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Research Article

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Synthesis of Two 7, 8-Dioxabicyclo[4.1.1]Octan-3-Yl)- Steroid Derivatives and Evaluation of Their Inotropic Activity in an Animal Model

İki 7, 8-Dioksabisiklo [4.1.1] Oktan-3-Yl) - Steroid Türevlerinin Sentezi ve Hayvan Modelinde İnotropik Aktivitelerinin Değerlendirilmesi

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ABSTRACT

The aim of this study was synthesizing two 7,8-dioxabicyclo[4.1.1]octan-3-yl)-steroid derivatives (compounds 3 or 4) to evaluate their inotropic activity in vitro. The first stage was achieved by the preparation of two 7,8-dioxabicyclo[4.1.1]octan-3-yl)-steroid derivatives using some chemical strategies. Then, the inotropic activity of both steroid derivatives against left ventricular pressure (LVP) was evaluated in an isolated rat heart model using Bay-k-8644, nifedipine, aucubin and L-NAME as controls. The results showed that compound 3 increased LVP in a dose-dependent manner and this effect was inhibited by nifedipine. Other results showed that compound 4 decreased LVP in a dose-dependent manner and this effect was blocked in presence of L-NAME. All these data indicate that 1) the positive inotropic activity exerted by compound 3 was through type L calcium channel activation; 2) the negative inotropic effect of 4 was via nitric oxide synthase activation; these phenomena could be due to the different functional groups involved in the chemical structure of compounds 3 and 4. Therefore, the inotropic activity that exerts these compounds could be translated as good candidates for the treatment of heart failure.

Key Words

Steroid, dioxabicyclo, inotropic, pressure.

ÖZ

Bu çalışmanın amacı, inotropik aktivitelerini in vitro olarak değerlendirmek için iki adet 7,8-dioksabisiklo [4.1.1] oktan-3-il) -steroid türevini (bileşik 3 veya 4) sentezlemekti. İlk aşama, bazı kimyasal stratejiler kullanılarak iki 7,8-dioksabisiklo [4.1.1] oktan-3-il) -steroid türevinin hazırlanmasıyla başarıldı. Daha sonra, her iki steroid türevinin sol ventrikül basıncına (LVP) karşı inotropik aktivitesi, kontrol olarak Bay-k-8644, nifedipin, aucubin ve L-NAME kullanılarak izole edilmiş bir sıçan kalp modelinde değerlendirildi. Sonuçlar, bileşik 3'ün LVP'yi doza bağlı bir şekilde arttırdığını ve bu etkinin nifedipin tarafından inhibe edildiğini gösterdi. Diğer sonuçlar, bileşik 4'ün LVP'yi doza bağlı bir şekilde azalttığını ve bu etkinin L-NAME varlığında bloke edildiğini gösterdi. Tüm bu veriler, 1) Bileşik 3 tarafından uygulanan pozitif inotropik aktivitenin, L tipi kalsiyum kanalı aktivasyonu yoluyla olduğunu; 2) 4'ün negatif inotropik etkisi, nitrik oksit sentaz aktivasyonu ile olmuştur. Bu fenomen, bileşik 3 ve 4'ün kimyasal yapısına dahil olan farklı fonksiyonel gruplar nedeniyle olabilir.

Anahtar Kelimeler

Steroid, dioksabisiklo, inotropik, basınç.

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INTRODUCTION

Heart failure (HF) is one of mainly risk factors of death worldwide [1, 2]. There are several drugs for treatment of heart failure such as diuretics [3], cardiac glycosides [4], angiotensin-converting enzyme inhibitors [5], angiotensin-receptor blockers [6], β1-adrenergic agonist [7], calcium sensitizer [8], and phosphodiesterase inhibitors [9]. However, some of these drugs can produce secondary effects such as arrhythmias [10], hypotension [11], hyperkalemia [12] and others. In the search of new therapeutic alternatives for treatment of HF, several compounds have been developed; for example, some steroid derivatives [13] were prepared which showed positive inotropic activity, via Na^+-K^+-ATPase inhibition. Another study has shown that 14β-hydroxyprogesterone increases the contractility of isolated cardiac tissue through glycoside receptor [14]. Additionally, a report indicate that compound 20R- 14βamino-3β-rhamnosyl-5β-pregnan-20β-ol can exert a positive inotropic action on a dog heart and this effect could be via Na^+,K^+-ATPase receptor activation [15]. In addition, recently a study show that a progesterone-dehydrotestosterone derivative exerts changes on perfusion pressure and vascular resistance through the thromboxane A2 synthesis and secretion [16]. Also, a steroid derivative (F90363) showed positive inotropic activity on cardiac muscle preparations [17]. All these data show that several steroid derivatives exert inotropic effects in the cardiovascular system; nevertheless, there is scarce information about the effects of steroid-dioxabicyclo at cardiovascular level. Therefore the aim of this study was to synthesize two new 7,8-dioxabicyclo[4.1.1]octan-3-yl)-steroid derivatives to evaluate its inotropic activity in a model of isolated rat hearts.

MATERIALS and METHODS

General methods

The compound 2-nitroestrone was prepared using a previously method reported [18]. In addition, all the reagents used in this study were purchased from Sigma-Aldrich Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were determined using KBr pellets on a Perkin Elmer Lambda 40 spectrometer.1H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR300/5 FT NMR spectrometer at 300 MHz (megahertz) in $CDCl_3$ (deuterated chloroform) using TMS (tetramethylsilane) as an internal standard. EIMS (electron impact mass spectroscopy) spectra were determined using a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were determined from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

Chemical Synthesis

3-((1R,3S,6R)-1,6-diphenyl-7,8-dioxabicyclo[4.1.1] octan-3-yl)aniline (2)

A solution of acethophenone (100 µl, 0.85 mmol), 3-ethynylaniline (100 µl, 0.88 mmol), and potassium hydroxide (40 mg, 0.71 mmol) dimethyl sulfoxide (3 ml) was stirring for 72 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water (4:1) system.; yielding 56%; m.p. 62-64°C; IR (V_{max}, cm^{-1}) 3460 and 1242: ^1H NMR (300 MHz, Chloroform-***d***) δ_H: 1.29-3.80 (m, 6H), 3.92 (broad, 2H), 4.14 (m, 1H), 6.56-6.80 (m, 4H), 7.24-7.30 (m, 10H) ppm. ^{13}C NMR (300 MHz, Chloroform-***d***) δ_C: 28.40, 35.92, 35.92, 45.75, 99.25, 100.61, 111.80, 117.82, 123.96, 125.35, 125.73, 126.80, 127.17, 127.40, 127.62, 145.40, 145.86, 146.22, 147.20 ppm. EI-MS m/z: 357.17. Anal. Calcd. for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92; O, 8.95. Found: C, 80.60; H, 6.44.

