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Design and synthesis of methylthiododec-5-en-7-yn-6-yl-steroid derivativewith positive inotropic activity

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ABSTRACT

There are several drugs for the treatment of heart failure; however, some of these drugs can produce several secondary effects such as hyperkalemia, arrhythmias, and others. Therefore, the aim of this study was synthesized a new methylthiododec-5-en-7-yn-6-yl-steroid derivative using some chemical strategies. In addition, the biological activity of the steroid derivative against heart failure was evaluated using an isolated heart model. Also, the effect produced by this steroid derivative on left ventricular pressure (LVP) was determinate using some positive inotropic drugs such as milrinone, digoxin, levosimedan and dobutamine as controls. The results showed that methylthiododec-5-en-7-yn-6-yl-steroid derivative increase LVP in a dose-dependent manner and this effect was like that produced by milrinone. In conclusion, the biological activity produced by steroid-derivative; 1) depends on the functional groups involved in their chemical structure; 2) could act as a positive inotropic agent similar to milrinone. All these data suggest that steroid-derivative may be a good candidate for the treatment of heart failure.

Keywords: *Steroid, synthesis, heart, failure, inotropic.*

1. INTRODUCTION

Heart failure is a main cause of death in patients with heart disease [1]. Several drugs have used for treatment of patients with heart failure; such as digoxin (ATP-ase inhibitor) [2], dobutamine (β_1-antagonist) [3], levosimendan (calcium sensitizer) [4], spironolactone (aldosterone-antagonist) [5], captopril (angiotensin-converting enzyme (ACE) inhibitor) [6], milrinone (phosphodies-terase 3-inhibitor) [7] and others. Unfortunately, the use of some of these agents is limited by their narrow therapeutic window and their propensity to cause life-threatening arrhythmias [8, 9]. In the search of new alternative therapyfor the treatment of heart failure, several drugs have been developed; for example, a series of trichloroacetamidine derivatives were prepared from trichloroacetonitrile, with positive inotropic activity in an animal model [10]. Other study showed the synthesis of androstane-3,6,17-trione(E,Z)-3-(2-aminoethyl)oxime from androstane-3,6,17-trione with positive inotropic activity in a heart failure model [11]. In addition, other data showed the preparation of a steroid-derivative from dihydrotestosterone and their inotropic activity using an isolated rat heart model [12]. All these studies suggest that several drugs can exert effects against heart failure; however, it is important to mention that differences of functional groups involved in their chemical structure could produce differences in biological activity against heart failure; therefore, in this study was prepared a methylthiododec-5-en-7-yn-6-yl-steroid derivative to evaluate their biological activity using a heart failure model.

2. MATERIALS AND METHODS

2.1. General methods.

The reagents used in this investigation were acquired from Sigma-Aldrich Co., Ltd. The melting point for compounds was determined on an Electrothermal (900 model). Infrared spectra (IR) were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer.^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR300/5 FT NMR spectrometer at 300 MHz in $CDCl_3$ using TMS as an 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 an indol-steroid

(12aR,14aS)-6-hydroxy-12a,14a-dimethyl-3,3a,3b,4,5,7,12,12a, 12b,13,14,14a-dodecahydrocyclopenta[5,6]naphtho[2,1-b]carbazol-1(2H)-one (2)

In a round bottom flask (10 ml), formestane (200 mg, 0.66 mmol), phenylhydrazine (80 mg, 0.74 mmol) in acetic acid (5 ml) were stirred to reflux for 12 h. The solution obtained was reduced pressure and purified through a crystallization using the methanol:water (3:1) system; yielding 44% of product; m.p. 120-122 ^{o}C; IR (V_{max}, cm^{-1}) 3430, 3400, and 1480: ^{1}H NMR (300

MHz, Chloroform-*d*) δ_H: 0.94 (s, 3H), 1.06 (s, 3H), 1.08-2.00 (m, 12H), 2.12-3.12 (m, 5H), 7.12-7.66 (m, 4H), 10.70 (broad, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 13.62, 18.90, 21.26, 21.67, 22.77, 30.86, 30.90, 33.72, 34.98, 35.56, 41.02, 47.52, 51.44, 53.70, 112.11, 116.93, 119.49, 119.78, 121.24, 121.53, 126.87, 132.35, 135.44, 149.50, 220.10 ppm. EI-MS m/z: 375.21. Anal.Calcd.for $C_{25}H_{29}NO_2$: C, 79.96; H, 7.78; N, 3.73; O, 8.52. Found: C, 79.90; H, 7.70.

