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ORIGINAL ARTICLE **OPEN ACCESS**

Design and Synthesis of a new Steroid-derivative with Inotropic activity

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ABSTRACT

Several drugs have been used for the treatment of heart failure; however the adverse effects induced by some drugs affects their use on a large scale. In the search for therapeutic alternatives it has been evaluated inotropic activity of some derivatives of steroids in several biological models; however, the cell site and mechanism of action at cardiovascular level is very confusing. The aim of this study was evaluate the biological activity of a new steroid derivative on heart failure in an isolated rat heart. In addition, to characterize the molecular mechanism involved in the inotropic activity induced by the estrone-derivative, several alternative experiments were carried out. In this sense, the effect exerted by the estrone derivative on left ventricular pressure in the absence or presence of propranolol, prazosin, metoprolol, nifedipine and indomethacin was evaluated. The results showed that the steroid derivative significantly increased the perfusion pressure and coronary resistance in comparison with the control conditions. Additionally, other data indicate that estrone derivative increase left ventricular pressure in a dose-dependent manner (0.001 to 100 nM); nevertheless, this phenomenon was significantly inhibited in presence of propranolol or metoprolol at a dose of 1 nM. These data suggest that positive inotropic activity induced by the steroid derivative is via activation of β_1-receptor adrenergic. This phenomenon is a particularly interesting because the positive inotropic activity induced by this steroid derivative involves a molecular mechanism different in comparison with other positive inotropic drugs.

Keywords: *Estrone derivative, inotropic activity, indomethacin, nifedipine.*

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INTRODUCTION

There are data which indicate that heart failure is a major cause of death and disability worldwide[1-3]. For several decades have been used positive inotropic drugs with different characteristics for the treatment of heart failure[4, 5]; For example, the digoxin has been used to this clinical pathology; however, its narrow therapeutic margin makes their use is decreasing[6-8]. Other data indicate that dobutamine may be helpful in severe heart failure associated with ischemic heart disease, myocardial infarction and cardiogenic shock; however, due to their poor oral bioavailability this drug has been administered intravenously; moreover, it has been observed that continuous administration for 24 to 72 hours leads to progressive loss of their effectiveness[9]. Other studies conducted[10], in patients with congestive heart failure who were administered a dose of levosimendan or dobutamine showed improvement in hemodynamic function with levosimendan compared with dobutamine-treated patients. It is important to mention, that the administration of high doses of levosimendan in patients with episodes of myocardial infarction may induce changes in the inotropic effect by inhibiting the activity of some phosphodiesterases[11].

On the other hand, it is noteworthy that there are other drugs for the treatment of heart failure such as milrinone. It has been observed that administration of milrinone at doses of 0,75µg/kg per minute in

patients with heart failure has beneficial effects inducing an increase in cardiac output and reducing systemic vascular resistance[12-15]; however, this drug can increase the incidence of ventricular arrhythmias in a manner dependent on the dose[16], so that the therapeutic management of this drug affects its large scale use, resulting in finding another therapeutic for the treatment of congestive heart failure alternatives. One of those pharmacological alternatives is the use of angiotensin-converting enzyme inhibitors and spironolactone in patients with congestive heart failure[17], which reduce the effects of ischemic heart disease and stroke recurrence[18-20]. However, there are data indicating that the sharp increase in prescribing spironolactone induces to a greater number of hospitalizations of patients with hyperkalemia which is associated to increased mortality from this cause[21]. All these data show different therapeutic management to heart failure; however the adverse effects induced by these drugs affects their use on a large scale, which results in the search for other therapeutic alternatives for the treatment of congestive heart failure.

In the search of a therapeutic alternative for heart failure, several steroid derivatives with positive inotropic activity has been evaluated in diverse biological models; for example, a study indicates that the strophanthidin (steroid derivative) increase the force of contraction by changes in the calcium levels[22, 23]. In addition, there are studies that show the synthesis of a steroid derivative (F90927) which exerts a positive inotropic activity in cardiac muscle via activation of the L-type Ca2+ channel[24]. Additionally, a series of steroid derivatives[25, 26] were synthesized which showed a positive inotropic effect, mainly by inhibition of Na+, K+-ATPase. Nevertheless, other reports indicate that 14-β hydroxyprogesterone [27] increases the contractility of isolated cardiac tissue via glycoside receptor. Additionally, it is important to mention that recently an estradiol-ethylenediamine derivative was synthetized which induce increase the perfusion pressure and vascular resistance via activation of the L-type Ca2+ channel[28]. All these data show that some steroid derivatives induce inotropic effects in the cardiovascular system; nevertheless, the cellular site and molecular mechanism involved in its inotropic activity are very confusing, perhaps this phenomenon is due to differences in the chemical structure of steroid derivatives or to the different pharmacological approaches used. Therefore, more pharmacological data are needed to characterize the activity induced by the steroid derivatives at cardiovascular level. To provide this information, the aim of this study was designed to investigate the inotropic effects of a new estrone derivative in isolated rat hearts using the Langendorff technique. In addition, some theoretical physicochemical parameters were evaluated to characterize the interaction of the estrone derivative with some bioactive substance involved in their biological activity.

METHODOLOGY

General methods

The reagents used in this study were purchased from Sigma-Aldrich Co. Ltd. The melting point was determined on an Electrothermal (900 model). ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace GCPolaris Q. spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/0 2400 elemental analyzer.

Chemical synthesis

17-(6-Hydroxy-hex-1-ynyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthrene-3,17-diol (3).