(13S,17S)-4-(((3-((1R,3S,6R)-1,6-diphenyl-7,8-dioxabicyclo[4.1.1]octan-3-yl)phenyl)amino)methyl)-13-methyl-7,8,9,11,12,13,14,15,16, 17-decahydro-6H-cyclopenta[a] phenanthrene-3,17-diol (3)

A solution of compound 2 (200 mg, 0.55 mmol), estradiol (150 mg, 0.55 mmol), and formaldehyde (5 ml) was stirring for 24 h to reflux. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:hexane:water (4:1:1) system.; yielding 48%; m.p. 80-82°C; IR (V_{max}, cm^{-1}) 3400, 3330 and 1242: ^1H NMR (300 MHz, Chloroform-***d***) δ_H: 0.66 (s, 3H), 0.80-1.18 (m, 4H), 1.28 (m, 1H), 1.34-1.40 (m, 3H), 1.51 (m, 1H), 1.70-1.90 (m, 4H), 1.96 (m, 1H), 2.10 (m, 1H), 2.18 (m, 1H), 2.46-2.52 (m, 3H), 3.58 (m, 1H), 3.66 (m, 1H), 3.81-4.14 (m, 2H), 4.22 (m, 2H), 5.86 (broad, 3H), 6.36-6.40 (m, 2H), 6.68 (m, 1H), 6.78 (m, 1H), 6.82 (m, 1H), 6.88-7.30 (m, 11H) ppm. ^{13}C NMR (300 MHz, Chloroform-***d***) δ_C: 11.30, 23.54, 26.22, 27.70, 28.00, 30.80, 31.10, 33.22, 35.50, 37.42, 39.02, 40.30, 43.64, 44.50, 47.75, 50.50, 81.80, 96.70, 98.12, 112.14, 112.54, 117.30, 122.56, 122.98, 126.30, 126.52, 128.00, 128.02, 128.14, 128.34, 131.75, 138.40, 141.70, 142.20, 145.67, 146.32, 150.10 ppm. EI-MS m/z: 641.35. Anal. Calcd. for $C_{43}H_{47}NO_4$: C, 80.47; H, 7.38; N, 2.18; O, 9.97. Found: C, 80.44; H, 7.34.

(13S)-4-(((3-((1R,3S,6R)-1,6-diphenyl-7,8-dioxabicyclo[4.1.1]octan-3-yl)phenyl)ami no) methyl)-3-hydroxy-13-methyl-6,7,8,9,11,12,13, 14,15,16-decahydro-17H-cyclopen- ta[a]phenanthren-17-one (4)

A solution of compound 2 (200 mg, 0.55 mmol), 2-nitroestrone (170 mg, 0.54 mmol), and formaldehyde (5 ml) was stirring for 24 h to reflux. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water (4:1) system.; yielding 56%; m.p. 72-74°C; IR (V_{max}, cm^{-1}) 3400, 3330, 1722, and 1512: ^{1}H NMR (300 MHz, Chloroform-***d***) δ_H: 0.92 (s, 3H), 1.20-1.26 (m, 3H), 1.28 (m, 1H), 1.37 (m, 1H), 1.50 (m, 1H), 1.54-1.92 (m, 3H), 1.94 (m, 1H), 2.10-2.12 (m, 3H), 2.18 (m, 1H), 2.20-3.02 (m, 5H), 3.58-4.14 (m, 3H), 4.40 (m, 2H), 6.36-7.26 (m, 140H), 7.76 (m, 1H), 9.15 (broad, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-***d***) δ_C: 13.90, 21.60, 25.39, 27.40, 28.00, 30.86, 31.10, 33.20, 34.66, 35.50, 37.19, 42.32, 47.74, 47.96, 48.40, 50.39, 96.70, 98.12, 112.12, 117.32, 122.57, 122.94, 125.02, 126.33, 126.57, 128.01, 128.17, 128.38, 134.36, 136.64, 141.72, 142.21, 143.05, 145.42, 146.32, 146.74, 219.80 ppm. EI-MS m/z: 684.31. Anal. Calcd. for $C_{43}H_{44}N_2O_6$: C, 75.42; H, 6.48; N, 4.09; O, 14.02. Found: C, 75.40; H, 6.44.

Physicochemical Parameters Evaluation

Some electronic parameters such as HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital) energy, orbital coefficients distribution, molecular dipole moment, HBD (hydrogen bond donor groups), HBA (hydrogen bond acceptor groups) and PSA (polar surface area) were evaluated using the SPARTAN'06 software[19].

Pharmacophore Evaluation

The 3D pharmacophore model for the compounds 2-4 was determinate using LigandScout 4.08 software [20].

Biological Methods

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 [21]. Male Wistar rats, weighing 200-250g, 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 (200-250g) 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, $NaHPO_4$; 1.2, $MgSO_4$; 24.2, $NaHCO_3$; 5, glucose; 7 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.

Induction of Congestive Heart Failure (CHF)

CHF was development mainly of method previous reported [22], in this process the pentobarbital (100/kg mg) was administered through of cannula inserted in the aorta to induce CHF. 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 (LVdP), using a saline-filled latex balloon (0.01 mm, diameter) inserted into the left ventricle via the left atrium. It is important to mention that latex balloon was bound to cannula which was linked to pressure transducer which is connected to MP100 data acquisition system.

Biological Evaluation

Effects of the compounds 3 and BAY-K-8644 on left ventricular pressure via calcium channel activation.

