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Synthesis of new azaindeno-acetonitrile derivative with inotropic activity against heart failure model

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ABSTRACT

Several steroid derivatives have prepared as inotropic drugs; however, there are few reports on azaindeno-steroid derivatives with inotropic activity. The objective of this investigation was to prepare some azaindeno-acetonitrile derivatives (compounds 3 to 7) to evaluate their biological activity on left ventricular pressure. The first step was achieved by preparation of azaindeno-steroid derivatives using reactions of etherification and addition. The second stage involves the evaluation of biological activity from azaindeno-steroid derivatives on left ventricular pressure in a heart failure model using either estrone or an enone-steroid derivative (compound 2) as controls. The results showed that only compound 6 increases left ventricular pressure compared with estrone, compounds 2-5 and 6. In conclusion, the positive inotropic effect exerted by compound 6 depends on the functional groups involved in its chemical structure.

Keywords: *Azaindeno, steroid, left ventricular pressure, inotropic*.

1. INTRODUCTION

Congestive Failure heart (CFH) is a risk factor to developed cardiovascular diseases in the worldwide; it is noteworthy that several drugs have been used for the treatment of CFH such as β-adrenergic blocking [1], calcium antagonist [2], diuretics [3], angiotensin-converting-enzyme inhibitors [4] and others; however some these drugs can produce some secondary effects [5-7]. In the search of a new therapeutic alternatives several compound have been developed to treatment of CHF; for example, the preparation of (2-Methylsulfonylethyl)1-(Isopropyl-amino)diazen-1-ium-1,2-diolate from a 2-Methylthio-ethyl derivative with positive inotropic activity in an isolated mouse heart model [8]. Another study showed the reaction of a cyanoacetamide with a carbonitrile derivative to 5-cyano-1,6-dihydro-2-methyl-6-oxo-3-pyridine carboxyl acid which exerted a positive inotropic effect on an isolated heart guinea pig model [9]. Also, the positive inotropic 3,6-Dimethyl-5-hydroxymethyl-2-pivaloyloxymethylpyrazine was prepared from 2,5-Dihydroxymethyl-3,6-dimethyl- pyrazine and pivaloyl chloride [10]. Other report indicates the reaction of 3,5-dinitre and hexyne to form a pirrol-indol derivative with positive inotropic activity in an isolated rat heart model [11]. In addition, a 7,7a-hexahydro-1H-indene derivative from aminoguanidine was prepared to evaluate their positive inotropic activity on isolated guinea pig atria [12]. Other data showed the preparation of 7-(1-H-Azol-1-yl)-2,4,4a,5-tetrahydro-3H-indeno[1,2-c]pyradazin-3-one from (5-Imidazol-1-yl-1-oxo-indan-2-yl)-acetic acid which increase the inotropic effect in a dog model [13]. In addition, some dihydro-indeno-pyridines were prepared from 1,3-indandione with positive inotropic effect on the left atria of guinea pig [14]. All these data indicate that several compounds can exert positive inotropic activity in several biological models; however, there are few data on inotropic activity of indeno-derivatives. Therefore, in this study an azaindeno-acetonitrile derivative was prepared to evaluate their inotropic activity on heart failure model.

2. MATERIALS AND METHODS

2.1. General methods.

All reagents used in this investigation were acquired from Sigma-Aldrich Co., Ltd. The melting point for compounds was evaluated on an Electrothermal (900 model). Infrared spectra (IR) were evaluated with a Thermo Scientific iSOFT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded using a Varian VXR300/5 FT NMR spectrometer at 300 MHz in $CDCl_3$ using TMS as internal standard. EIMS spectra were obtained with a Finnigan Trace Gas Chromatography Polaris Q-Spectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

2.2. Chemical Synthesis.

Preparation of 16-Benzylidene-3-hydroxy-13-methyl-6,7,8,9, 11,12,13,14,15,16-decahy-dro-cyclopenta[a]phenanthren-17-one (2)

A solution of estrone (200 mg, 0.74 mmol), sodium hydroxide (20 mg, 0.5 mmol mmol) and 3 ml of acetophenone were stirred to reflux for 4 h. Then, the solvent of the mixture was evaporated to pressure reduced and following the product was purified via crystallization using the methanol:water:hexane (3:1:1) system; yielding 64 % of product; m.p. 120-122 ^{o}C; IR (Vmax, cm^{-1}) 2124 and 1712: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.90 (s, 3H),

1.24-1.80 (m, 5H), 2.11-2.90 (m, 8H), 4.82 (broad, 1H), 6.30 (d, 1H, J = 0.33 Hz), 6.60-6.66 (m, 2H), 7.10-7.24 (m, 3H), 7.32 (m, 1H), 7.60 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 18.50, 26.12, 27.38, 29.82, 31.70, 37.02, 38.50, 44.70, 46.22, 48.95, 113.42, 115.92, 126.30, 127.00, 128.50, 129.16, 129.82, 132.63, 137.30, 137.90, 140.52, 155.73, 214.90 ppm. EI-MS m/z: 358.19. Anal. Calcd. for $C_{25}H_{26}O_2$: C, 83.76; H, 7.31; O, 8.93. Found: C, 83.72; H, 7.30.