A mixture of estrone (135 mg, 0.50 mmol), 5-Hexyn-1-ol (60 µl, 0.54 mmol), potassium hydroxide (30 mg, 0.53 mmol), in 5ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (4:1)

5,5´-(((12aS)-1-(6-(3-carboxy-5-nitrophenoxy)hex-1-yn-1-yl)-12a-methyl-1,2,3,4,4a,4b, 5, 6,10b,11,12,12a-dodecahydrochrysene-1,8-dihyl)bis(oxy))bis(3-nitrobenzoic acid)

A mixture of 3 (200 mg, 0.52 mmol), 3,5-dinitrobenzoic acid (60 µl, 0.54 mmol), potassium hydroxide (30 mg, 0.53 mmol), in 5 ml of dimethyl sulfoxide was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:water (4:2).

Biological activity

The experimental methods 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 [29]. Animals Male Wistar rats; weighing 200-250 g were obtained from University Autonomous of Campeche

Reagents

The compounds involved in this study were dissolved in methanol and different dilutions were obtained using Krebs-Henseleit solution (≤ 0.01%, v/v).

Experimental design

Male rats (200-250 g) were anesthetized by injecting them with pentobarbital at a dose rate of 50 mg/Kg body weight. After, the chest was opened, and a loose ligature passed through the ascending aorta. Then, the heart was removed and immersed in ice cold physiologic saline solution*. The hearts were trimmed of non-cardiac tissue and retrograde perfused via a non-circulating perfusion system at a constant flow rate. Following, 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.

*Physiologic saline solution (Krebs-Henseleit solution) was composed of; NaCl, 117.8 mmol; KCl, 6 mmol; $CaCl_2$, 1.75 mmol ; NaH_2PO_4, 1.2 mmol ; $MgSO_4$, 1.2 mmol ; $NaHCO_3$, 24.2 mmol ; glucose, 5 mmol; and sodium pyruvate 5 mmol; and the coronary flow was adjusted with a variable speed peristaltic pump.

*Krebs-Henseleit solution was actively bubbled with a mixture of O_2/CO_2 (95:5/5 %) and regulated at a pH of 7.4 and 37°C.

Evaluation of left ventricular pressure

The contractile activity was evaluated 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[30]. It is important to mention that the latex balloon was linked to a cannula which was bound to a pressure transducer that was connected with the MP100 data acquisition system.

Biological evaluation

First stage

Ischemia/Reperfusion model

After of 15-minute equilibration time, the hearts were subjected to ischemia for 30 minutes by turning off the perfusion system [28] After this period, the system was restarted and the hearts were reperfused by 30 minutes with Krebs-Henseleit solution. The hearts were randomly divided into 3 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 estrone at a dose of 0.001 nM before ischemia period (for 10 minutes) and during the entire period of reperfusion.

Group III. Hearts were subjected to ischemia/reperfusion and treated with the compounds **2** at a dose of 0.001 nM before ischemia period (for 10 minutes) and during the entire period of reperfusion.

Group III. Hearts were subjected to ischemia/reperfusion and treated with the compound **3** at a dose of 0.001 nM before ischemia period (for 10 minutes) and during the entire period of reperfusion.

At the end of each experiment, the perfusion pump was stopped, and 0.5 ml of fluorescein solution (0.10%) was injected slowly through a sidearm port connected to the aortic cannula. The dye was passed through the heart for 10 sec 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 methods previously reported (Figueroa et al., 2011-b). Total area at risk was expressed as the percentage of the left ventricle.

Second stage.

Effect induced by estrone and the compound 2 or 3 on perfusion pressure: Changes in perfusion pressure as a consequence of increase in time (3 to 18 min) in the absence (control) and presence of estrone or **2** or **3** 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 estrone and the compound 2 or 3on coronary resistance: Coronary resistance in the absence (control) and presence of estrone or **2** or **3** 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.

Third stage

Effects of the compound 3 on left ventricular pressure through the calcium channel activation: Intracoronary boluses (50 µl) of the compound **3** [0.001 to 100 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 was 10 min).

Effect exerted by the compounds 3 on left ventricular pressure through synthesis of prostanglandins. The boluses (50 µl) of the compound **3** [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The bolus was injected at 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 10 min).

Effects induced by the compound 3 on left ventricular pressure through adrenergic receptors. Intracoronary boluses (50 µl) of the compound **3** (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 prazosin or propanolol or metoprolol at a concentration of 1 nM (duration of preincubation with prazosin or metoprolol was for 10 min equilibration period).

Docking evaluation

Docking calculations were carried out using DockingServer[31]. The MMFF94 force field[32] was used for energy minimization of ligand molecule (compound **3**) using DockingServer. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations[31] were carried out on 2ycw-B1 receptor protein model (Protein Data Bank) . Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools[33]. Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program[34].

AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method[35]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 4 were applied.

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[36] using the SPSS 12.0 program. The differences were considered significant when ***p*** was equal or smaller than 0.05.

RESULT AND DISCUSSION

There are several data which indicate that some steroid derivatives induce inotropic effects in the cardiovascular system [22-23]; nevertheless, the cellular site and molecular mechanism involved in its inotropic activity are very confusing. The aim of this study was synthesize a new steroid derivative to evaluate their biological activity in an isolated rat heart model.

