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Synthesis and evaluation of biological activity from two steroid-diazacyclododecin derivatives on left ventricular pressure

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ABSTRACT

There are several studies which indicate that some cardiovascular diseases are relationship with biological activity of estrogens through of its receptors activation. The objective of this work was synthesizing two new steroid-diazacyclododecin derivatives (compounds 11 or 12) to evaluate their effect on left ventricular pressure in Langendorff model using as control to 17β-estradiol. In addition, a theoretical study was carried out to evaluate the interaction of compound 11 or 12 with the estrogen receptor (3os8 protein) using a docking model. The experimental results showed that only the compound 12 decreased left ventricular pressure in a similar form to 17β-estradiol. In addition, other experimental data showed that effect exerted by 17β-estradiol was inhibited in presence of 12. Finally, theoretical results indicated that interaction of 12 with 3os8 protein involved some aminoacid residues such as Pro_{224}, Leu_{319}, Leu_{320}, Leu_{327}, Asp_{321}, Ala_{322}, Glu_{323}, Pro_{324}, Pro_{325}, Ile_{326} and Leu_{327}. All these data suggest that compound 12 may act as a selective agonist of estrogen receptor which translated as changes on left ventricular pressure.

Keywords: *Steroid-diazacyclododeci; derivatives; 17β-estradiol; left ventricular pressure; docking.*

1. INTRODUCTION

Cardiovascular diseases are one of the main health problems in the world; several reports suggest that exogenous sex hormones have an influence on these clinical pathologies. For example, one study showed that estrogens can reduce the development of coronary heart diseases [1]. Another data indicates that 17β-estradiol; protect blood vessels from atherosclerotic lesion formation [2, 3]. It is noteworthy that the cellular effects exerted by estrogen can be controlled by the expression of nuclear receptors [4]. In this sense, several studies carried out in patients with cardiovascular diseases (coronary artery disease and atherosclerosis) and estrogen therapy showed an association between these clinical pathologies and estrogen receptor activation [5-10]. In addition to evaluate this association, a series of studies have been carried out using various estrogen receptor agonists or antagonist. A study showed that 16α-lactone-estradiol activates estrogen receptor which results as decrease cardiac hypertrophy using an animal model [11]. Other data indicated that propyl pyrazole and diarylpropiolnitrile exert cardioprotection through estrogen receptor activation in a hemorrhage injury model [12]. Also, a study showed that raloxifene relaxes coronary arteries via estrogen receptor activation *in vitro* [13]. However, other studies indicate that 27-hydroxycholesterol (endogenous oxysterol) inhibits the cardiovascular effects of estrogen [14]. Also, a study showed that tamoxifen and fulvestrant induce changes on cell growth via estrogen receptor [15]. All these data indicate that some drugs exert their biological activity as agonist or antagonist of estrogen receptors; this phenomenon can be due to the different functional groups involved in the chemical structure of each compound or to different protocols used. Analyzing this hypothesis, in this study two steroid-diazacyclododecin derivatives were synthesized to evaluate their biological activity using a Langendorff model. In addition, a theoretical study was conducted to characterize its interaction with estrogen receptor.

2. EXPERIMENTAL SECTION

Chemical synthesis. The compounds 2-nitro-estrone and 2-nitroestradiol were prepared using previously methods reported [16, 17]. Additionally, 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.^{1}H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR300/5 FT NMR spectrometer at 300 and 75.4 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.

General method for preparation of tert-Butyl-dimethyl-silanyloxy-nitro-steroid derivatives (3 or 4).

A solution of compound 1 or 2 (0.50 mmol), *tert*-butyldimethylsilyl chloride (200 µl, 1.07 mmol) in 3 ml of chloroform was stirring for 12 h at room temperature. The mixture obtained was dried under reduced pressure and purified by crystallization using the methanol:water (4:1) system.

3-(tert-Butyl-dimethyl-silanyloxy)-13-methyl-17-(1-methyl-1-trimethylsilanyl-ethyl)-2-nitro-7,8,9,11,12,13,14,15,16,17-deca-hydro-6H-cyclopenta[a]phenanthrene (3)

Yielding 68 % of product, m.p. 66-68 ^{o}C; IR (V_{max}, cm^{-1}) 1352 and 1080: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.08 (s, 6H), 0.22 (s, 6H), 0.80 (s, 3H), 0.86 (s, 9H), 0.98 (s, 9H), 1.06-1.92 (m, 10H), 2.10-7.80 (m, 7H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: -4.50, -4.40, 15.22, 17.80, 18.22, 25.34, 25.62, 25.70, 25.74, 27.76, 29.84, 32.97, 35.07, 37.28, 43.58, 43.74, 51.49, 82.62, 115.62, 122.73, 132.52, 133.34, 144.24, 151.72 ppm. EI-MS m/z: 545.33 Anal. Calcd. for $C_{30}H_{51}NO_4Si_2$: C, 66.10; H, 9.42; N, 2.57; O, 11.72; Si, 10.29. Found: C, 66.02; H, 9.36.

3-(tert-Butyl-dimethyl-silanyloxy)-13-methyl-2-nitro-6,7,8,9, 11,12,13,14,15,16-deca- hydro-cyclopenta[a]phenanthren-17-one (4)

Yielding 68 % of product, m.p. 50-52 ^{o}C; IR (V_{max}, cm^{-1}) 1350 and 1082: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.22 (s, 6H), 0.92 (s, 3H), 1.00 (s, 9H), 120-1.90 (m, 7H), 2.10-7.90 (m, 10H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: -4.44, 13.82, 18.22, 21.70, 25.51, 25.74, 26.40, 29.84, 31.06, 35.00, 37.24, 46.40, 48.34, 50.12, 115.62, 122.34, 132.00, 133.34, 143.94, 151.72, 219.70 ppm. EI-MS m/z: 429.23 Anal. Calcd. for $C_{24}H_{35}NO_4Si$: C, 67.10; H, 8.21; N, 3.26; O, 14.90; Si, 6.54. Found: C, 67.02; H, 8.16.