Synthesis of 6a-Methyl-7,9-diphenyl-5,6,6a,11,11a,11b,12,13-octahydro-4bH-10-aza-indeno[2,1-a]phenanthren-2-ol (3)

A solution of **2** (200 mg, 0.56mmol), 1-(2-oxo-2-phenyl-ethyl)-pyridinium Iodide (200 mg, 0.58 mmol), ammonium acetate (40 mg, 0.52 mmol) in 5 ml of dimethyl sulfoxide was stirred to reflux for 24 h. Then, the solvent of the mixture was evaporated to pressure reduced and following the product was purified via crystallization using the methanol:water (3:1) system; yielding 64 % of product; m.p. 98-100 ^{o}C**;** IR (Vmax, cm^{-1}) 2124 and 1712: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 1.22 (s, 3H), 1.30-2.06 (m, 7H), 2.44-3.34 (m, 6H), 5.56 (broad, 1H), 6.60-6.66 (m, 2H), 7.04 (m, 2H), 7.20 (m, 1H), 7.26-7.70 (m, 6H), 7.88 (m, 1H), 7.96 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 20.82, 26.84, 27.92, 31.32, 34.20, 35.66, 37.72, 41.62, 45.40, 55.95, 113.20, 115.54, 119.80, 125.86, 126.32, 126.84, 128.37, 128.82, 128.94, 129.32, 133.22, 137.74, 138.60 140.34, 146.60, 154.33, 155.00, 155.26, 182.80 ppm. EI-MS m/z: 457.24. Anal. Calcd. for $C_{33}H_{31}NO$: C, 86.61; H, 6.83; N, 3.06; O, 3.50. Found: C, 86.58; H, 6.80.

Preparation of 4-(6a-Methyl-7,9-diphenyl-5,6,6a,11,11a,11b, 12,13-octahydro-4bH-10-aza-indeno[2,1-a]phenanthren-2-yl-oxy)-benzoyl azide (4)

A solution of **3** (200 mg, 0.43 mmol), *p*-nitrobenzoyl azide (100 µl, 0.52 mmol), potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirred to room temperature for 72 h. Then, the solvent of the mixture was evaporated to pressure reduced and following the product was purified via crystallization using the methanol:water:hexane (3:1:2) system; yielding 64 % of product; m.p. 128-130 ^{o}C; IR (vmax, cm^{-1}) 2124 and 1712: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 1.22 (s, 3H), 1.30-1.94 (m, 4H), 2.02-3.32 (m, 9H), 6.66-6.76 (m, 2H), 6.90 (m, 2H), 7.04 (m, 1H), 7.06-7.70 (m, 8H), 7.80 (m, 2H), 7.86 (m, 1H), 7.97 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C:20.82, 26.84, 27.92, 29.72, 34.20, 35.64, 37.72, 41.62, 45.40, 55.95, 114.23, 114.55, 115.32, 119.80, 123.76, 124.30, 125.86, 126.87, 128.34, 128.82, 128.92, 129.30, 132.54, 133.90, 138.58, 139.22, 140.34, 146.60, 154.33, 154.80, 155.30, 163.80, 171.62, 182.74 ppm. EI-MS m/z: 602.26. Anal. Calcd. for $C_{40}H_{34}N_4O_2$: C, 79.71; H, 5.69; N, 9.30; O, 5.31. Found: C, 79.68; H, 5.66.

[4-(Hydroxy-phenyl-methyl)-[1,2,3]triazol-1-yl]-[4-(6a-methyl-7,9-diphenyl-5,6,6a,11,11a,11b,12,13-octahydro-4bH-10-aza-indeno[2,1-a]phenanthren-2-yloxy)-phenyl]-me- thanone (5)

A solution of **4** (200 mg, 0.33 mmol), 1-Phenyl-2-propyn-1-ol (50 µl, 0.41 mmol) and Copper(II) chloride anhydrous (60 mg, 0.45 mmol) in 5 ml of methanol was stirred to room temperature for 72 h. Then, the solvent of the mixture was evaporated to pressure reduced and following the product was purified via crystallization using the methanol:water (3:1) system; yielding 77 % of product; m.p. 188-190 ^{o}C; IR (vmax, cm^{-1}) 2124 and 1712: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 1.22 (s, 3H), 1.30-3.30 (m, 13H), 5.74 (m, 1H), 6.30 (broad, 1H), 6.66-7.03 (m, 3H), 7.06 (m, 2H), 7.24 (m, 2H), 7.26 (m, 1H), 7.27-7.29 (m, 4H), 7.43-7.60 (m, 4H), 7.70 (m, 2H), 7.74 (m, 1H), 7.88 (m, 1H), 7.96 (m, 2H), 8.38 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 20.82, 26.84, 27.92, 29.66, 34.20, 35.66, 37.74, 41.62, 45.40, 53.56, 55.98, 114.22, 114.52, 117.70, 118.55, 119.84, 124.30, 125.86, 126.12, 126.86, 127.50, 128.36, 128.82, 128.94, 129.00, 129.32, 130.40, 133.90, 138.58, 139.25, 140.37, 146.22, 146.60, 150.12, 154.33, 154.80, 155.26, 161.32, 169.20, 182.76 ppm. EI-MS m/z: 734.32. Anal. Calcd. for $C_{49}H_{42}N_4O_3$: C, 80.08; H, 5.76; N, 7.62; O, 6.53. Found: C, 80.02; H, 5.72.