3.1 Synthesis chemical.

Propargylic-alcohols derivatives via reaction of terminal Alkynes with ketone group (2)

There are several reports which showed the preparation of some propargylic-alcohols using different methods and reagents such as disulfide-oxazolidine [37], Ti(O-i-Pr)$_4$-BINOL complex [38], chiral diamine-coordinated tin(II) triflate [39], P($PhCH_2NCH_2CH_2$)$_3$N [40] and others; however some of these reagents are difficult to handle require and special conditions. Therefore, in this work the estrone was reacted with 5-hexyn-1-ol in basic medium (Figure 1). The yield of the reaction product (compound **2**) was 55 % with melting point of 146-148 °C. In addition, the chemical shifts of the spectroscopic analyses of ^{1}H NMR and ^{13}C NMR for the propargylic-alcohol-estrone derivative is showed in the table 1 and 2. Finally, the results of mass spectroscopy (m/z) showed a value of 368.23 for the compound **3**. Additionally, the elementary analysis data for the propargylic-alcohol-estrone derivative ($C_{24}H_{32}O_3$) were calculated (C, 78.22; H, 8.75; O, 13.02) and found (C, 78.16; H, 8.64). Yielding 55 % of product, m.p. 182-184 ^{o}C; IR (V_{max}, cm^{-1}): 3400 and 2192.

The following stage was achieved by the formation of an eter group involved in the compound **3**. It is important to mention that there are several methods for preparation of ether derivatives which involve the use of different reagents such as *N*-methyl-2-pyrrolidone[41] and sulfolane[42] and others. In addition, other data indicate the preparation of ether derivatives via displacement of nitro groups using methoxy groups[43], fluoride ion[44], nitropropane or nitrocyclohexanone[45], sodium phenoxide[46], nitrobenzamide in DMSO[47]. In this study, the compounds **3** was prepared via displacement of nitro group from 3,5-dinitro benzoic acid in presence of K_2CO_3 (Scheme 3). The yield of the reaction product (compound **3**) was 44 % with melting point of 174-176 °C. In addition, the chemical shifts of the spectroscopic analyses of ^{1}H NMR and ^{13}C NMR for the propargylic-alcohol-estrone derivative is showed in the table 3 and 4. Finally, the results of mass spectroscopy (m/z) showed a value of 877.26 for the

compound **3**. Additionally, the elementary analysis data for the propargylic-alcohol-estrone derivative ($C_{46}H_{43}N_3O_{15}$) were calculated (C, 62.94; H, 4.94; N, 4.79, O, 27.34) and found (C, 62.86; H, 4.88).

Biological evaluation

Several drugs have been used to treat the infarction/reperfusion injury resulting from myocardial ischemia[19-21]; nevertheless, there is scarce information about of activity exerted by the steroid derivatives on this phenomenon. Therefore, in this study the activity of two estrone derivatives (compounds **2** or **3**) on the infarction/reperfusion injury was evaluated. The results showed in the Figure 2 indicate that compound **3** reduced infarct size compared with estrone, the compound **2** and the vehicle-treated hearts (control). This phenomenon could be because the compound **3** exerts some influence on blood pressure which could result reduction in the infarct size, and decrease the myocardial injury after ischemia-reperfusion. In order to evaluate this hypothesis in this study the biological activity exerted by the compounds **2** or **3** on the perfusion pressure in an isolated rat heart model was evaluated. The results (Figure 3) showed that the compound **3** at a dose of 0.001 nM significantly increase the perfusion pressure over time compared with the control, estrone and the compound **2**. Here it is important to mention that the effect induced by compound **3** on vascular tone may lead to the synthesis or release of vasoactive substances involved in the regulation of blood pressure, in form similar to biological activity exerted by other substances[48].

Analyzing this hypothesis and some reports which indicate that some steroid derivatives induces its effect on blood pressure via *type-L* calcium channel activation[49]; in this study, the activity induced by the compound **3** (Figure 4) on left ventricular pressure was evaluated in the absence or presence of nifedipine. The results showed that effect exerted by the compound **3** was not inhibited in the presence of nifedipine; these results indicate that effect exerted by the compound **3** was not via *type-L* calcium channel activation.

Analyzing these results and other studies[50], which indicate that some steroid derivatives can exert their effect on left ventricular pressure through of prostaglandins synthesis; in this study the biological activity induced by the compound **3** on left ventricular pressure was asses in absence or presence of indomethacin (Figure 5). The results showed that effect exert by the compound **3** was not blocked by indomethacin; these data indicated that molecular mechanism involved in the activity of steroid derivative was not via prostaglandins.

In the search of a molecular mechanism involved in the inotropic activity of the compound **3**, and analyzing some reports which indicate that some steroid derivative exert an indirect tonic effect on adrenal catecholamine concentration[51, 52]. In this study the biological activity induced by the compound **3** on left ventricular pressure was evaluated (Figure 6 and 7) in absence or presence of prazosin, propanolol or metoprolol. The results showed that, the effect induced by the estrone-derivative was only inhibited ($\boldsymbol{p}$ = 0.05) in presence of propranolol or metoprolol. All these data indicate that the molecular mechanism involved in the effects of this steroid-derivative on left ventricular pressure is via β_1-adrenergic receptor activation. To test this premise, in this study some strategies were conducted using a molecular theoretical model.

Molecular Docking Analysis

Several studies have been reported for predicting the ligand-protein interaction in some biological models[43, 44]. Therefore, in this study a molecular docking model[45] was used to evaluate the interactions theoretical of the compound **3** with the human β_1-receptor adrenergic (PDB ID:2YCW)[31]. The results indicate that interaction (hydrogen bonds) between compound **3** and the β_1-receptor (Figure 8, 9 and Table 5) involves several amino acid residues such as Gli_{98}, Thr_{126}, Ser_{169}, Tyr_{207}, Ser_{211}, and Ser_{215}. In addition, other results showed the interaction energies involved between the compound **3** and the β_1-androgen receptor (table 6); these data indicate higher interaction energy with the residues Gli_{98} (12.227), Thr_{126} (2.7292), Ser_{169} (0.2119), Tyr_{207} (0.3525), Ser_{211} (0.3854), and Ser_{215} (0.7537). (Table 6). This phenomenon is conditioned by the physicochemical properties of the compound **3**; Furthermore, this interaction could be a key for biological activity exerted by compound **3** on left ventricular pressure.