Preparation of an indol-steroid-alkyne derivative

(12aR,14aS)-1-(6-hydroxyhex-1-yn-1-yl)-12a,14a-dimethyl-1,2,3,3a,3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta-[5,6]naphtho[2,1-b]carbazole-1,6-diol (3)

In a round bottom flask (10 ml), compound **2** (200 mg, 53 mmol), 5-hexyn-1-ol (70 µl 0.63 mmol), sodium azide (35 mg, 0.53 mmol) and 5 ml of methanol were stirred to reflux for 12 h. The solution obtained was reduced pressure and purified through a crystallization using the methanol:water(2:1) system; yielding 67% of product; m.p. 148-150 ^oC; IR (V_{max}, cm^{-1}) 3432, 3400 and 21094: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.86 (s, 3H), 0.96-1.04 (m, 2H), 1.08 (s, 3H), 1.36-1.50 (m, 2H), 1.58-1.60 (m, 4H), 1.62-2.10 (m, 9H), 2.18 (m, 2H), 2.19-3.12 (m, 4H), 3.64 (m, 2H), 7.12-7.66 (m, 3H), 7.30 (broad, 3H), 7.68 (m, 1H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 12.74, 18.86, 18.90, 21.96, 22.78, 22.93, 25.50, 30.84, 31.82, 33.02, 33.72, 36.72, 38.90, 41.02, 47.02, 52.23, 53.13, 62.10, 80.62, 80.72, 83.40, 112.10, 116.93, 118.92, 119.47, 119.78, 121.24, 126.86, 132.33, 135.44, 149.50 ppm. EI-MS m/z: 473.29. Anal.Calcd.for $C_{31}H_{39}NO_3$: C, 78.61; H, 8.30; N, 2.96; O, 10.13. Found: C, 78.56; H, 8.26.

Preparation of a methylthio-steroid derivative

(12aR,14aS)-1-((E)-1-chloro-6-hydroxy-2-(methylthio)hex-1-en-1-yl)-12a,14a-dimethyl-1,2,3,3a,3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtho[2,1-b]carbazole-1,6-diol (4)

In a round bottom flask (10 ml), compound **3** (200 mg, 0.42 mmol), hydrochloric acid (0.5 ml) in 10 ml of dimethyl sulfoxide was stirred to reflux for 6 h. The solution obtained was reduced pressure and purified through a crystallization using the methanol:water:hexane (3:1:1) system; yielding 56 % of product; m.p. 166-168 ^oC; IR (V_{max}, cm^{-1}) 3430, 3402 and 1480: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.84 (s, 3H),0.96-1.04 (m, 2H), 1.08 (s, 3H), 1.48-1.50 (m, 3H), 1.51 (m, 2H), 1.56-1.70 (m, 2H), 1.72 (m, 2H), 1.76-2.02 (m, 5H), 2.16 (s, 3H), 2.18-2.30 (m 3H), 2.32 (m, 2H), 2.64-3.12 (m, 2H), 3.64 (m, 2H), 7.12-7.20 (m, 2H), 7.23 (broad, 4H), 7.22-7.66 (m, 2H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 15.60, 16.11, 18.92, 21.02, 22.77, 24.66, 25.12, 30.85, 31.22, 31.70, 32.24, 33.21, 33.72, 36.99, 41.02, 44.44, 49.96, 52.24, 62.20, 90.95, 112.10, 116.90, 118.94, 119.48, 119.76, 121.25, 126.88, 130.97, 132.34, 135.46, 137.43, 149.50 ppm. EI-MS m/z: 555.25. Anal.Calcd.for $C_{32}H_{42}ClNO_3S$: C, 69.10; H, 7.61; Cl, 6.37; N, 2.52; O, 8.63; S, 5.77. Found: C, 69.02; H, 7.56.