Intracoronary boluses (50 µl) of compounds 3 or BAY-K-8644 [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The biological activity of 3 was repeated in the presence of nifedipine at a concentration of 1 nM (duration of preincubation with nifedipine was by a 10 min equilibration period).

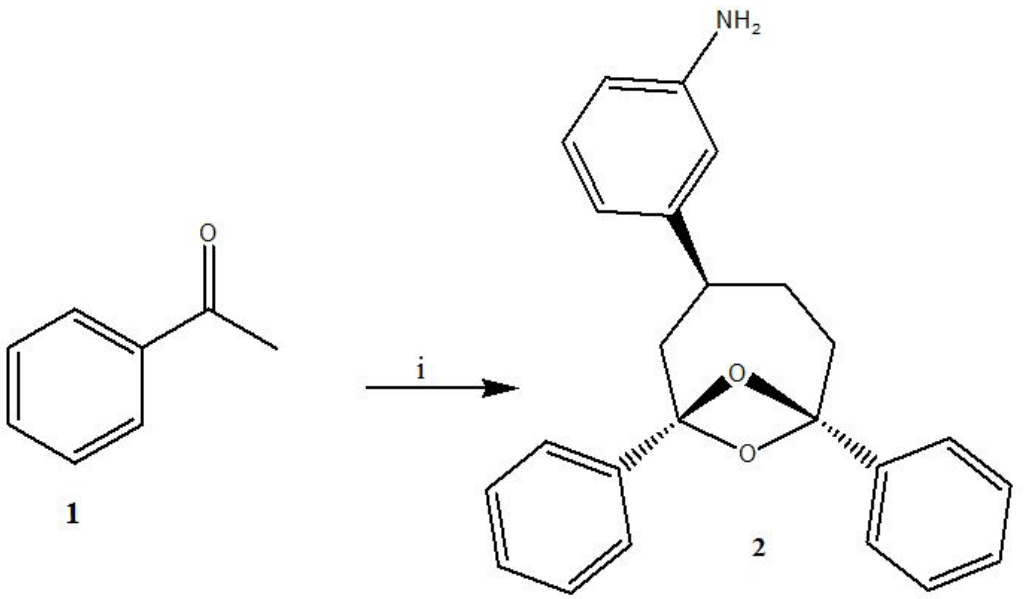

Figure 1. Preparation of a 7,8-dioxabicyclo-aniline derivative (2). Reaction of acetophenone (1) with 3-ethynylaniline to form the compound 2. Conditions: i = dimethyl sulfoxide.

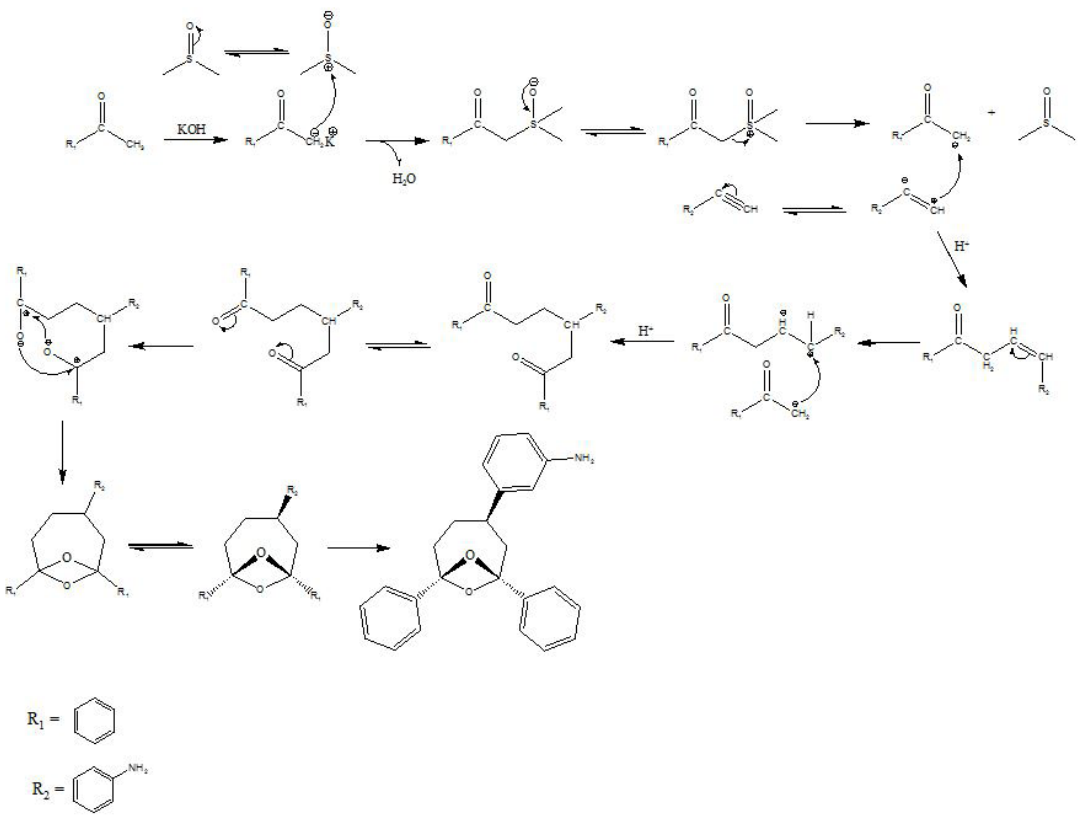

Figure 2. Reaction mechanism involved in the synthesis of a 7,8-dioxabicyclo-aniline derivative (2).