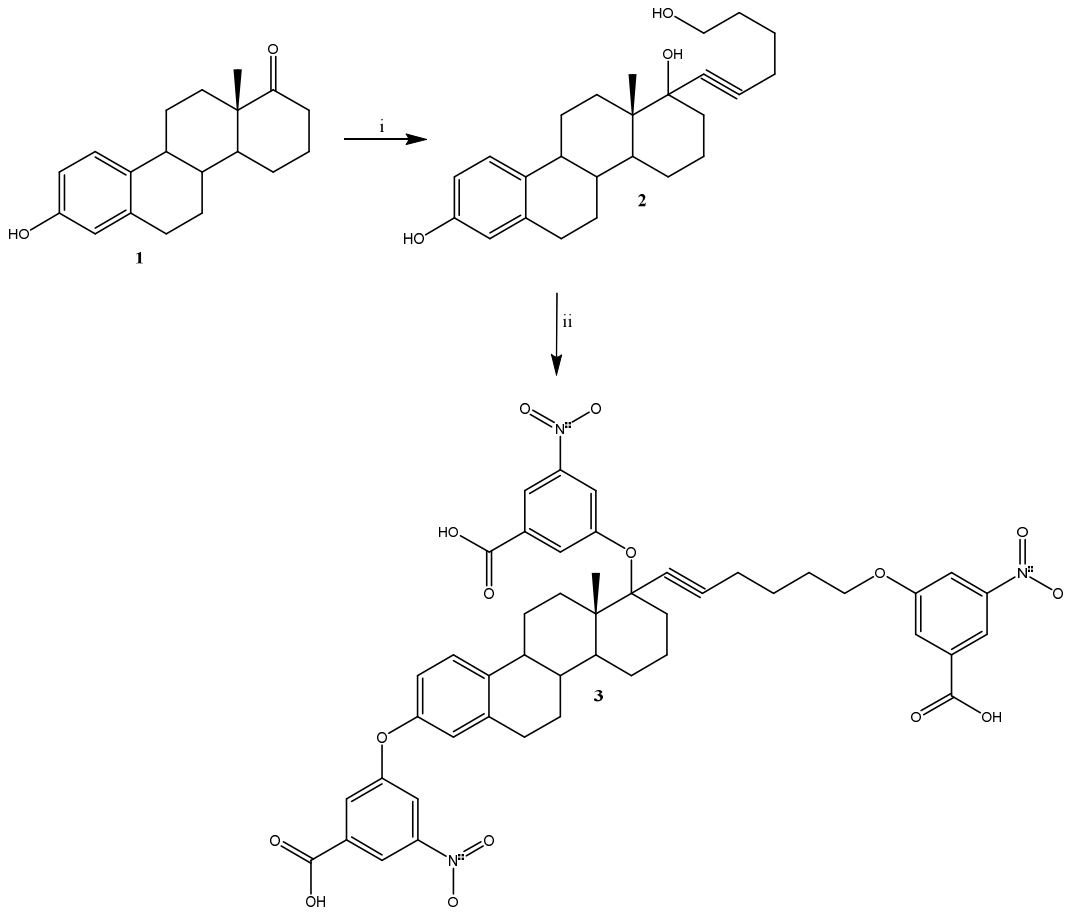

Figure 1 Synthesis of estrone-derivative (**3**). Reaction of estrone (1) with 5-hexyn-1-ol to (i) form the hydroxy-hexynyl-steroid-3,17-diol derivative (**2**). Then, 3 was reacted with 3,5-dinitrobenzoic acid (ii) to synthesize the compound **3**.

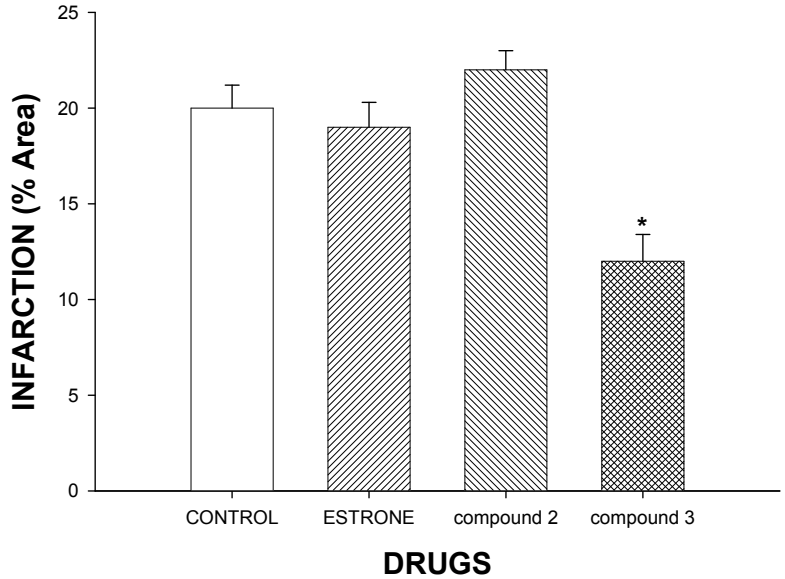

Figure 2. Effect exerted by the estrone and their derivatives (compounds **2** and **3)** at a dose of 0.001 nM on the functional recovery of rat hearts subjected to ischemia and reperfusion. The results showed that compound **3** decrease the infarction area in comparison with the compound **2**, estrone and control conditions. Each bar represents the mean ± S.E. of 9 experiments.