Synthesis of two tert-Butyl-dimethyl-silanyloxy-steroid-carbaldehyde derivatives (5 or 6)

In a round bottom flask (10 ml), the compound 3 or 4 (0.50 mmol), 2-hydroxy-1-naphthaldehyde (100 mg, 0.58 mmol) and potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide were stirring for 72 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. After, the residue was purified by crystallization from methanol:hexane:water (4:2:1)

8-[3,17-Bis-(tert-butyl-dimethyl-silanyloxy)-13-methyl-7,8,9, 11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenan-thren-2-yloxy]-naphthalene-1-carbaldehyde (5)

Yielding 54 % of product, m.p. 140-142 ^{o}C; IR (V_{max}, cm^{-1}) 1724, 1350 and 1082: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.06 (s, 6H), 0.18 (s, 6H), 0.80 (s, 3H), 0.86 (s, 9H), 0.98 (m, 1H), 1.00 (s, 9H), 110-1.90 (m, 10H), 2.10-6.52 (m, 7H), 7.10-8.40 (m, 6H), 9.96 (broad, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 9.96 (broad, 1H) ppm. -4.55, -4.50, 15.22, 17.84, 18.22, 25.32, 25.56, 25.74, 25.92, 27.76, 29.65, 32.97, 35.07, 37.24, 43.74, 44.00, 51.46, 82.60, 112.50, 114.94, 118.12, 120.92, 121.14, 126.72, 126.94, 128.44, 132.74, 135.62, 136.84, 137.62, 138.44, 143.64, 144.33, 147.92, 197.82 ppm. EI-MS m/z: 670.38 Anal. Calcd. for $C_{41}H_{58}O_4Si_2$: C, 73.28; H, 8.71; O, 9.54; Si, 6.54. Found: C, 73.20; H, 8.66.

8-(((13S)-3-((tert-butyldimethylsilyl)oxy)-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-2-yl)oxy)-1-naphthaldehyde (6)

yielding 54 % of product, m.p. 140-142 ^{o}C; IR (V_{max}, cm^{-1}) 3466, 3402, 1712 and 1620: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.18 (s, 6H), 0.90 (s, 3H), 1.00 (s, 9H), 120-1.90 (m, 7H), 2.10-6.52 (m, 10H), 7.10-8.40 (m, 6H), 9.96 (broad, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 9.96 (broad, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: -4.54, 13.80, 18.22, 21.78, 25.84, 25.92, 26.40, 29.64, 31.50, 35.40, 37.52, 46.84, 48.12, 50.40, 112.24, 114.94, 118.12, 120.93, 121.14, 126.60, 126.74, 128.40, 132.74, 135.62, 136.82, 137.12, 138.50, 143.64, 144.38, 147.90, 197.80, 220.70 ppm. EI-MS m/z: 554.28 Anal. Calcd. for $C_{35}H_{42}O_4Si$: C, 75.77; H, 7.63; O, 11.54; Si, 5.06. Found: C, 75.66; H, 7.58.

Deprotection of hydroxyl group. In a round bottom flask (10 ml), the compound 5 or **6** (0.50 mmol), 10 ml of Hydrofluoric acid were stirring for 72 h at room temperature. The product obtained was dried under reduced pressure. Then, the mixture was purified through of crystallization with methanol:benzene:water (4:2:1) system

8-(3,17-Dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-deca-hydro-6H-cyclopenta[a]phenanthren-2-yloxy)-naphthalene-1-carbaldehyde (7)

yielding 54 % of product, m.p. 308-310 ^{o}C; IR (V_{max}, cm^{-1}) 3400, 1724, 1222 and 1080: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.82-1.90 (m, 11H), 2.10-3.64 (m, 5H), 5.90 (broad, 2H), 6.04-6.70 (m, 2H), 7.12-8.40 (m, 6H), 9.96 (d, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.76, 24.22, 25.24, 27.76, 29.64, 32.74, 33.68, 37.24, 44.00, 44.42, 50.78, 82.42, 113.50, 115.00, 116.24, 120.92, 121.08, 126.74, 128.00, 128.40, 132.74, 136.80, 137.94, 138.52, 143.44, 143.94, 144.38, 151.60, 197.82 ppm. EI-MS m/z: 442.21 Anal. Calcd. for $C_{29}H_{30}O_4$: C, 78.71; H, 6.83; O, 14.46. Found: C, 78.64; H, 6.76.

8-(3-Hydroxy-13-methyl-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-2-yloxy)-naphtha-lene-1-carbaldehyde (8)

yielding 54 % of product, m.p. 234-236 ^{o}C; IR (V_{max}, cm^{-1}) 1728, 1725 and 1220: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H), 120-1.90 (m, 7H), 2.10-2.80 (m, 8H), 5.36 (broad, 1H), 6.14-6.70 (m, 2H), 7.12-8.40 (m, 6H), 9.96 (broad, 1H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_H: 13.80, 21.74, 25.84, 26.44, 29.64, 31.48, 35.42, 37.54, 46.87, 48.11, 50.41, 113.14,115.00, 16.28, 120.92, 121.11, 126.74, 127.60, 128.41, 132.77, 136.84, 137.50, 138.50, 143.48, 143.94, 144.38, 151.54, 197.80, 220.70 ppm. EI-MS m/z: 440.19 Anal. Calcd. for $C_{29}H_{28}O_4$: C, 79.07; H, 6.41; O, 14.53. Found: C, 79.00; H, 6.36.