Synthesis of a methylthiododec-5-en-7-yn-6-yl-steroid derivative

(12aR,14aS)-1-((Z)-1,12-dihydroxy-5-(methylthio)dodec-5-en-7-yn-6-yl)-12a,14a-dimethyl-1,2,3,3a,3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtho[2,1-b]carbazole-1,6-diol (5)

In a round bottom flask (10 ml), compound **4** (200 mg, 0.36 mmol), 5-hexyn-1-ol (70 µl 0.63 mmol), Copper(II) chloride (105 mg 0.78 mmol) and 5 ml of methanol were stirred to reflux for 12 h. The solution obtained was reduced pressure and purified through a crystallization using the methanol:water (4:1) system; yielding 64% of product; m.p. 132-134 ^oC; IR (V_{max}, cm^{-1}) 3430, 3400, 2194 and 1482: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.76 (s, 3H), 0.96-1.04 (m, 2H), 1.08 (s, 3H), 1.28-1.50 (m, 3H), 1.52 (m, 2H), 1.56 (m, 1H), 1.58-1.62 (m, 4H), 1.70-1.76 (m, 3H), 1.80 (m, 2H), 1.82-2.18 (m, 6H), 2.19 (m, 2H), 2.22 (m, 2H), 2.28 (s, 3H), 2.29-3.12 (m, 2H), 3.64 (m, 2H), 3.65 (m, 2H), 6.20 (broad, 5H), 7.16-7.66 (m, 4H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 14.90, 16.44, 18.70, 18.90, 21.05, 22.79, 24.50, 26.44, 27.54, 30.23, 30.86, 31.82, 32.97, 33.20, 33.72, 36.99, 41.00, 46.10, 51.88, 52.24, 62.09, 62.20, 80.02, 86.12, 112.14, 113.30, 113.90, 116.92, 118.94, 119.45, 119.76, 121.24, 132.32, 135.44, 149.50, 150.90 ppm. EI-MS m/z: 617.35. Anal.Calcd.for $C_{38}H_{51}NO_4S$: C, 73.87; H, 8.32; N, 2.27; O, 10.36; S, 5.19. Found: C, 73.80; H, 8.26.

Preparation of an azete-steroid derivative

(12aR,14aS)-1-(3-(4-hydroxybutyl)-4-(methylthio)cyclobut-2-en-1-yl)-12a,14a-dimethyl-1,2,3,3a,3b,4,5,7,12,12a,12b,13,14,14a-tetradecahydrocyclopenta[5,6]naphtho[2,1-b]carbazole-1,6-diol (6)

In a round bottom flask (10 ml), compound **5** (200 mg, 1.15 mmol), 1-Phenyl-2-propyn-ol (100 µl 0.82 mmol), Copper(II) chloride (105 mg 0.78 mmol) and 5 ml of methanol were stirred to reflux for 12 h. The solution obtained was reduced pressure and purified through a crystallization using the methanol:water:hexane (3:1:2) system; yielding 45% of product; m.p. 176-178 ^oC; IR (V_{max}, cm^{-1}) 3432, 3402 and 1482: ^{1}H NMR (300 MHz, Chloroform-*d*) δ_H: 0.92 (s, 3H), 0.97-1.04 (m, 2H), 1.08 (s, 3H), 1.30-1.44 (m, 3H), 1.50 (m, 2H), 1.52 (m, 2H), 1.63 (m, 1H), 1.66 (m, 2H), 1.68-1.69 (m, 3H), 1.70 (m, 1H), 1.74 (m, 1H), 1.78 (m, 1H), 1.82 (m, 1H), 1.86-1.96 (m, 2H), 2.04 (s, 3H), 2.12 (m, 2H), 2.18-3.12 (m, 5H), 3.60 (m, 2H), 3.66 (m, 2H), 5.70 (broad, 5H), 6.00 (d, 1H, J = Hz), 7.14-7.66 (m, 4H) ppm. ^{13}C NMR (300 MHz, Chloroform-*d*) δ_C: 10.44, 17.24, 18.90, 21.26, 21.88, 22.76, 24.06, 24.42, 30.88, 32.52, 33.87, 34.10, 34.40, 34.77, 35.12, 35.64, 36.67, 37.60, 40.83, 43.52, 44.80, 51.87, 52.05, 62.52, 62.84, 82.04, 112.11, 116.90, 118.66, 119.46, 119.78, 121.24, 126.88, 132.34, 134.40, 135.44, 149.50, 151.50 ppm. EI-MS m/z: 619.36. Anal.Calcd.for $C_{38}H_{53}NO_4S$: C, 73.63; H, 8.62; N, 2.26; O, 10.32; S, 5.17. Found: C, 73.58; H, 8.58.

2.3. Evaluation of biological activity.

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

Animals. Male Wistar rats (weighing 200-250 g) used were obtained from the pharmacochemical laboratory of the Autonomous University of Campeche. In addition, the animals were managed in accordance with the guide for the care and use of laboratory animals [13]. All rats 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 trimmed of non-cardiac tissue and retrograde perfused via a non-circulating perfusion system at a constant flow rate (10 ml/min). The perfusion medium was the Krebs-Henseleit solution (pH = 7.4; 35-37 0C) bubbled with gas mixture (CO_2, 5% and O_2, 95%). Experimental data were done after an equilibration period (10 min).