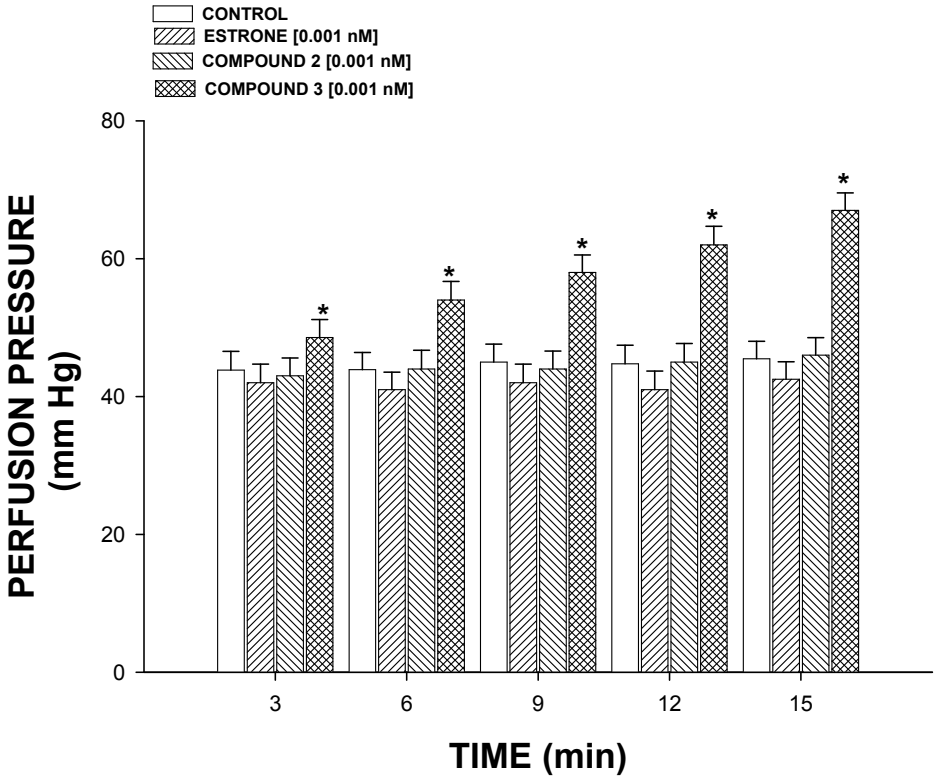

Figure 3. Effect induced by estrone and their derivatives (compounds **2** or 3) on perfusion pressure. The results show that compound **3** significantly increase perfusion pressure (***p*** = 0.05) through time in comparison with the control conditions, estrone and the compound **2**. Each bar represents the mean ± S.E. of 9 experiments.

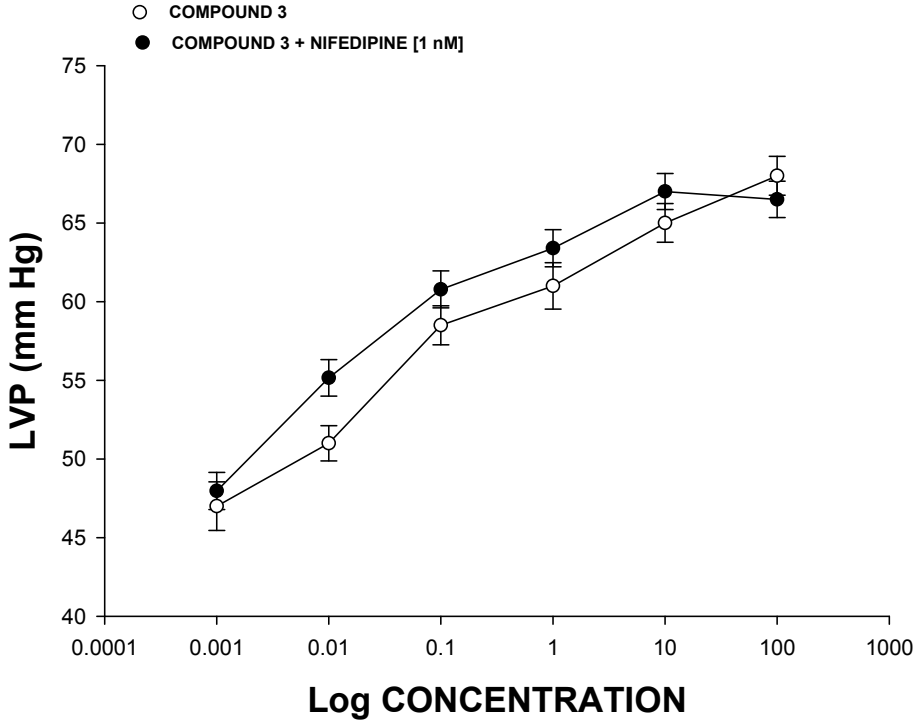

Figure 4. ***Effects induced by the compound 3 on LVP through calcium channel activation.*** Intracoronary boluses (50 µl) of the compound **3** [0.001 to 100 nM] were administered and the corresponding effect on the LVP was determined. The results showed that the compound **3** increase the LVP in a dependent dose manner and this effect was not inhibited in the presence of nifedipine. Each bar represents the mean ± S.E. of 9 experiments. LVP = left ventricular pressure.