[4-(6a-Methyl-7,9-diphenyl-5,6,6a,11,11a,11b,12,13-octahydro-4bH-10-aza-indeno[2,1-a]phenanthren-2-yloxy)-phenyl]-acetonitrile (6)

A solution of **3** (200 mg, 0.43 mmol), (4)-Nitro-phenyl)-acetomitrile (90 mg; 0.55 mmol) potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirred to room temperature for 72 h. Then, the solvent of the mixture was evaporated to pressure reduced and following the product was purified via crystallization using the methanol:water:hexane (3:1:1) system; yielding 64 % of product; m.p. 188-190 ^{o}C; IR (vmax, cm^{-1}) 2124 and 1712: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 1.22 (s, 3H), 1.30-3.30 (m, 13H), 3.62 (m, 2H), 6.66-6.76 (m, 2H), 6.96 (m, 2H), 7.04 (m, 1H), 7.06-7.29 (m, 6H), 7.34 (m, 2H), 7.70 (m, 2H), 7.88 (m, 1H), 7.96 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 20.82, 23.42, 26.82, 27.92, 29.72, 34.20, 35.66, 37.72, 41.63, 45.40, 55.95, 114.22, 114.52, 117.02, 117.40, 119.80, 122.48, 124.30, 125.85, 126.87, 128.24, 128.34, 128.86, 128.92, 129.30, 133.90, 138.55, 139.22, 140.34, 146.60, 154.32, 154.95, 155.32, 155.90, 182.80 ppm. EI-MS m/z: 572.28. Anal. Calcd. for $C_{41}H_{36}N_2O$: C, 85.98; H, 6.34; N, 4.89; O, 2.79. Found: C, 85.94; H, 6.31.

2-{4-[4-(6a-Methyl-7,9-diphenyl-5,6,6a,11,11a,11b,12,13-octa-hydro-4bH-10-aza-indeno[2,1-a]phenanthren-2-yloxy)-benzyl]-2,3-dihydro-azet-2-yl}-1-phenyl-ethanol (7)

A solution of **6** (200 mg, 0.35 mmol), 4-Phenyl-1-buten-4-ol (60 µl, 0.40 mmol), Copper(II) chloride anhydrous (60 mg, 0.45 mmol) in 5 ml of methanol was stirred to reflux for 72 h. Then, the solvent of the mixture was evaporated to pressure reduced and following the product was purified via crystallization using the methanol:water(3:1 system; yielding 67 % of product; m.p. 118-120 ^{o}C; IR (vmax, cm^{-1}) 2124 and 1712: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 1.22 (s, 3H), 1.30-1.85 (m, 3H), 1.90 (m, 1H), 1.94-2.04 (m, 4H), 2.20 (m, 1H), 2.44-2.66 (m, 2H), 2.68 (broad, 1H), 2.70-3.30 (m, 4H), 3.56 (m, 1H), 4.20-4.50 (m, 3H), 5.13 (m, 1H), 6.66-6.76 (m, 2H), 6.80-6.86 (m, 4H), 7.04 (m, 1H), 7.06 (m, 2H), 7.25 (m, 2H), 7.27 (m, 3H), 7.28 (m, 1H), 7.29 (m, 1H), 7.32 (m, 2H), 7.70 (m, 2H), 7.88 (m, 1H), 7.96 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 20.82, 26.84, 27.92, 29.66, 33.08, 34.20, 35.64, 37.72, 41.20, 41.62, 44.22, 45.40, 45.96, 55.96, 70.52, 114.22, 114.52, 114.72, 119.80, 124.30, 125.84, 126.02, 126.60, 126.84, 126.94, 128.02, 128.34, 128.82, 128.92, 129.32, 130.00, 133.90, 138.56, 139.22, 140.32, 145.30, 146.60, 154.32, 155.22, 155.27, 155.90, 165.96, 182.80 ppm. EI-MS m/z: 720.37. Anal. Calcd. for $C_{51}H_{48}N_2O_2$: C, 84.96; H, 6.71; N, 3.89; O, 4.44. Found: C, 84.90; H, 6.70.