Determination of perfusion pressure

Perfusion pressure Changes in perfusion pressure and left ventricular pressure produced by the administration of the compounds involved in this investigation were determined using a pressure transducer that was bound to both chamber (where the hearts were mounted) and computerized data capture system (MP-100).

Left ventricle pressure evaluation

To evaluate the biological activity of drugs involved in this study against left ventricle pressure, a latex balloon filled with saline solution (0.01 mm, diameter) was inserted into the left ventricle through the left atrium. It is important to mention 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) [14].

Effects exerted by noradrenaline, milrinone, dobutamine, levosimedan and the compound 6 on left ventricular pressure. Intracoronary boluses (50 μL) of either noradrenaline or milrinone or dobutamine or levosimedan or the compound **6** at dose of 0.001 to 100 nM were administered and the corresponding effect on the left ventricular pressure was evaluated.

Statistical analysis

The obtained values are expressed as average ± SE, using each heart as its own control. The data obtained were put under an analysis of variance (ANOVA) using the Bonferroni correction factor [15]. The differences were considered significant when p was equal or smaller than 0.05.

Pharmacophore evaluation

The 3D pharmacophore model for the compounds **1-6** was determinate using LigandScout 4.0 software [16].

3. RESULTS

3.1. Chemical synthesis.

Indol-steroid synthesis

Several indole derivatives have been prepared using some reagents such as $NaAuCl_4$ [17], CuI/Pd [18], trimethylsilyltrifluoromethanesulfonate [19], $Mn_2(CO)_{10}$ [20], Co(III) [21] and others. In this study, formestane (compound **1**) reacted with phenylhydrazine using Fisher reaction in acid medium (Figure 1) to form an indol-steroid derivative (**2**).

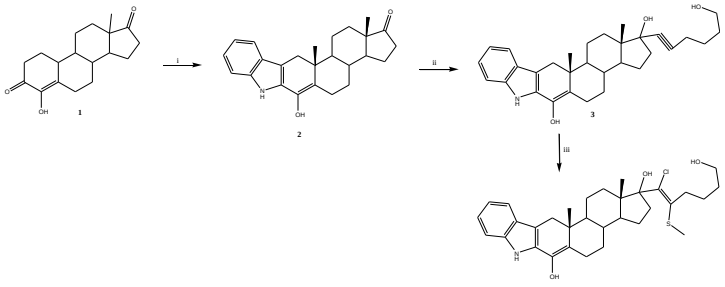

Figure 1. Preparation of a methylthio-steroid derivative (**4**).Reaction of formestane (**1**) with phenylhydrazine (i) to form an indol-steroid analog (**2**). Then, **2** reacted with 5-hexyn-1-ol (ii) to synthesis of a propargylic-steroid (**3**). Finally, **4** was prepared viareaction of **3** with dimethyl sulfoxide (iii).

The ^{1}HNMR showed several signals for **2** at 0.94 and 1.06 ppm for methyl groups bound to steroid nucleus; at 1.08-2.00 and 2.12-3.12 ppm for steroid moiety; at 7.12-7.66 ppm for indole fragment; at 10.70 ppm for both amino and hydroxyl groups. ^{13}C NMR spectra showed chemical shifts at 13.62-18.90 ppm for methyl groups bound to steroid nucleus; at 21.26-53.70, 121.53 and 149.52 ppm for steroid moiety; at 112.12-121.24 and 126.87-135.44 ppm for indole fragment; at 220.10 ppm for ketone group. In addition, the mass spectrum from **2** showed a molecular ion (m/z) 375.21.