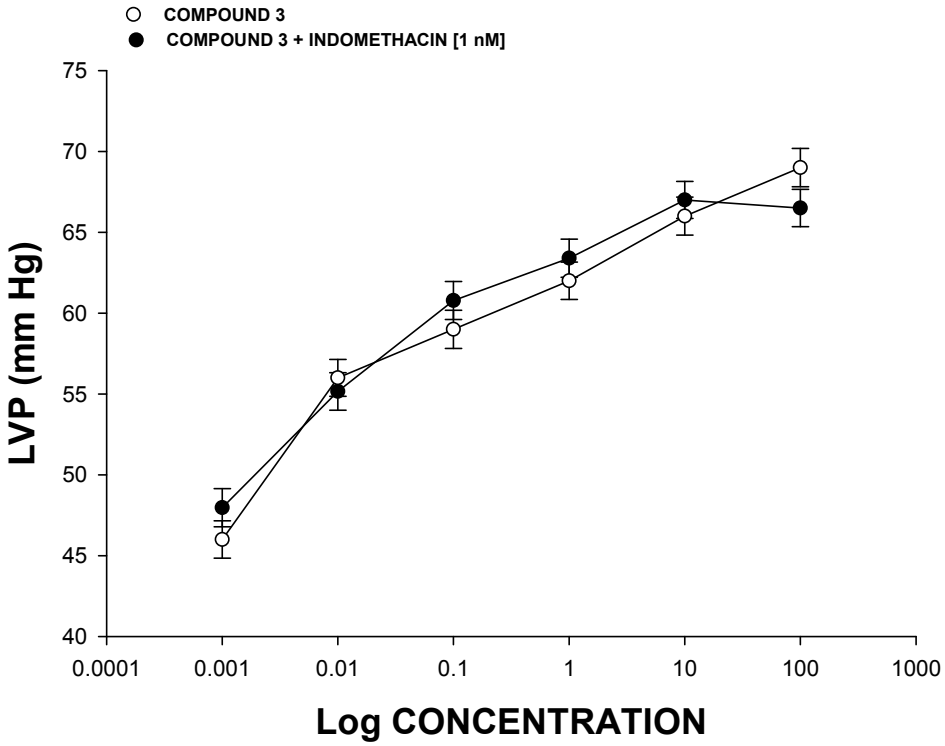

Figure 5. ***Effects induced by the compound 3 on LVP through prostaglandins synthesis.*** Intracoronary boluses (50 µl) of the compound **3** [0.001 to 100 nM] were administered and the corresponding effect on the LVP was determined. The results showed that compound **3** increase the LVP in a dependent dose manner and this effect was not inhibited in the presence of indomethacin [1 nM]. Each bar represents the mean ± S.E. of 9 experiments. LVP = left ventricular pressure.

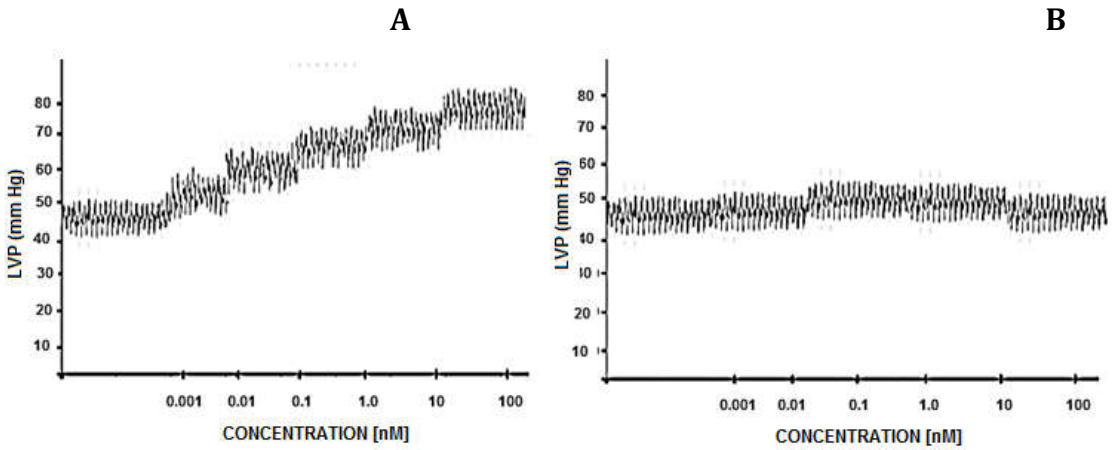

Figure 6. Effect exerted by compound **3** on LVP at dose of 0.001 to 100 nM in absence (A) or presence of metoprolol (B).