2.3. Biological method.

All experimental methods used were reviewed and approved by the Animal care and use committee of University Autonomous of Campeche (UAC) and were in accordance with the guide for the care and use of laboratory animals [15].

2.4. Methodology general.

The male rats (200-250 g) were anesthetized through administration with pentobarbital (50 mg/Kg). Then the chest was opened, and a loose ligature passed *via* the ascending aorta; following the heart was removed and immersed in a Krebs-Henseleit solution* (pH 7.4, 37°C and bubbled with a mixture of O_2/CO_2 [5%/95%]). After, the heart was perfused with a Krebs-Henseleit solution trough a non-circulating perfusion system at a constant flow rate (10 ml/min).

* Krebs-Henseleit solution (mmol); 117.8 NaCl; 6 KCl; 1.75 $CaCl_2$;1.2 NaH_2PO_4; 1.2 $MgSO_4$; 24.2 $NaHCO_3$; 5 glucose and 5 sodium pyruvate. The coronary flow was adjusted with a variable speed peristaltic pump and perfusion rate of solution was of 10 ml/min.

2.5. Heart failure (HF).

HF was carried out using a previously reported method [16] as follows; the pentobarbital (100 Kg^{-1}/mg) is administered through a cannula which was inserted into the aorta to decrease the cardiac work.

2.6. Inotropic activity.

Contractile activity was determined by measuring left ventricular pressure; this process involves a saline-filled latex balloon (0.01 mm, diameter) inserted into the left ventricle via the left atrium. It is noteworthy that latex balloon was linked to a cannula which is bound to a pressure transducer connected at a MP100 data acquisition system.

2.7. Evaluation of effect exerted by estrone and compounds 2 to 6 on left ventricular pressures. Intracoronary boluses (50 µl) of estrone or either of compounds 2 to 6 at a dose of 0.001 nM were administered and their biological activity on the left ventricular pressure was determined.

2.8. Statistical Analysis.

The results were expressed as average ± SE, using each heart (n = 7) as its own control. The results were put under analysis of variance with the Bonferroni correction factor [19] using the SPSS 12.0 software and the differences were considered significant when $p = 0.05$

3. RESULTS

It is noteworthy that several indene analogs have been prepared using some protocols which require different reagents and special conditions [12-14]. Therefore, in this study some indene derivatives were synthesized to evaluate their biological activity using a heart failure model.

3.1. Preparation of an enone-steroid derivative.

The first reaction involved the synthesis of a 16-Benzylidene-3-hydroxy-steroid17-one (**2**); it is noteworthy that several enone derivatives have developed using some reagents such as N-bromosuccinamide [18], L-tartaric acid [19], PPh_3AuNTf_2 [20], (IPr)AuCl]/$AgBF_4$ [21], diethyl acetal [22]. In this investigation, the compound **2** was prepared from estrone and benzaldehyde in basic conditions (Figure 1).

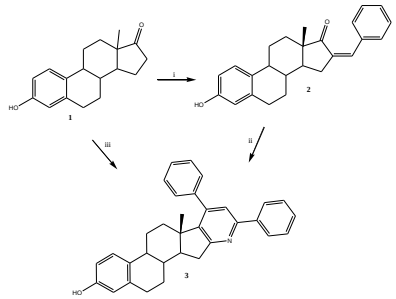

Figure 1. Preparation of a pyridine-steroid derivative (**3**). Reaction of estrone with acetophenone to form an enone-steroid derivative (**2**). Then, **2** reacted with 1-(2-oxo-2-phenyl-ethyl)-pyridinium Iodide to synthesis of **3**. Conditions: i = NaOH/reflux; ii = ammonium acetate/reflux.

^{1}H NMR spectrum for compound 2 (Figure 2) was found at 0.90 ppm for methyl group linked to steroid nucleus; at 1,24-2.90, 6.60-6.66 and 7.32 ppm for steroid moiety; at 4.82 ppm for hydroxyl group; at 6.30 ppm for alkene group; at 7.10-7.24 and 7.60 ppm for phenyl linked to alkene group. Additionally, other signals involved in the

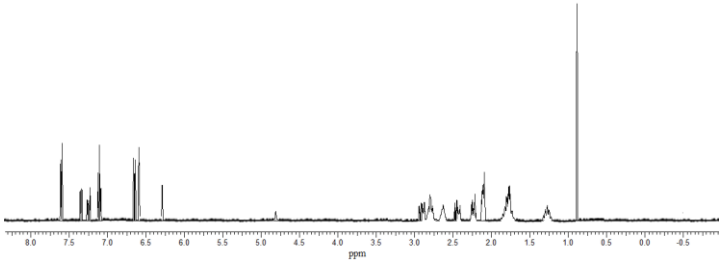

Figure 2. The scheme showed ^{1}HNMR spectrum from compound **2**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

^{13}C NMR spectrum were showed at 18.50 ppm for methyl group; at 26.12-126.30, 132.63, 137.30 and 140.52-155.73 ppm for steroid moiety; at 127.00-128.50, 129.82 and 137.90 ppm for phenyl bound to alkene group; at 129.16 ppm for alkene group; at 21.90 ppm for ketone group. In addition, the mass spectrum (m/z) from compound 2 was found to 358.19.