Preparation of a propargylic-steroid derivative

There are several reports which showed the preparation of some propargylic-alcohols using different methods and reagents such as disulfide-oxazolidine [22], Ti(O-i-Pr)$_4$-BINOL complex [23], chiral diamine-coordinated tin(II) triflate [24], $P(PhCH_2NCH_2CH_2)_3N$ [25] and others. In this investigation, **2** reacted with 5-hexyn-1-ol using Copper(II) as catalyst to form a propargylic-steroid derivative (**3**). The ^{1}HNMR showed several signals for **3** at 0.86 and 1.08 ppm for methyl groups bound to steroid nucleus; at 0.96-1.04, 1.36-1.50, 1.62-2.10 and 2.19-3.12 ppm for steroid moiety; at 1.58-1.60, 2.18 and 3.64 ppm for arm bound to both hydroxyl and alkyne groups; at 7.12-7.22 and 7.68 ppm for indole fragment; at 7.30 ppm for amino and both hydroxyl groups. ^{13}C NMR spectra showed chemical shifts at 12.74 and 18.90 ppm for methyl groups; at 18.86, 25.50, 31.82 and 62.10 ppm for arm bound to both alkyne and hydroxyl groups; at 21.96-22.93, 30.84, 33.02-53.13, 80.62, 118.92 and 149.50 ppm for steroid moiety; at 80.72-83.40 ppm for alkyne group; at 112.10-116.93 and 119.47-135.44 ppm for indole fragment. Finally, the mass spectrum from **3** showed a molecular ion (m/z) 473.29.

Synthesis of a methylthio-steroid derivative

Some studies have shown the preparation of methyl-thio analogs using several reagents such as CuO/I_2 [26], KOH [27], CS_2 [28] dimethyl sulfoxide/HCl [29] and others. In this study, a methylthio-steroid derivative was prepared by reaction of compound **3** with dimethyl sulfoxide in acid conditions to reflux. The ^{1}HNMR showed several signals for **4** at 0.84 and 1.08 ppm

for methyl groups bound to steroid nucleus; at 0.96-1.04, 1.48-1.50, 1.56-1.70, 1.76-2.02, 2.18-2.34 and 2.64-3.12 ppm for steroid moiety; at 1.51, 1.72, 2.32 and 3.64 ppm for arm bound to both alkene and hydroxyl groups: at 2.16 ppm for methyl group bound to sulfur; at 7.12-7.20 and 7.26-7.66 ppm for indole fragment; at 7.23 ppm for amino and hydroxyl groups. ^{13}C NMR spectra showed chemical shifts at 16.11-18.92 ppm for methyl groups bound to steroid nucleus; at 15.60 ppm for methyl linked to sulfur; at 21.02-24.66, 30.85-31.22, 32.24, 33.72-52.24, 90.95, 118.94 and 149.50 ppm for steroid moiety; at 25.12, 31.70, 33.21 and 62.20 ppm for arm bound to both alkene and hydroxyl groups; at 112.10-116.90, 119.48-126.88 and 132.34-135.46 ppm for indole fragment; at 130.97 and 137.43 ppm for alkene group. In addition, the mass spectrum from **4** showed a molecular ion (m/z) 555.25

Preparation of methylthiododec-5-en-7-yn-6-yl-steroid derivative (5)

The compound **5** was prepared by the reaction of **4** with 5-hexyn-1-ol using Copper(II) as catalyst (Figure 2). The ^{1}HNMR showed several signals for **5** at 0.76 and 1.08 ppm for methyl groups bound to steroid nucleus; at 2.28 ppm for methyl group bound to sulfur; at 0.96-1.04, 1.28-1.50, 1.56, 1.70-1.76, 1.82-2.18 and 2.29-3.12 ppm for steroid moiety; at 1.52, 1.80, 2.22 and 3.65 ppm for arm bound to both alkene and hydroxyl groups; at 1.58-1.62, 2.19 and 3.64 ppm for arm bound to both alkyne and hydroxyl groups; at 6.20 ppm for amino and hydroxyl groups; at 7.16-7.66 ppm for indole fragment. ^{13}C NMR spectra showed chemical shifts at 14.90 for methyl bound to sulfur; at 16.44 and 18.90 for methyl groups bound to steroid nucleus; at 18.70, 26.47, 31.82 and 62.09 ppm for arm bound to both alkyne and hydroxyl groups; at 26.44, 27.54, 33.21 and 62.20 for arm bound to both alkene and hydroxyl groups; at 21.05-24.50, 30.23-30.86, 32.97, 33.72-52.24, 86.12, 116.92-118.94 and 149.50 ppm for steroid moiety; at 80.02 and 113.30 ppm for alkyne group; at 113.90 and 150.90 ppm for alkene group; at 112.14 and 119.45-135.44 ppm for indole fragment. Finally, the mass spectrum from **5** showed a molecular ion (m/z) 617.35.