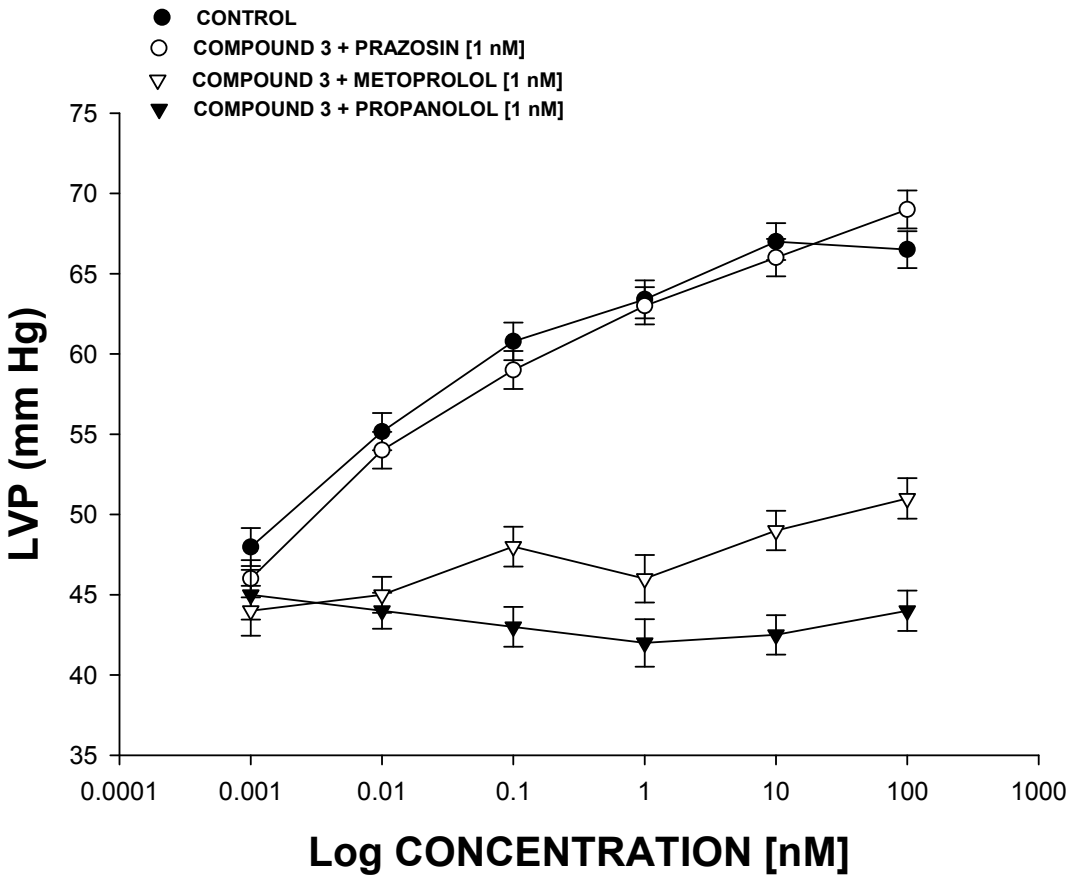

Figure 7. ***Activity exerted by the compound 3 on LVP through of adrenergic receptors.*** The compound **3** [0.001 to 100 nM] was administered (intracoronary boluses, 50 µl) and the corresponding effect on the LVP was evaluated in the absence and presence of prazosin, propranolol or metoprolol at a dose of 1 nM. The results showed that activity induced by the estrone-derivative on LVP was inhibited (***p*** = 0.05) only in the presence of propanolol or metoprolol. Each bar represents the mean ± S.E. of 9 experiments. LVP = left ventricular pressure.

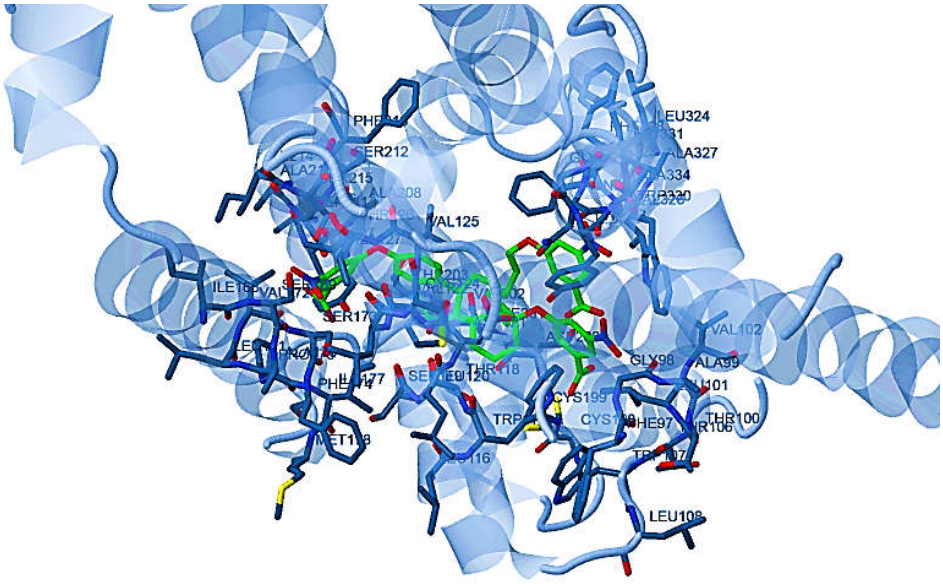

Figure 8. Site of binding for compound **3** with human adrenergic β_1-receptor (PDB ID: 2YCW, PDB Protein Data Bank) visualized with GL mol Viewer after docking analysis with Docking Server [31].