3.2. Synthesis of a pyridine-steroid derivative.

There are some protocols for preparation of pyridines using several reagents such as $Fe(CF_3SO_3)_2$ complex [23], [RhCp-$Cl_2]_2$ complex [24], pyrrolidine/Copper(I) [25], Tf_2O [26], $LiN(TMS)_2$ [27]. In this study, the compound **3** (Figure 1) was prepared using two methods; in the method A, the compound **2** reacted with pyridinium iodide salt in presence of NH_4OAc to synthesis of an aza-indeno[2,1-a]phenanthren-2-ol derivative (**3**); the reaction mechanism involves condensation of pyridinium salt to enone (**2**) to form **3**. ^{1}H NMR spectrum (Figure 3) for compound **3** were found at 1.22 for methyl bound to steroid nucleus; at 1.30-3.34, 6.60-6.66 and 7.20 for steroid moiety; at 5.56 ppm for hydroxyl group; at 7.04, 7.26-7.70 and 7.96 for phenyl groups bound to pyridine ring; at 7.88 ppm for pyridine ring.

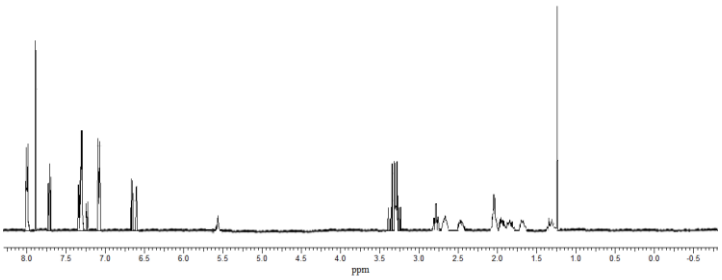

Figure 3. The scheme showed ^{1}HNMR spectrum from compound **3**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

Other signals found in the ^{13}C NMR spectrum for **3** showed bands at 20.82 ppm for methyl group; at 26.84-115.54, 126.32, 133.22-137.74 and 155.00 ppm for steroid moiety; at 119.80, 146.60-154.33 and 155.26-186.80 ppm for pyridine ring; at 125.86, 126.84-129.32 and 138.60-140.34 ppm for phenyl groups bound to pyridine ring. Additionaly, the mass spectrum (m/z) from compound **3** was found to 457.24. The second method (B) was achieved using a three-component system (estrone, benzaldehyde and acetophenone) to form the compound **3**. It is noteworthy that yielding was higher with method A, possible due to conditions of reaction used.

3.3. Synthesis of an ether derivative.

In the literature has several protocols for preparation of ether derivatives using some reagents such as aluminum oxide [29], $ZnCl_2$ [29], dinitrobenzophenone derivative [30], DMSO [31]. In this study, the compound **4** was prepared through of displacement from nitro group of p-nitrobenzoil azide of hydroxyl group of compound **3** in the presence of dimethyl sulfoxide at mild conditions (Figure 4). ^{1}H NMR spectrum for compound 4 (Figure 5) was found at 1.22 for methyl bound to steroid nucleus; at 1.30-3.32, 6.60-6.76 and 7.04 for steroid moiety; at 6.90 and 7.80 ppm for phenyl bound to both ether and carbonyl groups; at 7.06-7.70

and 7.97 ppm for phenyl groups bound to pyridine ring; at 7.86 ppm for pyridine ring.

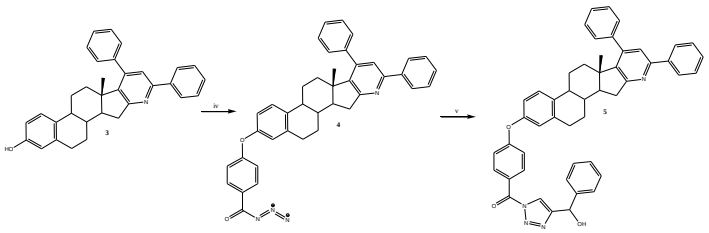

Figure 4. Synthesis of a triazole-steroid analog (**5**). Reaction of a pyridine-steroid derivative (**3**) with p-nitrobenzoyl azide to form an ether-derivative (**4**). Then, **4** reacted with 1-Phenyl-2-propyn-1-ol to synthesis of **5**. Conditions: iv = potassium carbonate/dimethyl sulfoxide/rt; v = Copper(II) chloride/rt.