Preparation of formyl-steroid-carbaldehyde complex. In a round bottom flask (50 ml), the compound **7** or **8** (0.50 mmol) and 10 ml of dimethyl sulfoxide were stirring for 72 h at room temperature. The product was dried under reduced pressure. Then, the mixture was purified by crystallization with the methanol:bencene:water (3:1) system

2-(8-Formyl-naphthalen-1-yloxy)-17-hydroxy-13-methyl-7,8,9, 11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3-carbaldehyde (9)

Yielding 54 % of product, m.p. 132-134 ^{o}C; IR (V_{max}, cm^{-1}) 3400, 1724 and 1220: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-3.62 (m, 16H), 6.40 (broad, 1H), 7.10 (m, 1H), 7.24-7.62 (m, 3H), 7.66 (m, 1H), 7.70-8.40 (m,3H), 10.00-10.44 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 15.80, 24.22, 25.34, 28.00, 29.61, 32.78, 33.71, 37.28, 44.02, 44.39, 50.76, 82.46, 107.02, 115.20, 119.92, 120.52, 121.12, 126.74, 127.17, 128.50, 129.24, 129.54, 135.62, 138.49, 144.36, 154.60, 154.76, 164.64, 189.50, 197.80 ppm. EI-MS m/z: 454.21 Anal. Calcd. for $C_{30}H_{30}O_4$: C, 79.27; H, 6.65; 0, 14.08. Found: C, 79.18; H, 6.56.

2-(8-Formyl-naphthalen-1-yloxy)-13-methyl-17-oxo-7,8,9,11, 12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3-carbaldehyde (10)

Yielding 54 % of product, m.p. 118-120 ^{o}C; IR (V_{max}, cm^{-1}) 1728, 1724 and 1220: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: s (0.88), 1.20-7.16 (m, 16H), 7.26-7.62 (m, 3H), 7.66 (m, 1H), 7.68-8.40 (m, 3H), 10.00-10.44 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_C: 13.82, 21.74, 25.87, 27.55, 29.61, 31.51, 35.43, 37.56, 46.87, 48.11, 50.41, 106.70, 115.24, 119.92, 120.52, 121.08, 126.74, 127.17, 128.14, 129.28, 129.54, 135.62, 138.49, 144.38, 154.12, 154.76, 164.66, 189.50, 197.80, 220.70 ppm. EI-MS m/z: 452.19 Anal. Calcd. for $C_{30}H_{28}O_4$: C, 79.62; H, 6.24; 0, 14.14. Found: C, 79.54; H, 6.18.

Preparation of imino-steroid derivatives. In a round bottom flask (50 ml), the compound **11** or **12** (0.50 mmol), ethylenediamine (50 µl, 0.74 mmol) and boric acid (26 mg, 0.42 mmol) 10 ml of methanol were stirring for 72 h to room temperature. The product obtained was dried under reduced pressure. Then, the residue was purified through the crystallization with the methanol:hexane:water (3:1:1) system

(4E,8E,15aS)-15a-methyl-7,11,12,12a,12b,13,14,15,15a,16,17, 17a-dodecahydro-6H-cyclopenta[7,8]phenanthro[3,2-b]naphtha[1,8-jk][1]oxa[5,8]diazacyclododecin-15-ol (11)

yielding 54 % of product, m.p. 140-142 ^{o}C; IR (V_{max}, cm^{-1}) 3380, 3322 and 1220: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.80-3.62 (m, 16H), 4.18 (m, 4H), 6.40 (broad, 2H), 6.88 (m, 1H), 7.18 (m, 1H), 7.46 (m, 1H), 7.50-7.70 (m, 5H), 8.30-8.50 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_H: 12.74, 23.87, 25.82, 27.62, 29.33, 31.16, 35.66, 38.90, 44.06, 44.15, 49.82, 58.96, 81.24, 111.00, 113.00, 120.16, 123.17, 126.02, 126.68, 128.15, 128.72, 128.95, 129.54, 130.63, 133.50, 136.55, 138.44, 144.63, 147.02, 158.64, 165.54 ppm.. EI-MS m/z: 544.35 Anal. Calcd. for $C_{37}H_{44}N_4$: C, 81.57; H, 8.14; N, 10.28. Found: C, 81.50; H, 8.08.

2-(((4E,8E,15E,15aS)-15a-methyl-6,7,11,12,12a,12b,13,14,15a, 16,17,17a-dodecahydro-15H-cyclopenta[7,8]phenanthro[3,2-b] naphtho[1,8-jk][1]oxa[5,8]diazacyclododecin-15-ylidene)amino) ethan-1-amine (12)

Yielding 54 % of product, m.p. 150-152 ^{o}C; IR (V_{max}, cm^{-1}) 3380, 3320 and 1222: ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H: 1.00 (s, 3H), 1.22-2.98 (m, 15H), 3.10-3.50 (m, 4H), 4.18 (m, 4H), 4.34 (broad, 2H), 7.10 (m, 1H), 7.18 (m, 1H), 7.44 (m, 1H), 7.52- 7.72- (m, 5H) 8.30-8.50 (m, 2H) ppm. ^{13}C NMR (500 MHz, Chloroform-*d*) δ_H: 15.96, 21.98, 26.00, 27.00, 27.53, 29.30, 32.42, 37.60, 41.02, 41.05, 44.56, 54.12, 54.29, 58.96, 111.00, 113.94, 120.18, 123.14, 126.00, 126.64, 128.19, 128.95, 129.56, 129.60, 130.63, 133.54, 136.55, 138.43, 144.62, 148.03, 158.68, 165.52, 176.82 ppm. EI-MS m/z: 518.30 Anal. Calcd. for $C_{34}H_{38}N_4O$: C, 78.73; H, 7.38; N, 10.80; O, 3.08. Found: C, 78.68; H, 7.30.