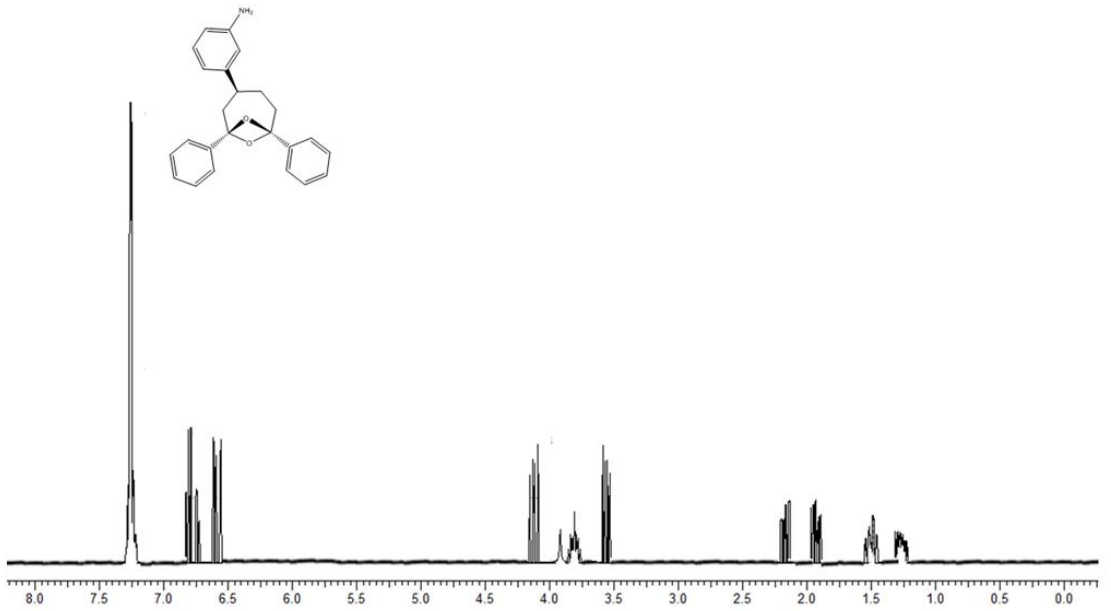

Figure 3. The scheme showed ^{1}HNMR spectrum from compound 2. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

Biological Activity of the Compounds 4 and Aucubin on Left Ventricular Pressure via Nitric Oxide Synthase Activation.

Intracoronary boluses (50 µl) of compounds 4 or aucubin [0.001 to 100 nM] were administered and the corresponding effect on the left ventricular pressure was evaluated. The biological activity of 4 was repeated in the presence of L-NAME (N_{ω}-Nitro-L-arginine methyl ester hydrochloride) at a concentration of 1 nM (duration of preincubation with nifedipine was by a 10 min equilibration period).

Statistical Analysis

The results were expressed as average ± SE, using each heart (n = 9) as its own control. In addition, the results were analyzed via analysis of variance using the SPSS 12.0 program [23]. The differences in the values found were determinates with p = 0.05.

RESULTS and DISCUSSION

There is little information on the biological activity of dioxabicyclo-steroids derivatives at cardiovascular level; therefore, in this study, two 7,8-dioxabicyclo[4.1.1] octan-3-yl)-steroid derivatives were synthesizing to evaluate their inotropic activity. The first stage was achieved by the preparation of some steroid derivatives as follows:

Chemical Synthesis

Preparation of a 7,8-dioxabicyclo[4.1.1]octan-3-yl) Aniline Derivative

The first stage was achieved for synthesis of a 7,8-dioxabicyclo[4.1.1]octan-3-yl)aniline derivative (2). It is important to mention that several dioxabicyclo derivatives have been prepared using some reagents such as ***p***-toluenesulfonic acid [24], Iodosobenzene [25], lithium [26], [Hydroxy(tosyloxy)iodo]benzene [27], dirhodium(II) [28] and others. In this study was synthesizing a new 7,8-dioxabicyclo[4.1.1]octan-3-yl)aniline derivative from acetophenone and 3-ethynylaniline in presence of dimethyl sulfoxide at mild conditions (Figure 1 and 2).

The ^{1}H NMR spectrum of 2 shows signals at 1.29-3.80 and 4.14 ppm for 7,8-Dioxa-bicyclo[4.1.1]octane; at 3.92 ppm for amino group; at 6.56-6.80 ppm for phenyl bound to amino group; at 7.24-7.30 ppm for phenyl groups bound to 7,8-Dioxa-bicyclo[4.1.1]octane (Figure 3).

^{13}C NMR spectra showed chemical shifts at 28.40-100.61 ppm for 7,8-Dioxa-bicyclo[4.1.1]octane; at 11.80-123.96, 126.80, 146.22 and 147.20 ppm for phenyl gro-

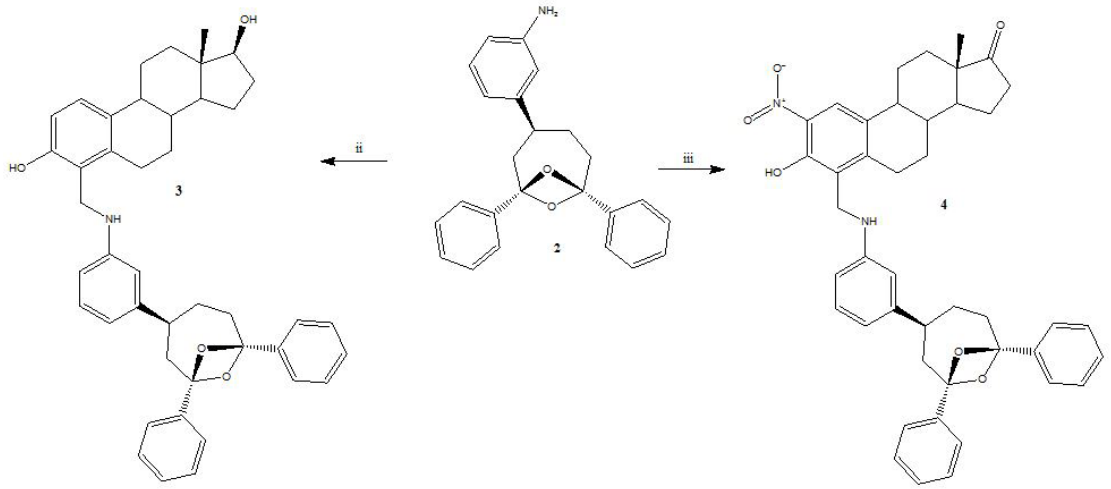

Figure 4. Preparation of two 7,8-dioxabicyclo[4.1.1]octan-3-yl)-steroid derivatives (3 or 4). Reaction of 7,8-dioxabicyclo-aniline derivative (2) with 2-nitro-estradiol to form a 7,8-dioxabicyclo[4.1.1]octan-3-yl)-estradiol derivative (3). Then, 2 reacted with 2-nitroestrone to synthesis of a 7,8-dioxabicyclo[4.1.1]octan-3-yl)-estrone derivative (4). Conditions: ii and iii = formaldehyde.

up bound to amino group; at 125.35-125.73 and 127.17-145.86 ppm for phenyl groups bound to 7,8-Dioxa-bicyclo[4.1.1]octane. In addition, the mass spectrum from 2 showed a molecular ion (m/z) 357.17.