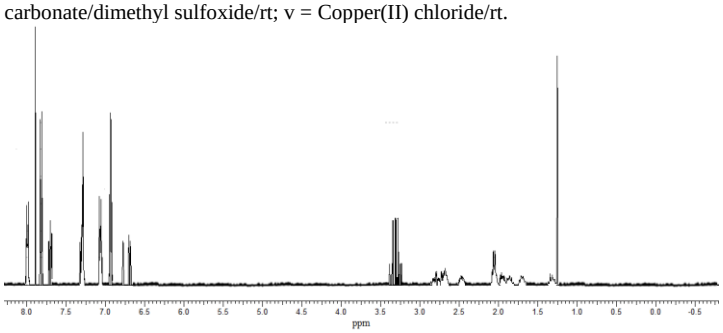

Figure 5. The scheme showed ^{1}HNMR spectrum from compound **4**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

Additionally, other signals involved in the ^{13}C NMR spectrum were showed at 20.82 ppm for methyl group; at 26.84-114.55, 124.30, 133.90, 139.22 and 154.80 ppm for steroid moiety; at 115.32, 123.76, 132.54 and 163,80 ppm for phenyl bound to both ether and carbonyl groups; at 119.80, 146.60-154.33, 155.30 and 162.74 ppm for pyridine ring; at 125.86-129.30, 138.58 and 140.34 ppm for phenyl groups bound to pyridine ring; at 171.62 ppm for carbonyl group. In addition, the mass spectrum (m/z) from compound 4 was found to 602.26.

3.4. Preparation of a triazole ring.

Several triazole rings have been synthetized using different reagents such as alkynyl/vinyl/azide(Cu(I) [32], Ag(I) [33], $Pd(OAc)_2$ [34], CuO [35], $PhCO_2$/Ag [36] and others. In this study the compound 5 was prepared via reaction of **4** with 1-Phenyl-2-propyn-1-ol using Copper(II) as catalyst (Figure 4). The ^{1}H NMR spectrum for compound **5** (Figure 6) was found at 1.22 for methyl bound to steroid nucleus; at 1.30-3.30 and 6.66-7.03 for steroid moiety; at 5.74 ppm for methylene bound to hydroxyl group; at 6.30 ppm for hydroxyl group; at 7.06, 7.27-7.29, 7.70 and 7.96 ppm for phenyl groups bound to pyridine ring; at 7.24 ppm for phenyl group bound to ether and carbonyl groups; at 743-7.60 ppm for methanol fragment; at 7.74 ppm for triazole ring; 7.88 ppm for pyridine ring.

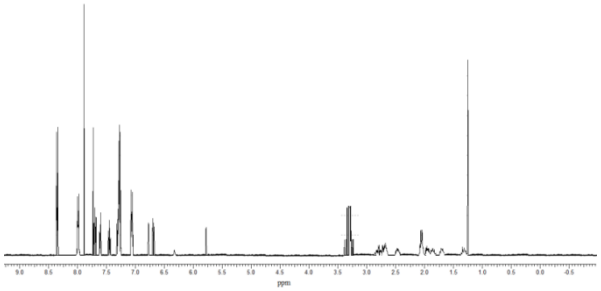

Figure 6. The scheme showed ^{1}HNMR spectrum from compound **5**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

Other signals found in the ^{13}C NMR spectrum for 5 showed bands at 20.82 ppm for methyl group; at 26.84-45.40, 55.98-114.52, 124.30, 133.90, 139.25 and 154.80 ppm for steroid moiety; at 53.36 for methylene group bound to hydroxyl group; at 117.70 and 150.12 ppm for triazole ring; at 118.55, 133.90 and 161.32 ppm for phenyl bound to both ether and carbonyl groups; at 119.84, 126.12, 146.60, 154.33, 155.26 and 182.76 ppm for pyridine ring; at 125.88, 126.86, 128.36-128.94, 129.32, 138.58, 140.37 and 182.76 ppm for phenyl groups bound to pyridine ring; at 127.50, 129.00, 130.40 and 146.22 ppm for phenyl bound to methanol fragment; at 169.20 ppm for carbonyl group ppm. Additionaly, the mass spectrum (m/z) from compound **5** was found to 734.32.

3.5. Second etherification.

This stage was achieved by the reaction of compound **3** with (4-Nitro-phenyl)-acetonitrile in the presence of dimethyl sulfoxide on mild conditions to form the compound 6 (Figure 7). The ^{1}H NMR spectrum for compound **6** (Figure 8) was found at 1.22 for methyl bound to steroid nucleus; at 1.30-3.30, 6.66-6.76 and 7.04 for steroid moiety; at 3.62 ppm for methylene bound to nitrile group; at 6.96, 7.34 ppm for phenyl bound to ether group; at 7.06-7.29, 7.70 and 7.96 ppm for phenyl groups bound to pyridine ring; at 7.88 ppm for pyridine ring.