Physicochemical properties of compounds 9 and 10. Theoretical electronic properties, such as HOMO (Highest Occupied Molecular Orbital), LUMO (Lowest Unoccupied Molecular Orbital) energy, orbital coefficients distribution, molecular dipole moment and HBD (hydrogen bond donor groups) and HBA (hydrogen bond acceptor groups) and TPSA (topological polar surface area) were evaluated using the SPARTAN'06 software [18].

Evaluation of biological activity. The rats male (Wistar; weighing 200-250 g) used were obtained from the pharmacochemical laboratory of the Autonomous University of Campeche. It is important to mention that animals were handled in accordance with international standards for the care of laboratory animals [19].

Reagents. The different compounds were dissolved in methanol; in addition, the dilutions were obtained using Krebs-Henseleit.

Experimental design. The animals were anesthetized with pentobarbital (50 mg/Kg body weight) via intraperitoneal injecting. After, the chest was opened to expose the heart, following descending aorta was cut and the heart was immediately flushed with Krebs-Henseleit solution. Then, the heart was removed and perfused with Krebs-Henseleit* solution via retrograde at a constant flow rate of 10 ml/min.

*Krebs-Henseleit solution (pH = 7.4; 35-37 ^{0}C) bubbled with gas mixture (CO2, 5% and O2, 95%). Experimental data were done after of an equilibration period (10 min).

Perfusion pressure. The perfusion pressure and left ventricular pressure produced by the administration of each compound were determined using a pressure transducer that was bound to a chamber (where was inserted the heart). In addition, the signals were obtained using a computerized data capture system (MP-100).

Inotropic activity. To evaluate the inotropic effect, a latex balloon filled with saline solution (0.01 mm, diameter) was inserted into the left ventricle through the left atrium. It is noteworthy, that latex balloon was bound to pressure transducer which was connected to a computerized data capture system (MP-100). After, inotropic effect produced by compounds involved in this study was evaluated by determining left ventricular developed pressure (LV/dP) [19].

Biological evaluation

First stage

Biological activity exerted by 17β-estradiol and the compounds 6 or 7 against perfusion pressure: Effect produced by the

compounds 17β-estradiol, 11 or 12 (0.001 nM) and the conditions control on perfusion pressure through of time (3 to 18 min) was determined.

Second stage

Effects exerted by the compound 12 on left ventricular pressure through estrogen-receptor. 50 µl of compound **12** at dose of 0.001 to 100 nM were administered and their effect induced on left ventricular pressure in absence or presence of tamoxifen* (1 nM) was determined.

*Duration of preincubation with tamoxifen was by a 10 min equilibration period.

Biological activity produced by 17β-estradiol via estrogen receptor. 50 µl of 17β-estradiol at dose of 0.001 to 100 nM were administered and biological activity induce on left ventricular pressure in absence or presence of compound **12**** (0.001 nM) was determined. **Duration of preincubation with compound **12** was by a 10 min equilibration period.

Statistical analysis

Experimental data are display as average ± SE, using each heart as its own control. The results were analyzed with a statistical package (ANOVA) with $p = 0.05$ [20].

Theoretical evaluation of the interaction between compounds 6 or 7 with phosphodiesterase-4B.

A theoretical analysis was carried out using a docking program (DockingServer) [21]. Estrogen receptor (3os8 protein) was used to determine the interaction of compounds 11 or 12 with the enzyme [22].

3. RESULTS SECTION

There are several methods for preparation of azabicyclic; however, some reagents used require special conditions [23-25]; analyzing this data, in this report, a new azabicyclic derivative was prepared using different chemical strategies.

Praparation of tert-butyldimethylsilanyloxy steroid-derivatives (3 or 4). The first stage was achieved by protecting hydroxyl group of estradiol estrone using a previous method reported. This process was carried out in order to avoid a possible reaction of the hydroxyl group with any substance involved in the following reactions. It is noteworthy, that several organosilyl groups have been used as protectors of hydroxyl groups such as tertbutyldimethylsilyl and tert-butyldiphenylsilyl [26]. In this study, hydroxyl groups of 17β- estradiol or estrone were protecting with tert-butyldimethylsilyl chloride to form **3** or **4** (Figure 1).

The ^{1}H NMR spectrum of the compound 3 showed signals at 0.08-0.22 and 0.86 ppm for terbuthyldimethylsylane fragment; at 0.80 ppm for methyl group bound to steroid nucleus; at 1.00 and 1.06-7.80 ppm for steroid moiety. The ^{13}C NMR spectra display chemical shifts at -4.50, -4.40, 17.80-18.22, 25.70-25.74 ppm for terbuthyldimethylsylane fragment; at 25.34-25.62, 27.76-132.52 and 144.24 ppm for steroid moiety; at 133.34 ppm for carbon bound to nitro group; at 151.72 ppm for ether group. Finally, the mass spectrum from 3 showed a molecular ion (m/z) 545.33.

Other result showed the ^{1}H NMR spectrum of the compound 4 at 0.22 and 1.00 ppm for terbuthyldimethylsylane fragment; at 0.92 ppm for methyl group bound to steroid nucleus; at 1.20-7.90 ppm for steroid moiety. The ^{13}C NMR spectra displays chemical shifts at -4.44, 18.22 and 25.74 ppm for terbuthyldimethylsylane fragment; at 13.82 ppm for methyl group bound to steroid nucleus; at 21.70-25.51, 26.40-132.00 and 143.94 ppm for steroid moiety; at133.34 ppm for carbon bound to nitro group; at 151.72 ppm for ether group; at 219.70 for ketone group. In addition, the mass spectrum from 4 showed a molecular ion (m/z) 429.23.