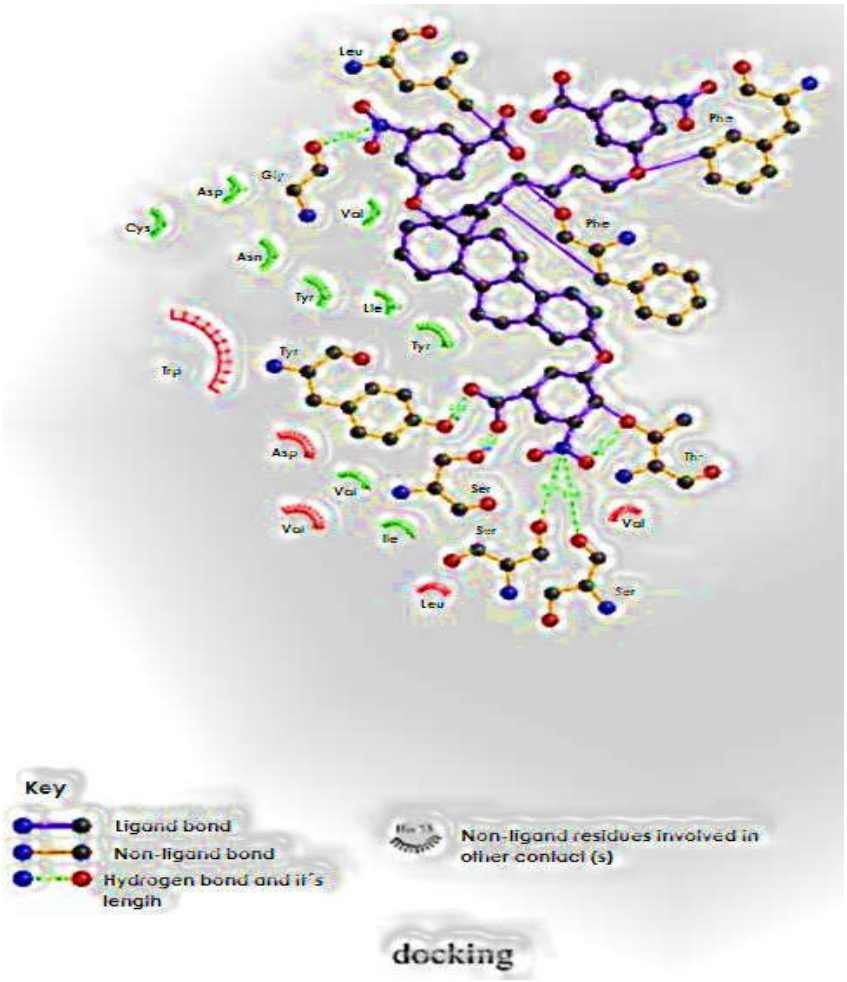

Figure 9. The scheme shown the contact site of amino acids residues involved in the androgen receptor (2YCW, PDB Protein Data Bank) with the compound **3**, viewer after docking analysis using Docking Server [31].

Table 1. ^{1}H NMR (300 MHz, $CDCl_3$) data for the compound **2**.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.92 (s, 3H), 1.22-1.50 (m,4H), 1.58-1.60 (m, 4H), 1.70-2.10 (m, 6H), 2.20 (t, 2H, J = 13.47 Hz), 2.24- 2.76 (m, 5H), 3.66 (t, 2H, J = 11.00 Hz), 5.54 (broad, 3H), 6.48-7.10 (m, 3H) ppm.

Table 2. ^{13}C NMR (300 MHz, $CDCl_3$) data for the compound **2**.

δ_C: 12.28, 18.90, 23.66, 25.54, 26.92, 27.70, 30.30, 31.82, 34.90, 36.62, 37.87, 44.89, 48.12, 52.80, 62.12, 80.10, 80.66, 83.42, 112.72, 115.34, 126.5, 132.24, 138.34, 153.02 ppm.

Table 3. ^{1}H NMR (300 MHz, $CDCl_3$) data for the compound **3**.

δ_H: 1.00 (1.00), 1.12-1.56 (m, 6H), 1.60 (t, 2H, J = 6.00 Hz), 1.68-1.84 (m, 4H), 1.88 (t, 2H, J = 6.50 Hz), 1.92-2.24 (m, 2H), 2.25 (m, 2H), 2.32-2.80 (5H), 4.10 (m, 2H), 7.04-7.18 (m, 3H), 7.40-8.50 (m, 9H), 11.60 (broad, 3H) ppm.

Table 4. ^{13}C NMR (300 MHz, $CDCl_3$) data for the compound **3**.

δ_C: 12.74, 19.20, 22.72, 26.72, 26.94, 27.25, 27.74, 28.92, 29.74, 34.32, 35.77, 37.79, 44.87, 46.1, 48.32, 68.72, 78.38, 80.35, 83.44,110.06, 111.23, 112.34, 112.66, 114.13, 114.42, 114.43, 117.43, 118.28, 119.10, 119.46, 124.79, 131.61, 132.18, 133.83, 134.41, 139.72, 144.68, 149.03, 149.60, 155.44, 157.17, 160.26, 164.16, 164.56, 164.56 ppm.

Table 5. Interaction of several aminoacids with the compound **3**.

Hydrogen bonds	***polar***	***Other***
Gly_{98}	Thr_{126}	Leu_{101}
Thr_{126}	Ser_{173}	Trp_{117}
Ser_{169}	Asp_{200}	Val_{122}
Tyr_{207}	Tyr_{200}	Val_{125}
Ser_{211}	Ser_{215}	Val_{172}
Ser_{215}	Asn_{329}	Cys_{199}
	Trp_{330}	Phe_{201}
	Tyr_{333}	Tyr_{207}
		Phe_{325}
		Val_{326}

Table 6. Decomposed interaction energies (Kcal/mol) for the compound **3**.

Hydrogen bonds	***Polar***	***Hydrophobic***	***Other***
Gly_{98} (-12.227)	Tyr_{333} (-11.320)	Leu_{101} (-27.802)	Ile_{177} (-0.6879)
Ser_{169} (0.2119)	Trp_{330} (-10.257)	Cys_{199} (-4.344)	Thr_{118} (-0.5192)
Tyr_{207} (0.3525)	Asn_{329} (-8.7885)	Val_{326} (-2.2876)	Asp_{121} (15.1837)
Ser_{211} (0.3854)	Asp_{200} (-4.1404)	Val_{125} (-1.6248)	
Ser_{215} (0.7637)	Ser_{173} (07331)	Phe_{201} (0.3963)	
Thr_{126} (2.7292)		Val_{172} (1.2361)	
		Phe_{325} (1.4542)	
		Val_{122} (15.0591)	
		Trp_{117} (105.667)	

CONCLUSION

All these data indicate that compound **3** increased induce positive inotropic activity in heart. This phenomenon is conditioned by the functional groups involved in their chemical structure and the interaction with the adrenergic β_1-receptor. In addition, it is important to mention that the estrone-derivative involves a molecular mechanism different in comparison with other positive inotropic drugs.

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None.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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