy-naphtalen-1-yl)-phenyl-methyl]-amino}-ethyl)-3,4-dinitro-benzamide. Oriental J. Chem. 29:17-22.

Figueroa-Valverde L, Díaz-Cedillo F, García-Cervera E (2013b). Design and Synthesis of an Aromatic-steroid Derivative.*Oriental* J. Chem. 29:465-468.

Graham R, Oates H, Stoker L, Stokes G (1977). Alpha blocking action of the antihypertensive agent, prazosin. J. Pharmacol. Exper.Ther. 201:747-752.

Henry PD (1980). Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. Am. J. Cardiol. 46:1047-1058.

Hocht C, Opezzo L, Gorzalczany S (1999). Una AproximaciónCinética y Dinámica de Metildopa en Ratas conCoartación Aórtica Mediante Microdiálisis. Rev. Argent. Cardiol. 67:769-773.

Jeanes H, Wanikiat P, Sharif I (2006). Medroxyprogesterone acetate inhibits the cardioprotective effect of estrogen in experimental ischemia-reperfusion injury.Menopause.13:80-86.

Klone R, Przyklener K, Whittaker P (1989). Deleterious effects of oxygen radicals in ischemia/reperfusion.Resolved and unresolved issues.Circulation. 80:1115-1127.

Lilley J, Golden J, Stone R (1976). Adrenergic regulation of blood pressure in chronic renal failure. J. Clin. Investig. 57:1190-1200.

Melcangi R, Magnaghi V, Cavarretta I (1999).Progesterone derivatives are able to influence peripheral myelin protein 22 and P_0 gene expression: Possible mechanisms of action. J. Neurosci. Res. 56:349-357.

Node K, Kitakaze M, Kosaka H (1997). Amelioration of Ischemia-and Reperfusion-Induced Myocardial Injury by 17β-Estradiol Role of Nitric Oxide and Calcium-Activated Potassium Channels. Circulation 96:1953-1963.

Owen T, Ehrhart I, Weidner W, Scott J, Haddy F (1975). Effects ofindomethacin on local blood flow regulation in canine heart and kidney. Exp. Biol. Med. 149:871-876.

Pfeffer M (1995). Left Ventricular remodeling after acute myocardial infarction. Ann. Rev. Med. 46:455-466 (1995).

Sheillan C, Ody C, Russo F, Duval D (1983). Differential aspects of sex steroids on prostaglandin secretion by male and female dultured piglet endothelial cells. Prostaglandins 26:3-12.

Shirayev A, Moiseev I, Karpeev S (2005). Synthesis and cis/trans isomerism of N-alkyl-1, 3-oxathiolane-2-imines. Arkivok 4:199-207.

Suparto I, Koudy W, Fox J (2005). A comparison of two progestins on myocardial ischemia-reperfusion injury in ovariectomized monkeys receiving estrogen therapy. Coronary Artery Dis. 16:301-308.

Thiemermann C, Bowes J, Myint F, Vane J (1997). Inhibition of the activity of poly(ADP ribose) synthetase reduces ischemia–reperfusion injury in the heart and skeletal muscle. Proc. Natl. Acad. Sci. 94:679-683.

Thygesen K, Alpert J, White H (2007). Universal Definition of Myocardial Infarction Free. J. Am. Coll. Cardiol. 50:2173-2195.

Tollan A, Oian P, Kjeldsen S, Eide I, Maltau J (1993). Progesterone reduces sympathetic tone without changing blood pressure or fluid balance in men. Gynecol. Obstet. Investig. 36:234-238.

Uppiah D, Gupta M, Jhaumeer S (2009). Solventless synthesis of imines derived from diphenyldisulphidediamine or p-vanillin. E-J Chem. 6:S195-S200.

Williams S, Sigler P (1998). Atomic structure of progesterone complexed with its receptor. Nature 393(6683):392–396.

Yusuf S, Hawken S, Ounpuu S (2005). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 366:1640-1649.