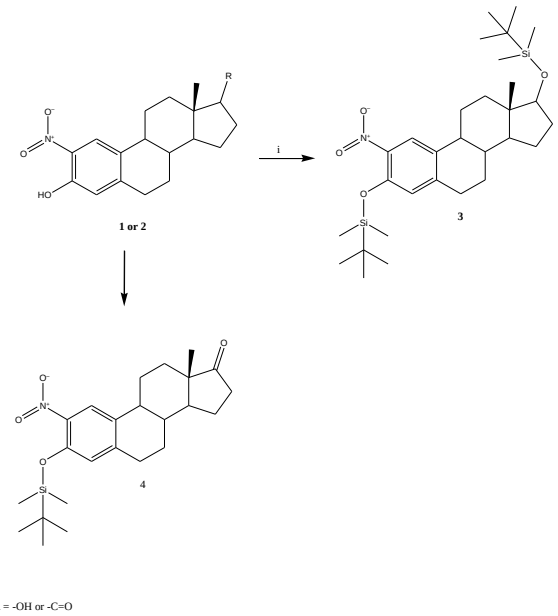

Figure 1. Preparation of two butyl-dimethyl-silanyloxy-nitro-steroid derivatives (3 or 4). Reaction of estradiol (1) or estrone (2) with *tert*-Butyldimethylsilyl chloride to form 3 or 4.

Synthesis of two steroid-naphtalen-carbaldehydederivatives*-tert-butyl-dimethyl-silanyloxy *complex (5 or 6).

Several carbaldehyde derivatives have been prepared using some reagent such as $POCl_3$ [27], nBuLi/THF [28], Cu/Fe [29], difluoro(phenylsulfanyl) methane [30], $RhCl_2$ [31] and others. However, some of these reagents are expensive and difficult to handle; therefore, in this study **5** or **6** were prepared using a previously method reported [32]. The compounds 3 or 4 were reacted with DMSO to form **5** or **6** (Figure 2). The ^{1}H NMR spectrum of the compound **5** showed signals at 0.06-0.18, 0.86 and 1.00 ppm for terbuthyldimethylsylane fragment; at 0.80 ppm for methyl group bound to steroid nucleus; at 0.98 and 1.10-6.52 ppm for steroid moiety; at 7.08-8.40 ppm for phenyl groups bound to both aldehyde and ether groups; at 9.96 ppm for aldehyde group.

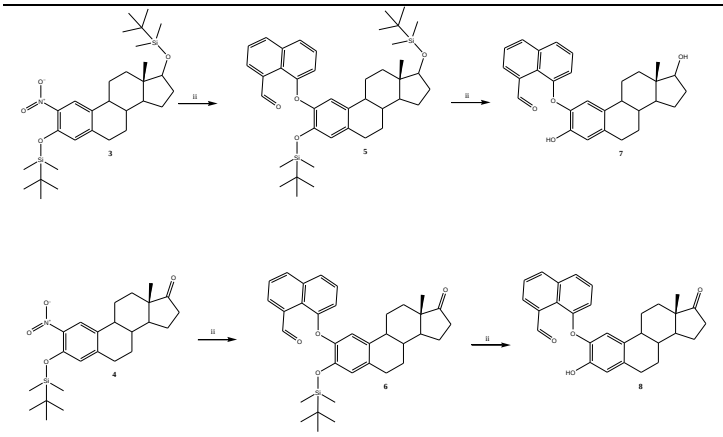

Figure 2. Synthesis of two steroid-naphtalen-carbaldehydederivatives (5 or 6). Reaction of 3 or 4 with 2-hydroxy-1-naphthaldehyde (ii) to form 5 or 6. Then 5 or 6 reacted with hydrofluoric acid (ii) to synthesis of 5 or 6.

The ^{13}C NMR spectra displays chemical shifts at -4.55, -4.50, 17.84-18.22 and 25.74-25.92 ppm for terbuthyldimethylsylane fragment; at 15.22 ppm for methyl group; at 25.32-25.56, 27.76-112.50, 118.12, 126.94, 135.62 and 137.62 ppm for steroid moiety; at 114.94, 129.92-126.72. 128.44-132.74, 136.84 and 138.44-144.33 ppm for phenyl groups bound to both aldehyde and ether groups; at 147.92 for ether group bound to diphenyl group; at 197.82 ppm for aldehyde group. Finally, the mass spectrum from **5** showed a molecular ion (m/z) 670.38.

On the other hand, the ^{1}H NMR spectrum of the compound **6** at 0.18 and 1.00 ppm for terbuthyldimethylsylane fragment; at 0.90 ppm for methyl group; at 1.20-6.52 ppm for steroid moiety; at 7.10-8.40 ppm for phenyl groups bound to both aldehyde and ether groups; at 9.96 ppm for aldehyde group. The ^{13}C NMR spectra displays chemical shifts at -4.54, 18.22 and 25.92 ppm for terbuthyldi- methylsylane fragment; at 13.80 ppm for methyl group bound to steroid moiety; at 21.78-25.84, 26.40-112.24, 118.12, 126.60 and 137.12 ppm for steroid moiety; at 114.94, 120.93-121.14, 126.74-132.74, 135.62-136.82, 138.50 and 144.38 ppm for phenyl groups bound to both aldehyde and ether groups; at 147.90 ppm for ether group bound to diphenyl groups; at 197.80 ppm for aldehyde group. In addition, the mass spectrum from 6 showed a molecular ion (m/z) 554.28.