Synthesis of azete-steroid derivative

Several azete derivatives have been synthesized using some reagents such as mesitonitrile oxide [30], α,α-bis-(alkylthio) oxime [31], acylisothiocyanate [32] and others. In this study, an azete-steroid derivative (**6**) was prepared via intramolecular 2 + 2 addition of alkyne group to alkene-derivative of compound **5** in the presence of Copper(II) chloride (Figure 2). The ^{1}HNMR showed several signals for **6** (Figure 3)at 0.92 and 1.08 ppm for methyl group bound to steroid nucleus; at 0.97-1.04, 1.30-1.44, 1.63, 1.68-1.69, 1.74, 1.86-1.96 and 2.18-3.12 ppm for steroid moiety; at 1.50, 1.52, 1.66, 1.70, 1.78, 2.12, 3.60 and 3.66 ppm for both arms bound to both hydroxyl group and azete ring; at2.04 ppm for methyl group linked to sulfur; at 1.82, and 6.00 ppm for azete ring; at 5.70 ppm for both amino and hydroxyl groups; at 7.14-7.66 ppm for indole fragment.

^{13}C NMR spectra showed chemical shifts at 10.44 ppm for methyl group bound to sulfur; at 17.24-18.90 ppm for both methyl groups bound to steroid nucleus; at 21.26, 22.76-24.06, 30.88, 33.87, 34.40, 35.12-35.67, 40.83, 51.87-52.05, 82.04, 118.66 and 149.50 ppm for steroid moiety; at 21.88, 24.42, 32.52, 34.10, 34.77, 37.60, 62.52 and 62.84 ppm for arms bound to both hydroxyl group and azete ring; at 43.52-44.80, 134.40 and 151.50 ppm for azete ring; at 112.11-116.90, 119.46-132.34 and 135.44 ppm for indole fragment. In addition, the mass spectrum from **6** showed a molecular ion (m/z) 619.36.

Physicochemical parameters of 1-6

There are some studies which suggest some physicochemical parameters such as molar volume (M_V) and molar refractory (M_R) are chemical tools correlate with different biological properties which may depend on the characteristics of each substituent involved in the chemical structure of a molecule. In this investigation, both M_V and M_R descriptors were determinate for the compounds **1** to **6** using a previous method reported [33]. The theoretical results showed (Table 1) that M_V and M_R values were different for compound **6** compared with **1**-**5**. This phenomenon suggests that steric hindrance, conformational preferences, and internal rotation may be two factors which influence the biological activity to exert by **6** on some biological model. However, it is important to mention that anothertype of physiochemical factors such as hydrogen bond donor groups (HBD) and hydrogen bond acceptor groups (HBA) has been used to predict the biological activity of some compounds in several theoretical models [34]. Therefore, in this study these physicochemical parameters (Table 1) were determinate using the LigandScout 4.0 software [16]. The theoretical data showed that the values of physicochemical parameters HBA and HBD were <10 for compounds **1** to **6**, this phenomenon suggests that these compounds could be well absorbed, as with other types of substances accordance to Lipinski's Rule [34].

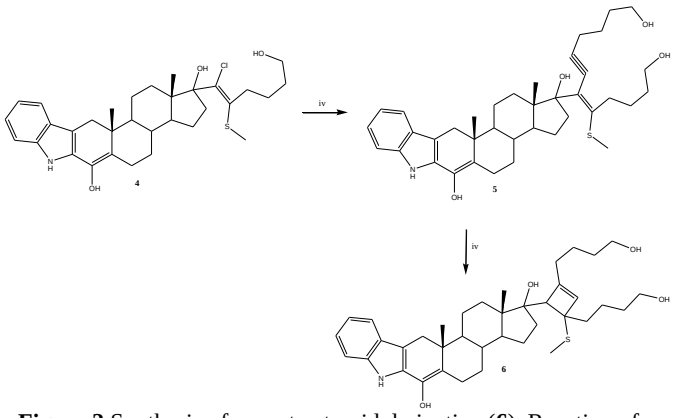

Figure 2.Synthesis ofan azete-steroid derivative (**6**). Reaction of a methylthio-steroid derivative (**4**) with 5-hexyn-1-ol (iv) to form a propargyl-steroid analog (**5**). Then, **6** was prepared via intramolecular 2+2 addition in presence of Copper(II) chloride (v).