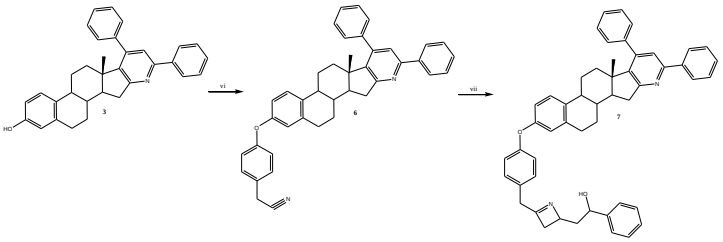

Figure 7. Preparation of an azete-steroid derivative (**7**). Reaction of Reaction of a pyridine-steroid derivative (**3**) with 4-Nitro-phenyl)-acetonitrile to form an ether-derivative (**6**). Then, **6** reacted with 4-Phenyl-1-buten-4-ol to form **7**. Conditions: vi = potassium carbonate/dimethyl sulfoxide/rt; vii = Copper(II) chloride/rt.

Additionally, other signals involved in the ^{13}C NMR spectrum were showed at 20.82 ppm for methyl group; at 23.42 ppm for methylene bound to nitrile group; 26.84-114.52, 124.30, 133.90, 139.22 and 155.90 ppm for steroid moiety; at 117.02, 122.48, 128.24 and 154.95 ppm for phenyl bound to ether group; at 117.40 ppm for nitrile group; at 119.80, 146.60-154.32, 155.32 and 182.80 ppm for pyridine ring; at 125.85-126.87, 128.34-129.30, 138.55 and 140.34 ppm for phenyl groups bound to pyridine ring. In addition, the mass spectrum (m/z) from compound **6** was found to 572.28.

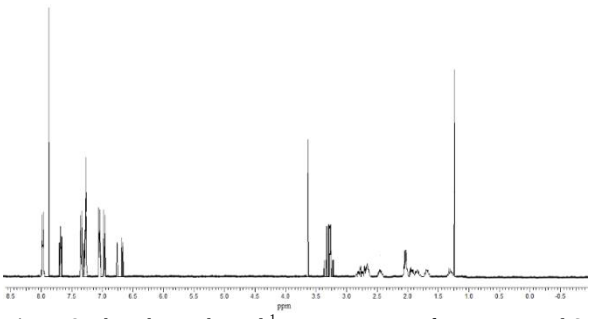

Figure 8. The scheme showed ^{1}HNMR spectrum from compound **6**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

3.6. Preparation of an azete derivative.

There are several reports on the synthesis of unsubstituted azete using several reagent such as Rh(II)/TBDMSO [37], CuI/Et_3N [38], $CuCl_2$ [39] and others. In this investigation, the compound **7** was prepared via reaction of **6** with 4-Phenyl-1-buten-4-ol using Copper(II) chloride as catalyst (Figure 7). The ^{1}H NMR spectrum for compound 7 (Figure 9) was found at 1.22 for methyl bound to steroid nucleus; at 1.30-1.85, 1.94-2.04, 2.44-

2.66, 2.70-3.30, 6.66-6.76 and 7.04 for steroid moiety; at 1.90 and 2.20 ppm for methylene group bound to 2,3-Dihydroazete ring; at 2.68 ppm for hydroxyl group; at 3.56 ppm for methylene group bound to both hydroxyl and phenyl groups; at 4,20-4.50 ppm for 2,3-Dihydroazete ring; at 5.13 ppm for methylene bound to both hydroxyl and phenyl groups; at 6.80-6.86 ppm for phenyl bound to ether group; at 7.06, 7.27, 7.29, 7.70 and 7.96 ppm for phenyl groups bound to pyridine ring; at 7,25, 7.28 and 7.32 ppm for phenyl bound to hydroxyl group; at 7.88 ppm for pyridine ring.

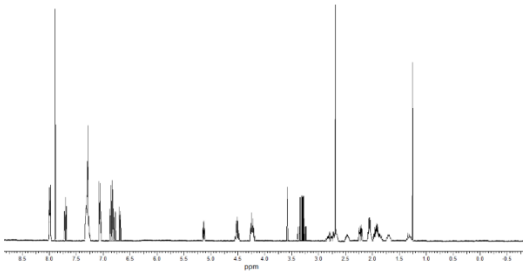

Figure 9. The scheme showed ^{1}HNMR spectrum from compound **7**. Analyzed with a Varian VXR300/5 FT NMR apparatus at 300 MHz in $CDCl_3$. ppm = parts per million.