***Removal of tert*-butyldimethylsilyl protecting groups.**

Some studies showed that hydrofluoric acid can be used to the removal of the *tert*-butyldimethylsilyl protecting groups [32, 33]. In this investigation, the compounds 5 or 6 were reacted with a hydrofluoric acid to form the compounds 7 or 8 (Figure 2). The ^{1}H NMR spectrum of the compound 7 at 0.76 ppm for methyl group bound to steroid nucleus; at 0.82-3.64 and 6.04-5.70 ppm for steroid nucleus; at 5.90 for hydroxyl groups; at 7.12-8.40 ppm for phenyl groups bound to both aldehyde and ether groups; at 9.96 ppm for aldehyde group. The ^{13}C NMR spectra displays chemical shifts at 15.76 ppm for methyl group; at 113.50, 116.24, 128.00, 137.94 and 143.44 for steroid moiety; at 115.00, 120.92-126.74, 128.40-136.80, 138.52 and 143.94-144.38 ppm for phenyl groups bound to both aldehyde and ether groups; at 151.60 for ether group; at 197.82 ppm for aldehyde group. Finally, the mass spectrum from 7 showed a molecular ion (m/z) 442.21.

The ^{1}H NMR spectrum of the compound **8** at 0.90 ppm for methyl group; at 1.202.80 and 6.16-6.70 ppm for steroid moiety; at 5.30 ppm for hydroxyl group; at 7.12-8.40 ppm for phenyl groups bound to both aldehyde and ether groups; at 9.16 ppm for aldehyde group. The ^{13}C NMR spectra displays chemical shifts at 13.80 ppm for methyl group; at 21.74-113.14, 116.28, 127.60, 137.50, 143.48 and 156.54 ppm for steroid moiety; at 115.00, 120.92-126.74, 128.41-138.84, 138.50 and 143.94-144.38 ppm for phenyl groups bound to both aldehyde and ether groups; at 197.80 for aldehyde group; at 220.70 ppm for ketone group. In addition, the mass spectrum from **8** showed a molecular ion (m/z) 440.19.

Preparation of formyl-steroid-carbaldehyde complex.

The compounds 7 or 8 were reacted (Figure 3) with dimethyl sulfoxide for the synthesis of new steroid-carbaldehyde derivatives (9 or 10).

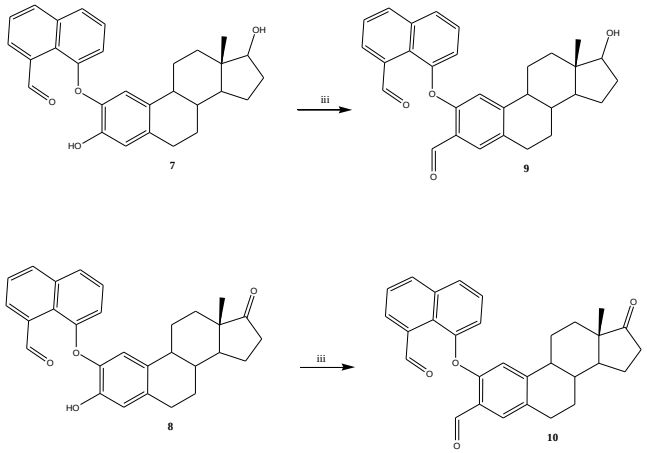

Figure 3. Preparation of di-carbaldehyde-steroid derivatives (9 or 10). Reaction of steroid-naphtalen-carbaldehydederivatives (**7** or **8**) with dimethylsulfoxide (iii) to form **9** or **10**.

The spectrum of the compound **9** at 0.76 ppm for methyl group; at 0.80-3.62, 7.10 and 7.66 ppm for steroid moiety; at 7.24-7.62 and 7.70-8.40 ppm for phenyl groups bound to ether group; at 10.00-10.44 for both aldehyde groups. The ^{13}C NMR spectra display chemical shifts at 15.80 ppm for methyl group; at 24.22-107.02, 120.52, 128.54, 129.54, 154.60 and 164.64 ppm for steroid moiety; at 115.20-119.92, 121.12-127.17, 129.24, 135.62-144.36 and 154.76 ppm for phenyl groups bound to ether group; at 189.50-197.80 ppm for both aldehyde groups. Finally, the mass spectrum from **9** showed a molecular ion (m/z) 454.21.

Other results showed the spectrum of the compound **10** at 0.88 ppm for methyl group; at 1.20-7.16 and 7.66 ppm for steroid moiety; at 7.26-7.62 and 7.68-8.40 ppm for phenyl groups; at 10.00-10.44 ppm for both aldehyde groups. The ^{13}C NMR spectra displays chemical shifts at 13.82 ppm for methyl group; at 21.74-106.70, 120.52, 128.14, 129.54, 154.12 and 164.66 ppm for steroid moiety; at 115.24, 121.08-127.17, 129.28, 135.62-144.38 and 154.76 ppm for phenyl groups bound to ether group; at 189.50-197.80 ppm for both aldehyde groups; at 220.70 ppm for ketone group. Additionally, the mass spectrum from **10** showed a molecular ion (m/z) 452.19.

Preparation of imino-steroid derivatives.

Several protocol have been used use some reagents for preparation of imino derivatives [34, 35]; however, some methods require special conditions; therefore, in this study, the compounds **9** or **10** were reacted with ethylenediamine using boric acid as catalyst [36] to form **11** or **12** (Figure 4).