Synthesis of 7,8-dioxabicyclo[4.1.1]octan-3-yl)-Estradiol via Mannich Reaction.

Several steroid derivatives have been prepared using the Mannich reaction, these compounds contain in its chemical structure an activated methyl group in ring A and B [29]. Therefore, in this study the Mannich reacti-

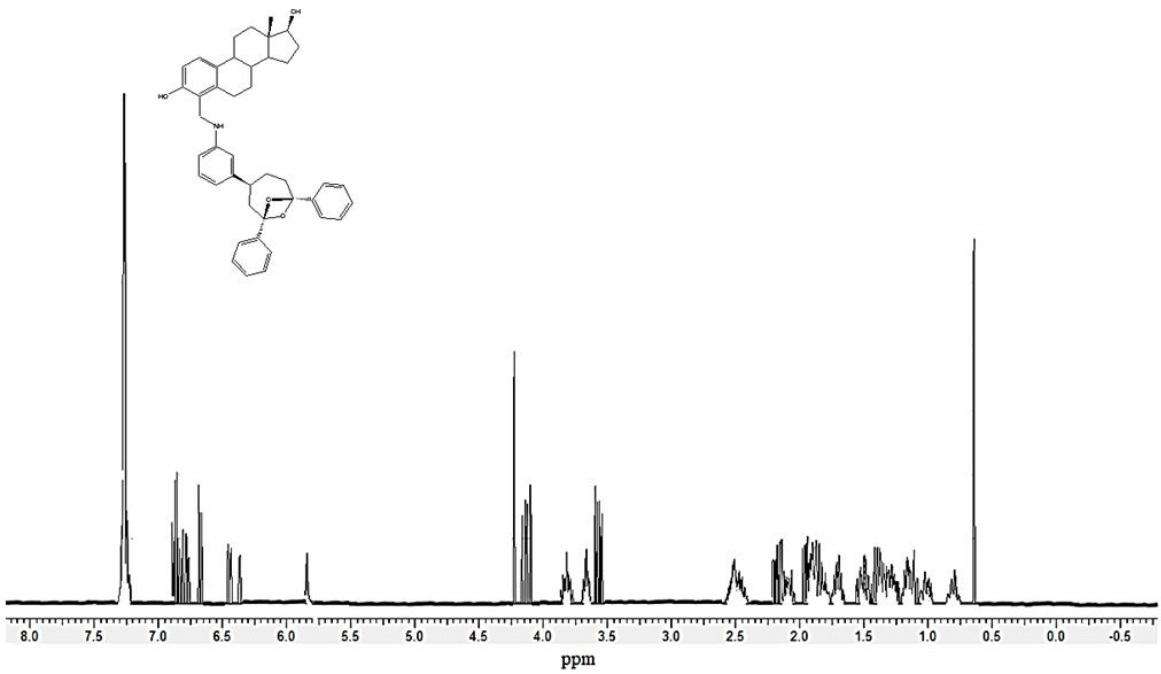

Figure 5. The scheme showed ^{1}HNMR spectrum from compound 3. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

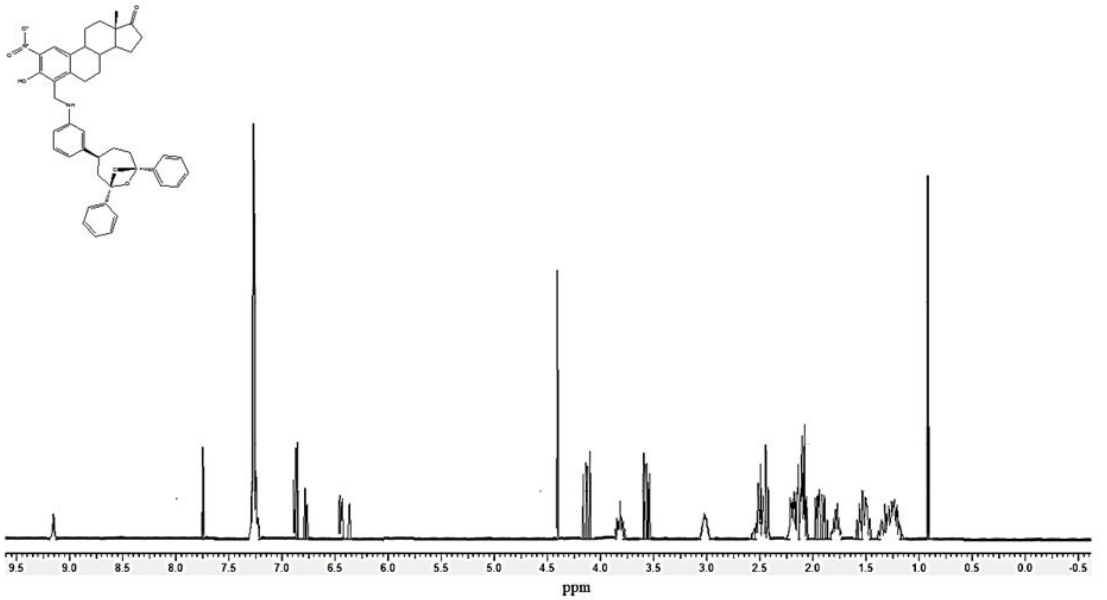

Figure 6. The scheme showed 1HNMR spectrum from compound 4. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