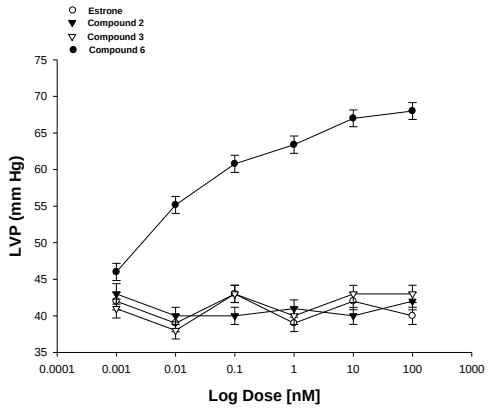

Figure 10. *Biological Activity exerted by estrone and compounds **2**, **3** and **6** on left ventricular pressure (LVP) in a heart failure model*. Intracoronary boluses (50 µl) of the compound estrone or compound **2**, **3** and **6** at a dose of 0.001 to 100 nM were administered and the corresponding effect on the LVP was determined. The results showed that compound **6** increases the LVP in a dependent dose manner (p = 0.05) compared with estrone and compound **2** and **3**. Each point represents the mean ± S.E. of 7 experiments.

Additionally, other signals involved in the ^{13}C NMR spectrum were showed at 20.82 ppm for methyl group; at 26.84-29.66, 34.20-37.32, 41.62, 45.40, 55.96, 114.22-114.52, 124.30, 133.90, 139.22 and 155.90 ppm for steroid moiety; at 33.08, 44.22 and 165.96 ppm for 2,3-Dihydroazete ring; at 41.20 ppm for methylene bound to 2,3-Dihydroazete ring; at 45.96 ppm for methylene bound to both phenyl and 2,3-Dihydroazete ring; at 70.52 ppm for methylene bound to both hydroxyl and phenyl groups; at 114.72, 126.60, 128.., 130.00 and 155.22 ppm for phenyl bound to ether group; at 125.84, 126.84, 128.34-129.32, 138.56 and 140.32 ppm for phenyl groups bound to pyridine ring; at 126.02, 126.94-128.02 and 145.30 ppm for phenyl bound to hydroxyl group; at 119.80, 146.60-154.32, 155.27 and 182.80 ppm for pyridine ring. Finally, the mass spectrum (m/z) from compound **7** was found to 720.37.

3.7. Biological activity.

The biological activity of estrone and their derivatives (**2** to **7**) on left ventricular pressure was evaluated using a failure heart model; the results showed that only compound **6** increase left ventricular pressure in a dose-dependent manner compared with estrone and compounds **2**-**4**, **5** and **7** (Figures 10 and 11).

This phenomenon suggests that nitrile group involved in the chemical structure of **6** is essential to biological activity exerted for this compound on left ventricular pressure compared with the compounds **2**-**4**, **5** and **7.**

However, other physicochemical parameters [40] could be involved in the biological activity of **6** such as lipophilicity degree; in this way, in this study this physicochemical parameter was determinate. The results showed in the table 1 indicate that lipophilicity degree for **6** was higher compared with estrone and the compounds **2**-**5** and **7**. This phenomenon could condition the biological activity of compound **6**.

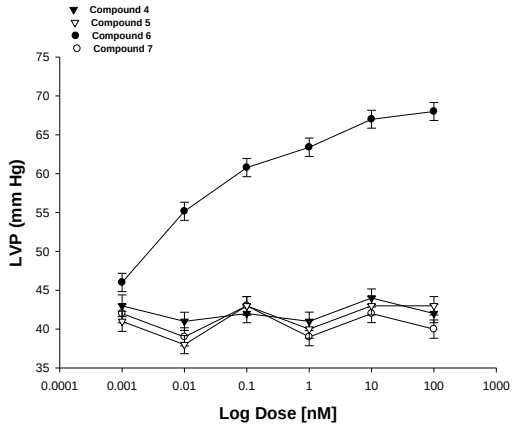

Figure 11. *Effects exerted by compounds **4** to **7** on left ventricular pressure (LVP) in a heart failure model*. Intracoronary boluses (50 µl) of either compounds **4** to **7** at a dose of 0.001 to 100 nM were administered and the corresponding effect on the LVP was determined. The results showed that compound **6** increases the LVP in a dependent dose manner (p = 0.05) compared with the compounds **4**, **5** and **7**. Each point represents the mean ± S.E. of 7 experiments.

Table 1. Lipophilicity of estrone and compounds C-2 to C7.

Parameter	Estrone	C-2	C-3	C-4	C-5	C-6	C-7
iLOGP	2.40	3.27	4.11	5.63	6.01	8.50	6.81
XLOGP	3.13	5.19	8.15	10.85	10.19	18.87	10.60
WLOGP	3.82	5.40	7.69	10.23	9.82	14.22	10.95
MLOGP	3.44	4.65	5.92	6.35	6.80	9.45	7.01
SILICOS-IT	3.84	5.48	7.53	7.76	9.00	18.50	11.91
Consnsus LogP	3.33	4.80	6.68	8.16	8.36	14.91	9.45

4. CONCLUSIONS

In this study is reported a facile synthesis of some azaindeno-acetonitrile derivative using some reagents which no require special conditions. In addition, compound **6** exert biological activity on left ventricular pressure in a heart failure model; this phenomenon could depend on the functional groups involved in their chemical structure.

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6. ACKNOWLEDGEMENTS

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