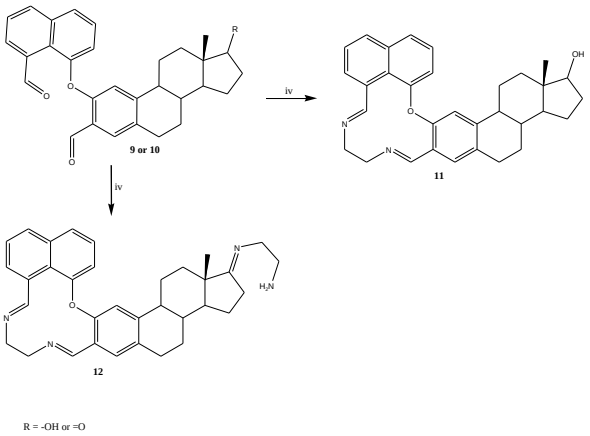

Figure 4. Preparation of amino-steroid derivative (11 or 12). Reaction di-carbaldehyde-steroid derivatives (9 or 10) with ethylenediamine (iv) to form 11 or 12.

The ^{1}H NMR spectrum of the compound 11 at 0.76 ppm for methyl group bound to steroid nucleus; at 0.80-3.62, 6.88 and 7.46 ppm for steroid moiety; at 4.18 ppm for methylene groups bound to both imino groups; at 7.18 and 7.50-7.70 for phenyl groups; at 8.30-8.50 ppm for both imino groups. The ^{13}C NMR spectra displays chemical shifts at 13.20 ppm for methyl group bound to steroid nucleus; at 12.74 ppm for methyl group at 23.87-49.82, 81.24, 113.00, 128.72, 130.63-133.50, 147.82 and 150.64 ppm for steroid moiety; at 111.00, 120.16-128.15, 128.95-129.54. 136.55 and 165.54 ppm for phenyl groups bound to ether group; at 138.44-144.63 for both imino groups. Finally, the mass spectrum from **11** showed a molecular ion (m/z) 544.35.

The ^{1}H NMR spectrum of the compound **12** at 1.00 ppm for methyl group bound to steroid nucleus; at 1.22-2.98, 7.10 and 7.44 ppm for steroid moiety; at 3.10-3.50 ppm for methylene groups bound to both imino and amino groups; at 4.18 ppm for methylene groups bound to both imino groups; at 4.34 ppm for amino group 7.18 and 7.52-7.72 ppm for both phenyl groups bound to ether group; at 8.30-8.50 ppm for both imino groups. The ^{13}C NMR spectra displays chemical shifts at 15.96 ppm for methyl group bound to steroid nucleus; at 21.98-37.60, 41.05-44.56, 54.29, 113.84, 129.60-133.54 and 148.03-158.68 ppm for steroid moiety; at 41.00 and 52.12 ppm for methylene groups bound to both amino and imino groups; at 58.96 ppm for methylene groups bound to both imino groups; at 111.oo, 112.18-129.56, 136.55 and 165.52 ppm for phenyl groups bound to ether group; at 135.43-144.62 and 176.82 ppm for imino groups. Additionally, the mass spectrum from 12 showed a molecular ion (m/z) 518.30.

Electronic parameters evaluation (HOMO and LUMO).

The molecular orbitals HOMO and LUMO (Figure 5, Table 1) for the compounds **11** and **12** were theoretically evaluated with SPARTAN'06 software, using Hartree-Fock method at 321-G level [37].

The results showed in Figure 5 indicated that LUMO values were lower for the compound 12 compared with 11; in addition, HBD and HBA values were different for two compounds (table 1), these data indicate that 12 have a different electron donation ability compared to 11.

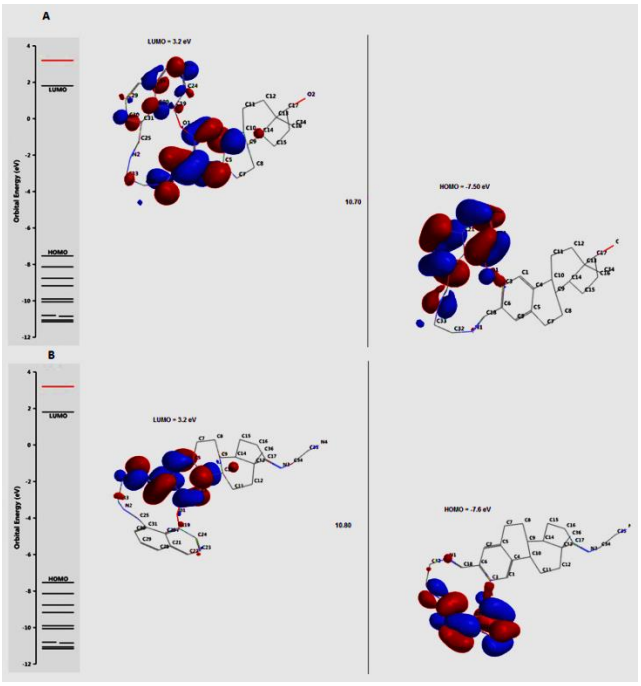

Figure 5. Molecular Orbitals (HOMO and LUMO) involved in the compounds 11 (A) and 12 (B). Visualized with SPARTAN'06 software.

Table 1. Structural properties

Parameter	Compound 11	Compound 12
logP	1.37	1.42
HBD count	1	0
HBA count	4	5
PSA	38.437 Å^2	52.188 Å^2
Polarizability	79.98	83.84

Biological activity

Effect induced by both compound 11 and 12 against perfusion pressure. The effect induced by the compounds **11** or **12** and β-estradiol against left ventricular pressure was evaluated in a Langendorff model. The experimental data showed that compound 12 decreased the left ventricular pressure in a similar form that estradiol.