on was used to evaluate the reactivity of hydrogen atom involved in the ring A of steroid nucleus. This stage was achieved by the reaction of estradiol or 2-nitroestrone with the compound 2 in presence of formaldehyde to form the compounds 3 or 4 (Figure 3). The ^{1}H NMR spectrum of 3 shows signals at 0.66 ppm for methyl group; at 0.80-1.18, 1.34-1.40, 1.70-1.90, 2.10, 2.46-2.52, 3.66, 6.68 and 6.82 ppm for steroid moiety; at 1.28, 1.51, 1.96, 2.18, 3.58 and 3.81-4.14 ppm for 7,8-Dioxa-bicyclo[4.1.1]octane; at 4.22 ppm for methylene group bound to steroid nucleus and amino group; at 5.86 ppm for both hydroxyl and amino groups; at 6.36-6.40, 6.78 and 6.88-7.30 ppm for phenyl groups. ^{13}C NMR spectra showed chemical shifts at 11.30 ppm for methyl group; at 23.54-30.80, 37.42-39.02, 43.64-44.50, 50.50-81.80, 112.54, 122.56, 128.02, 131.75-138.40 and 150.10 ppm for steroid moiety; at 31.10-35.50, 47.75 and 96.70-98.12 ppm for 7,8-Dioxa-bicyclo[4.1.1]octane; at 40.30 ppm for methylene group bound to steroid nucleus and amino group; at 112.14, 117.30, 122.98-128.00, 128.14-128.34 and 141.70-145.32 ppm for phenyl groups. Additionally, the mass spectrum from 3 showed a molecular ion (m/z) 641.35.

On the other hand, the ^{1}H NMR spectrum of 4 showed several signals at 0.92 ppm for methyl group; at 1.20-1.26, 1.37, 1.54-1.92, 2.10-2.12, 2.20-3.02 and 7.76 ppm for steroid moiety; at 1.28, 1.50, 1.94, 2.18 and 3.58-4.14 ppm for 7,8-Dioxa-bicyclo[4.1.1]octane; at 4.40 ppm for methylene group bound to steroid nucleus and amino group; at 6.36-7.26 ppm for phenyl groups; at 9.15 ppm for both amino and hydroxyl groups.

^{13}CNMR spectra showed chemical shifts at 13.90 ppm for methyl group; at 21.60-30.86, 34.66, 37.19, 47.96-50.39, 122.57, 125.02, 134.36-136.64, 145.42 and 146.74 ppm for steroid moiety; at 31.10-33.20, 35.50, 47.74 and 96.70-98.12 ppm for 7,8-Dioxa-bicyclo[4.1.1] octane; at 42.32 ppm methylene group bound to steroid nucleus and amino group; at 112.12-117.32, 122.94, 126.33-128.38, 141.72-143.05 and 146.32 ppm for phenyl groups; at 219.80 ppm for ketone group. Finally, the mass spectrum from 4 showed a molecular ion (m/z) 684.31.

Electronic Parameters

There are several studies which have shown that molecular orbitals and frontier electron density can be used to predict the most reactive position in some electron system on several types of reactions [30, 31]. In this way, the values of highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO) and their energy gap reflect the chemical activity of a molecule [32]. It is important to mention that some methods have been used to evaluate the relation bet-

Table 1. Physicochemical parameters involved in the chemical structure of compounds 2 (C-2), 3 (C-3) and 4 (C-4).

Parameter	C-2	C-3	C-4
HOMO (eV)	-7.95	-7.72	-8.12
LUMO (eV)	3.68	3.58	-0.14
Moment dipolar (debye)	1.55	3.92	6.36
PSA (Å^2)	41.935	65.88	97.08
LogP	6.12	9.80	8.58

ween HOMO and LUMO with the biological activity of some compounds [33, 34].

Therefore, in this study, the Hartee-Fock method (method of approximation for the determination of the wave function and the energy of a quantum many-body system in a stationary state) was used to determinate both HOMO and LUMO orbitals (Figure 6 and Table 1) with SPARTAN'06 program19. The results showed changes in both HOMO and LUMO values for the compounds 2 to 4; this phenomenon could be conditioned by the difference in π orbitals density that is located in chemical structure these compounds.

Pharmacophore Ligand Model

There are several chemical models to determine the three-dimensional orientation adopted by the functional groups of a compound to predict its interaction with some proteins [35]; for example, the use of a pharmacophore model which can furnish a new insight to design novel molecules that can enhance or inhibit the function of a biological target which can be useful in new drug discovery. Analyzing this premise in this study, the LigandScout software [36] was used to develop a pharmacophore model for compounds 2 to 4 (Figure 7). The results showed that some functional groups of 2 to 4 may interact through hydrophobic contacts or as hydrogen bond acceptors or as hydrogen bond donor with some proteins.

Biological Activity Evaluation "in vitro"

There are some studies which indicate that some steroid derivatives exert inotropic activity in some biological models [13-17]; however, their molecular mechanism is not clear; therefore, in this study the biological activity of two steroids derivatives was determinate using an isolated rat heart model. In this way, the inotropic effect exerted by steroid-derivatives (compounds 3 or