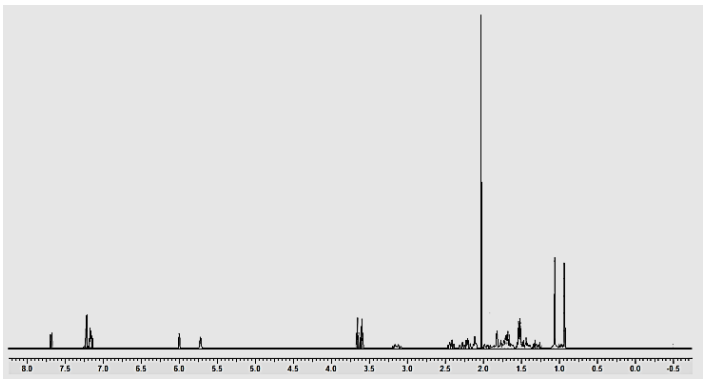

Figure 3. The scheme showed ^{1}H NMR of compound **6**. Analyzed with a Variant VXR300/5FT NMR Apparatus at 300 Hz in $CDCl_3$.

Table 1. Physicochemical parameters of compounds **1-6**. The values were calculated using both ACDLabs and Spartan software's.

Parameters	1	2	3	4	5	6
Molar Refractivity (cm^3)	78.36	109.83	136.59	157.51	180.01	180.17
Molar Volume(cm^3)	237.00	295.20	373.30	424.00	489.6	488.70
Polarizability (cm^3)	31.06	43.54	54.94	62.44	71.36	71.42
Parachor (cm^3)	628.70	820.10	1062.90	1197.20	1393.00	1384.20
Index of refraction	1.57	1.66	1.66	1.66	1.65	1.65
Surface Tension (dyne/cm)	49.40	59.50	65.70	63.50	65.50	64.30
Density g/cm^3		1.27	1.26	1.31	1.26	1.26
HBD	1	2	4	4	5	5
HBA	3	2	3	4	5	5

3.3 Pharmacophore evaluation.

The pharmacophore model can furnish a new insight to design novel molecules that can enhance or inhibit the function of the target and will be useful in drug discovery strategies.

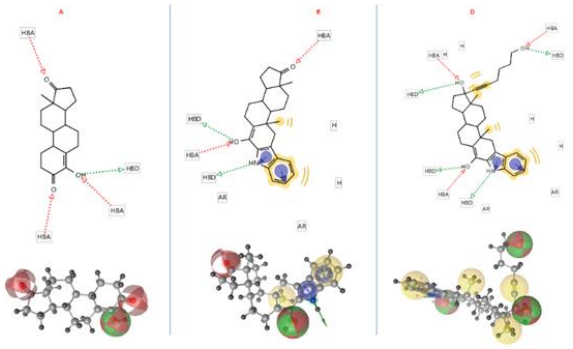

Figure 4. Scheme represents a pharmacophore from both compounds **1** (A), **2** (B) and **3**(C)using the LigandScout 4.0 software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red) and hydrogen bond donor (HBD, green).

Therefore, in this study, LigandScout 4.0 software [33] was used to develop a pharmacophore model of compounds **1-6**. The results showed in Figures 4 and 5 indicated that there are different types of functional groups involved in the compounds **1-6** which could interact via hydrophobic contacts or as hydrogen bond acceptors or as hydrogen bond donor with several biomolecules which can be translated as changes in the biological activity of some target cells of their action.

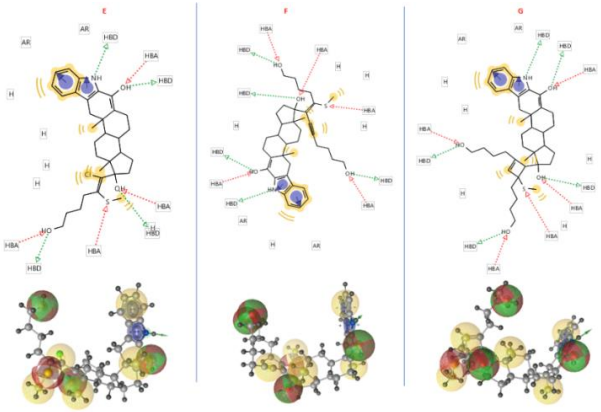

Figure 5. Scheme represents a pharmacophore from both compounds **4** (D), **5** (E) and **6** (F)using the LigandScout 4.0 software. The model involves a methyl group (yellow) hydrogen bond acceptors (HBA, red) and hydrogen bond donor (HBD, green).

Biological activity

Analyzing the hypothesis above mentioned; in this study, the biological activity of compounds **1-6** on perfusion pressure was evaluated in an isolated rat heart model. The results showed that only compound **6** increases the perfusion pressure in a time-dependent manner compared with the compounds **1-5** and conditions control (Figure 6).