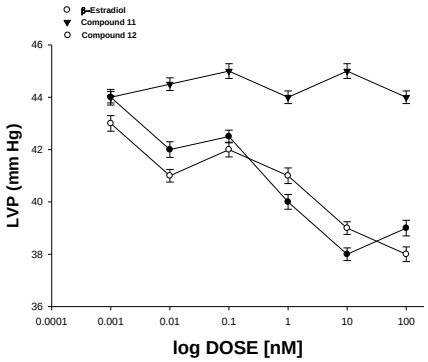

Figure 6. Effects induced by 17β-estradiol, compounds **11** and **12** on left ventricular pressure (LVP). 50μL of 17β-estradiol, **11** and **12** at dose of 0.001 to 100 nM were administered and the biological activity against LVP was evaluated. Experimental data obtained shown that compound **12** decreased LVP (P = 0.06) in a similar form that 17β-estradiol. Each bar represents the mean ± SE of 9 experiments.

To evaluate the possibility that compound **12** could exert its activity through the activation of the estrogen receptor, in this study the effect induced by compound **12** on left ventricular pressure (LVP) in the absence or in presence of an estrogen antagonist (tamoxifen) was evaluated (Figure 7). The results indicated that compound **12** decrease the **LVP** in a dose-dependent

manner and their biological activity was blocked by tamoxifen; this phenomenon suggests that molecular mechanism involved in produced by compound 12 was via estrogen receptor activation.

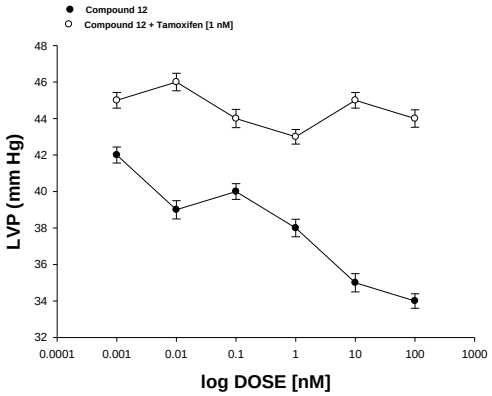

Figure 7. Effect produced by compounds 12 on left ventricular pressure (LVP) in absence or presence of tamoxifen. Intracoronary boluses (50μL) of **12** [0.001 to 100nM] were administered in absence or presence of tamoxifen and their biological activity against LVP was evaluated. The results showed that compound **12** decreased LVP (P = 0.06) and this effect was inhibited with tamoxifen. Each bar represents the mean ± SE of 9 experiments.

Analyzing this data and other reports, which showed that fulvestrant (steroid derivative) inhibit the biological activity of 17β-estradiol exerted on left ventricular pressure [15]; therefore, in this investigation the effect induced by 17β-estradiol against left ventricular pressure was evaluated in presence or absence of compound **12** (Figure 8).

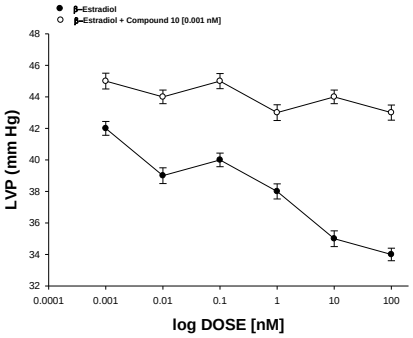

Figure 8. Biological activity induced by 17β-estradiol on left ventricular pressure (LVP). Intracoronary boluses (50μL) of 17β-estradiol [0.001 to 100nM] were administered in absence or presence of compound **12** [0.001 nM] and their biological effect against LVP was evaluated. The results showed that 17β- estradiol decreased LVP (***p*** = 0.05) and this effect was inhibited by the compound **12**. Each bar represents the mean ± SE of 9 experiments.

The results indicated that 17β-estradiol decreased the left ventricular pressure; however, this phenomenon was inhibited by the compound 12. These results suggest that compound 12 could be an estrogen selective agonist. It is noteworthy, that there are several theoretical studies which have been used to predict the biological activity of several substances as estrogen agonist [38-40].

Theoretical analysis.

To evaluate the interaction of compound 12 with estrogen receptor (3os8 protein) [22] a Docking model was used [21]. The results showed (Figure 9 and Table 2) the interaction of compound 12 with the amino acid residues of 3os8 protein such as Pro_{224}, Leu_{319}, Leu_{320}, Leu_{327}, Asp_{321}, Ala_{322}, Glu_{323}, Pro_{324}, Pro_{325}, Ile_{326} ,and Leu_{327}. It is important to mention of amino group free of compound **12** could bound to Leu_{327}, Ile_{326} and Pro_{324} aminoacid residues.

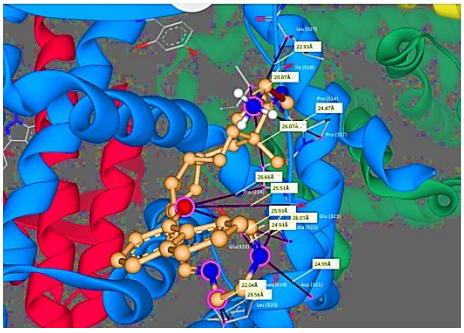

Figure 9. Distance between compound 10 and aminoacid residues of estrogen receptor (3os8 protein).

Table 2. Distance between the aminoacid residues of 3os8 protein and both nitrogen and oxygen atoms

Aminoacid Residues	Nitrogen (Å)	Oxygen (Å)
Pro_{224}	-	26.66
Leu_{319}	22.04	-
Leu_{320}	29.56	-
Asp_{321}	24.99	-
Ala_{322}	24.93	26.01
Glu_{323}	26.01	25.51
Pro_{324}	24.07	-
Pro_{325}	26.07	-
Ile_{326}	20.07	-
Leu_{327}	22.93	-

4. CONCLUSIONS

The experimental results showed that; 1) compound 12 decreased left ventricular pressure via estrogen receptor; 2) the effect exerted by estradiol was inhibited in presence of 12; 3) the data indicate that compound 12 could exert their biological activity which the interaction with could involves some aminoacids residue of the estrogen receptor.

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