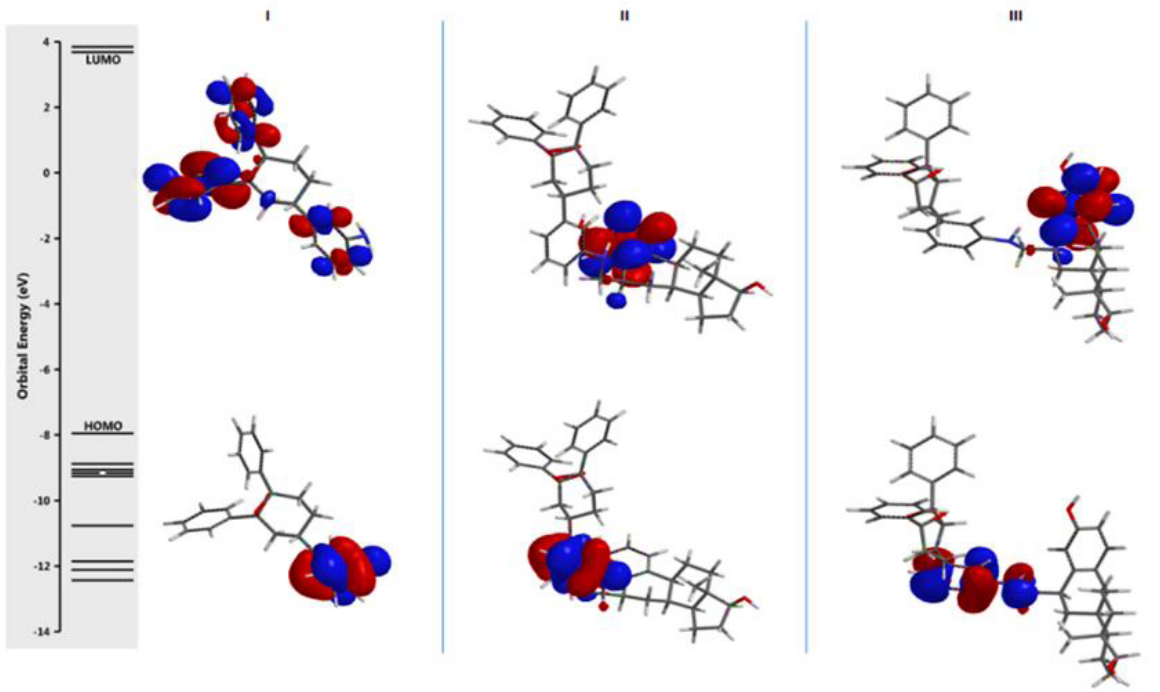

Figure 7. Molecular orbitals (HOMO and LUMO) involved in the compounds 2 (I), 3 (II) and 4 (III). Visualized with SPARTAN'06 software.

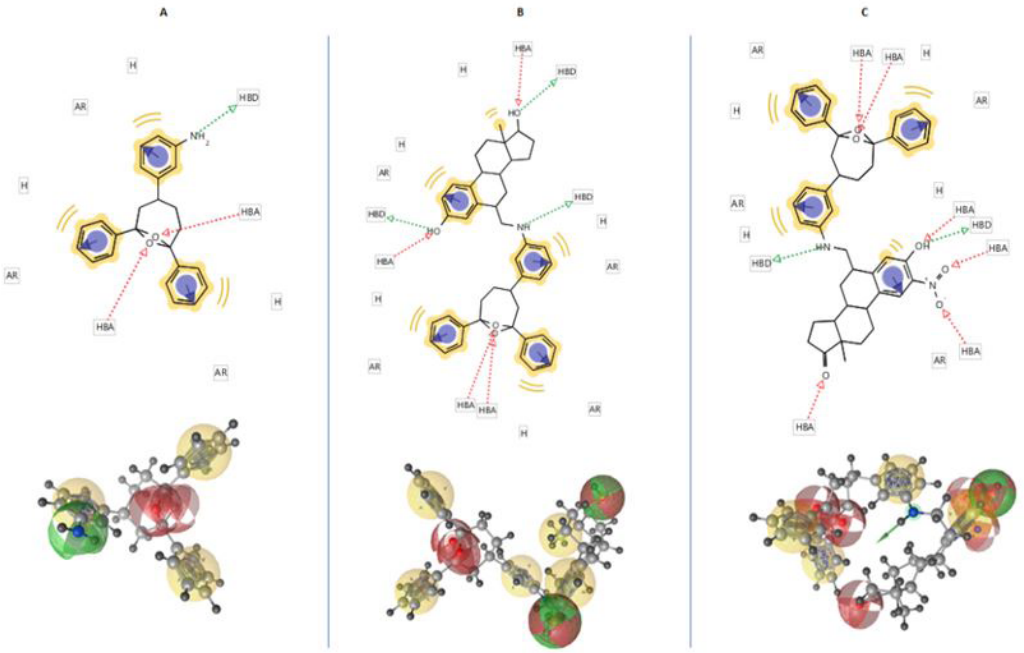

Figure 8. Theoretical pharmacophore from both compounds 2 (A), 3 (B) and 4 (C) using the LigandScout software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red) and hydrogen bond donor (HBD, green).

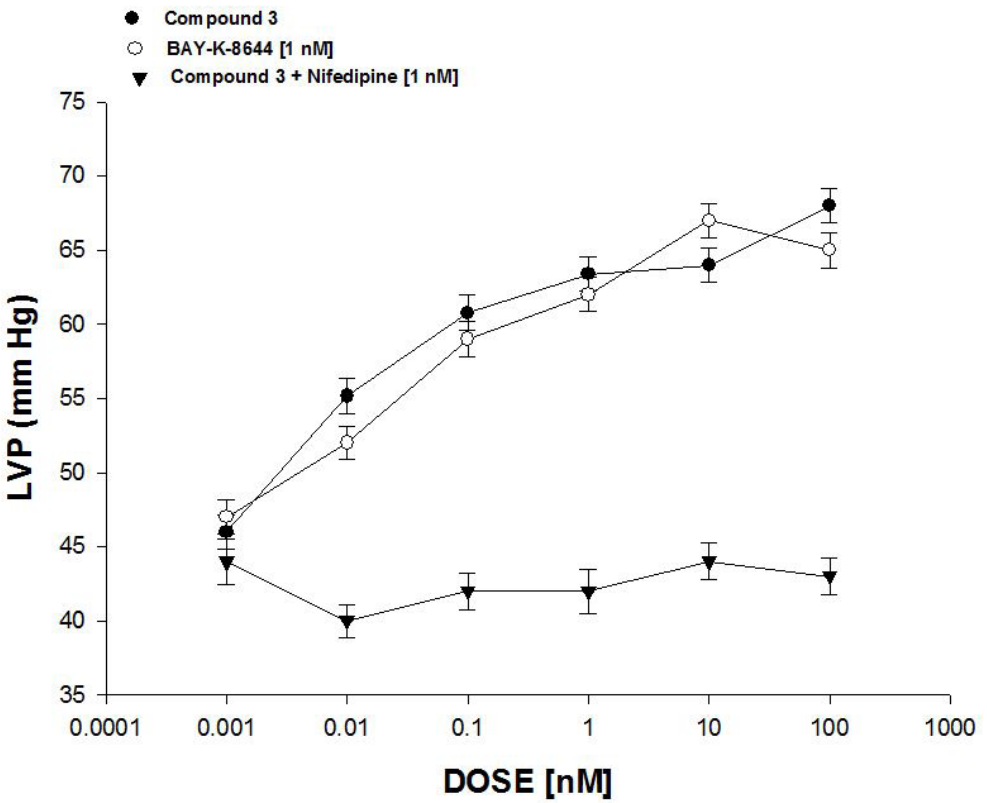

Figure 9. Effect exerted by the compounds 3 and BAY-K-8644 on left ventricular pressure (LVP). The results showed that 3 and BAY-K-8644 increase the LVP in a dose-dependent manner. In addition, the positive inotropic activity of 3 was inhibited by nifedipine. Each point represents the mean ± S.E. of 9 experiments.