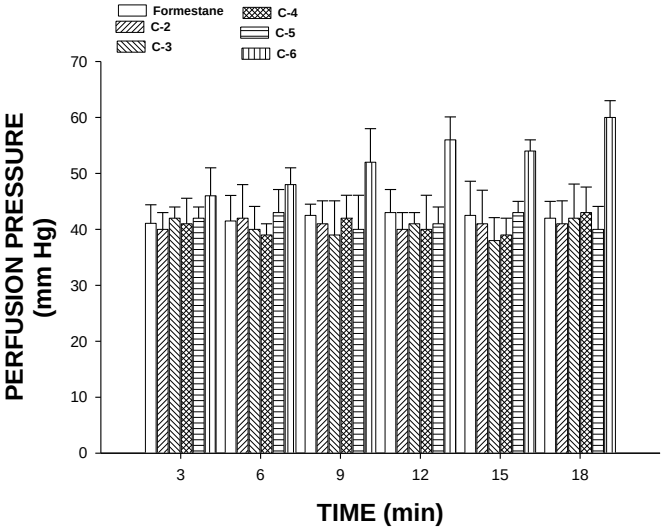

Figure 6. Effect exerted by compound **1-6** on perfusion pressure. The results showed that only compound **6** significantly increased perfusion pressure (p = 0.05) through time (3-18 min) compared with compounds **1-5** at a dose of 0.001 nM and the control conditions. Each bar represents the mean ± S.E. of 9 experiments.

Analyzing these data experimental alternative was carried out to characterize the inotropic activity of **6** using digoxin (Na^+/K^+-ATPase inhibitor) [2], milrinone (phosphodiesterase III antagonist) [7], dobutamine (β_1-adrenergic receptor inhibitor) [3] and levosimedan (calcium sensitizer) [4] as controls. The results shown in Figure 7 indicate that the biological activity of compound **6** was in a similar manner to milrinone.

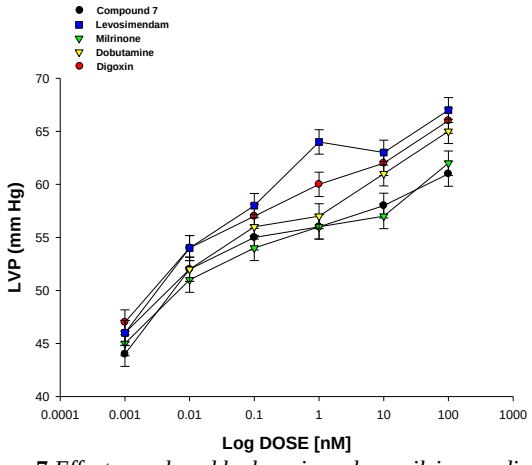

Figure 7.*Effects produced by levosimendan, milrinone, digoxin, dobutamine and compound 6 against left ventricular pressure (LVP).* The scheme showed that compound **6** increase LVP in a similar manner that milrinone. Nevertheless, the biological activity of **6** was lower compared with dobutamine, digoxin and levosimendan.Each bar represents the mean ± SE of 9 experiments.

All these data indicate that compound **6** could exert their positive inotropic activity via phosphodiesterase III inhibition which depend their functional groups involved in their chemical structure. However, it is important to perform some toxicity

studies to evaluate if there are any side effects in the biological activity of the compounds involved in this stud

Theoretical analysis of toxicity

Analyzing the premise above mentioned, in this study also was evaluated the possible toxicity induced by compound **6** using the GUSAR software [35]. The results (Table 2) showed that toxicity could be higher via oral administration of compound 6 compared to the other types of administration routes such as intravenous, intraperitoneal and subcutaneous.

Table 2.Theoretical analyses of Ld50 from compound 6 using GUSAR software.

Rat IP LD50 Log10(mmol/kg)	Rat IV LD50 log10(mmol/kg)	Rat Oral LD50 log10(mmol/kg)	Rat SC LD50 log10(mmol/kg)
0,271	-1,838	0,290	-0,464

Routes of administration:
IP (Intraperitoneal)
IV (Intravenous)
Oral
SC (Subcutaneous)

4. CONCLUSIONS

In this study was reported a facile synthesis of a methylthiododec-5-en-7-yn-6-yl-steroid derivative (compound **6**)using some chemical strategies. In addition, the results indicate that: 1) the functional groups involved in the chemical structure of are responsible forbiological activity exerted by **6** against left ventricular pressure; 2) the compound **6** could act as a positive inotropic agent similar to milrinone. All these data suggest that steroid-derivative may be a good candidate for the treatment of heart failure.

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