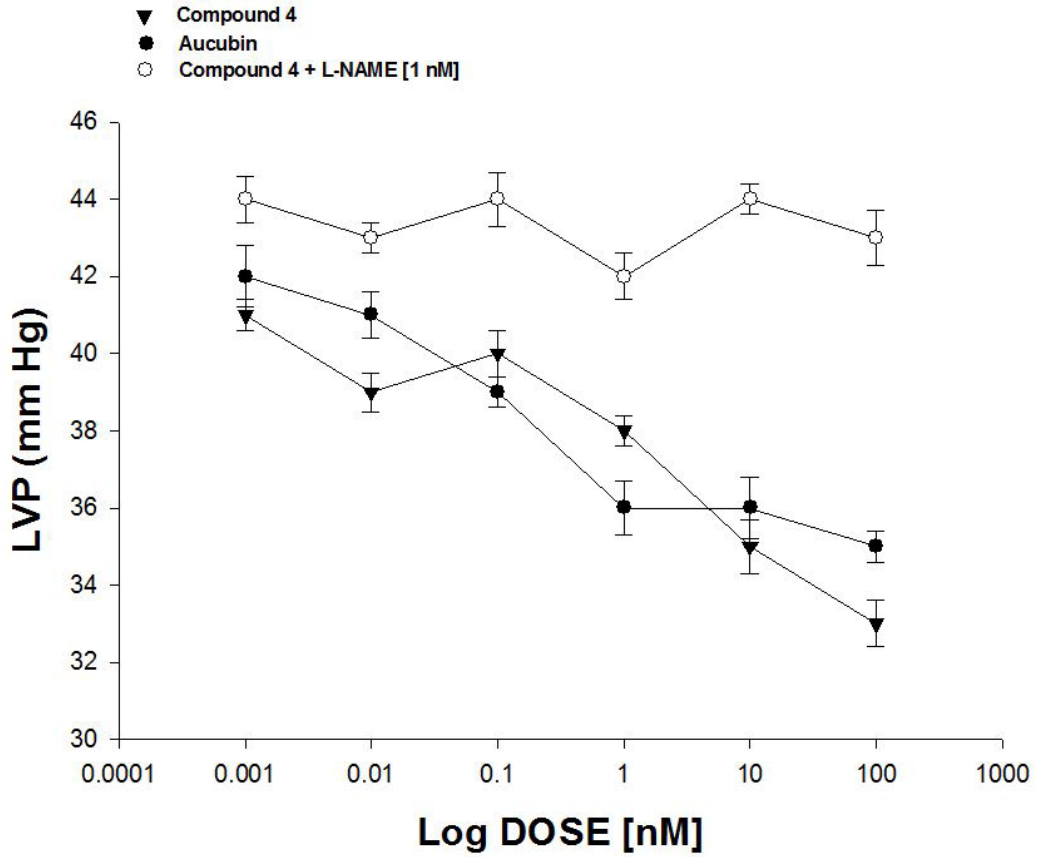

Figure 10. Effect exerted by compounds 4 and aucubin on left ventricular pressure (LVP). The results showed that 4 and aucubin decreased the LVP in a dose-dependent manner. Additionally, the negative inotropic activity of 4 was inhibited by L-NAME. Each point represents the mean ± S.E. of 9 experiments. L-NAME = Nω-Nitro-L-arginine methyl ester hydrochloride.

4) against left ventricular pressure (LVP) was evaluated. The results showed that compound 3 increase the LVP in a dose-dependent manner (Figure 7). Analyzing these data and other reports which indicate that some steroid derivatives can exert their effect through calcium channels activation [37], in this study the biological activity of compound 3 was determinate in the presence of nifedipine (calcium channel antagonist) [38].

The results showed that 3 increase LVP in a dose-dependent manner effect; however, this effect was inhibited by nifedipine; these data suggest that molecular mechanism involved in the inotropic activity of 3 was via type L calcium channel activation. To determine this hypothesis, also the biological activity of BAY-K-8644 (calcium channel activator) [39] against LVP was evaluated; the results indicated that BAY-K-8644 increase the LVP in a similar manner that compound 3; these data confirm that inotropic activity of compound 3 was via type L calcium channel activation.

On the other hand, other alternative experiments were carried out to evaluate the inotropic activity exerted by compound 4 against LVP; the results showed in the Figure 8 indicate that compound 4 decreased the LVP in a dose-dependent manner; however, this effect was inhibited by L-NAME (nitric oxide synthase inhibitor)[40].

These data indicate that negative inotropic activity induced by the compound 4 could be via nitric oxide synthase activation. To evaluate this hypothesis, also the effect exerted by aucubin (nitric oxide synthase activator) was evaluated; the results showed that aucubin decreased the LVP in a similar manner that compound 4; this phenomenon suggest that negative inotropic activity exerted by the compound 4 was through nitric oxide synthase activation.

Conclusions.

In this study is reported a facile synthesis of two 7,8-dioxabicyclo[4.1.1]octan-3-yl)-steroid derivatives using some chemical strategies. In addition, the results of biological activity of these steroid derivatives showed that; 1) the positive inotropic activity exerted by compound 3 was through type L calcium channel activation; 2) the negative inotropic effect of 4 was via nitric oxide synthase activation. These phenomena could be due to the different functional groups involved in the chemical structure of compounds 3 and 4.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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